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Full Length Research Paper

Serological and molecular detection and prevalence of *Cucurbit aphid-borne yellows virus* in the Sistan region, Iran

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During the 2009 growing seasons, virus-like symptoms were noticed on cucurbit crops (melons (*Cucumis melo* L.) and watermelons [*Citrullus lanatus* (Thunb.) Matsum and Nakai)] grown in the Sistan region. The symptoms were widespread and included initial chlorotic lesions followed by yellowing of whole leaves and thickening of older leaves. 100 melon samples from symptomatic plants from 32 melon fields were collected for virus detection during 2009 and 2010. In a preliminary serological study, extracts of diseased leaves did not react with antisera to viruses reported from area, namely *Cucumber mosaic virus*, *Watermelon mosaic virus*-2 and *Zucchini yellow mosaic virus*. During the entire survey, *CABYV* was detected by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in all samples. Analysis of these samples were done with reverse transcription-polymerase chain reactions with a pair of primers designed to amplify the coat protein region of *CABYV* which resulted in the expected product (approximately 479 bp), confirming that all melon samples were infected by *CABYV*. The high incidence of *CABYV* in infections suggests that this virus may well become an important threat for cucurbit crops in the Sistan region.

Key words: Yellowing disease, melon virus, loss, incidence, double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA), reverse transcription polymerase chain reaction (RT-PCR).

INTRODUCTION

Melon and watermelon cultivation reaches 15,000 ha of crop surface in the Sistan region where they form the most important cucurbit crops. Viral diseases of cucurbit crops cause important economic losses in Sistan region. According to Provvidenti (1996), more than 35 viruses are isolated from cucurbits. These viruses cause complex and dynamically changing problems, as was described by Nameth et al. (1986). Approximately 35 viruses reported on the Cucurbitaceae worldwide (Provvidenti, 1996; Lovisolo, 1980), *Zucchini yellow mosaic virus* (*ZYMV*), *Watermelon mosaic virus* (*WMV)*, *Cucumber green mottle mosaic virus* (*CGMMV*), *Watermelon chlorotic stunt virus (WmCSV)*, *Cucumber mosaic virus (CMV*), *Cucumber yellow stunting disorder virus* (*CYSDV*), *Papaya ringspot virus type W (PRSV-W)*, *Squash mosaic virus (SqMV)*, *Ourmia melon virus* (*OuMV*), *Cucurbit aphid-borne yellows virus* (*CABYV*) and *Cucumber vein yellowing virus* (*CVYV*) have been reported from field-grown cucurbit crops in Iran (Danesh, 1969; Ebrahim-Nesbat, 1972; 1974; Rahimian and Izadpanah, 1978; Ghorbani, 1986, 1988; Lisa et al., 1988; Parvizy, 1989; Bananej et al., 1998; Keshavarz and Izadpanah, 2004; Bananej et al., 2006a, 2006b). *CMV*, *WMV*-2 and *ZYMV* have been reported from cucurbit crops in the Sistan region (Massumi et al., 2007). *CABYV* is one of several viruses causing yellowing symptoms in cucurbit crops. *CABYV* was first described in 1992 in France and early infections with *CABYV* may lead to a ca. 50% yield loss in cucumber, and 40% in melon (Lecoq et al., 1992). *CABYV* is a member of the genus Polerovirus in the family Luteoviridae (Mayo and D'Arcy, 1999), and causes severe yellowing in cucurbit crops in France (Lecoq et al., 1992) and in the United States (Lemaire et al., 1993). *CABYV* multiplies in phloem tissue and is transmitted persistently from plant to plant by aphids, mainly by *Aphis gossypii* Glover and *Myzus persicae* Sulzer. It cannot be

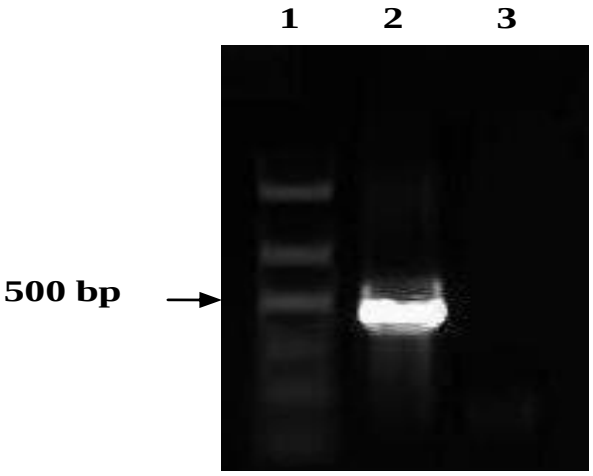

Figure 1. Agarose gel electrophoresis (1%) of reverse transcription–polymerase chain reaction (RT-PCR) products obtained after amplification with two specific primers. Lane 1: Molecular weight marker; lane 2: various collected sample; lane 3: healthy plant.

transmitted mechanically (Lecoq et al., 1992; Dogimont et al., 1996). Typical symptoms in cucumber, melon, squash, and watermelon include yellowing and thickening of the older leaves. *CABYV* severely reduces yield in melon and cucumber by reducing the number of fruits per plant as a result of a high percentage of flower abortions (40 and 51%, respectively) but it does not alter fruit shape or quality (Dogimont et al., 1996). Researches revealed that *CABYV* is the most common and widespread virus in field-grown cucurbit crops in Iran (Bananej and Vahdat, 2008). Extensive surveys have shown that *CABYV* is one of the most common viruses in open-field crops in many regions having a variety of ecologies (Lecoq, 1999; Lecoq et al., 2003).

In this study, a prevalent virus infecting melon crops in the Sistan region during two consecutive growing seasons (2009 and 2010) was identified.

MATERIALS AND METHODS

Surveys and sample collection

Surveys were performed in open field melon of the Sistan region during 2009 and 2010. In the Sistan region, melon is grown during the spring and summer seasons. Samples were obtained from 32 melon fields randomly distributed in a geographical area of approximately 3,500 km^2. 100 hundred melon samples from symptomatic plants from 32 melon fields were collected for virus detection. Each field was visited and sampled at least twice, before and at the beginning of the harvest. Incidence of viral symptoms was visually evaluated in each field by examining 300 to 400 plants following a W-shaped itinerary, and expressed as the percentage of the total plants. Viral symptoms observed included chlorotic lesions followed by yellowing of whole leaves and thickening of older leaves. A maximum of five samples per field from symptomatic plants were collected. A sample consisted of two to three symptomatic leaves per plant.

Virus detection

Samples were analyzed to determine the presence of prevalent viruses such as *CABYV*, *CMV*, *WMV* and *ZYMV*. The samples were used immediately after collection for the detection of viruses by the double-antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA (Clark and Adams, 1977) for the presence of viruses. IgGs and alkaline phosphatase conjugated IgGs were used for important viruses. Leaf samples were ground in a pre-cooled mortar and pestle with an extraction buffer (PBST: 0.13 M NaCl, 0.003 M KCl, 0.008 M Na_2HPO_4, 0.001 M KH_2PO_4, pH 7.4) containing 0.05% Tween 20 and 0.1% nonfat dry milk and were placed in wells that had been precoated with specific polyclonal antisera diluted in a carbonate buffer (pH 9.6). Plates (Nunc Microwell, Roskilde, Denmark) were incubated at 4°C overnight and washed three times with PBST-Tween 20 buffer. Plates were then coated with alkaline phosphatase conjugated antibody diluted in extraction buffer and incubated for 2 h at 37°C. After washing, *p*-nitrophenyl phosphate in diethanolamine substrate buffer (0.5 mg ml^{-1}, pH 9.8) was added to each well and incubated at room temperature for 30 to 120 min. The reaction was detected colorimetrically at A_{405} nm using an ELISA reader (MCC-340, Multiscan Labsystem, Finland). Two wells were used per sample. Virus-free cucurbit species grown in insect-proof cages were used as negative controls. Positive and healthy controls were included in all tests. Samples were considered to be positive if the A_{405} nm values were more than three times those of the healthy control. To confirm *CABYV* identification, total RNA extracts were obtained from leaves of infected plants that were positive in DAS-ELISA, using TRI-Reagent (Sigma Chemical, St Louis, MO, USA). RNA resuspended in 20 µl DEPC-treated H_2O was heated to 65°C for 5 min before reverse transcription. The primers used in this study were as follows:

CABYV-CP-5´: 5´-CGCGTGGTTGTGGTCAACCC-3´
CABYV-CP-3´: 5´-CCYGCAACCGAGGAAGATCC-3´

4 µl of RNA were submitted to reverse transcription in a final volume of 20 µl, using the *CABYV*-CP-5´ (Guilley et al., 1994) primer, for 1 h at 42°C with M-MuLV reverse transcriptase (Fermentas, Vilnius, Lethuania). 2½ µl of the RT reactions were used for PCR. PCR reactions were carried out in an Eppendorf Mastercycler 5330 with the following conditions: initial denaturation of 94°C for 3 min (×1 cycle), and then 35 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, followed by a final extension of 7 min at 72°C. All amplifications were performed in volumes of 25 µl containing 10 mM Tris-HCl (pH 9), 50 mM KCl, 3 mM $MgCl_2$, 250 µM dNTPs 1.5 µM each primer, 100 ng of genomic DNA, and 2.5 U of Taq polymerase. PCR products were electrophoresed (30 to 60 min at 90 volts) in 1% agarose gel in Tris-acetate-EDTA buffer, pH 8. Gels were stained with ethidum bromide (1.5 µg) and viewed with a UV transilluminator.

RESULTS

We analyzed 100 samples of symptomatic plants taken from 36 melon fields in the Sistan region. In a preliminary serological study, extracts of diseased leaves did not react with antisera to viruses reported from the area, namely *CMV*, *WMV*-2 and *ZYMV.* All samples were found infected by *CABYV*. Samples that were positive using DAS-ELISA were checked by RT-PCR to confirm *CABYV* identification. One DNA amplification product of approximately 479 bp was observed in samples that were positive to *CABYV* in DAS-ELISA. No DNA product was amplified in healthy plant extracts (Figure 1). RT-PCR

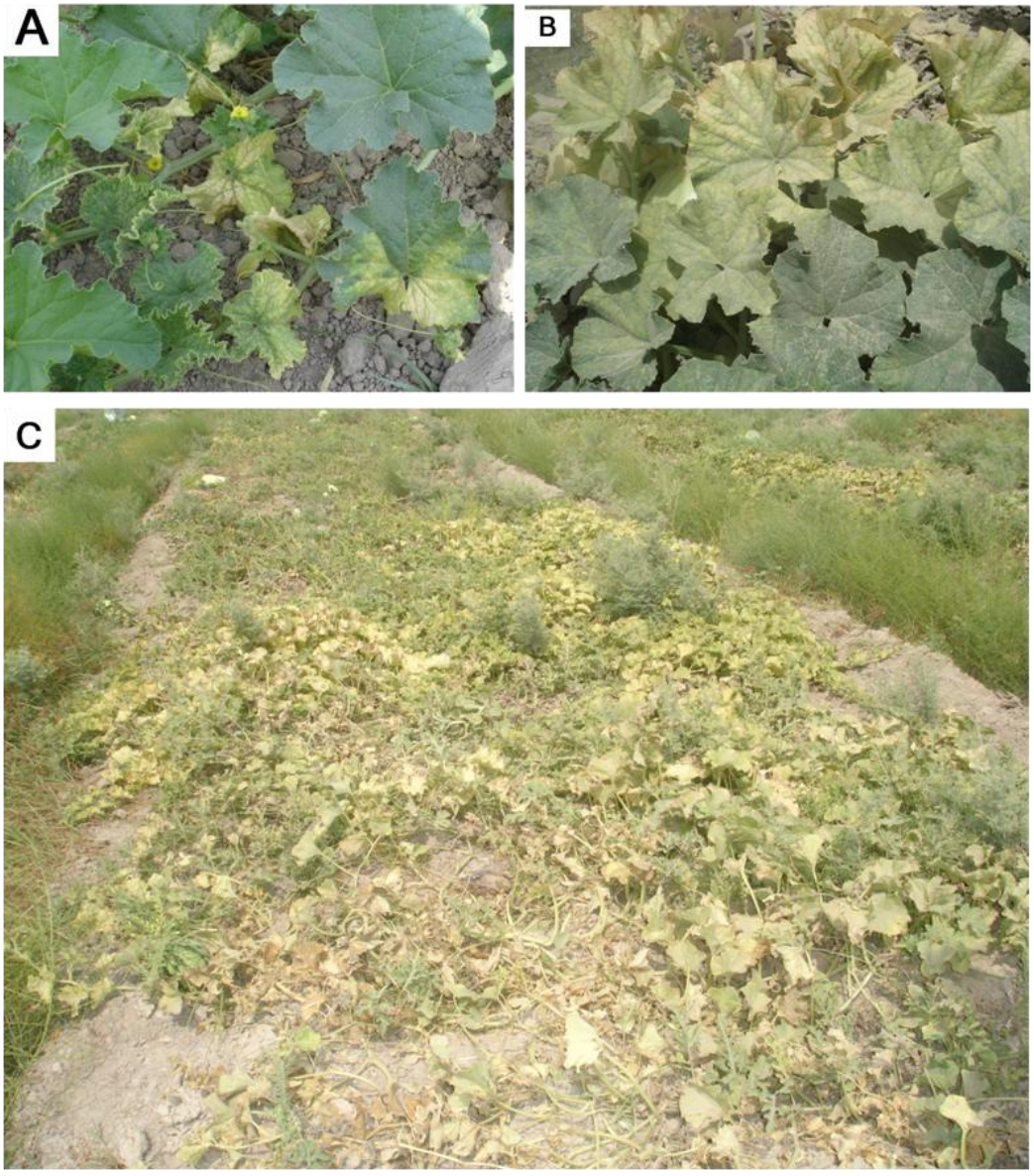

Figure 2. Symptoms in melon plants infected with *Cucurbit aphid-borne yellows virus* (*CABYV*). Yellowing and thickening of the basal leaves (A) and necrosis of basal leaves of older plants (B) was frequent. Melon (C) crops of Sistan region often showed *CABYV* incidences close to 100%.

results confirmed that *CABYV* occurred on all samples.

During the surveys, the proportion of plants showing yellowing symptoms was visually estimated for each plot. These observations, together with the results of the analysis of virus detection in samples, provided an estimation of *CABYV* incidence in the plots considered. 74% virus was present in all plots. Symptoms on infected melon plants included yellowing and thickening of the basal and older leaves, which occasionally also showed slight epinasty, or resulted in flower abortion and a reduction in the number of fruits per plant (Figure 2A to C). Frequently, the chlorotic basal leaves of older plants developed large interveinal necrotic areas.

DISCUSSION

Cucurbit viruses have always caused major losses in the quantity and quality of cucurbit crops worldwide and they represent one of the most important limiting factors for growers (Provvidenti, 1996). In the Sistan region, cucurbits have a high incidence of symptoms suggestive of viral infection. This is the first report of a survey using serological and molecular diagnostic procedures to identify the *CABYV* of melon crops in the Sistan region. The data presented here show that *CABYV* was one of the most prevalent and widespread virus in open field crops of the Sistan region during 2009 to 2010. *CABYV*

was first described by H. Lecoq and co-workers in 1992 in France, where it affected open field cucurbit crops (Lecoq et al., 1992). *CABYV* was later detected in Italy, Greece, Tunisia, Algeria, Lebanon, Turkey, Sudan, Nepal, Taiwan, China, Reunion Island, Swaziland, Brazil, Honduras, Spain, and California (U.S.A) (Abou-Jawdah et al., 1997; Juarez et al., 2004; Lecoq, 1999; Lecoq et al., 1992; 2003; Lemaire et al., 1993; Mnari-Hattab et al., 2005). Extensive surveys conducted by Lecoq and co-workers have shown that *CABYV* is one of the most common cucurbit viruses in open field crops in a great diversity of areas and environments (Lecoq, 1999; Lecoq et al., 2003). Bananej and Vahdat (2008) also showed that *CABYV* is the most common and widespread virus in field-grown cucurbit crops in Iran and an important threat for cucurbit crops in that country.

Due to the similarity of symptoms induced by *CABYV* with factors including abiotic stress, we suppose that in the Sistan region, the presence of this virus has remained unnoticed during some years. The data presented here show that it was one of the most prevalent and wide-spread viruses in open field crops of the Sistan region. In agreement with our findings, field-grown cucurbit crops (cucumber, melon, squash, and watermelon) from Lebanon also showed high *CABYV* incidence (Abou-Jawdah et al., 2000).

This survey provides essential basic information useful for *CABYV* control strategies in the Sistan region. The avoidance of aphid vectors is of fundamental importance in controlling this virus. An important option for disease control would be the use of cultivars with genetic resistance to the virus. Several resistance sources for *CABYV* in melon have been described (Dogimont et al., 1996, 1997). Evaluation of cucurbit germplasm of Sistan for disease resistance as well as for other traits such as fruit quality could prove very promising. This paper is the first report of *CABYV* in the Sistan region.

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