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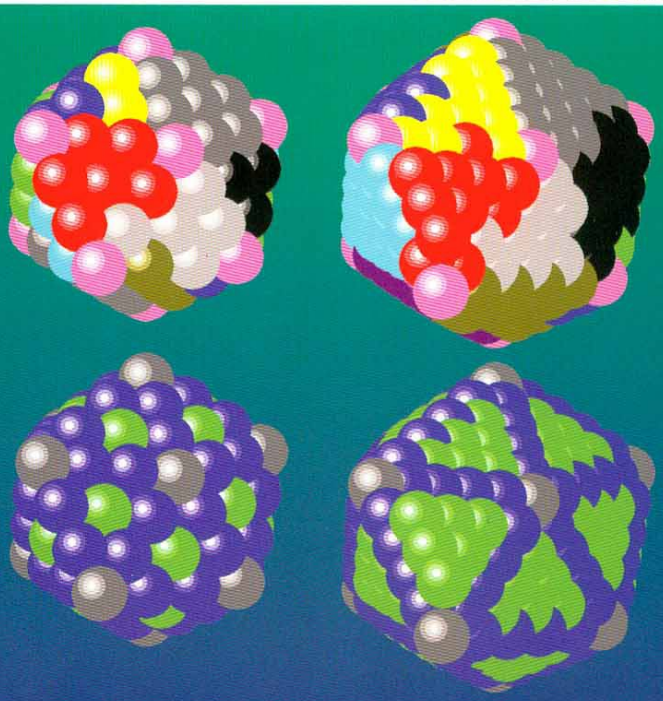

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A New Isolate of Iris Severe Mosaic Virus Causing Yellow Mosaic in *Iris ensata* Thunb.

Shijie Yan†, Zuodong Qin†, Leilei Jin, and Jishuang Chen*

Institute of Bioengineering, Zhejiang Sci-Tech University, Hangzhou 310018, China

Severe mosaic disease was observed on *Iris ensata* Thunb. in Spring 2008, in Hangzhou, China and it was found to be widely distributed in that region. Detection of viruses by electron microscopy resulted in the occurrence of a potyvirus in most symptomatic seedlings. Sequencing 1745 nucleotides of the 3′-terminal region of the genome of the typical viral isolate revealed that it was a new isolate of Iris severe mosaic potyvirus (ISMV), tentatively named ISMV-PHz .This strain shared high nucleotide identity with ISMV. Phylogenetic analysis also showed that this isolate clustered with iris severe mosaic potyvirus (ISMV) and onion yellow dwarf virus (OYDV) into a monophyletic group, and was closest in similarity to ISMV. The divergence of potyvirus-infecting iris species had a higher degree of relevance with natural host but not with the region from which it was isolated. This is the first report of ISMV isolated from *I. ensata* in China.

Keywords: *Iris ensata* Thunb., Iris Severe Mosaic Virus, Phylogenetic Analysis, Host Relevance.

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1. INTRODUCTION

Iris ensata Thunb., a bulbous ornamental plant native to China, is widely cultivated in Northeast China, Shandong, and Zhejiang Provinces.[1] Potyvirus is the largest genus of the family *potyviridae*, with nearly 200 definite and tentative species.[2] The typical virus in this family has a single-stranded RNA genome (~10 Kb), containing a 5′ untranslated region (5′ UTR), a single open reading frame (ORF) and a 3′ UTR which has a polyadenylated (polyA) tail. The ORF encodes a large polyprotein which is cleaved into ten functional proteins.[3,4] Members of potyvirus infect over 500 plant species in more than 60 plant families, including many important ornamentals.[5] In common with other perennial ornamentals, iris plants are often infected by potyvirus, sometimes in the form of complex infections.[6] Filamentous viruses, including: iris mild mosaic virus (IMMV), iris severe mosaic virus (ISMV), Ornithogalum mosaic virus (OrMV), Ornithogolum virus 2 (OV-2) and narcissus latent virus (NLV) have all been reported to infect iris plants.[6–10] Recently, yellow mosaic symptoms indicative of viral infection were observed in *I. ensata* in Hangzhou, Eastern China. In the present work, the 3′ end of the pathogenic potyvirus was sequenced and compared to those of other potyviruses.

*Author to whom correspondence should be addressed.
†These authors contributed equally to this paper.

2. MATERIALS AND METHODS

2.1. Virus Source and Electron Microscopy

The virus isolate (PHz) was obtained from fresh leaves of *I. ensata* exhibiting severe yellow mosaic symptoms from Hangzhou, China. The specimens were examined with a JEM-1230 transmission electron microscope after first fixing with glutaraldehyde and osmium tetroxide, embedding in Epon 812, and prepared in thin sections using standard protocols.[11]

2.2. RT-PCR Amplication and Gene Cloning

RNA isolation, first-strand cDNA synthesis and the amplification of the 3′-end partial genome of potyvirus, cloning, and sequencing were performed as per our previous work.[12] Three independent clones were sequenced in both directions using an ABI PRISMTM 3730 DNA Sequencer.

2.3. Phylogenetic Analysis

The molecular evolutionary genetics analysis program (MEGA) Version 4.0 was used to analyze the nucleotide and deduced amino acid sequences, after similarity searching from the NCBI database. All the potyvirus isolates used for comparison and their corresponding detailed information are listed in Table I.

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Table I. Isolates of partial potyviruses used for phylogenetic analysis.

Virus name and strain	Detection sites	Host	Sequence accession
ISMV-N	Netherlands	*Crocus vernus*	X75939
ISMV-K	Korean	*Iris*	AF034839
ISMV-I	India	*Iris x hollandica*	AJ549755
IMMV-DC4a	New Zealand	*Iris x hollandica cv.* Wedgewood	DQ436918
IMMV-DC4b	New Zealand	*Iris x hollandica cv.* Wedgewood	DQ436919
IMMV-PB	Netherlands	*Iris*	EF203682
OrMV-1	New Zealand	*Iris tingitana*	AY994107
OrMV-2	New Zealand	*Iris* ' Wedgewood	AY994106
ChiRSV	Vietnam	*Chilli*	DQ925438
OYDV	Japan	*Garlic*	AB000836
SbMV-N	Beijing, China	*Soybean*	X96665
BFMV	Zhejiang, China	*Iris japonica*	AM774001
NLSYV	Zhejiang, China	*Narcissus*	AJ493579
ISMV-PHz	Zhejiang, China	*Iris ensata* Thunb.	FJ481099
ZYMV	Taiwan, China	*Luffa cylindrica*	NC_003224

3. RESULTS AND DISCUSSION

3.1. Symptoms and Electron Microscopy Results

Naturally-diseased *I. ensata* plants were found to exhibit chlorosis and yellow spots in the early spring and were also observed to develop large yellow spots in spring and in autumn, as shown in Figure 1. Ultra sectioning under electron microscopy revealed the presence of cytoplasmic cylindrical inclusions characteristic of infection by

Fig. 1. Large yellow spots and mosaic symptoms on naturally-diseased *I. ensata* plants.

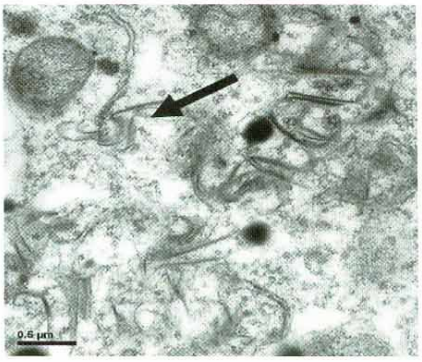

Fig. 2. Electron micrographs of thin sections of infected leaf tissue of *I. ensata*. Pinwheels (arrows), typical of potyvirus infection, are present in the cytoplasm. Length scale bar = 500 nm.

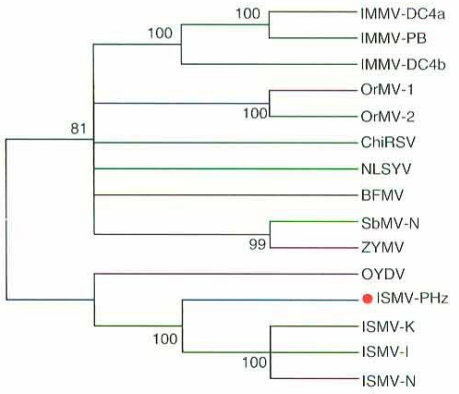

Fig. 3. Phylogenetic analysis of the deduced amino acid sequences of CP for PHz aligned with those related potyviruses. All branches with <70% bootstrap support were collapsed. Bootstrap percentage values are shown at the nodes. The dendrogram was constructed using Maximum Parsimony Mega 4.0 and bootstrapped 1,000 times. Abbreviations: ISMV: Iris severe mosaic virus; IMMV: Iris mild mosaic virus; ChiRSV: Chili ringspot virus; ISMV: Iris severe mosaic virus; OYDV: Onion yellow dwarf virus; SbMV: Soybean mosaic virus; NLSYV: Narcissus late season yellow virus; BFMV: Butterfly flower mosaic virus; ZYMV: Zucchini yellow mosaic virus. The isolate numbers are given for description convenience according to the detection sites of each virus. The GenBank accession numbers of these viruses are as follows: ChiRSV DQ925438; ISMV-N X75939; ISMV-K AF034839; ISMV-I AJ549755; OYDV AB000836; SbMV-N X96665; IMMV-DC4a DQ436918; IMMV-DC4b DQ436919; IMMV-PB EF203682; OrMV-1 AY994107; OrMV-2 AY994106; NLSYV AJ493579; BFMV AM774001; ISMV-PHz FJ481099 and ZYMV NC_003224.

potyvirus, as indicated in Figure 2. Morphological analysis of these inclusions indicated that they belonged to type II according to the classification of Edwardson,[13] with a pinwheel-like configuration in cross section and parallel lines in longitudinal section.[14]

3.2. Phylogenetic Analysis

After RT-PCR, cloning, and sequencing, a total of 1,745 nucleotides sequenced at the 3′ terminal viral genome was deposited in the EMBL/Genebank/DDBJ databases with the accession number FJ481099. It was found to be composed of the 3′-untranslated region and the capsid protein (CP) gene of a potyvirus. The deduced CP gene was found to be composed of 714 nt potentially encoding a protein of 237 acids.

All the potyvirus isolates used for comparison and phylogenetic tree construction in the present work were selected after similarity searches from the NCBI database. To date, the most closely related sequences identified by a BLASTN search were: 98% identity with ISMV-N (X75939), 98% identity with ISMV-I (AJ549755) and 97% identity with ISMV-K (AF034839).[15] Unrooted phylogenetic trees constructed by the Neighbor-joining Method in MEGA 4.0 and their robustness were evaluated with 1,000 bootstrap replicates. The phylogenetic analysis of the relationship between ISMV and other potyviruses based on the deduced amino acid sequences of the putative viral CP is shown in Figure 3. For an alignment of the deduced amino acid sequences of the putative viral CP, the Neighbor-joining tree was almost fully resolved, with bootstrap values greater than 70%. The topology of the phylogenetic tree revealed that ISMV-PHz was closely clustered with ISMV and OYDV into a monophyletic group, showing that the ISMV-PHz was much closer to ISMV than to OYDV. No specific relationship was found between ISMV isolates previously reported in China and other iris-infecting potyviruses such as IMMV, NLSYV, and BFMV, as the analysis did not indicate any apparent similarities between them.

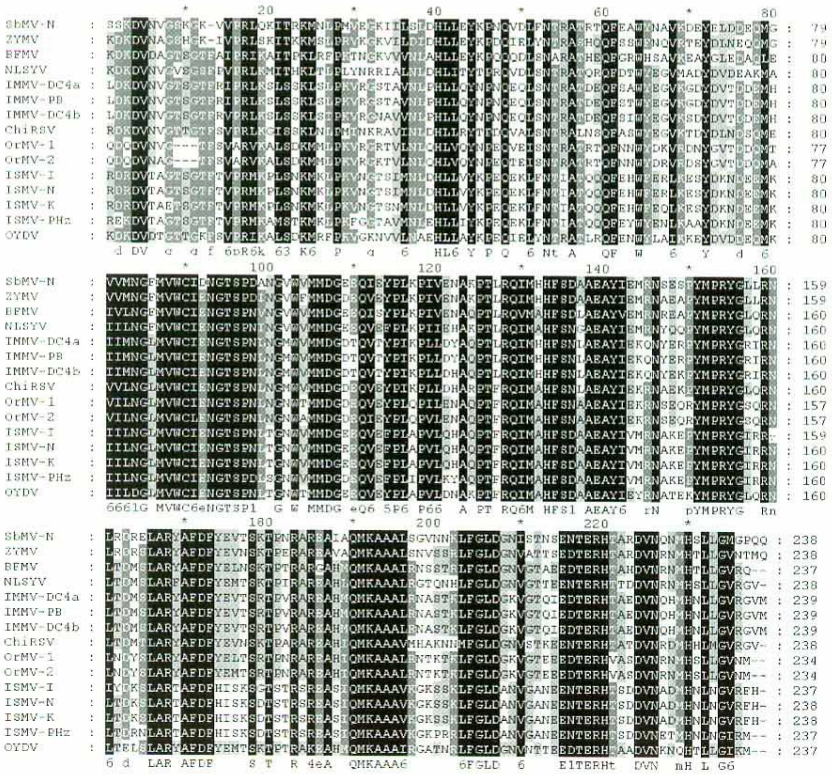

Fig. 4. Aligment of the deduced amino acid sequences of CP with CLUSTAL X 1.83.

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Table II. Divergency analysis of the deduced amino acid sequences of the coat protein. The lower left diagonal half of the table corresponds to the sequence diversity.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. SbMV-N		0.036	0.047	0.045	0.051	0.051	0.051	0.049	0.049	0.050	0.056	0.055	0.056	0.056	0.050
2. ZYMV	0.263		0.044	0.047	0.051	0.051	0.051	0.048	0.049	0.050	0.056	0.055	0.055	0.057	0.048
3. BFMV	0.427	0.376		0.044	0.045	0.045	0.045	0.046	0.048	0.048	0.054	0.053	0.053	0.054	0.045
4. NLSYV	0.399	0.418	0.380		0.044	0.044	0.044	0.042	0.046	0.048	0.052	0.051	0.051	0.053	0.048
5. IMMV-DC4a	0.482	0.475	0.399	0.385		0.004	0.013	0.041	0.041	0.043	0.052	0.051	0.051	0.052	0.050
6. IMMV-PB	0.489	0.475	0.399	0.379	0.004		0.013	0.041	0.041	0.043	0.052	0.051	0.051	0.052	0.050
7. IMMV-DC4b	0.489	0.475	0.399	0.385	0.038	0.038		0.039	0.041	0.043	0.051	0.050	0.051	0.052	0.049
8. ChRSV	0.444	0.431	0.412	0.348	0.336	0.336	0.313		0.045	0.048	0.051	0.050	0.050	0.050	0.048
9. OrMV-1	0.440	0.447	0.431	0.399	0.337	0.337	0.337	0.393		0.015	0.053	0.052	0.053	0.053	0.044
10. OrMV-2	0.454	0.454	0.431	0.425	0.355	0.355	0.355	0.425	0.048		0.052	0.051	0.052	0.053	0.044
11. ISMV-I	0.559	0.551	0.522	0.491	0.491	0.491	0.484	0.478	0.502	0.488		0.007	0.010	0.027	0.049
12. ISMV-N	0.548	0.541	0.505	0.475	0.475	0.475	0.468	0.462	0.492	0.479	0.013		0.007	0.025	0.048
13. ISMV-K	0.563	0.548	0.512	0.482	0.482	0.482	0.475	0.468	0.499	0.492	0.026	0.013		0.026	0.048
14. ISMV-PHz	0.551	0.566	0.526	0.505	0.498	0.498	0.491	0.471	0.507	0.507	0.156	0.140	0.145		0.047
15. OYDV	0.467	0.440	0.399	0.431	0.464	0.464	0.444	0.431	0.374	0.380	0.447	0.431	0.438	0.425	

3.3. Amino Acid Sequence Alignment

Based on amino acid sequence alignment with the 15 related potyviruses presented in Figure 4, the structure of the CP amino acid sequence can be divided into three major domains, including: CP core domain (81–196), C-terminal conserved domain (197–239) and N-terminal variable domain (1–80). Most variations occurred in the variable domain, while few variations occurred in the CP core domain. However, the presence of identical motifs specifically for ISMV, IMMV and OrMV, as shown in Figure 4, such as the 152–158 motif and the 174–189 motif, was noted. Little difference in sequence was found between PHz and other ISMV strains, with only a small single mutation occurring in random regions. When comparing the CP core domain of ISMV-PHz to other ISMV isolates reported previously, there were eight amino acid mutations present, including the 98, 100, 109, 117, 121, 122, 164, and 165 sites. Among these eight locations, only random mutations were observed. Therefore, it appears that mutations other than segment recombination between ISMV and other iris-infecting potyviruses occurred in the region of the deduced amino acid sequences.

3.4. Divergence Analysis

ISMV-PHz sequence divergence in the group ranged from 14.0 (pairwise distance between ISMV-PHz and ISMV-N) to 56.6% (pairwise distance between ISMV-PHz and ZYMV), revealing that ISMV-PHz was closer to ISMV than to the other related potyviruses listed in Table II. When isolates of IMMV and ISMV infected iris in different regions, the minimum divergence distance was 0.4% (pairwise distance between IMMV-DC4a and IMMV-PB) and the second–minimum divergence distance was 1.3% (pairwise distance between ISMV-N and ISMV-K). By comparison, when isolates of OrMV infected different iris species in the same region, the divergence distance was 4.8% (pairwise distance between OrMV-1 and OrMV-2). Therefore, it appears that the divergence distance of potyvirus-infecting iris species had a higher degree of relevance with the host but lower degree with the region from which it was isolated.

4. CONCLUSIONS

When taking all the above factors into consideration, it can be concluded that ISMV-PHz, which was isolated from *I. ensata* Thunb., is the first report of ISMV in China. Similarity and homology of nucleotide sequences for ISMV CP genes demonstrated high host correlation and low partial habitat correlation, and divergency analysis of the deduced amino acid sequences of the coat protein also showed that the host correlation was more pronounced than the habitat correlation. Early reports from our previous work revealed the evolutionary strategies of such viruses, especially the rapid variation or recombination of SMV of *P. ternata*, in order to adapt to the particular host.[12] In the present work, no segment recombination between ISMV and other iris-infecting potyviruses was observed using the same methodology as per our previous work. Many plants of *I. ensata* were found to exhibit yellow mosaic symptoms in parks in Hangzhou, Zhejiang Province, China and were determined, after RT-PCR, cloning, and sequencing, to be infected with ISMV. However, ISMV was not detected in two other iris species, namely as *I. japonica* and *I. gerinanica*, using the same methodology. Questions as to why ISMV-PHz infected only *I. ensata* plants, as well as on the origin of ISMV, should be further studied. The present work provides theoretical evidence on the origin of ISMV infecting iris species and characteristics of molecular ecology, as well as on the evolution and variation of ISMV of iris in China.

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