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ANNOTATED SEQUENCE RECORD

Nucleotide sequence and genome organization of the medium RNA of Iris yellow spot virus from the United States

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Abstract Iris yellow spot tospovirus (IYSV) of the family *Bunyaviridae* causes a serious disease in onion in the USA and other parts of the world. Inspite of its economic importance, the complete genomic sequence of IYSV from the USA is not available. The genome structure and organization of the medium (M) RNA of a Washington (WA) isolate of IYSV were determined and compared to the corresponding region of two isolates previously described from Brazil and The Netherlands. Sequence analysis showed that the M-RNA was 4,817 nucleotides long and potentially coded for the movement protein (NSm) in the viral sense and the glycoprotein precursor (Gn and Gc) in the viral complementary sense. The predicted sizes of NSm and Gn/Gc precursor were 34.7 and 128.84 kDa, respectively. The two open reading frames are separated by a 380 nucleotide intergenic region. Phylogenetic analysis of the NSm and Gn/Gc genes from the WA isolate showed grouping that reflected their respective serogroups. The WA isolate formed a close cluster with the two previously reported IYSV isolates and the IYSV cluster was distinguishable from other tospovirus species. This is the first report of complete genomic sequence of the M-RNA of IYSV from the US.

Introduction

Thrips-transmitted Iris yellow spot virus (IYSV) is one of the most economically important constraints to onion production in the United States [4]. Most recent reports of IYSV include Yuma County, Arizona [12], and Nevada (S. Bag, unpublished) in the US, and Serbia [2], New Zealand [17], and France [5] in other parts of the world. IYSV, of the genus *Tospovirus* and family *Bunyaviridae*, is presumed to share the genomic features of other tospoviruses: a segmented RNA genome of three RNAs referred to as large (L), medium (M), and small (S). The L-RNA is in the negative sense, and the M and S RNAs are ambisense in their genome organization. The M-RNA uses an ambisense coding strategy and codes for the precursor for the Gn and Gc glycoproteins in the viral complementary (vc) sense and a non-structural protein (NSm) in the viral (v) sense. Considerable data are available on the sequence variability of the N gene of IYSV [10]. However, no information is available on the sequence of the M-RNA of IYSV from the USA. In the case of TSWV, it was shown that the gene products of M-RNA play an important role in virus movement in plants and thrips transmission [8, 15]. The only reports on the genome structure and organization of IYSV M-RNA have been from Brazil [14] and The Netherlands [3]. The present study was undertaken to determine the complete sequence of the M-RNA of an onion isolate of IYSV collected from Washington State, a region that has been experiencing severe outbreaks of the virus in both bulb and seed onion crops, and to conduct comparative sequence analyses with those of other known tospoviruses.

Sequences reported here is available in NCBI GenBank under the following accession no.: FJ361359.

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Provenance of the virus material

The IYSV isolate was collected from a commercial onion crop grown near Pasco, Franklin County, Washington, USA. Virus infection was confirmed using a commercially available ELISA kit (Agdia Inc., Elkhart, IN, USA). The

identity of IYSV was further verified by cloning and sequencing of the N gene using previously published primers and procedures. To clone the M-RNA, total plant RNA was extracted from IYSV-infected onion tissue using the RNeasy kit (Qiagen, Valencia, CA, USA). A total of three sets of primers that would provide overlapping amplicons were initially designed using the M-RNA sequences available in GenBank. Following RT-PCR, the resulting overlapping amplicons were cloned into TOPO-TA vector (Invitrogen, Carlsbad, CA, USA). Recombinant clones were identified using standard molecular biology techniques, and plasmid DNA templates were sequenced at the sequencing facility of Washington State University, Pullman. At least three clones were sequenced for each of the amplicons. From the overlapping clones, the complete sequence was assembled by using Vector NTI (Invitrogen). Pair-wise and multiple alignments were done using CLUSTAL W [16]. Cluster dendrograms were constructed using the full optimal alignment and neighbor-joining method options with 1,000 bootstrap replications contained within this software and TreeView [9]. Peptide cleavage sites, transmembrane domains and *N*- and *O*-linked glycosylation sites were predicted using SignalP 3.0 [1], TMHMM Server v. 2.0 [7], NetNGlyc 1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/) and the NetOGlyc 3.1 Server [6], respectively. Tospovirus M-RNA sequences available in GenBank were used for comparisons. Accession numbers for the M-RNA are: Capsicum chlorosis virus (CaCV) NC008303; Chrysanthemum stem necrosis virus (CSNV) AF213675, AB274026; *Groundnut bud necrosis virus* (GBNV) AY871097; *Groundnut ringspot virus* (GRSV) AF213673, AY574055; Iris yellow spot virus (IYSV-Brazil) AF213677, (IYSV-The Netherlands) AF214014; (IYSV-USA) FJ361359; *Impatiens necrotic spot virus* (INSV) NC003616; Melon yellow spot virus (MYSV) NC008307; *Tomato chlorotic spot virus* (TCSV) AF213674, AY574054; *Tomato spotted wilt virus* (TSWV) AY870389; Tomato zonate spot virus (TZSV) EF552434; *Watermelon silver mottle virus* (WSMoV) DQ157768; and Zucchini lethal chlorosis virus (ZLCV) AF213676, AB274027.

Sequence properties

The M-RNA was 4,821 nucleotides long, and sequence analysis revealed two non-overlapping open reading frames (ORF) with an ambisense arrangement. The smaller ORF of 935 nucleotides was located at the 5′ end of the v-sense strand, potentially encoding a 311-amino-acid protein with a predicted molecular mass of 34.7 kDa. The deduced amino acid sequence of this ORF showed the highest sequence identity with the non-structural protein (NSm) of an IYSV isolate from Brazil and The Netherlands and thus appeared to be the NSm protein. The larger ORF of 3,410 nt was located at the 5′ end of the viral complementary (vc) strand, potentially encoding an 1,136-amino acid protein with a predicted molecular mass of 128.84 kDa, assumed to be the glycoprotein precursor (Gn/Gc) based on the sequence comparison with known tospovirus Gn/Gc gene sequences. The two ORFs were separated by an intergenic region (IGR) of 395 nucleotides. IYSV-USA IGR was 15 nt longer than those reported for The Netherlands isolate and shared 69% identity at the nucleotide level.

The ORF coding for the NSm started at nucleotide position 64 and terminated at nucleotide position 999. Analysis of the amino acid sequence did not reveal any hydrophobic regions that might function as signal or transmembrane spanning segments. There were three *N*-glycosylation sites predicted in the IYSV-USA isolate as compared to six in other tospoviruses. At the nucleotide level, the NSm gene of IYSV-US isolate was 97% identical to both IYSV-The Netherlands and IYSV-Brazil isolates, whereas it shared 73–76% identity with the corresponding region of other known tospoviruses. The amino acid sequence of the NSm protein shared 94 and 95% identity with the isolates from The Netherlands and Brazil, respectively, while it shared 33–68% identity to NSm protein of other tospoviruses. A cluster dendrogram based on the amino acid sequences of NSm of known tospoviruses showed the formation of two major clusters: IYSV isolates formed a close cluster with CaCV, GBNV, MYSV, WSMoV and TZSV. The second cluster consisted of CSNV, GRSV, INSV, TCSV, TSWV, and ZLCV, forming a clear distinction from the IYSV cluster (Fig. 1a).

The Gn/Gc ORF in the vc strand of IYSV M-RNA was from nt 1,394 to 4,804 (numbered from the 5′ end of viral RNA), was 3,410 nt in length, potentially coding for a 1,136-amino-acid protein. Comparison of the nucleotide sequence of the IYSV Gn/Gc ORF with that of other tospoviruses revealed that it is 69% identical to IYSV-The Netherlands. In contrast, the sequence identity with other tospoviruses ranged from 53 to 74%. Similarly, at the amino acid level, it shared 92% identity with IYSV-The Netherlands and 33–61% with other tospoviruses. The protein domain prediction showed that the topology of the Gn/Gc precursor is similar to that of other tospoviruses. The Gn/Gc precursor of IYSV-USA possessed two peptide cleavage sites located at the N-terminus and five transmembrane domains and seven putative *N*-glycosylation sites as compared to five in The Netherlands isolate. The IYSV-USA isolate's Gn/Gc precursor had two *O*-glycosylation sites as compared to five in the case of The Netherlands isolate and other tospoviruses [3, 13]. The dendrogram based on the Gn/Gc ORF showed clustering similar to the one based on NSm (Fig. 1b).

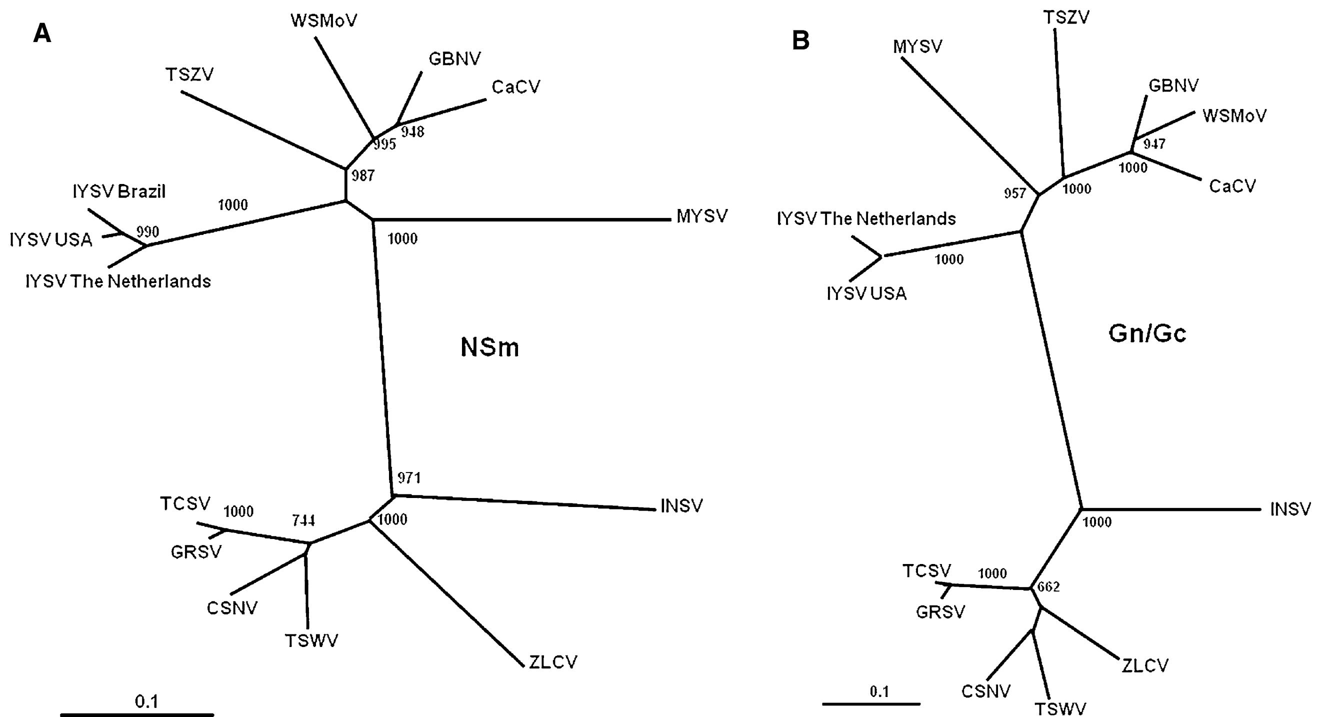

Fig. 1 Clustal dendrogram showing the relationship of iris yellow spot virus (IYSV) to representatives of other species within the genus *Tospovirus* based on **a** the amino acid sequence of the non-structural protein NSm and **b** the precursor of the Gn/Gc glycoprotein. Virus acronyms are explained in the text

The intergenic region of the IYSV-USA isolate shared 69% sequence identity with that of the isolate from The Netherlands. IGRs of S and M-RNAs of tospoviruses were found to be useful markers for classifying tospoviruses at the species level [11]. The predicted secondary structures of both the NSm and Gn/Gc genes were found to be similar to those reported for other tospoviruses. The genome organization of the M-RNA of IYSV-USA isolate was similar to that reported for other tospoviruses. However, some differences were observed in terms of the length and sequence identity of the IGR among IYSV isolates. A definite biological function is yet to be established for IGRs. Besides the IGR, some major differences were also observed in the topology of Gn/Gc of IYSV and other tospoviruses, which are considered to be transmission determinants. The effect of these differences on thrips transmission remains to be seen.

IYSV continues to be a major constraint to onion production in the USA and is becoming increasingly important in other parts of the world [4]. A better understanding of the genome structure, organization, and sequence divergence of IYSV isolates from different production systems and geographic regions would potentially provide tools and technologies for developing management options for reducing the impact of this economically important virus.

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