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Sequence diversity of the nucleoprotein gene of iris yellow spot virus (genus *Tospovirus*, family *Bunyaviridae*) isolates from the western region of the United States

Brief Report

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Summary. Iris yellow spot virus (IYSV), a tentative virus species in the genus *Tospovirus* and family *Bunyaviridae*, is considered a rapidly emerging threat to onion production in the western United States (US). The present study was undertaken to determine the sequence diversity of IYSV isolates from infected onion plants grown in California, Colorado, Idaho, Oregon, Utah and Washington. Using primers derived from the small RNA of IYSV, the complete sequence of the nucleoprotein (NP) gene of each isolate was determined and the sequences compared. In addition, a shallot isolate of IYSV from Washington was included in the study. The US isolates of IYSV shared a high degree of sequence identity (95 to 99%) with one another and to previously reported isolates. Phylogenetic analyses showed that with the exception of one isolate from central Oregon and one isolate from California, all the onion and shallot isolates from the western US clustered together. This cluster also included onion and lisianthus isolates from Japan. A second distinct cluster consisted of isolates from Australia (onion), Brazil (onion), Israel (lisianthus), Japan (alstroemeria), the Netherlands (iris) and Slovenia (leek). The IYSV isolates evaluated in this study appear to represent two distinct groups, one of which largely represents isolates from the western US. Understanding of the population structure of

IYSV would potentially provide insights into the molecular epidemiology of this virus.

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Iris yellow spot virus (IYSV, genus *Tospovirus*, family *Bunyaviridae*) is a pathogen of onion (*Allium cepa* L.) and was reported from Australia [6], Brazil [34], Chile [37], India [36], Israel [16, 23], Japan [47], Slovenia [24], and the United States (US) [19, 29]. Infection of Lisinathus and Alstroemeria was also reported [10, 17, 22, 47]. Although not yet named as IYSV at the time, the virus was first reported in onion seed crops in the Treasure Valley of Idaho in the 1990s [19], where it has remained endemic. Since 2000, the virus has spread rapidly in the western regions of onion production in the US, with recent first reports or observations in California [Rick Watson, personal communication; 2], Colorado [38], Georgia [30], Nevada [H. F. Schwartz, *unpublished data*], New Mexico [8], Oregon [9], Texas [M. Black, *personal communication*], Utah [S. Thomson, *personal communication*], and Washington [12].

Considered an emerging virus disease, iris yellow spot has caused considerable losses in onion bulb crops in Colorado [15], and in bulb and seed crops in Idaho [25, 26], Oregon [9] and Washington [13, 14]. The latter three states produce up to 50% of the US supply and up to 20% of the world supply of onion seed [44]. IYSV is of concern to the onion bulb and seed industries in the US because few effective management options are currently available for control of the virus and/or the thrips vector, *Thrips tabaci* [31]. Onion thrips populations have developed resistance to most classes of insecticides used by onion growers, resulting in damaging thrips populations in onion crops [1, 7, 40]. The recent rapid spread of IYSV in the US may be due to proliferation of the vector, production and dissemination of transplants infected with IYSV or infested with viruliferous thrips, the continuous presence of susceptible host plants [volunteers or overlapping annual bulb and biennial seed crops], and the presence of alternative hosts [18, 40].

IYSV is a member of the genus *Tospovirus.* Members of this genus are characterized by a segmented RNA genome of three RNAs: large (L), medium (M), and small (S) [27, 28, 41]. The L RNA is in negative sense, while the M and S RNAs are ambisense in character. Based on the genome organization of tomato spotted wilt virus (TSWV), the L RNA codes for the RNA dependent RNA polymerase; M RNA codes for G1 and G2 glycoproteins and a non-structural protein, designated as NSm; and S RNA codes for one non-structural protein, designated as NSs, and the nucleoprotein, NP. Each of the RNA species is tightly bound by the NP and encapsulated in a lipid envelope [27, 28, 41]. Tospoviruses are transmitted by several species of thrips and the virus-vector relationship involves a close biological association between the virus and the thrips vector [48].

The genomes of several tospoviruses have been sequenced and the genome organization elucidated [27]. The most common gene sequenced for tospoviruses is the NP gene. Sequence comparisons of NP genes of tospoviruses have proven

useful in the identification and classification of tospoviruses. Based on sequence comparisons, identity of the causal agent of iris yellow spot of onion was unequivocally established as IYSV [5, 25].

Considering the rapid spread of IYSV in the western US in recent years, and especially its emergence as a serious threat to the onion crop in Washington State within two years of its finding in that state [14], the present study was undertaken as part of a regional effort to better understand the population structure and epidemiology of IYSV in this region. The specific objectives were to obtain sequences of the NP gene of IYSV isolates from onion crops in several states in the western US, and to assess the sequence diversity among them and determine the phylogenetic relationships with known IYSV isolates from other parts of the world. Isolates studied were from the Columbia Basin of Washington State, the primary region of onion production in that state, and from California, Colorado, Idaho, Oregon, and Utah.

Table 1. List of Iris yellow spot virus (IYSV) isolates for which the nucleoprotein gene sequences were determined. Isolate designations used in this table are the same as those in Fig. 1. Year collected and the county of origin are given only for those isolates sequenced in this study

Isolate designation	Host	Year collected	Region or country of origin[1]	GenBank accession number[2]
WARR9	Onion	2004	Grant Co., WA, USA	DQ233468*
WAPasco	Onion	2004	Franklin Co, WA, USA	DQ233469*
WAGrant	Onion	2003	Grant Co., WA, USA	DQ233470*
Shallot	Shallot	2004	Grant Co., WA, USA	DQ233471*
IDNampa	Onion	2005	Canyon Co., ID, USA	DQ233472*
IDNew Plymouth	Onion	2005	Payette Co., ID, USA	DQ233473*
IDParma	Onion	2004	Canyon Co., ID, USA	DQ233474*
California1	Onion	2005	Imperial Co., CA, USA	DQ233475
California2	Onion	2005	Los Angeles Co., CA, USA	DQ233476*
Colorado	Onion	2004	Weld Co., CO, USA	DQ233477*
Utah	Onion	2004	Davis Co., UT, USA	DQ233478*
Oregon	Onion	2004	Jefferson Co., OR, USA	DQ233479*
Australia	Onion		Australia	AY341825
Brazil	Onion		Brazil	AF067070
Israel	Lisianthus		Israel	AF271129
Japan	Onion		Japan	AB180921
Japan – Alstroemeria	Alstroemeria		Japan	AB121025
Japan – Lisianthus	Lisianthus		Japan	AB121026
Netherlands	Iris		The Netherlands	AF001387
Slovenia	Leek		Slovenia	AY377428

[1] *Co* County; *CA* California; *CO* Colorado; *ID* Idaho; *OR* Oregon; *WA* Washington; *UT* Utah

[2] GenBank accession numbers with asterisks were obtained in this study

Onion plants with symptoms of IYSV infection [11, 20] were collected from the following counties in Washington in 2003 and 2004: Walla Walla (bulb crops), Grant (bulb and seed crops), Franklin (seed crop) and Adams (bulb crop). In addition, shallot plants [*Allium cepa* Aggregatum Group] with symptoms suggestive of IYSV infection were sampled in Grant Co. from a trial immediately adjacent to an onion cultivar trial that displayed a high incidence of IYSV [14]. These counties represent the primary region of onion production in Washington, which ranks third in the US for acreage of storage onions. IYSV isolates also were obtained from onion plants collected in Imperial Co. and Los Angeles Co., CA; Weld Co., CO; Canyon Co., ID; Jefferson Co., OR; and Davis Co., UT (Table 1). NP gene sequences determined for these isolates were compared with published sequences of IYSV isolates from other countries, as listed in Table 1.

Total nucleic acids for each isolate were obtained using the method described by Presting et al. [35]. RT-PCR was carried out as described by Jain et al. [21], using the following primer pair derived from the S-RNA of IYSV and flanked the NP gene: 5′-TAA AAC AAA CAT TCA AAC AA-3′ and 5′-CTC TTA AAC ACA TTT AAC AAG CAC-3′. The PCR product (ca. 1.2 kb) consisted of the complete NP gene, and was cloned into the TOPO vector (Invitrogen) and sequenced. At least two clones were sequenced for each isolate and no sequence polymorphisms were observed for a given isolate. Sequences were compiled and compared to related sequences found in GenBank [4] using the BLAST software [3]. Multiple alignments were made using CLUSTAL W [45]. Maximum likelihood trees were then constructed using the PUZZLE program [42] included within the PAUP version 4.0 phylogenetic package [43]. The trees were constructed using 1000 puzzling steps. The program implements a fast tree search algorithm that automatically assigns estimations of support to each internal branch. In the first step, all possible quartet maximum likelihood trees are reconstructed, which are then combined to an overall tree in the puzzling step. Finally, a majority rule consensus of all intermediate trees is computed resulting in the quartet puzzling tree [42]. The unrooted trees were generated using Treeview [32].

The primer pair used in this study was specific to the S-RNA of IYSV and thus amplified the NP gene of all the IYSV isolates tested. The resulting PCR product was approximately 1.2 kb in size, and consisted of the complete NP gene and about 100 bp on either side of the NP gene. The NP gene was 822 bp long, potentially coding for a 273 amino acid nucleoprotein. These results support previous studies on the NP gene sequence of IYSV isolates [2, 12, 15, 34]. When the sequences of all available isolates were compared, nucleotide sequence identity ranged from 92 to 99%. All Washington isolates shared a high degree of nucleotide sequence identity (96 to 99%) when compared to each other. The shallot isolate from Washington shared 98% sequence identity to the onion isolates from that state, suggesting that the same IYSV population may be infecting shallot and onion.

Cluster dendrograms based on the nucleotide sequences of the NP gene and the deduced amino acid sequences were similar, and only the dendrogram based on the deduced amino acid sequences is shown (Fig. 1). A distinct divergence of IYSV isolates into two clusters was observed. One cluster consisted of all

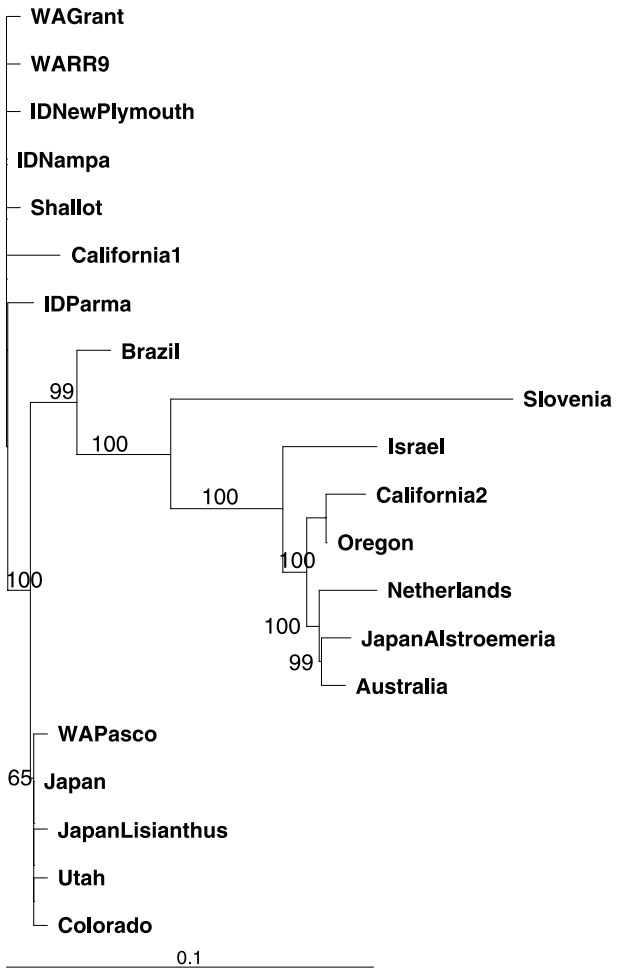

Fig. 1. Cluster dendrogram based on the amino acid sequences deduced from the nucleoprotein gene sequences of isolates of Iris yellow spot virus from the western United States and other countries. Description of each isolate designation is given in Table 1. The PUZZLE program [42] available in PAUP 4.0 [43] was used to construct the phylogenetic tree (based on 1000 puzzling steps). The phylogram was generated as an unrooted tree using TreeView [32]. The percent reliability value given above each branch indicates how often the corresponding cluster was found among the 1000 intermediate trees. Vertical length of the branches was arbitrary

the isolates from the western US (California, Colorado, Idaho, Oregon, Utah and Washington) as well as an onion isolate from Japan and a Lisianthus (*Eustoma russellianum* [Hook] G. Don) isolate from Japan (Fig. 1). Within this cluster, Washington isolates grouped with the onion isolates from California, Colorado, Idaho and Utah, forming a tight cluster comprised of IYSV isolates from the western US (Fig. 1).

The other distinct cluster largely consisted of IYSV isolates from other countries: onion isolates from Australia, Brazil, and Israel; an *Alstroemeria* isolate

from Japan; an iris isolate from the Netherlands; and a leek (*Allium porrum* L.) isolate from Slovenia. Interestingly, the Oregon isolate and one California isolate from Los Angeles Co. showed significant sequence divergence from the rest of the isolates from the western US, and clustered with isolates from Australia, Brazil, Israel, Japan, Netherlands and Slovenia. Within this cluster, isolates from Australia, Japan and the Netherlands were closer to each other than to those from Brazil, Israel and Slovenia (Fig. 1). At the amino acid level, these two isolates had 98% similarity, whereas the isolates from California (Imperial Valley and Lancaster) shared only 90% similarity.

Based on the data obtained on the population structure of several IYSV isolates from the western US, it appears that IYSV NP gene sequences fall into two broad and distinct groups, one of which represents the majority of the isolates from the western US. Such a divergence based on the geographic origin of a virus was reported for another tospovirus, TSWV [33]. Abad et al. [2] reported sequence divergence in the NP gene of selected IYSV isolates from the US. Clustering of TSWV isolates based on geographic origin was attributed to founder effects [46]. The high degree of sequence identity and the resulting close clustering of a majority of the US isolates potentially indicate the relative homogeneity of the NP gene sequences in the virus population. Exceptions to this were one isolate from California and one from Oregon. Interestingly, of the two isolates from California that were studied, one clustered with a majority of the US isolates while the other clustered with those from Australia, Japan, The Netherlands, and Oregon. The onion fields from which these two isolates were collected in California were about 180 miles apart. More isolates from these two areas need to be studied to better understand the nature and extent of the sequence divergence within the IYSV populations in the western US. Such exceptions to geographical structuring of virus populations were reported for TSWV [46].

During the past five years, IYSV has spread rapidly in the Pacific Northwest and other western states in the US where onion bulb and/or seed crops are produced. The rapid emergence of the virus as a potential yield reducing factor in onion crops has resulted in growers, industry personnel, and researchers exploring alternative management options [39]. As a contribution to this effort, we have determined the sequence variability of a sample of IYSV isolates from this region. This sequence information may serve a number of practical applications. The conserved region of the NP gene could be used for engineering resistance against IYSV into onion cultivars. The primers used in this study amplified the NP gene from all the isolates tested and, as such, the primer pair should be useful for the detection of IYSV by PCR.

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