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MOLECULAR CHARACTERIZATION OF GEOGRAPHICALLY DIFFERENT CUCURBIT APHID-BORNE YELLOWS VIRUS ISOLATES

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Summary. – Cucurbit aphid-borne yellows virus (CABYV) causes yellowing symptoms in cucurbit crops worldwide. In this work, the sequences for the coat protein (CP) gene for CABYV isolates from Iran and Slovakia are reported for the first time. Three Iranian isolates shared 96.2–99.3% and the Slovak isolate 98.9% of nucleotide identity in comparison with the reference French CABYV-N isolate. Phylogenetic analysis showed that the sequence of CP gene of examined CABYV isolates differed only slightly. Relatively close relationship and sequence similarity of geographically distant CABYV isolates could reflect the epidemic outbreak and rapid expansion of the virus throughout the world due to the host and vector abundance.

Keywords: CABYV; coat protein gene; Cucurbitaceae; molecular variability

Introduction

CABYV is an important pathogen inducing yellowing symptoms in cucurbit crops worldwide. It is a member of the genus *Polerovirus,* the family *Luteoviridae* (Mayo and D'Arcy, 1999). The isometric virion contains a single-stranded RNA of 5.6 kb (Guilley *et al*., 1994; Xiang *et al*., 2008). CABYV is obligately transmitted by aphids in a persistent and vector-specific manner (Herrbach, 1999). Typical symptoms in cucumber, melon, squash, and watermelon include yellowing and thickening of the older leaves (Lecoq *et al*., 1992). Although CABYV is an important cucurbit pathogen occurring worldwide, the data concerning its molecular variability are rather limited. The incidence of CABYV in Slovakia has not been reported yet, although the virus has been sporadically detected since 2002 (M. Glasa, unpublished results). In 2004, the virus was detected for the first time in Iran (Bananej *et al*., 2006). However, the isolated Iranian and Slovakian viruses have not been characterized at the molecular level.

Whole genome sequences of 2 CABYV isolates from France and China are available in the public database together with partial sequences of a few CABYV isolates from other geographical locations (Guilley *et al*., 1994; Xiang *et al*., 2008).

This study reports the sequences of CP gene of CABYV isolates from Iran and Slovakia. The obtained data allowed us to compare their genetic variability and to establish phylogenetic relationship with the known CABYV isolates from other parts of the world.

Materials and Methods

Virus isolates. The Iranian CABYV isolates analyzed in this study were collected from three major cucurbit growing areas in Iran, during 2005 to 2007, namely 1) Khorasan province: squash field in Mashad region (IR-1), 2) Esfahan province: melon field in Gorgab region (IR-2), and 3) Tehran province: squash field in Varamin region (IR-3). Slovak SK-1 isolate collected in 2008 in Western Slovakia (Dunajska Streda) originated from greenhouse-cultivated cucumber.

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Abbreviations: CABYV = Cucurbit aphid-borne yellows virus; CP = coat protein; DAS-ELISA = double-antibody sandwich ELISA

The presence of CABYV and other common cucurbit viruses in samples was ascertained by using double-antibody sandwich (DAS) ELISA (Clark and Adams, 1977) with polyclonal antibodies kindly provided by Dr. H. Lecoq (INRA Montfavet, France).

RT-PCR and phylogenetic analysis. The original field samples were used to RNA extraction (TRI-Reagent, Sigma or RNeasy® Plant Mini Kit, Qiagen) and cDNA synthesis. RT-PCR were carried out using CAB forward (5'-CGCGTGGTTGTGGTCAACCC-3') and CAB reverse (5'-CCYGCAACCGAGGAAGATCC-3') primers under following cycling conditions: initial denaturation of 94°C for 3 mins, 35 cycles of (94°C for 30 secs, 55°C for 30 secs, 72°C for 1 min), final extension step of 7 mins at 72°C. The amplified region encompasses almost the entire CP gene (nt 3580–4058 numbered according to the sequence of a CABYV-N reference isolate) (NC_003688, Guilley *et al*., 1994). The PCR products were purified (Wizard PCR Preps DNA Purification System, Promega) and directly sequenced with the same oligonucleotides as used for PCR. Sequence analyses and comparisons were conducted using the MEGA4 (Tamura *et al*., 2007) and DnaSP v.3.5 programmes (Rozas *et al*., 2003). Phylogenetic relationships were determined by a distance method (corrected by Kimura-2 parameter methods) and tree was reconstructed by the neighbor-joining method implemented in MEGA4. A Barley yellow dwarf virus-GPV isolate (EU386821) was used to root the tree. The available CP sequences were retrieved from the GenBank database (http://www.ncbi.nlm.nih.gov).

Results and Discussion

A survey of the CABYV occurrence was done in recent years in Iran and Slovakia using DAS-ELISA. The results indicated that the virus is naturally distributed in all three major cucurbit-growing areas in Iran and represented an important cause of the yellowing diseases of cucurbits. In Slovakia, the CABYV infection remained less frequent and the percentage of infected plants was relatively low (Table 1).

In this study, three Iranian (IR-1, IR-2, IR-3) and 1 Slovak (SK-1) CABYV isolate collected from the naturally infected cucurbit crops were analyzed. In the collected leaf samples, a multiple infections of CABYV with common cucurbit viruses as Watermelon mosaic virus, Cucumber mosaic virus, and/or Zucchini yellow mosaic virus were frequently serologically detected (data not shown).

In order to determine the molecular variability of CABYV isolates, an RT-PCR with specific primers was performed. An expected fragment (479 bp) was obtained from all analyzed isolates. The obtained sequences were deposited in GenBank under Acc. Nos. FJ428797-FJ428800.

In the sequenced region, the Iranian isolates shared 96.2–99.3% and Slovak isolate 98.9% nucleotide identity with reference French CABYV-N isolate (Guilley *et al*., 1994). Overall, the average nucleotide sequence divergence within all available CABYV sequences (n = 29) was 0.073. The nucleotide diversity among Iranian isolates reached 0.029 (calculated using Kimura´s 2 parameter). This is in contrast with very close relationship within Tunisian or Italian CABYV dataset that showed nucleotide diversities of 0.007 and 0.011, respectively.

It is interesting from the analysis of deduced amino acid sequences that SK-1, IR-3 and two Italian isolates (EF029113 and EF029114) share 100% homology. IR-2 shows 100% homology with Chinese isolates EF003706 and EU091148.

The phylogenetic tree reconstructed from the partial CP sequence for 29 CABYV isolates showed the splitting into two groups, the first one being further divided into two subgroups 1a and 1b. Our isolates were placed in the large group 1 (Fig. 1). SK-1 and IR-3 clustered in the 1a subgroup together with European and Mediterranean isolates. Interestingly, two Iranian isolates (IR-1 and IR-2) were found to belong to subgroup 1b that contained only Chinese isolates (Xiang *et al*., 2008). Overall, phylogenetic analysis showed only partial geographical divergence of the CABYV isolates. The genetic divergence and phylogenetic relationship of Iranian isolates suggested occurrence of at least 2 independent CABYV populations in this area.

Relatively close relationship and sequence similarity of geographically various CABYV isolates could be due to the epidemic outbreak and rapid expansion of the virus throughout the world due to the host and vector abundance. This is in accordance with recent occurrence of CABYV in different localities of the world (Juarez *et al*., 2004; Hattab *et al*., 2005; Tomassoli and Meneghini, 2007).

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Table 1. Occurrence of CABYV infection in Iran (2005–2007) and Slovakia (2002–2008) detected by DAS-ELISA

Locality	Total number of tested samples	Number (%) of CABYV-infected samples
Iran (Khorasan province)	68	46 (67.6%)
Iran (Esfahan province)	387	245 (63.3%)
Iran (Tehran province)	79	36 (45.6%)
Slovakia	140	26 (18.6%)

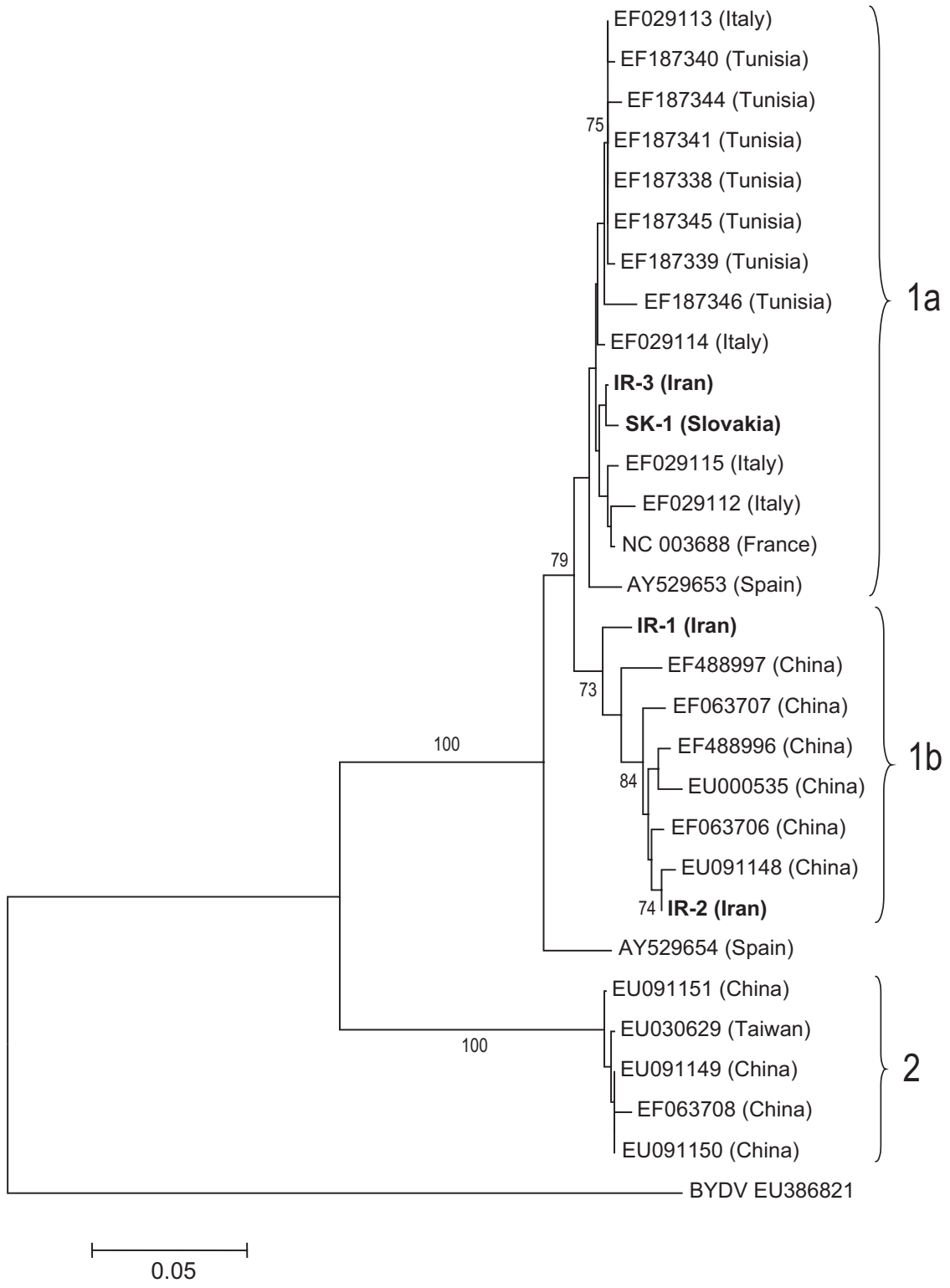

Fig. 1

Phylogenetic tree of 29 CABYV isolates based on partial nucleotide sequence of CP

The scale bar indicates a genetic distance of 0.05. Bootstrap values >70 (1000 bootstrap samples) are indicated as percentage.

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