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## Characterization of an Isolate of *Cucumber mosaic cucumovirus* from Radish (*Raphanus sativus*) in Iraq

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**Abstract:** New symptoms of systemic mosaic on radish (*Raphanus sativus*) were noticed by farmers in the middle of Iraq in 2009. This led to think that the causal agent may be a new virus. An isolate of the causal agent has been isolated and characterized as a *Cucumber mosaic virus* (CMV) from the infected Radish. Leaves from radishes showing systemic mosaic were collected from several locations that cultivate radish in Iraq in 2011. The virus was identified by symptoms on indicator plants, immunochromatography and the molecular weight of viral coat protein. The virus caused systemic severe mosaic on *Cucumis sativus*, *Cucumis melo*, *Cucurbita pepo*, mild systemic mosaic on *Raphanus sativus*, *Nicotiana tabaccum* and induced the formation of leaf strip and filliforme shaped on tomato mechanically inoculated by an extract from symptomatic radish leaves. Local lesion developed on the leaves of *Datura stramonium*, *Vigna unguiculata*, *Chenopodium amaranticolor*, *Chenopodium quinoa* following mechanical inoculation by virus extracts. Positive reaction were developed on the immunostrip containing anti-CMV polyclonal antibodies treated with extracts from infected radish leaves by immunochromatography. One protein of 24.5 kd was detected by electrophoresis on polyacrylamide gel containing SDS. It was found that the virus is transmitted through radish seeds at rate of 20%, which plays an essential role in virus epidemiology and acts as a continuous source of virus inoculum.

**Key words:** *Cucumber mosaic virus*, *Raphanus sativus*, immunochromatography, Iraq

### INTRODUCTION

The radish (*Raphanus sativus* L.) the type species belongs the family of Cruciferae. It is one of the most famous vegetables in Iraq, which is grown for its nutritious roots that are eaten fresh usually. Radish has a chilled and refreshing effect. Baloch (1994) stated that radish also prevents constipation, increases appetite and it contains sugar, several proteins, minerals and vitamin C. In traditional medicine, people who have piles or liver trouble are encouraged to take it and it is effective to reduce the pain (Baloch, 1994). Radish can be infected by many pathogens, one of which is *Cucumber mosaic virus*, that severely decrease its leaves yield.

*Cucumber mosaic cucumovirus* (CMV), which belongs the genus Cucumovirus and the Family Bromoviridae, is one of the most important viruses worldwide having the widest host range among all plant viruses. This virus has more than 1200 host plant species in families of dicotyledonous and monocotyledonous in the temperate region of the world (Kaper and Waterworth, 1981; Edwardson and Christie, 1991; Palukaitis and Garcia-Arenal, 2003).

CMV induces various kinds of symptoms, which depend on the host plant species, ranging from little or invisible symptoms to severe mosaic associated with stunting and leaf deformations (Choi *et al.*, 2004;

Jeon *et al.*, 2006; Lee *et al.*, 2007; Oh *et al.*, 2008; Grube *et al.*, 2000; Adhab and Alani, 2011) and reported that the virus infects many weeds and ornamental plants, which serves as a store source the primary virus inoculum.

It has been reported that CMV could be transmitted efficiently by about 80 species of aphids and that the more efficient species are *Myzus persicae* and *Aphis gossypii* in non-persistent manner (Palukaitis *et al.*, 1992; Edwardson and Christie, 1991; Perry *et al.*, 1998). The virus is also reported to be transmitted through seeds of more than twenty plant species with transmission percent varying from 1% up to 50% based on plant species (Palukaitis *et al.*, 1992). Seeds transmission of CMV by certain hosts is of epidemiological significance (Quiot *et al.*, 1983; Rist and Lorbeer, 1991).

Symptoms of mild mosaic was observed on radish plants in the vicinity of cucumber plants suspected to be of *Cucumber mosaic virus* (CMV); therefore, the study aimed to characterize the causal virus of these symptoms using biological, serological and molecular techniques.

### MATERIALS AND METHODS

**Virus source and mechanical inoculation:** The virus isolate used in this study was obtained from naturally infected radish plants (*Raphanus sativus* L.).

Leaves of the plant showing mosaic symptoms were collected and triturated in 0.01 M phosphate buffer, pH 7.5 containing 0.2% Na-diethyldithiocarbamate (DIECA) (1 g: 4 mL) with a mortar and a pestle. Leaves of indicator plants previously sprayed with carborandom 600 mesh were rub-inoculated by the virus extract and maintained in a green house and the plants were monitored for symptoms. The presence of the virus in the inoculated plants was tested 2 weeks after the inoculation by immunochromatography protocol.

**Seed transmission:** Radish plants were mechanically inoculated at 3-5 leaf stage and kept in insect proof cages under greenhouse conditions. Seeds were collected at maturity and sown in pots containing mixed soil. The virus in seedling raised from these seeds was first determined visually and then confirmed serologically by immunochromatography.

**Immunochromatography test:** This test was carried out using immunostrips containing anti-CMV polyclonal antibodies purchased from Agdia biofords, France. A part of symptomatic and asymptomatic radish and test plants leaves (0.1 g) was homogenized by a pestle in a sac extraction buffer (Phosphate Buffered Saline (PBS) provided by Agdia with the immunochromatographic kit. The end of immunostrips, that contain polyclonal antibodies to CMV, was immersed to 0.5 cm in the homogenate for 3-5 min. The development of a band on the strip indicates a positive reaction.

**Gel electrophoresis:** Young symptomatic leaves of infected and non-infected radish plants were ground in 0.1 M phosphate buffer pH 8.0 contains 1.5% Sodium Dodecyl Sulfate (SDS). One volume of the extract was mixed with one volume of Laemmli dissociation buffer (0.125 M Tris-HCl, pH 6.8, 30% glycerol, 2% SDS, 2% B-mercaptoethanol and 0.5% Bromophenol blue as tracking tincture). The mixture was boiled for 2 min and analyzed on 10% SDS-polyacrylamide gel by electrophoresis at 125V for 4-5 h. The electrophoresis was stopped when the tracking tincture reached the bottom of the resolving gel. Then, the gel was stained in 0.25% commassie brilliant blue in methanol: water: acetic acid (5:5:1) (v: v: v) for 30 min and destained in a mixture of 10% acetic acid and 5% methanol at 37°C. Markers of known molecular weights were used for gel calibration.

## RESULTS

**Symptomatology:** Results showed that the virus produced symptoms on indicator plants at various degrees depending on the plant species (Table 1). Systemic mild mosaic on *Raphanus sativus* and *Nicotiana tabacum* cv. Turkish, was developed following mechanical inoculation

by an extract from symptomatic radish leaves (Fig. 1). The virus induced severe mosaic, stunting and deformation on cucurbits. *Cucumis sativus*, *Cucumis melo* and *Cucurbita pepo* that were sub inoculated with virus extract of symptomatic radish leaves (Fig. 2). Chlorotic local lesions were formed on the inoculated leaves of *Datura stramonium*, *Chenopodium amaranticolor*, *Chenopodium quinoa* and *Tetragonia expansa* Murr. Necrotic local lesions were developed on the inoculated leaves of *Vigna unguiculata* and *Gomphrena globosa* (Fig. 3). The virus induced the formation of leaf strip and filliform on tomato mechanically inoculated by extract from symptomatic radish leaves. The symptoms on the indicator plants were similar to those that reported previously by Palukaitis *et al.* (1992) concerning *Cucumber mosaic virus* (CMV).

**Seed transmission:** One thousand seeds from infected radish plants from different locations were collected. One hundred seeds from each location, surface-sterilized by sodium hypochloride 2%, were sown. The virus was



Fig. 1(a-b): (a) Systemic mild mosaic induced on new leaves of *Raphanus sativus* and (b) *N. tabacum* cv. Turkish mechanically inoculated by sap from radish plants showing mosaic symptoms

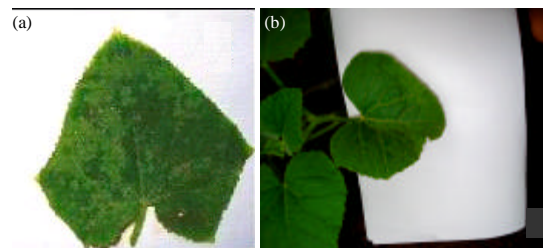


Fig. 2(a-b): Systemic severe mosaic induced on new leaves of (a) *Cucumis sativus* and (b) *Cucumis melo* mechanically inoculated by sap from radish plants showing mosaic symptoms

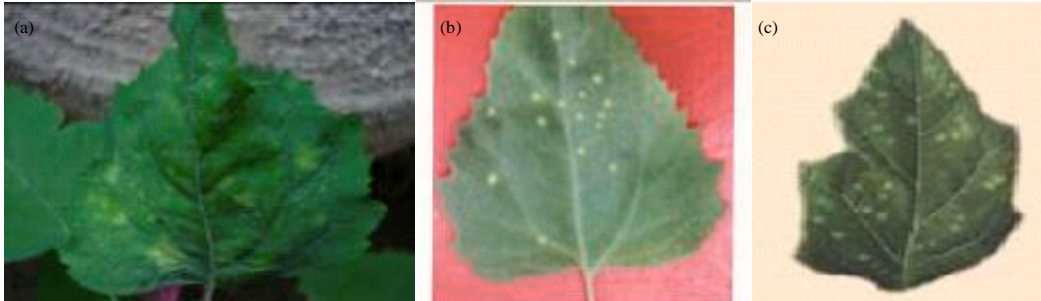


Fig. 3(a-c): Chlorotic local lesions induced on (a) *Chenopodium quinoa*, (b) *Ch. amaranticolor* and (c) *Datura stramonium* leaves mechanically inoculated by sap from radish plants showing mosaic symptoms

Table 1: Reaction of indicator plants, which mechanically inoculated by extracts from symptomatic radish leaves

Indicator plants	Immunochromatography	
	Symptoms	reaction
<i>Raphanus sativus</i>	SM	+
<i>Cucumis sativus</i>	SM	+
<i>Cucumis melo</i>	SM	+
<i>Cucurbita pepo</i>	SM	+
<i>Nicotiana tabacum</i> cv. Turkish	SM	+
<i>Lycopersicon esculentum</i>	SM, fil	+
<i>Datura stramonium</i>	CLL	+
<i>Chenopodium amaranticolor</i>	CLL	+
<i>Chenopodium quinoa</i>	CLL	+
<i>Tetragonia expansa</i>	CLL	+
<i>Vigna unguiculata</i>	NLL	+
<i>Ghompsonia globosa</i>	NLL	+

SM: Systemic mosaic, MS, fil: Mosaic, Filiforme, CLL: Chlorotic local lesions, NLL: Necrotic local lesions, +: Positive reaction

detected in 20 seedlings out of 95 with seed transmission rate 20%. Several previous studies had reported that CMV is transmitted through seeds of more than 20 plant species with a transmission rate ranging from 1% up to 50% based on plant species (Palukaitis *et al.*, 1992).

**Serological test:** A positive reaction was developed on the immunostrip which containing anti-CMV antibodies upon treating with an extract from symptomatic radish leaves as proved by the appearance of a band on the strip within 3 min. No reaction was observed on the strip treated with an extract from healthy plants. This technique was successfully used for detecting CMV in various hosts in plant virology lab-university of Baghdad (data not shown).

**Gel-electrophoresis:** The analysis of total proteins of CMV-infected and non-infected plants by SDS-PAGE revealed the presence of a protein of approximately 24.5 kd in the profile of protein preparations from infected plants. No corresponding protein was observed in the profile of healthy plant proteins (Fig. 4). This protein represents CMV coat protein. SDS-PAGE was frequently

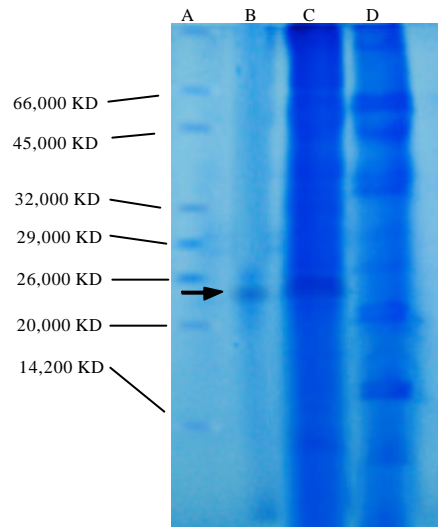


Fig. 4(A-D): Profile of Slab SDS-gel electrophoresis for samples of CMV on 10% polyacrylamide gel with 0.1% SDS. Arrow referred to principle 24.5 kd protein band of the virus observed in samples from infected radish plants only, (A) Protein markers of known molecular weight used to determine the MW of virus proteins, (B) Sample of partially purified CMV (control), (C) Total proteins extracted from virus infected plants, (D) Total proteins from healthy plant (control)

used to detect and identify plant viruses (Andreeva *et al.*, 1994; Dardick and Culver, 1997; Singh *et al.*, 1998; Mavrodieva *et al.*, 1998).

## DISCUSSION

A virus naturally infecting radish plants in the vicinity of cucumber plastic houses cultivation was

observed. The symptoms induced by the virus on the indicator plants mechanically inoculated by symptomatic radish leaves extract, the positive reaction with anti-CMV antibodies on immunostrip by immunochromatography and the molecular weight of viral coat protein, as determined by electrophoresis on polyacrylamide gel indicate that the virus isolated from radish plants is a strain of *Cucumber mosaic virus* (CMV). The symptoms, which was developed on the indicator plants, were found as similar as those reported by Palukaitis *et al.* (1992) concerning CMV.

Analysis of viral coat protein on SDS-PAGE was frequently used to differentiate virus and virus strains. It was reported that it is possible to differentiate CMV, ZYMV and WMV-2 using the analysis of their coat protein on SDS-Polyacrylamide gel by electrophoresis (Fuchs *et al.*, 1998). Several strains of CMV were differentiated and classified to groups using this technique (Mavrodirova *et al.*, 1998; Singh *et al.*, 1998). Unlike the strains previously identified in the Plant Virology laboratory-University of Bagdad, the molecular weight of coat proteins subunit of the present isolate was found to be 24.5 kd, while two subunit were identified, 24 and 26 kd, for the previous strains (Adhab, 2010). The present isolate infects radish causing systemic mosaic, whereas it was not reported that CMV infects radish in the previous.

In addition, the current isolate was found to be transmitted through radish seeds at a rate of 20%. It has been reported that CMV can be transmitted by the seeds of more than 20 plant species with a percent varying from 1% up to 50% depending on plant species (Palukaitis *et al.*, 1992; Wang and Maule, 1992). It was reported that the property of seed-borne transmission varies with virus strain and host cultivar. A virus may be transmitted through seeds of cultivar but not through seeds of others. At the same time, a variation in seed transmission rate was registered in the same cultivar for different strains of the same virus (Johansen *et al.*, 1996).

The high rate of the virus transmission through seeds may be responsible for its spread on radish plants and serve as a reservoir and as a primary source for the virus inoculum that disseminated to cucumber and other economic crops by the aphid vectors and play an essential role in virus epidemiology. Quiot *et al.* (1983) and Rist and Lorbeer (1991) reported that seed transmission of CMV by certain hosts is of epidemiological significance.

In the end, this study provides supporting information on the epidemiology of Cucumber mosaic virus in Iraq.

## CONCLUSION

According to the presented data, the causal agent of the new systemic mosaic symptoms on radish may be an isolate of *Cucumber mosaic virus*, which has just appeared on radish. Consequently, depending on our database, this is the first record of this virus on radish in Iraq.

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