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Study on distribution and detection of *cauliflower mosaic virus* (CaMV) in Dezful region of Iran

Maryam Ebrahim-Ghomi*

Department of Plant Protection, Dezful Branch, Islamic Azad University, Dezful, Iran

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Abstract

Cauliflower mosaic virus (CaMV) belong to *Caulimovirus* is one of the most important causal agent of viral diseases. During autumn and late winter of 2012, 180 canola (*Brassica napus*) leaf samples were randomly collected from fields around Dezful region in Iran. DAS-ELISA test proved CaMV presence serologically in some samples. RT-PCR reaction using specific primers confirmed ELISA tests. Seven samples out of total samples (3.9%) showed CaMV infection using serological and RT-PCR assays, hence incidence of CaMV in canola fields in Dezful area was confirmed.

* **Corresponding Author:** Maryam Ebrahim-Ghomi ✉ e_qomi@iaud.ac.ir

Introduction

Canola (*Brassica napus*) is one of the most important oil-seed crop worldwide. Its seeds containing 40-45% oil and 23-35% protein. Canola oil is among the most qualified oil because of its suitable composition of unsaturated fatty acids and low percentage of saturated fatty acids. Due to its importance, cultivation is increasing annually in Iran, and in majority of agricultural regions it is cultivated as alternate crop after wheat or rice.

Viral diseases are among the most destructive diseases of canola, and some of them can cause up to 90% yield losses (Goheen, 1998).Canola is the suitable host of overwintering of some viruses which infect Brassicaceae, thus viral diseases of canola have special importance (Lattan *et al.*, 2003). Several viruses can infect canola, and among them *Cauliflower mosaic virus* (CaMV) is one of the most prevalent and important viruses which can cause yield losses, seriously (Lathan *et al.*, 2003).

CaMV belongs to *caulimovirus* and Caulimoviridae is an isometric and dsDNA virus. CaMV is the first identified plant with DNA genome. Host rang of this virus is limited to Bracicaceae and, it is transmitted semi-dominantly with different species of aphids such as *Myzus persica* (Holling and Brunt, 1981). Obvious symptoms of this disease are vein banding, vein clearing, mottling, wrinkling, deformation of leaves, disorders in formation of seed sheath and decrease in plant growth (Shepherd, 1981).

For the first time in Iran, *Cauliflower mosaic virus* was reported from cauliflower fields in Isfahan (Bahar, 1986), then it was reported from Varamin, Shiraz and Sari (Shahraeen *et al.*, 2003). Some of biological and serological characterizations of the virus were studied in canola fields of West-Azarbaijan and 60% of symptomatically sample were reported as infected, using ELISA method. In addition, purification and preparation antiserum from Iranian isolates of CaMV have been performed (Sahandi *et al.*, 2006). Study of symptomatically samples in Fars province of Iran using ELISA method showed that 70% of symptomatically samples were infected to both CaMV and *Beet west yellows virus* (Sadeghi, 2003). In years 2004 and 2005, incidence and distribution of CaMV were determined in Cauliflower, Cabbage, Raphanus, Turnip and kohlrabi samples collected from 10 provinces of Iran using ELISA and, results were confirmed with study of host range, specific PCR and sequencing of virus genome. Also, virus particles in collected samples were observed by electron microscopy (Farzadfar *et al.*, 2007). Zahedi Taberstani *et al.*, (2010) studied presence and distributions of *Turnip mosaic virus*, *Cauliflower mosaic virus* and *Beet west yellows virus* using ELISA method. They showed 2.5% disease incidence of CaMV in Golestan province of Iran.

Dezful region in south west of Iran (Khuzestan province), one of the most canola cultivated crop and reported that CaMV is one of the damaging diseases causing decrease in quality and quantity of this crop. Therefore this research, due to lack of information about CaMV in Dezful, aimed to study distribution and detection of virus presence using serological and specific PCR methods.

Material and methods

Plant materials

In two steps, 180 symptomatically canola samples were collected from canola fields around Dezful region in January and March 2012. Sampling was done randomly from leaves and stalks of canola plants and in second step, sampling was synchronous with flowering stage, therefore some samples were collected from flowers and seed sheaths of different plants. In addition, leaves of canola plants with symptoms such as mosaic, deformation of leaves and dwarfing were also collected (Fig. 1). During sampling, weed and volunteer plants around canola fields were collected and transferred to lab. Unusual symptoms in plants were noted during sampling time.

Disease evaluation

To prove infection to CaMV in different canola samples, double antibody sandwich -enzyme linked immonosorbent assay (DAS- ELISA) method was performed using antiserums synthesized by Bioreba

company based on method Clark and Adams (1977). Polyclonal antibody of CaMV was diluted based on company instructions. Rate of dye change in different Wells was measured by 40 ELISA Reader in 405 nm.

Specific PCR

For detection of DNA of CaMV in infected plants specific PCR was carried out. DNA extraction was performed based on Callaway *et al.*, (1996) method with slightly modifications. One gram of leaves of canola plants were grounded in liquid nitrogen using pastel and mortar. Then 750 µl of extraction buffer (250 mM NaCl, 30mM EDTA, 100Mm Tiris pH=8, 0.25% SDS) was added to any microtubules and shook. Afterwards, 10 µl proteinas K10mgl was mixed with microtube contents and incubated in 55°C for 60 minutes and 100°C for 10 minutes, respectively. After cooling, microtubes were centrifuged at 13000 rpm for 5 minutes. DNA pellets were diluted with 70 µl dd water and transferred to -20°C. PCR reaction was performed based on method of Agma *et al.*, (2002). PCR reaction contained 2.5 µl 10X PCR buffer, 0.75 µl $MgCl_2$ (50mM), 0.5 µl dNTPs (10mM) , 1 µl of each forward and reverse primers, 0.5 µl Taq DNA polymerase (2.5u/ µl) and 2.5 µl of each extacted DNA samples. PCR conditions were adjusted in 95 C for 3 min and then 35 cycles of 95 C for 1 min, 54 C for 45S and 72 C for 50S followed by final extension in 72 C for 10 min. Specific primers with sequence of R= 5' GGAAACAGTGCTTCATCCTC 3' and F= 5' ACGCTGCAACGGCTTCTAAG 3' were performed to amplify a 720 bp fragment for CaMV specific amplification. PCR products were electrophoresed in 1.2% agarose gel at 70v for 1 hour.

Results and discussion

ELISA

Seven samples among canola plants showed infection using ELISA method.

Specific PCR

Twenty seven samples were determined as healthy or infected by ELISA method were assayed by PCR using specific primers, 7 infected samples showed 720bp PCR products, while no amplification of product was observed in health samples (Fig. 2).

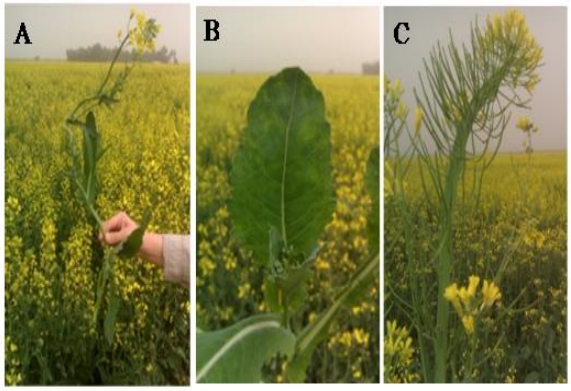

Fig. 1. Different symptoms of Cauliflower mosaic virus in canola plants. A) Stem deformation, B) leaf mosaic, c) stem fasciation and sheath sterilation.

Generally, 3.9% disease incidence was observed in Dezful canola fields. Obtained results were similar to study of Zahedi Tabarestani *et al.*, (2010) that observed 2.5% disease incidence in fields of Golestan province.

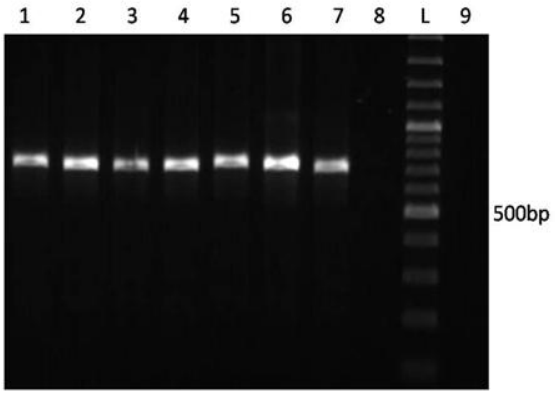

Fig. 2. Electrophoresis of PCR products obtained from specific amplification of genome of *Cauliflower mosaic virus* (CaMV) in different samples. Lane1-lane7 Infected samples showed 720bp PCR product specific for CaMV, lane8-lane9 Health samples without infection to CaMV didn't show any amplification after PCR, L 100bp DNA ladder.

Two of symptomless samples show infection response using ELISA and PCR methods. Appearance of symptoms in plants infected by viruses depends on virus concentration, virulence severity of virus and susceptibility of canola varieties. Some of plant samples with symptoms such as yellows, mosaic or leaf deformation, were recognized as uninfected to CaMV using PCR or ELISA method, therefore these

symptoms in canola plants are not only caused by CaMV and, other disease agents may cause these symptoms. Sometimes unfavorable environmental conditions, deficiency of some nutrients, feeding of some insects and some genetic disorders may show symptoms very similar to viral disease symptoms, too. Also, amplification of 720bp PCR product using specific primers was in accordance to results of Farzadfar *et al.*, (2007) and Agma *et al.*, (2002). Therefore, there are strong evidences that CaMV is present in canola fields of Dezful region.

Comparison of ELISA and specific PCR results showed more accuracy of second method so as a few samples had doubtful results using ELISA method, showed infection by specific PCR method. Although, there is a little information about CaMV in Iran, but results of this research show this virus is now distributing in this country. High rainfalls during spring and autumn help the survival of host plant and aphids, vectors which can help in widespread infection in Brassicaceae species such as canola.

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