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Molecular characterization of a *Bean yellow mosaic virus* isolate from Syria

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Summary. *Bean yellow mosaic virus* (BYMV, genus *Potyvirus,* family *Potyviridae*) was studied by comparing sequences from the coat protein (CP) gene of a Syrian isolate with sequences of six other isolates from the NCBI database. A homology tree of the CP sequences was developed using DNAMAN Software. BYMV isolates were grouped into two clusters of which the first comprised the Syrian isolate together with the Indian, Australian and Japanese isolates, and the second the BYMV isolates from China, the Netherlands and the USA. Moreover, the homology tree showed that the Syrian isolate was very close to the Indian one, with 99% homology.

Key words: Sequencing, BYMV, RT-PCR, Syria.

Introduction

Bean yellow mosaic virus (BYMV, genus *Potyvirus,* family *Potyviridae*) (Fauquet *et al.*, 2005) occurs worldwide. It naturally infects a wide range of legume species as well as some non-legumes. The virus is transmitted in a non-persistent manner by many aphid species (Bos, 1970; Boswell and Gibbs, 1983; Edwardson and Christie, 1991; Berlandier *et al.,* 1997).

Many strains of BYMV have been reported from different parts of the world and from different crops, but several have not been properly characterized (Doolittle and Jones, 1925; Barton *et al.*, 1964; Schroeder and Provvidenti, 1966; Bos *et al.*, 1974, Thottappilly *et al.*, 1976; Randles *et al.*, 1980; Abu-Samah and Randles, 1983; Tsuji *et al.*, 1996). Pea mosaic isolates and most BYMV isolates from faba bean cause mostly weak systemic symptoms such as slight chlorotic spotting with little or no growth reduction, but this is not the case with the Syrian isolate SV205-85. Severe or top-necrosis strains of the virus may cause irregular necrosis of the vein tissues and apical stem necrosis which may kill the plants. However, with mosaic symptoms in new growth, the plants often recover. With pea necrosis isolates, leaves may drop but here too the plants often recover. Pods may become mottled and malformed. In faba bean, common strains cause a mild green mosaic or vein-banding which are often associated with some leaf distortion, and the affected parts of the plant remain stunted. With the pea mosaic strain the mosaic is more distinctly green and yellowish. Pea necrosis strains cause

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systemic necrosis in faba bean (Bos *et al*., 1974). The virus may also cause flower bud necrosis and ovule abortion (Bailiss and Senanayake, 1984). Thus proper characterization of the virus isolates is essential for comparative studies, especially those related to screening crops for virus resistance.

Materials and methods

Virus isolates

BYMV Syrian isolate SV205-85 (BYMV-SY) was collected from Tel-Hadya (30 km south of Aleppo), characterized by its host reations, serological tests and electron microscopy, and maintained in faba bean (cultivar Syrian local large) in a glass house by serial mechanical inoculation (Skaf and Makkouk, 1988). The other isolates, from nine geographical areas, were used to compare symptoms induced by them and the RT-PCR products, with those of the Syrian isolate. These isolates were: SUV254-88 (Sudan), EV34-91 (Egypt), LYV61-91 (Libya), YeV48-96 (Yemen), ATCC PV89 (USA), G (Netherlands), AUV8-04 (Australia), ChV657-04 (China), and JV175-96 (Jordan).

Mechanical transmission

BYMV-infected plant tissues were ground in 0.01 M phosphate buffer, pH 7.2, and the extract was mixed with 'Celite' and inoculated by gently rubbing it on the leaves of faba bean seedlings at the 4-leaf stage, sprayed with an insecticide (Actara, 1 g l^{-1}) and grown in a temperature-controlled greenhouse.

Tissue-blot immunoassay (TBIA)

Twenty days after inoculation, all inoculated plants were tested by tissue-blot immunoassay (TBIA) following the method described by Makkouk and Kumari (1996). The BYMV-specific polyclonal antibody used was produced against SV205-85 isolate in the virology laboratory of ICARDA.

RNA extraction and RT-PCR assay

Total RNA was extracted from infected samples (those that reacted positively to the serological test) using a Plant Total RNA Mini Kit (Real Biotech Corporation, Banqiao City, Taiwan) following manufacturer's instructions. One-step RT-PCR was carried out using a Reverse iT TM One-step RT-PCR Kit (Applied Biosystems gene, Epsom, Surrey, UK) and the BYMV-specific primer pair (BYMV-CPU: 5'-GTCGATTTCAATCCGAACAAG-3' and BYMV-CPD: 5'-GGAGGTGAAACCTCACTAATAC-3') designed by Wang Xiaoming and Zhu Zhendong (Institute of Crop Sciences, CAAS, Beijing, China) and based on the CP sequence of BYMV (accession No. AB079782) to amplify a fragment 907 bp in size.

Amplification was performed in an automated thermal cycler (Applied Biosystems gene) programmed for the following thermo-cycling conditions: 47°C for 30 min for cDNA synthesis, 5 min at 94°C for reverse transcriptase inactivation and initial denaturation, followed by 35 cycles of 60 s at 94°C, 1 min at 50°C and 2 min at 72°C, and a final extension for 5 min at 72°C.

Electrophoresis analysis of PCR products

Aliquots of the PCR products were analyzed by electrophoresis on agarose gel (1%) in TBE buffer (90 mM Tris borate, 2 mM EDTA) at 120 V for 45 min. The amplified DNA fragments were stained with ethidum bromide (0.5 μg ml^{-1}), visualised under a UV transilluminator, and photographed using Gel DOC™ XR System (Bio-Rad, Hercules, CA, USA). The size of fragments was determined by comparison with 100 bp ladder DNA Marker (Real Biotech Corporation, Taipei, Taiwan)

Sequencing

PCR amplification products of the expected size were excised from agarose gel following electrophoresis, and purified using a QIAquick Gel Extraction Kit (Qiagen, Germantown, MD, USA). Amplicon from BYMV-SY was sequenced by automatic sequencing (Macro gene, Seoul, South Korea).

Computer-assisted analysis of nucleotide sequences

Multiple alignments of nucleotide sequences and homology analysis with 1000 bootstrap trials were done using the DNAMAN Software package (452 av St-Louis, Pointe-Claire, Quebec, Canada). A search for homologies with proteins deposited in the GenBank was carried out with the BLASTAX and BLASTAN (Altschul *et al*., 1990) programs. The sequence obtained was employed to further study homology among the BYMV isolates. The DNAMAN Sequence Analysis Software package (Lynnon BioSoft, Quebec, Canada) was applied to estimate the nucleotide distances between pairs of sequences (number of nucleotide differences per site). To compare the BYMV isolates with isolates

from other countries, 6 nucleotide sequences were retrieved from Genbank entries: India (AY845012), Australia (AF185961), Japan (AB079887), China (DQ060521), Netherlands (X63358) and USA (AY192568). Homological relationships were inferred using the DNAMAN software based neighbour-joining method with a 1,000 replicate bootstrap value.

Results and discussion

No differences in the leaf symptoms were observed in any of the plants inoculated with the BYMV isolates. The results of serological test were confirmed by RT-PCR, since all BYMV-infected samples (except for the Yemeni isolate) gave amplicons of

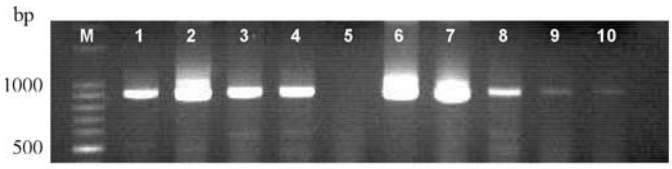

Fig. 1. Agarose gel electrophoresis of RT-PCR amplifications of the coat protein from ten BYMV isolates in faba bean. Lane 1. SV205-85 (Syria); 2. SUV254-88 (Sudan); 3. EV34-91 (Egypt); 4. LYV61-91 (Libya); 5. YeV48-96 (Yemen); 6. ATCC PV89 (USA); 7. G (Netherlands); 8. AUV8-04 (Australia); 9. ChV657-04 (China); 10. JV175-96 (Jordan). M, 100 bp Ladder DNA marker (Real Biotech Corporation, Taiwan).

the expected size (907 bp) (Fig. 1).

The BYMV-SY amplicon obtained was sequenced successfully and showed an open reading frame that encoded for a polypeptide with a high homology to the amino acid sequences of the capsid protein of BYMV. The cDNA fragment (907 bp) amplified by the BYMV-specific primers (W. Xiaoming and Z. Zhendong, Institute of Crop Science, CAAS, Beijing, China), encoded a polypeptide of 302 amino acids. The nucleotide sequence of the CP gene of the Syrian isolate and those of the comparable gene of six other BYMV isolates from GenBank were used for multiple sequence alignments and then a homology tree was constructed using DNAMAN program software (Fig. 2). In this tree, BYMV isolates were grouped in two clusters, of which the first comprised the Syrian isolate together with Indian, Australian and Japanese isolates, and the second the Chinese, Dutch and USA isolates. The homology tree also showed that the Syrian isolate was very close to the Indian one, with a high level of homology (99%) between the two isolates.

The alignments of nucleotide sequences of the Syrian isolate and the other isolates showed a homology in the CP region that was 99% with the Indian isolate and 98% with the Australian and Japanese isolates, while it was 71% with the isolates from China, Netherlands and USA. Variations in the amino acid sequence between the isolates was mainly located in the N-terminal region of the CPs (Frenkel *et al.*, 1989; Shukla and Ward, 1989; Fauquet *et al.*, 2005); the similarity among amino acid sequences of the CPs in the genus *Potyvirus* ranged from 71 to 99%. The results strongly underline the importance of sequence information for the taxonomic classification of viruses, such information is probably necessary for the exact identification and classification of viruses that belong to closely related groups.

Work is in progress to sequence the CP gene of BYMV isolates collected from other countries in the region (Sudan, Egypt, Libya, Yemen and Jordan) and to compare them with the sequence of the CP gene of the Syrian isolate.

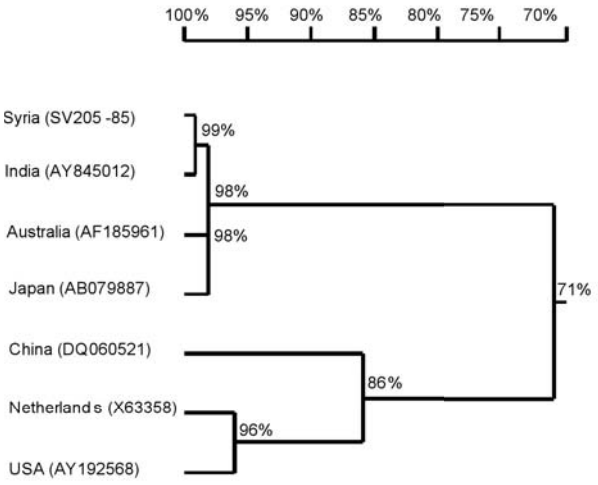

Fig. 2. Homology tree showing the relationship between different BYMV isolates including the Syrian isolate based on coat protein sequences. The tree was constructed using DNAMAN software with 1000 bootstrap replicates. Values at nodes indicate significance in bootstrap analysis.

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