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Molecular Diagnosis of *Iris Yellow Spot Virus* (IYSV) on Onion in Iran

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ABSTRACT

Viral symptoms indicative of *Iris yellow spot virus* (IYSV) were observed on onion in several fields near Chenaran in Khorasan Razavi Province. Mechanical inoculation of herbaceous hosts with onion sap extracts from symptomatic plants showed similar symptoms to those described for IYSV. The mechanically transmitted virus reacted only with antisera specific to IYSV in DAS-ELISA but not with antisera specific to seven other tospoviruses. In RT-PCR, a DNA fragment approximately 822 bp in size was amplified from infected *Nicotiana benthamiana* by using primers specific to the nucleocapsid (N) gene of IYSV. After cloning and sequencing, the deduced N protein sequence of two isolates (GenBank accession no. HQ148173 and HQ148174) showed 98% amino acid identity with a Sri Lankan isolate, 96% with a Dutch isolate and 92% with a Brazilian isolate. To our knowledge, this is the first molecular characterization of IYSV in Iran.

Keywords: Iran, *Iris yellow spot virus*, Molecular diagnosis, Onion.

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INTRODUCTION

Tospoviruses are among the most destructive and widespread plant viruses, causing severe damage in a broad range of crops throughout the world, both in field and in greenhouses (Mumford *et al*., 1996). All tospoviruses are transmitted by thrips in a propagative-circulative manner and in recent years, several species have caused severe damage in tropical and subtropical regions (Persley, 2006). Today, 21 tospovirus species are approved or proposed based on serological and phylogenetic relationships of nucleoprotein (N proteins), host ranges and vector specificity (Persley *et al*., 2006). In tospoviruses, the most common gene sequenced is the nucleocapsid (N) gene and sequence comparisons have proven useful in the identification and classification of the viruses (Pappu *et al*., 2006).

Hall *et al*. (1993) first described a new tospovirus infecting onion (*Allium cepa*) in the USA. This virus was later isolated from iris (*Iris hollandica*) and leek (*A. porrum*) in the Netherlands and, after characterization, was named *Iris yellow spot virus* (IYSV; genus: *Tospovirus*; family: *Bunyaviridae*) (Cortêz *et al*., 1998; Smith *et al*., 2006). Today, IYSV has a worldwide distribution in onion and leek crops, and in various ornamentals, including iris, alstroemeria, lisianthus and amarilis (Kritzman *et al*., 2000; Mitsuru *et al*., 2005; Smith *et al*., 2006). The virus has been reported from several countries around the world (Pozzer *et al*., 1999; Bulajić *et al*., 2009; Gent *et al*., 2004; Ghotbi and Shahraeen, 2005; Pappu *et al*., 2006). Disease symptoms caused by IYSV include chlorotic and necrotic eye-like or diamond-shaped lesions on onion stalks (Gent *et al*., 2006). Once IYSV is established in an area, it spreads rapidly in

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onion crops. For example, in Colorado, its incidence increased from 6% in 2001 to 73% two years later (Gent *et al*., 2004).

In Iran, onion is one of the most important leafy vegetables. It is cultivated in open fields on approximately 50,000 ha with a production of 1.85 million tons per year (Anon., 2009). Using ELISA, the virus was first reported on onion in Iran in 2005 (Ghotbi and Sharaeen, 2005). Also, other studies reported the presence of IYSV in onion and some ornamentals in Iran using ELISA (Jafarpour *et al*., 2009). To date, no information is available on the nucleotide sequence of the N gene of IYSV isolates from Iran. In this study, the virus was diagnosed using molecular methods and its phylogenetic situation was determined.

MATERIALS AND METHODS

Plant Materials

Onion plants with characteristic symptoms of tospoviruses were collected and analysed for the infecting virus.

Bioassay

Infected onion leaves collected from the fields were used for sap inoculation on *Nicotiana benthamiana*, *Petunia hybrida* and *Datura stramonium*. Infected areas of the leaves were harvested and macerated using sterilized and chilled pestle and mortar adding 0.01 M phosphate buffer (pH 7) containing 0.1% sodium sulfite. The extracted sap was used for inoculation (Hassani-Mehraban *et al*., 2005; Bulajić *et al*., 2009) and applied directly by rubbing gently onto the leaves. The inoculated plants were then kept in an insect-proof glasshouse.

Serological Test

Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed to detect the infection of tospovirus in the collected onion samples. Polyclonal antibodies directed against the N protein of Tomato yellow ring virus (TYRV), *Iris yellow spot virus* (IYSV), *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), *Groundnut bud necrosis virus* (GBNV), *Groundnut ring spot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV) and Chrysanthemum stem necrosis virus (CSNV) that were previously prepared at Wageningen University were used to check the serological identity of the onion tospovirus isolates. Symptomatic leaf samples of *N. benthamiana* were extracted in extraction buffer (1:30 w/v). The immunogammaglobulin (IgG) and alkaline phosphatase conjugate (1 mg ml^{-1}) were diluted 1,000 times and the substrate solution was used in a concentration of 0.1 mg ml^{-1}. After incubating the substrate at room temperature for 1 hour, absorbance values were recorded at 405 nm using a plate reader (FLUOstar OPTIMA, BMG LABTECH GmbH, Germany), (Clark and Adams, 1977; de Ávila *et al*., 1990; Gent *et al*., 2004).

Total RNA Extraction and RT-PCR

Total RNA from healthy and infected *N. benthamiana* leaves was extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. RT-PCR was used to characterize the IYSV N gene in samples that showed a positive reaction in DAS-ELISA. The first strand cDNA synthesis was performed in the presence of specific primers IY1 (5'-ATGGCTACCGTTAGGG-3') and IY2 (5'-TTAATTATATCTATCTTTCTTGG-3') (Cortêz *et al*., 1998; Pozzer *et al*., 1999), total RNA, AMV Reverse Transcriptase (22 U μl^{-1}, Promega, USA), 5x buffer, RNase inhibitor (40 U μl^{-1}, Promega), dNTPs (10 mM) and sterile RNase-free water at 60^{O}C for 1 hour after a 3 min denaturation at 85^{O}C. PCR amplifications were carried out using a modification of a published method (Pozzer *et al*., 1999) in a 50 µl reaction mixture containing 5x GoTaq buffer, 25 mM $MgCl_2$, 10mM dNTPs, GoTaq polymerase (5 U μl^{-1}, Promega), primers and cDNA. All PCR reactions were performed in an automated thermal cycler (Peqlab, Primus 25) by pre-heating at 92^{o}C for 2 minutes followed by 30 cycles of 30 seconds of denaturation at 92^{o}C, 30 seconds of annealing at 55^{o}C and 1 minute for extension at 72^{o}C. Finally, the amplified DNA was incubated at 72^{o}C for 7 minutes to accomplish a final extension. Amplified products were analyzed by 1% agarose gel electrophoresis in TAE buffer and stained with ethidium bromide.

Cloning, Digestion and Phylogenetic Analysis

DNA fragments of the IYSV N gene of the expected size were excised from the gel and extracted using the GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare UK Limited) according to the manufacturer's instructions. The purified fragment was ligated into the pGEM-T Easy vector (Promega) and cloned in *Escherichia coli* DH5α. Isolation of recombinant plasmid DNA was done using GeneJET™ Plasmid Miniprep Kit (Fermentas, Germany) according to the manufacturer's instructions. The presence of an insert in transformants was confirmed by restriction enzyme *NotI* digestion. Finally, the selected recombinant clones were sequenced at the automatic DNA sequencing facility (Sanger *et al*. 1977) using SP6 primer. The nucleotide sequences were translated to N protein sequences using Expasy software (http://expasy.org/tools/dna.html). The sequence data were compiled, analyzed, and compared with those available in the GenBank using NCBI/BLAST.

Data from a Clustal W alignment (Thompson *et al*., 1994) of N protein sequences were used as input for phylogenetic tree construction using MEGA 4.0 software (Tamura *et al*., 2007). Genetic distances (the average number of nucleotide substitutions between two randomly selected sequences in a population) between IYSV isolates from Asia, Europe, Oceania, and America continents were calculated using MEGA 4.0 based on the Kimura two parameter model. The average number of non-synonymous substitutions per non-synonymous site (dN) and the average number of synonymous substitutions per synonymous site (dS) were estimated using DnaSP (Rozas and Rozas, 1999; Yang and Bielawski, 2000; Wei *et al*., 2009). The dN/dS ratio was used to estimate natural selection pressure.

RESULTS AND DISCUSSION

Plant Samples

In July 2009, symptoms indicative of tospovirus infection such as chlorotic and necrotic eye-like or diamond-shaped lesions on onion stalks were observed in several fields near Chenaran in Khorasan Razavi Province. Symptoms were similar to those caused by IYSV. Leaves and stalks with well pronounced symptoms were used in our study to confirm the presence of IYSV.

Bioassay

Bioassay studies revealed that the tospovirus from symptomatic onion plants could be mechanically transmitted to indicator plants. Necrotic local lesions were observed on the inoculated leaves of *P. hybrida.* The inoculated leaves of *N. benthamiana* showed chlorotic spots followed by systemic necrosis on newly developed upper leaves and branches. The symptoms were similar to those of other IYSV isolates (Pozzer *et al*., 1999; Kritzman *et al*., 2001). Mechanical inoculation on *D. stramonium* induced necrotic local lesions on the inoculated leaves. The symptoms caused by our IYSV isolates on *D.*

stramonium were identical to those described by Gera *et al*. (2002). The various IYSV isolates cause different symptoms on *D. stramonium*. Some of them cause systemic infections (Cortêz *et al*., 1998; Pozzer *et al*., 1999), whereas some cause only local infection (Gera *et al*., 2002). However, Bulajić *et al*. (2009) did not observe symptoms on plants of this species. These differences may be related to different IYSV strains, environmental conditions, or differences in the susceptibility of the *D. stramonium* accessions (Pozzer *et al*., 1999). These data suggested that the pathogen causing the observed symptoms was likely IYSV. However, attempts to verify Koch's postulates and back-inoculate the virus onto onion failed. Similar observations were also made earlier for IYSV (Pozzer *et al*., 1999).

Serology

Of the eight polyclonal antisera directed against the N protein of different tospoviruses, onion samples reacted only to IYSV antiserum in DAS-ELISA (Figure 1). No reaction was observed with antisera raised against the other seven tospoviruses tested. The onion tospovirus isolate was designated IYSV-O based on the serological data.

RT-PCR

According to the DAS-ELISA data, only specific primers designed in the IYSV N gene were used in RT-PCR to characterize the virus in symptomatic *N. benthamiana*. A fragment of approximately 822 bp in size was obtained from samples O5, O9, and O18. No DNA bands were observed from healthy tissue (Figure 2). Primer IY1 was complementary to the first 16 nucleotides at the 5'-end of the N gene of a Dutch IYSV isolate (GenBank accession no. AF001387) and primer IY2 was identical to the first 23 nucleotides at the 3'-end (Cortêz *et al*., 1998). The IYSV identity of the PCR products was confirmed by cloning and sequencing.

Cloning and Phylogenetic Analysis

PCR amplicons were cloned and sequenced. The sequence of IYSV isolates from onion samples O5 (GenBank accession no. HQ148173) and O18 (GenBank accession no. HQ148174) was determined. Comparative sequence analyses revealed that the two IYSV isolates shared 98% sequence identity with a Sri Lankan IYSV isolate (GenBank accession no. GU901211), 96% sequence identity with a Dutch IYSV

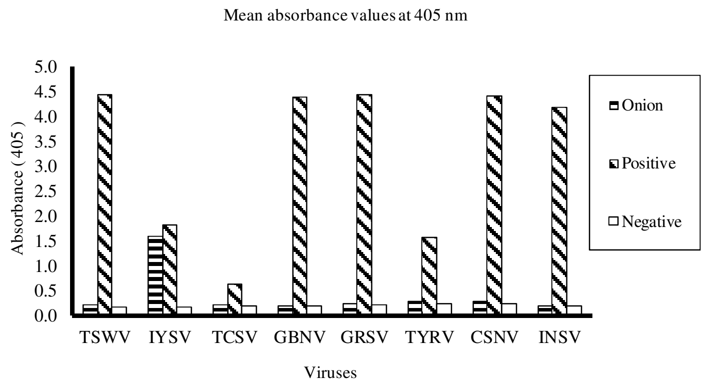

Figure 1. Serological reactions between eight tospoviruses and the onion tospovirus isolate showing amount of relatedness using polyclonal antisera against their respective N proteins. Infected plant extracts were used as antigen sources.

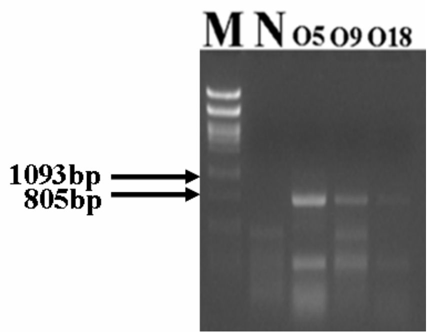

Figure 2. RT-PCR amplification of the IYSV N genes of onion samples: Lane M: Lambda DNA/PstI marker; Lane N: Healthy control, Lanes O5, O9 and O18: Samples of three different symptomatic onion samples.

isolate (GenBank accession no. AF001387) and 92% sequence identity with a Brazilian IYSV isolate (GenBank accession no. AF067070). To consider a tospovirus isolate as a distinct species, N protein amino acid sequence identity (%) should be below 90% (de Ávila *et al*., 1990) compared to other described tospovirus N proteins, therefore, both IYSV-O5 and IYSV-O18 could not be considered as a distinct species.

To determine the phylogenetic relationship of the two IYSV isolates with tospovirus species, the amino acid sequences of both isolates and 19 tospovirus sequences available in the GenBank were aligned using Clustal W (Thompson *et al*., 1994). Moreover, amino acid sequences of the IYSV-O5 and IYSV-O18 isolates and 18 IYSV isolates in GenBank (Table 1) were aligned. Data from the multiple sequence alignment of N protein sequences were used for the construction of a phylogenetic tree (consensus phylogenetic tree) using the neighbor-joining method of MEGA 4.0 software. Bootstrap values are shown as percentages derived from 500 replicates (Tamura *et al*., 2007) (Figures 3 and 4). The results clearly showed that the tospoviruses were distributed in two major clades (Figure 3). One clade (American tospovirus clade) contained all tospovirus species that were isolated and primarily distributed in American countries, while the second clade (Eurasian tospovirus clade) contained all species that were isolated and primarily distributed in Eurasian countries (Hassani-Mehraban *et al*., 2010). IYSV-O5 and IYSV-O18 clustered with IYSV within the second clade, as expected. This analysis is in agreement with the close relationship of

Table 1. IYSV isolates and their GenBank accession number.

Acc. No.	Host	Geographical Origin
AF001387	Iris	Netherlands
EU287943	Onion	Canada
FJ785835	Onion	Greece
FJ713700	Onion	America
FJ514257	Garlic	America
GU901211	Leek	Sri Lnaka
AB378751	*Allium tuberosum*	Japan
EU750697	Onion	Serbia
DQ838590	Onion	Guatemala
DQ150107	Onion	Chile
AY377428	Leek	Slovenia
AY345226	Onion	Australia
AF067070	Onion	Brazil
EU477515	Onion	New Zealand
AB121026	Lisianthus	Japan
AB181370	Alstroemeria	Japan
FJ185142	Onion	Italy
EU310299	Onion	India
HQ148173	Onion	Iran
HQ148174	Onion	Iran

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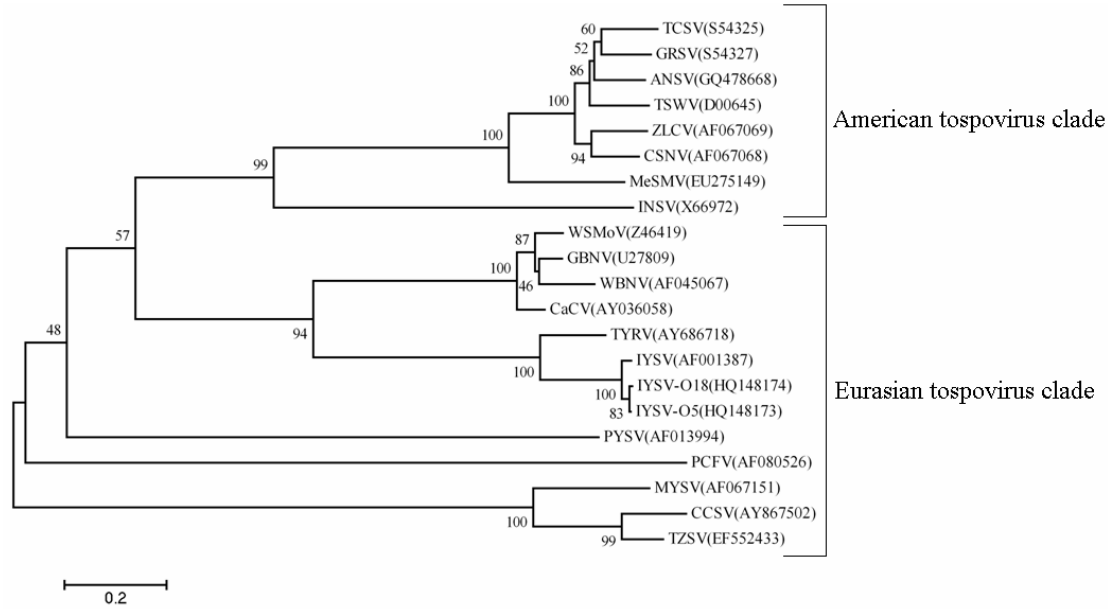

Figure 3. Cluster dendrogram based on the amino acid sequences deduced from the complete N gene sequences of tospovirus species: (Abbr. ASNV: *Alstroemeria necrotic streak virus*; CCSV: *Calla lily chlorotic spot virus*; CaCV: *Capsicum chlorosis virus*; MeSMV: *Melon severe mosaic virus*; MYSV: *Melon yellow spot virus*; PCFV: *Peanut chlorotic fan-spot virus*; PYSV: *Peanut yellow spot virus*; TZSV: *Tomato zonate spot virus*, WBNV: *Watermelon bud necrosis virus*; WSMoV: *Watermelon silver mottle virus*, and ZLCV: *Zucchini lethal chlorotic virus).*

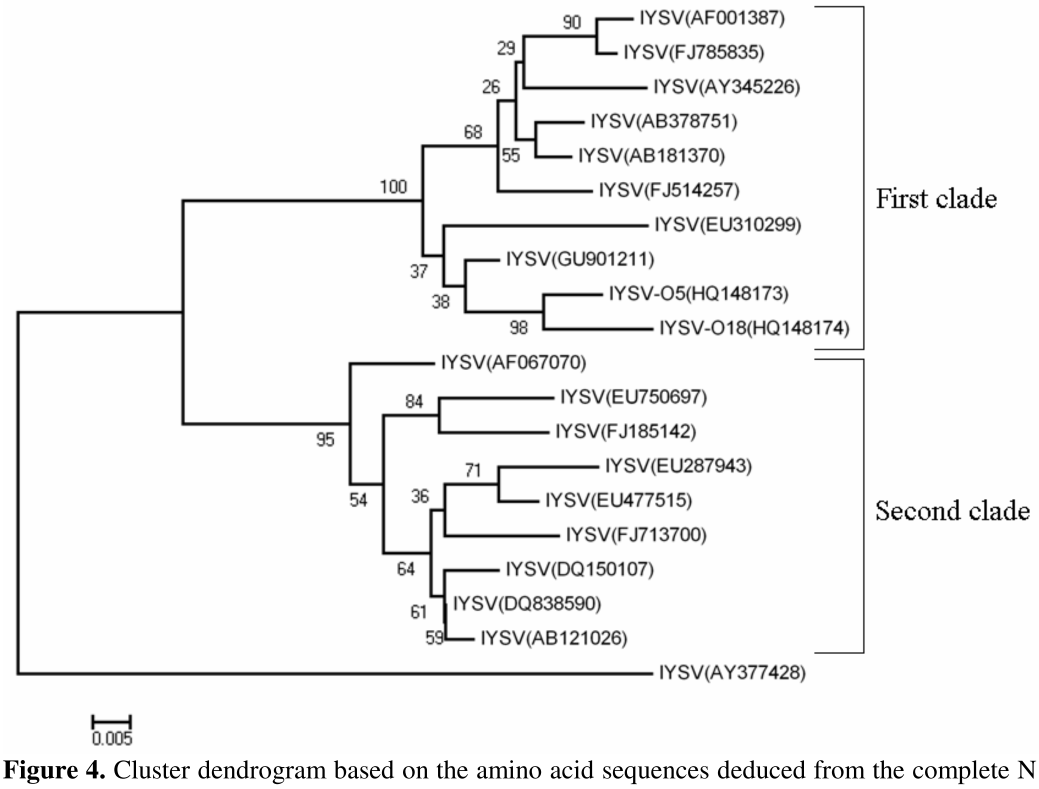

Figure 4. Cluster dendrogram based on the amino acid sequences deduced from the complete N gene sequences of IYSV isolates.

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Table 2. Genetic distances within districts for *N* gene. Standard error (SE) was calculated by using a bootstrap of 500 replicates.

District	Genetic distance					
	Sequence No.	Pi(s)J and C (dS)	Pi(a)J and C (dN)	dN/dS	Genetic distance within each district	SE
World	20	0.33670	0.03829	0.1137	0.094	0.007
American	6	0.22229	0.02384	0.1072	0.0621	0.0055
Asian	7	0.23077	0.02659	0.1152	0.0667	0.0057
European	5	0.41754	0.05407	0.1294	0.1211	0.0096
Oceanian	2	0.53842	0.05647	0.1048	0.1444	0.0147

TYRV and IYSV (Hassani-Mehraban *et al*., 2005) and the fact that IYSV is more closely related to the Asian tospovirus species GBNV and WSMoV than to any of the other tospovirus species (Cortêz *et al*., 1998).

Phylogenetic relationships of IYSV-O5 and -O18 with other 18 IYSV isolates are shown in Figure 4. The IYSV isolates from four geographic origins *i.e.* America, Asia, Europe and Oceania (Table 2) grouped into two major clades. Clade 1 contained several Asian isolates, two European isolates, one Oceanian isolate (from Australia) and an American isolate (from Northern America). Nine isolates were found in clade 2 containing several American isolates, two European isolates, one Oceanian isolate (from New Zealand) and one Asian isolate. The Slovenia isolate (GenBank accession no. AY377428) was equally distant from the isolates in clade 1 and 2. Southern American isolates only clustered within clade 2. As shown in a previous study on *Rice stripe virus* (RSV) (Wei *et al*., 2009), IYSV isolates clustering within two distinct clades (e.g. Japanese and Oceanian isolates) are more diverse than other isolates. IYSV-O5 and IYSV-O18 isolates reported from Iran (Iranian isolates) in Asia clustered within clade 1 and showed a distant phylogenetic relationship with the Southern American isolates. Both Iranian isolates were more closely related to a recent published Sri Lankan isolate and clustered together. The phylogenetic relationship shown in Figure 4 confirmed amino acid sequence identities of the N proteins of the both Iranian isolates with that of Sri Lankan (98%), Dutch (96%) and Brazilian isolates (92%). Also, the analyses indicated that both Iranian isolates and Sri Lankan isolate shared a common ancestor, providing a clue to the origin of the Iranian isolates, although more sequences data are needed. Furthermore, the analyses raise a question over the taxonomic status of the Slovenian isolate (Smith *et al*., 2006) because it did not fit in any of the two clades and its amino acid identities with those of other IYSV isolates had a wider difference of 84-87%.

The results shown in Table 2 indicate that the mean genetic distance within all IYSV isolates is 0.094 and the maximum and minimum genetic diversity can be found within the Oceanian and American isolates, respectively. The geographical origin of a virus can be inferred from the extent of its genetic diversity. If a viral population shows a high genetic diversity, it is usually considered to be more ancient (Wei *et al*., 2009). Although a larger number of isolates should be analyzed for a more accurate estimation of IYSV genetic diversity, based on our data, we can conclude that the Oceanian isolates are older than the American isolates. The historical record of disease may or may not be consistent with the extent of genetic diversity. For example, the genetic diversity of the Indonesian *Rice tungro bacilliform virus* (RTBV) population is higher than its population in the Philippines. The virus was reported in 1840 in Indonesia and in 1940 in the Philippines. Therefore, its genetic diversity is consistent with historic records of rice tungro disease. On the other hand, RSV was initially reported in eastern China, but southwest China could be the geographical origin of the virus in China due to its higher genetic diversity in that region (Wei *et al*., 2009).

IYSV was initially reported in Australia and New Zealand in 2003 and 2008, respectively (Ward *et al*., 2008), but its genetic diversity is higher than the American and European isolates. In spite of iris yellow spot disease being initially described in 1993 (Hall *et al*., 1993) and then its cause designated *Iris yellow spot virus* in the Netherlands in 1998 (Cortêz *et al*., 1998), Oceania could be considered as IYSV geographical origin due to its high genetic diversity in the continent.

Genetic distances between four districts of IYSV isolates (*i.e.* American, Asian, European and Oceanian) were calculated (Table 3). According to the results, genetic diversity varied between the districts, ranging from 0.0801 to 0.1052. Host-virus interaction, vector-virus interaction, and transmission by mechanical ways may explain the high or low genetic diversity in plant virus populations (Wei *et al*., 2009). IYSV infects monocotyledonous and dicotyledonous plants (Kritzman *et al*., 2000) and can be transmitted by mechanical inoculation and by *Thrips tabaci* in a persistent/propagative manner (Cortêz *et al*., 1998). Therefore, the host range, insect-vector, and transmission by mechanical ways of IYSV may explain the observed high genetic diversity in some districts in this study. *T. tabaci* is the only known vector of IYSV (Gent *et al*., 2006), but since the specificity of virus-vector interaction impose significant constraints on the evolution of plant viruses rather than the specificity of virus-host plant interactions (Wei *et al*., 2009), transmission of IYSV by other thrips species may also explain the high genetic diversity. Also, low genetic diversity of IYSV in some districts in the study may be due to founder effect, which is often invoked to explain the low genetic diversity of certain populations of various plant viruses (Wei *et al*., 2009).

Nucleotide diversity and dN/dS ratio is cited in Table 2. The mean ratio is 0.1137 for all IYSV isolates. The ratio is maximum and minimum within European and Oceanian isolates, respectively. For most coding genes, the dN/dS ratio is below unity, which is consistent with negative selection against protein change. In contrast, a ratio above unity may be an indication that adaptive or positive selection is driving gene divergence (Yang and Bielawski, 2000; Nei and Gojobori, 1986). In this study, the dN/dS ratio is below unity, implying that *N* gene in the IYSV isolates is under negative selection. High dS in Oceanian isolates indicates that IYSV is under a strong negative selection pressure in this region and older than other IYSV isolates. However, more nucleotide sequences should be analyzed for a more accurate estimation of the selection effect on IYSV evolution.

Multiple sequences alignment between *N* protein amino acid sequences of IYSV-O5 and IYSV-O18 isolates revealed that the isolates differed by 6 amino acids at positions 46 (Thr/Met), 121 (Tyr/His), 254 (Ala/Val), 267 (Ser/Pro), 269 (Lys/Glu) and 270 (Asp/Arg). The significance of these amino acid substitutions is not known. To get a better insight in the genetic diversity of IYSV in Iran, a large number of isolates from different onion producing regions have to be analyzed.

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Table 3. Genetic distances between districts for *N* gene. Standard error (SE) was calculated by using a bootstrap of 500 replicates.

Districts	American	Asian	European	Oceanian
American		SE: 0.0092	SE: 0.0076	SE: 0.007
Asian	0.1052		SE: 0.0076	SE: 0.0074
European	0.0997	0.103		SE: 0.0078
Oceanian	0.0801	0.0877	0.1051	

(Wageningen University, Wageningen, The Netherlands) for helpful suggestions and technical assistance.

REFERENCES

1. Anon. 2009. Ministry of Jihad-e-Agriculture. Retrieved from http://www.maj.ir/portal/File/ShowFile.aspx?ID=101202ae-46ff-4b27-b115-b59dfcbfa294.
2. Bulajić, A., Djekić, I., Jović, J., Krnjajić, S., Vučurović, A., and Krstić, B. 2009. Incidence and Distribution of *Iris Yellow Spot Virus* on Onion in Serbia. *Plant Dis.,* **93:** 976-982.
3. Clark, M. F. and Adams, A. N. 1977. Characteristics of the Microplate Method of Enzyme Linked Immunosorbent Assay for the Detection of Plant Viruses. *J. Gen. Virol.,* **34:** 475-483.
4. Cortêz, I., Livieratos, I. C., Derks, A., Peters, D. and Kormelink, R. 1998. Molecular and Serological Characterization of *Iris Yellow Spot Virus*: A New and Distinct Tospovirus Species. *Phytopathol.,* **88:** 1276-1282.
5. de Ávila, A. C., Huguenot, C., Resende, R. de O., Kitajima, E. W., Goldbach, R. W. and Peters, D. 1990. Serological Differentiation of 20 Isolates of *Tomato Spotted Wilt Virus*. *J. Gen. Virol.,* **71:** 2801-2807.
6. Gent, D. H., du Toit, L. J., Fichtner, S. F., Mohan, S. K., Pappu, H. R. and Schwartz, H. F. 2006. *Iris Yellow Spot Virus*: An Emerging Threat to Onion Bulb and Seed Production. *Plant Dis.,* **90:** 1468-1480.
7. Gent, D. H., Schwartz, H. F. and Khosla, R. 2004. Distribution and Incidence of *Iris Yellow Spot Virus* in Colorado and Its Relation to Onion Plant Population and Yield. *Plant Dis.,* **88:** 446-452.
8. Gera, A., Kritzman, A., Beckelman, H., Cohen, J. and Raccah, B. 2002. Detection of *Iris Yellow Spot Virus* in Lisianthus. *Proc. 10th IS Virus Diseases Ornamentals*, (Ed): Hammond, J., Acta Hort., ISHS, 568 PP.
9. Ghotbi, T. and Shahraeen, N. 2005. Reports on Occurrence of Three Tospoviruses in Onion (*Allium Cepa*) from Markazi Province. Iran. *J. Plant Path.,* **41:** 57-58.
10. Hall, J. M., Mohan, K., Knott, E. A. and Moyer, J. W. 1993. Tospoviruses Associated with Scape Blight of Onion (*Allium cepa*) Seed Crops in Idaho. *Plant Dis.,* **77:** 952.
11. Hassani-Mehraban, A., Botermans, M., Verhoeven, J. Th. J., Meekes, E., Saaijer, J., Peters, D., Goldbach, R. and Kormelink, R. 2010. A Distinct Tospovirus Causing Necrotic Streak on *Alstroemeria Sp.* in Colombia. *Arch. Virol.,* **155:** 423-428.
12. Hassani-Mehraban, A., Saaijer, J., Peters, D., Goldbach, R. and Kormelink, R. 2005. A New Tomato-Infecting Tospovirus from Iran. *Phytopathol.,* **95:** 852-858.
13. Jafarpour, B., Sabokkhiz, M. A. and Falahati Rastgar, M. 2009. *Iris Yellow Spot Virus* (IYSV) A New Viral Disease of Onion in Iran. *J. Plant Protec.*, **23:** 106-107.
14. Kritzman, A., Beckelman, H., Alexandrov, S., Cohen, J., Lampel, M., Zeidan, M., Raccah, B. and Gera, A. 2000. Lisianthus Leaf Necrosis: A New Disease of Lisianthus Caused By I*ris Yellow Spot Virus. Plant Dis.,* **84:** 1185-1189.
15. Kritzman, A., Lampel, M., Raccah, B. and Gera, A. 2001. Distribution and Transmission Of I*ris Yellow Spot Virus*. *Plant Dis.,* **85:** 838-842.
16. Mitsuru, O., Kaoru, H., Seiji, U., Fumio, F., Tosaku, M., Katsusuke, E. and Tooru, I. 2005. Necrotic Streaks of Alstroemeria (*Alstroemeria Spp*.) Caused By *Iris Yellow Spot Virus* (IYSV). *Jpn. J. Phytopathol.,* **71:** 119-122.
17. Mumford, R. A., Barker, I. and Wood, K. R. 1996. The Biology of the Tospoviruses. *Ann. Appl. Biol.,* **128:** 159-183.
18. Nei, M. and Gojobori, T. 1986. Simple Methods for Estimating the Numbers of Synonymous and Nonsynonymous Nucleotide Substitutions. *Mol. Biol. Evol.,* **3:** 418-426.
19. Pappu, H. R., duToit, L. J., Schwartz, H. F. and Mohan, S. K. 2006. Sequence Diversity of the Nucleoprotein Gene of *Iris Yellow Spot Virus* (Genus *Tospovirus*, Family *Bunyaviridae*) Isolates from the Western Region of the United States. *Arch. Virol.,* **151:** 1015-1023.
20. Persley, D. M., Thomas, J. E. and Sharman, M. 2006. Tospoviruses: An Australian Perspective. *Aust. Plant Pathol.,* **35:** 161-180.
21. Pozzer, L., Bezerra, I. C., Kormelink, R., Prins, M., Peters, D., Resende, R. de O. and de Ávila, A. C. 1999. Characterization of a Tospovirus Isolate of *Iris Yellow Spot Virus* Associated with a Disease in Onion Fields in Brazil. *Plant Dis.,* **83:** 345-350.

22. Rozas, J. and Rozas, R. 1999. DnaSP version 3: An Integrated Program for Molecular Population Genetics and Molecular Evolution Analysis. *Bioinformatics,* **15:** 174-175.
23. Sanger, F., Nicklen, S. and Coulson, A. R. 1977. DNA Sequencing with Chain-terminating Inhibitors. *Proc. Natl. Acad. Sci. USA,* **74:** 5463-5467.
24. Smith, T. N., Jones, R. A. C. and Wylie, S. J. 2006. Genetic Diversity of the Nucleocapsid Gene of I*ris Yellow Spot Virus*. Australas. *Plant Pathol.,* **35:** 359-362.
25. Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.,* **24:** 1596-1599.
26. Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice. *Nucleic Acids Res.,* **22:** 4673-4680.
27. Ward, L. I., Perez-Egusquiza, Z., Fletcher, J. D., Ochoa Corona, F. M., Tang, J. Z., Liefting, L. W., Martin, E. J., Quinn, B. D., Pappu, H. R. and Clover, G.R.G. 2008. First Report of I*ris Yellow Spot Virus* on *Allium Cepa* in New Zealand. *Plant Dis.,* **17:** 38.
28. Wei, T. -Y., Yang, J. -G., Liao, F. -L., Gao, F. -L., Lu, L. -M., Zhang, X. -T., Li, F., Wu, Z. -J., Lin, Q. -Y., Xie, L. -H. and Lin, H. -X. 2009. Genetic Diversity and Population Structure of *Rice Stripe Virus* in China. *J. Gen. Virol.,* **90:** 1025-1034.
29. Yang, Z. and Bielawski, J. P. 2000. Statistical Methods for Detecting Molecular Adaptation. *Trends Ecol. Evol.,* **15:** 496-503.

شناسایی مولکولی ویروس لکه زرد زنبق (*Iris yellow spot virus*) روی پیاز در ایران

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چکیده

علائم ویروسی شبیه آلودگی های ناشی از ویروس لکه زرد زنبق(*Iris yellow spot virus*; IYSV) در چندین مزرعه پیاز در شهرستان چناران از استان خراسان رضوی، مشاهده گردید. تلقیح مکانیکی برخی گیاهان آزمون با استفاده از عصاره تهیه شده از نمونه های پیاز دارای علائم آلودگی، نشان داد که علائم ایجاد شده شبیه علائم توصیف شده برای این ویروس هستند. در آزمون الایزا، ویروسی که به طریق مکانیکی منتقل شده بود، فقط با آنتی سرم اختصاصی ویروس لکه زرد زنبق واکنش مثبت نشان داده و در برابر آنتی سرم اختصاصی ۷ گونه توسپوویروس دیگر هیچ گونه واکنش مثبتی مشاهده نگردید. در آزمون RT-PCR، فقط در حضور پرایمرهای اختصاصی برای تکثیر ژن نوکلئوپروتئین (Nucleoprotein; N) ویروس لکه زرد زنبق، یک قطعه DNA با اندازه تقریبا ۸۲۲ جفت باز از بوته های آلوده *Nicotiana benthamiana* تکثیر گردید. بعد از همسانه سازی و تعیین توالی، توالی آمینواسیدی پروتئین N این دو جدایه (ثبت شده در GenBank با رس شمارHQ148173 وHQ148174) نشان داد که این دوتوالی، ۹۸ درصد با توالی آمینواسیدی پروتئین N یک جدایه از ویروس لکه زرد زنبق در سری لانکا، ۹۶ درصد با یک جدایه در هلند و ۹۲ درصد با یک جدایه در برزیل شباهت دارد. این اولین شناسایی مولکولی این ویروس در ایران می باشد.