

## First Nearly Complete Genome Sequence of *Onion Yellow Dwarf Virus* Infecting Garlic in Iran

A. Entezari<sup>1</sup>, M. Mehrvar<sup>1\*</sup>, and M. Zakiagh<sup>1</sup>

### ABSTRACT

For the first time, the nearly complete genome sequence of onion yellow dwarf virus (OYDV) was found in Iran from garlic (*Allium sativum* L.) by deep RNA sequencing. Complete coding sequence of the Iranian isolate of OYDV (MN528769) consists of 10,212 nucleotides (nt), encodes a polyprotein with 3,403 amino acids (aa). Pairwise sequence comparisons showed that IR-Kh2 shares 74.84-97.39% identity at the nt level and 75.67-98% identity at the amino acids level, respectively with other OYDV isolates deposited in the GenBank previously. According to the phylogenetic analysis, the OYDV isolates were divided into two main groups based on the coding sequence of genome and the Iranian OYDV isolate cluster together with the Australian (MS/SW1), Spanish (SG1), Chinese (G78 and G37-2), and Indian (RR1) isolates. Furthermore, a genetic recombination analysis was also performed, in which a putative recombination event was detected in the nuclear inclusion body b (NIb) gene.

**Keywords:** *Allium sativum* L., OYDV, Phylogenetic analysis, Recombination

### INTRODUCTION

Garlic (*Allium sativum* L.) is one of the medicinal and economically important crop plants belonging to the Amaryllidaceae family and well known for its worldwide applications. It is widely used as a spicy vegetable (Sevik *et al.*, 2018). In Iran, despite agricultural potential, we are still facing of garlic shortage in the market (Baghalian *et al.*, 2010), mainly due to the high prevalence of plant diseases, particularly viral infections (Shahraeen *et al.*, 2008; Baghalian *et al.*, 2010). Most garlic plants are infected by several viruses often in complex mixtures and belonging to different taxonomic groups known as “garlic viral complex”, which includes mainly viruses from the genera *Potyvirus*, *Carlavirus*, and *Allexivirus* and have significantly reduced bulb weight and yield all around the world (Gawande *et al.*, 2013;

Da Silva *et al.*, 2019). Common among these are members of the genus *Potyvirus*, namely, onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), and shallot yellow stripe virus (SYSV) (Abraham *et al.*, 2019). Nonetheless, the most prevalent garlic viruses are OYDV and LYSV, which cause a noticeable foliar damage and reduce bulb quality and yield (Katis *et al.*, 2012). It should be noted that OYDV acts as a major element of the garlic virus complex (Arya *et al.*, 2006; Gawande *et al.*, 2013; Manglli *et al.*, 2014). This virus has a narrow host range [Onion (*Allium cepa*), garlic (*A. sativum*), shallot (*A. ascalonicum*) and a few ornamental *Allium*] and survives in bulbs and sets, so, can be transmitted during vegetative reproduction (Takaki *et al.*, 2006; Mahmoud *et al.*, 2008). The green peach aphid, *Myzus persicae*, as well as other aphids, spreads the virus from plant to plant in a non-persistent manner (Melhus *et al.*,

<sup>1</sup> Department of Plant Pathology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran.

\* Corresponding author: e-mail: mehrvar@um.ac.ir

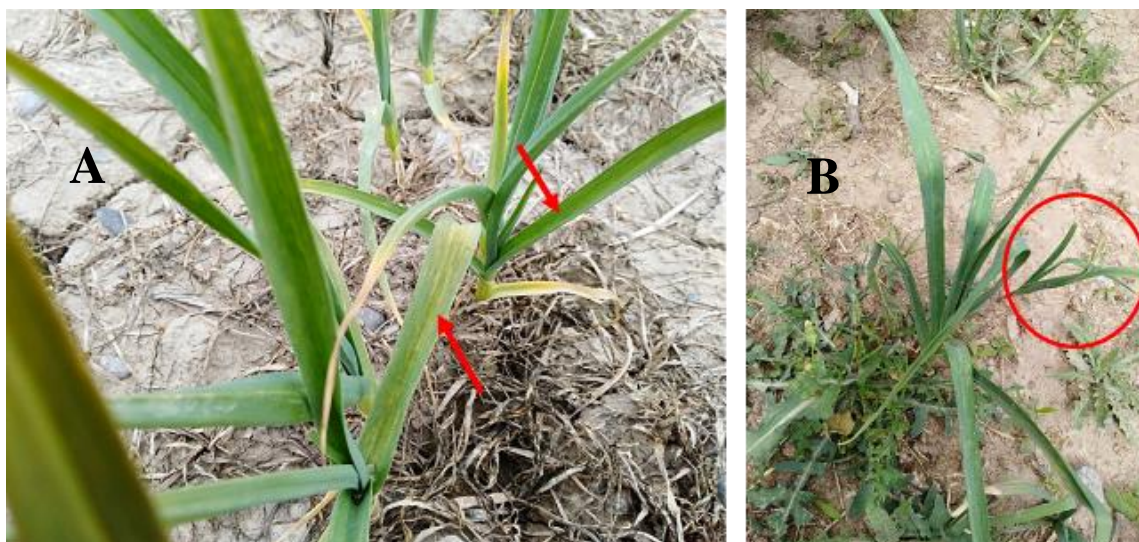
1929; Bos, 1976; Manglli *et al.*, 2014; Verma *et al.*, 2015). For the first time in Iran, OYDV was reported via serological and molecular methods from garlic (Shahraeen *et al.*, 2008; Baghalian *et al.*, 2010).

However, due to the shortage of research on garlic pathogenic viruses, we conducted a study on OYDV, which is one of the most widely distributed and important viruses in garlic. In this study, we aimed to determine the first nearly complete genome sequence of Iranian OYDV isolate with *de novo* assembly and annotation of contigs to better understand the diversity, biology, and evolutionary history of the virus, which is vital for its management.

## MATERIALS AND METHODS

This work was performed on a pooled sample of the garlic leaves (ten fresh leaf samples were combined) showing yellow striping, dwarfing, and mosaic symptoms on young leaves. Samples were collected from one of the major areas under the cultivation of garlic in Khorasan Razavi Province, Iran (Figure 1). Leaves were held together in a cluster and sliced with a scalpel blade so that 100 mg of leaf tissue, representing nearly

equal amounts of tissue from each leaf, was removed. Total RNA was extracted using SV Total RNA Isolation Kit (Promega Madison, WI, USA). Quality, concentration and integrity of RNA were confirmed by using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Also, RNA quality was checked using agarose gel electrophoresis. Samples that had the entire 18S and 28S subunits on agarose gel, without genomic DNA contamination, were selected for library preparation and sequencing. Ribosomal RNA was depleted from the purified total RNA with the Plant Ribo-Zero rRNA depletion Kit (Epicentre, Madison, WI, USA). For cDNA library construction, Illumina TruSeq Stranded Total RNA Sample Preparation Kit (Illumina, San Diego, CA, USA) was used. Sequencing was performed with the Illumina NovaSeq 6000 technology with paired-end read length at 151 bp provided by Macrogen Inc (Seoul, Republic of Korea). Low quality reads and adaptor sequences were removed from the raw reads. Then clean reads were mapped to reads from healthy-looking plants. Further unmapped reads were *de novo* assembled using CLC Genomics Workbench (version 12, QIAGEN, Venlo,



**Figure 1.** Most common virus-induced symptoms (see arrows and circle) found in garlic plants grown in Kadcan region of Razavi Khorasan Province, Iran: (A) mosaic, (B) stunting.

The Netherlands) and Geneious Prime 2019 (Biomatters, Auckland, New Zealand). Preliminary viral sequences were identified using BLASTn and BLASTx (<https://blast.ncbi.nlm.nih.gov>) against non-redundant nucleotide database of the GenBank to identify sequences which shared identity to known garlic viruses available in the NCBI database. Moreover, putative open reading frames (ORFs) of OYDV was identified by ORF finder

(<https://www.ncbi.nlm.nih.gov/orffinder>).

Finally, Consensus sequence for IR-Kh2 isolate was deposited in the GenBank. In order to confirm integrity of the consensus genome sequence of the virus, RT-PCR using specific primers of conserved region of RNA-dependent RNA polymerase gene and 3'-UTR regions (Arya *et al.*, 2006) and potyvirus-degenerate primer pairs targeting the CI and NIb coding region (Ha *et al.*, 2008; Zheng *et al.*, 2010) was done followed by Sanger sequencing of amplicons (Table S1). The consensus sequence of OYDV obtained from previous step was subjected to sequence analyses. Multiple sequence alignments were conducted using Clustal Omega (Sievers *et al.*, 2011). Evolutionary analysis of putative virus-derived sequence was inferred using the Neighbor-joining method with 1000 replicate in bootstrap test as implemented within Mega X software (Kumar *et al.*, 2018). Moreover, to identify the possible recombination event, the IR-Kh2 genome and 55 other OYDV isolates retrieved from the GenBank (Table S2) were subjected to recombination analysis using the RDP4 (Recombination detection program) (Martin *et al.* 2015), with various recombination detection methods including RDP, GENECONV, Chimaera, MaxChi, BOOTSCAN, and SISCAN. Default settings and a Bonferroni-corrected *P*-value cut off of 0.05 were used throughout the analysis.

## RESULTS

### OYDV Genome Analysis

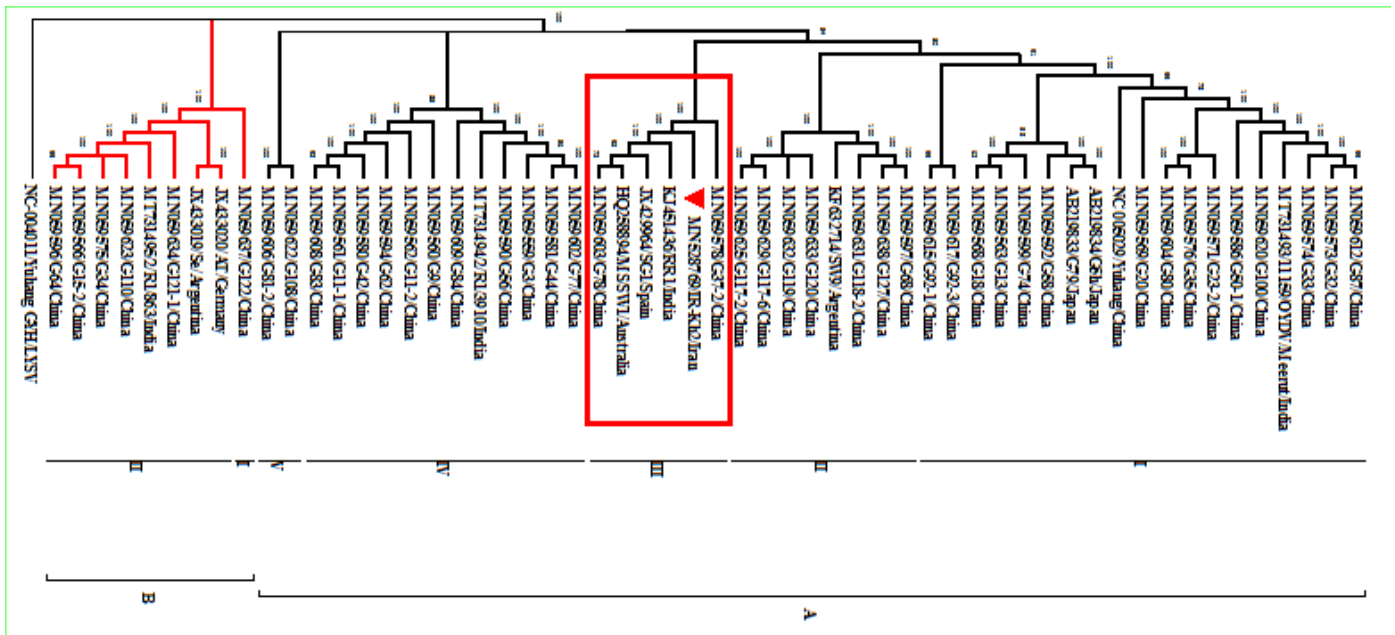
In the current study, consensus sequence of Iranian OYDV isolate has been deposited in the GenBank under accession number MN528769 and designated as OYDV isolate IR-Kh2. The complete coding sequence of IR-Kh2 contained 10,212 nts in length, encoding 3,403 amino acids, with an estimated molecular mass of 385.1 kDa. It has typical genomic organization and structural characteristic of potyviruses. The first initiator for translation of single large ORF is AUG codon at nucleotides 94–96 and the UAA termination codon located at nt position of 10303 – 10305. The genome flanked to an untranslated region (UTR) with 214 nt length at 3'end. In the partial sequence of 5' UTR, there are a large number of CAA recurring sequences which are involved in enhancing genomic translation as described by previous studies (Gallie and Walbot, 1992). Moreover, the putative P3N-PIPO ORF (3864-4088 nt) which encodes a protein of 74 AA residues, starting within the highly conserved G1A6 motif (Adams *et al.*, 2005; Chung *et al.*, 2008; Yaghmaiean, 2015), was found embedded within the P3 cistron of IR-Kh2. Comparison of the polyprotein of Iranian isolate with other OYDV isolates showed that nine putative protease cleavage sites were exist based on diagnosis motifs for the viral proteases (Chen *et al.*, 2003; Adams *et al.*, 2005; Celli *et al.*, 2013; Manglli *et al.*, 2014) (Table 1), so, the polyprotein was also cleaved into ten functional proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP) (Figure S1) with the assistance of P1, HC-Pro, and NIa-Pro which are three viral-encoded proteases (Chen *et al.*, 2003; Adams *et al.*, 2005; Celli *et al.*, 2013; Manglli *et al.*, 2014). Several potyvirus-conserved motifs were identified in the polyprotein sequence of IR-Kh2 isolate. For example: the serine-type protease domain at the 3'-end of P1 is H<sub>366</sub> (X)<sub>39</sub> G<sub>406</sub>XSG<sub>409</sub>

**Table 1.** Genomic structure of IR-Kh2 isolate and putative cleavage sites.

Untranslated regions and of functional regions of polyprotein	Start-position (nt)	End	Size in nt/aa	Predicted cleavage sites (C-terminus)
Partial 5'UTR	1-93		93	
P1	94-1467		1374-458	IEHY/S
HC-Pro	1468-2847		1380-460	YNVG/G
P3	2848-4437		1590-530	VIYQ/A
6kl	4438-4593		156-52	VHYQ/A
PIPO	3864-4088		225-74	PIPO
CI	4594-6504		1911-637	VNYQ/S
6K2	6505-6663		159-53	VKYQ/A
NIa-VPg	6664-7251		588-196	VSFE/A
NIa-Pro	7252-7977		726-242	VTFQ/S
NIb	7978-9531		1554-518	VRYQ/A
CP	9532-10302		771-257	
3'UTR	10306-10519		214	

(X)<sub>22</sub> V<sub>432</sub>RG (X)<sub>5</sub> I<sub>440</sub> (X)<sub>17</sub> Y<sub>458</sub>, while G<sub>406</sub>XSG<sub>409</sub> motif is seems to be the active site of a serine protease which cleaves the protein downstream of the final Y (or F) residue (Chen *et al.*, 2003; Valli *et al.*, 2007, 2015). Besides the proteolytic motif, M<sub>430</sub>VVRGR<sub>435</sub> was found in P1 (Valli *et al.*, 2007, 2015). In the N-terminal region of HC-Pro, the highly conserved motif K<sub>512</sub>ITC<sub>515</sub> which is located in cysteine (Cys)-rich region, and associated with binding of virions to aphid stylet was found (Llave *et al.*, 2002; Takaki *et al.*, 2006). Two conserved motifs associated with aphid transmission, C<sub>752</sub>CCVT and P<sub>770</sub>TK was located near the C-terminal region of HC-Pro (Takaki *et al.*, 2006). A cysteine proteinase motif, GYCY, was located at position 802–805 in the polyprotein of IR-Kh2 isolate. In the central region of HC-Pro, there was another conserved motif, F<sub>641</sub>RNK<sub>644</sub>, which is associated with aphid transmission, symptom expression and suppression of RNA silencing (Shiboleth *et al.*, 2007). Furthermore, near the PTK motif there is another conserved motif, I<sub>777</sub>GN<sub>779</sub>, which is thought to be essential in genome amplification (Urcuqui-Inchima *et al.*, 2001; Plisson *et al.*, 2003). CI is an RNA helicase protein with various motifs (Urcuqui-Inchima *et al.*, 2001). In OYDV CI protein, the characteristic RNA helicase domains, D<sub>1495</sub>ECH<sub>1498</sub>, L<sub>1754</sub>VYV<sub>1757</sub>, V<sub>1805</sub>ATNIIENGVTL<sub>1816</sub>, and

G<sub>1849</sub>ERIQLGRVGR<sub>1860</sub> were found (Riechmann *et al.*, 1992; Kadare and Haenni, 1997). The NIb gene sequences of OYDV isolates included all the conserved motifs C<sub>2831</sub>VDDFN<sub>2836</sub>, G<sub>2935</sub>NNSGQ<sub>2940</sub>, G<sub>2939</sub>QPSTVVD<sub>2946</sub>, F<sub>2817</sub>TAAPIE<sub>2823</sub> and D<sub>2876</sub>GSRFDS<sub>2882</sub> (Mangli *et al.*, 2014). In addition, in NIb protein the putative active site of RNA-dependent RNA polymerase, SG(X)<sub>3</sub>T(X)<sub>3</sub>NT(X)<sub>30</sub>GDD (Urcuqui-Inchima *et al.*, 2001; Mangli *et al.*, 2014), was conserved at the amino acid position 2938–2981. Previous studies showed that NIb-CP interaction is sensitive to changes of the highly conserved motif GDD in NIb (Urcuqui-Inchima *et al.*, 2001). N-terminal region of OYDV CP gene which is exposed on the virion surface contains a highly conserved DAG motif (López-Moya *et al.*, 1999; Arya *et al.*, 2006). Previous studies showed that the DAG motif is essential for aphid transmission, and a particular communication between CP and HC-Pro with the involvement of the DAG and KITC motifs in each component, respectively, was important in the virus transmission by aphids (López-Moya *et al.*, 1999; Llave *et al.*, 2002). Conversely the DAG motif was replaced by D<sub>3172</sub>TG motif in IR-Kh2 isolate. Another conserved motif, M<sub>3253</sub>VWCIENGTSPT<sub>3263</sub>, A<sub>3336</sub>FDF<sub>3339</sub>, and Q<sub>3356</sub>MKAAA<sub>3361</sub>, which identified in the CP of most potyviruses (Dujovny *et al.*, 2000), were found in IR-Kh2 as well.



**Figure 2.** Neighbor-joining phylogenetic tree based on the nucleotide sequence of polyprotein of OYDV isolates was generated with a 1000 replicate of bootstrap test. Nodes with bootstrap values below 50% were collapsed. *Leek yellow stripe virus* (NC-004011) selected as an outgroup.

### Sequence Comparisons of Iranian OYDV

Pairwise polyprotein comparison between IR-Kh2 and 55 other isolates of OYDV from different geographical locations of the world retrieved from the GenBank, revealed that it shared 74.84-97.39% and 75.67-98% similarities at the nt and aa sequence level, respectively. Iranian isolate of OYDV shared the highest similarity with Australian isolate, MS/SW1 at the nt (97.39%) and aa (98%) sequence level, respectively. Moreover, amino acids sequence comparison of each mature protein between IR-Kh2 and 55 other OYDV isolates was performed. The result showed that P1 and P3 protein sequences were pointedly more variable than other mature protein which maybe somehow due to mutation in these regions. The similarities of P1 and P3 are as low as 45.56-93.01% and 61.69-98.68%, respectively.

### Phylogenetic Analysis

The coding sequence of IR-Kh2 and other 55 OYDV isolates from different geographical areas were used for phylogenetic analysis. The neighbor-joining phylogenetic tree divided the OYDV isolates into two main groups: A and B (Figure 2). The first group included 47 isolates from China, Japan, Iran, Australia, Spain, India, and Argentina. These isolates were further divided into five subgroups (I, II, III, IV and V); subgroup I was composed of 16 isolates from China, 2 from Japan, and 1 from India, while subgroup II comprised 7 isolates from China and 1 from Argentina. Subgroup III was created by an OYDV isolate from Iran along with the isolates from China (G78 and G32-7), Australia (MS/SW1), Spain (SG1), and India (RR1). Subgroup IV consisted of 11 isolates from China and 1 isolate from India whereas subgroup V was composed of only two isolates from China. However, group B was further divided into two



subgroups (I and II) comprising 7 garlic infecting isolates from China (G122, G121-1, G110, G34, G15-2 and G64) and India (2/R1/863/India), followed by 2 onion infecting isolates from Germany (OYDV-AT) and Argentina (OYDV-Se). It should be noted that all the isolates in the first group were isolated from garlic, except for RR1 from India, which was isolated from onion (Figure 2).

### Recombination Analysis

To estimate the number and location of recombination breakpoints in IR-Kh2 isolate, the recombination detection analysis with seven algorithms in RDP4.0 was done for the 56 aligned sequences. Only a strong recombination signal detected by six method (RDP: 7.01E-32, GENECONV: 4.14E-28, Bootscan: 4.67E-35, Maxchi: 2.83E-4, Chimaera: 3.75E-14, SiSscan: 2.98E-33, Se: 4.58E-6) in IR-Kh2 isolate, was localized from 8994 to 9204 in N1b gene (Table 2). The recognized major and minor parents of the recombination were the Australian isolate, MS/SW1 (HQ258894) and Chinese isolate, G44 (MN059581), respectively. In addition to IR-Kh2 isolate, recombination signals were detected in 11 other isolates of OYDV in N1b gene or flanking genes (Table 2).

### DISCUSSION

Nearly complete genome sequence of Iranian isolate of OYDV (MN528769) consists of 10,519 nt length. It consists of 10212 nt of coding region which flanked to partial sequences of 5' (93 nt) and 3' (214 nt) of untranslated regions (UTRs). Other viral species belonging to the potyviruses, carlaviruses, and allexiviruses were also identified from the sample (unpublished data), in addition to OYDV. Hence, the observed disease symptoms could not be completely associated with a single virus in a mixed nature of the infection. Phylogenetic tree constructed based on the entire genomic nucleotide sequences demonstrated that Iranian isolate was located in group A, subgroup III,

alongside isolates from Australia, Spain, China, and India. According to the pairwise sequence comparisons based on the nucleotides and amino acid sequence, IR-Kh2 shared the highest similarity with Australian MS/SW1 isolate. IR-Kh2 polyprotein includes the previously identified and extremely conserved motifs described in potyviruses (Chen *et al.*, 2003; Valli *et al.*, 2007, 2015; Takaki *et al.*, 2006). However, some of these motifs were not conserved in IR-Kh2. The CP of potyviruses is the most extensively characterized gene product playing important roles in virus life cycle and virus-vector interaction (Urcuqui-Inchima *et al.*, 2001). DAG, a critical motif near the N terminus of the CP gene, is found in some OYDV isolates as well as numerous other potyviruses (López-Moya *et al.*, 1999; Dombrovsky *et al.*, 2005; Manglli *et al.*, 2014). Though, it is not completely conserved in all potyviruses and it can occasionally be substituted. Analysis of OYDV isolates retrieved from the GenBank along with IR-Kh2, revealed that the aphid transmissibility aspartic acid alanine-glycine (DAG) motif found in the putative CP protein of all OYDV isolates from onion in Argentina (JX433019), Germany (JX433020) or India (KJ451436) or from garlic in Argentina (KF632714), India (MT731495, MT731494) and China (MN059559, MN059581, MN059566, MN059568, MN059575, MN059563, MN059562, MN059599, MN059596, MN059594, MN059590, MN059602, MN059634, MN059623, MN059622, MN059613, MN059609, MN059608, MN059606, MN059561). Interestingly, alanine (Hydrophobic and nonpolar) in second position of the DAG motif was replaced by threonine (Hydrophilic and polar) in OYDV isolates from Australia (HQ258894), Spain (JX429964), Japan (AB219834, AB219833), Iran (MN528769), India (MT731493) and some Chinese isolates from garlic. Reports of naturally occurring DTG motifs were also found in some other potyviruses such as the *Wild potato mosaic virus* (WPMV) (Hasan, 2004). However, several previous reports indicate the lower aphid-transmissibility of potyviruses holding motifs other than DAG (DAS, DAA, or DAL). This may be the case for DTG holding OYDV isolates with potential low aphid transmissibility power (López-Moya *et al.*,

**Table 2.** Characteristics of recombination events detected in some isolates of *Onion yellow dwarf virus*.

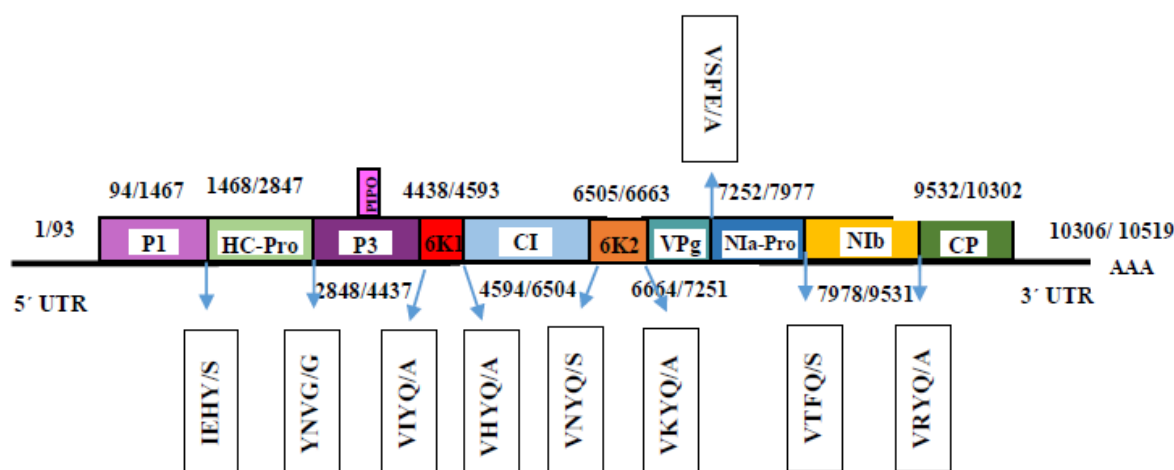
Event	Recombinant isolate	recombination site	Recombinant length	Parental sublineage <sup>a</sup>	Recombination detected by <sup>b</sup>	P-value <sup>c</sup>
1	MN528769/IR-Kh2	8994-9204	210	HQ258894*MN059581	<b>R.G.B.M.C.S.Se</b>	4.67E-35
2	MN059609/G84	5931-9045	3114	MN059602*KJ451436	<b>R.G.B.M.C.S.Se</b>	2.025E-32
3	MN059617/G92-3	8167-10211	2044	AB219833*NC-005029	<b>R.G.B.M.C.S.Se</b>	4.677E-35
4	MN059581/G44	7058-9958	2900	MN059596*NC_005029	R.G.B.M.C.S.Se	1.649E-24
5	MN059559/G3	7097-9955	2858	MN059596*NC_005029	R.G.B.M.C.S.Se	1.649E-24
6	MN059602/G77	7097-9955	2858	MN059596*NC_005029	R.G.B.M.C.S.Se	1.649E-24
7	MN059590/G56	7097-9598	2501	MN059596*NC_005029	R.G.B.M.C.S.Se	1.649E-24
8	MN059581/G44	8630-9789	1159	AB219834*MN059594	R.G.B.M.C.S.Se	1.140E-10
9	MN059559/G3	8630-9783	1153	AB219834*MN059594	R.G.B.M.C.S.Se	1.140E-10
10	MN059602/G77	8642-9783	1141	AB219834*MN059594	R.G.B.M.C.S.Se	1.140E-10
11	MN059562/G11-2	8786-9921	1135	AB219834*MN059594	R.G.B.M.C.S.Se	1.140E-10
12	MN059590/G56	8579-9634	1055	AB219834*MN059594	R.G.B.M.C.S.Se	1.140E-10

<sup>a</sup> Parental sublineage estimated by RDP Program; <sup>b</sup> Recombination-detecting programs representing significant signal showed in bold; **R:** RDP, **G:** GENECONV, **B:** Bootscan, **M:** Maxchi, **C:** Chimaera, **S:** SiSscan, **Se:** 3Seq; <sup>c</sup> The greatest P-value calculated by the program for the recombination event.



1999; Dombrovsky *et al.*, 2005; Mangli *et al.*, 2014). The variety in this motif (DAG to DTG) may be resultant from the natural vegetative propagation of the garlic or adaptation of the virus to its host plant. Recombination is an important driving force in evolution and divergence of viruses, such as potyviruses (Nagy, 2008). It has been recently proposed as a key factor associated with increasing host range to adapting to new hosts (Kehoe *et al.*, 2014). This study provide evidence for a strong potential recombination event in an Iranian isolate of OYDV. Analysis demonstrated a strong unique recombination event in IR-Kh2 isolate in Nib gene. This gene plays an important role in life cycle and genetic diversity of potyviruses, concurrently, it can be

a major evolutionary way to adapt to the new hosts and new environmental conditions (Lian *et al.*, 2013; Verma *et al.* 2016). Finally, the accurate identification of viruses present in garlic plants will help to use the appropriate strategies to reduce viral incidence in garlic-growing areas. To the best of our knowledge, this is the first report of the nearly complete genome sequence of garlic infecting OYDV isolate in Iran. OYDV is the major element of the garlic virus complex which could be a limiting factor for successful allium production along with other viruses of this complex (Lot *et al.*, 1998; Bagi *et al.*, 2012). Thus, further investigation is necessary to prevent the spread of these pathogens to new locations and hosts.



**Figure S1.** Schematic representation of the genomic structure of *onion yellow dwarf virus* and the predicted proteolytic cleavage sites of the OYDV polyprotein. The cleavage sites are shown inside the rectangle. P1, the first protein; HC-Pro, helper component-proteinase; P3, the third protein; 6K1 and 6K2, 6 kDa protein 1 and 2; CI, cytoplasmic inclusion protein; VPg, viral genome-linked protein; NIa-Pro, 49 kDa proteinase; Nib, nuclear inclusion protein b; CP, coat protein. PIPO (nucleotides 3864 to 4088) derived from RNA polymerase slippage on the P3 cistron.

**Table S1.** PCR primers used for the OYDV detection by RT-PCR.

Primer Code	Primer Sequence (5' to 3')	Size of amplified fragment (bp)	Reference
OYDV-F/ R	F- ATAGCAGAAACAGCTCTTA R- GTCTCYGTAATTCACGC	1111 bp	Arya <i>et al.</i> 2006
NIB2-F/ NIB3-R	F- GTITGYGTIGAYGAYTTYAAYAA R- TCIACIACIGTIGAIGGYTGNCC	350 bp	Zheng <i>et al.</i> 2010
CIFor/ CIREv	F-GGIVVIGTIGGIWSIGGIAARTCIAC R-ACICCRTTYTCDATDATRTTIGTIGC	700 bp	Ha <i>et al.</i> 2008



**Table S2.** Isolates of onion yellow dwarf virus (OYDV) used in this study.

Isolate	Country	Collection-Date	GenBank accession no.
RR1	India	2013	KJ451436
Yuhang	China	Unknown	NC-005029
OYDV-AT	Germany	1994	JX433020
OYDV-Se	Argentina	2004	JX433019
G5h	Japan	Unknown	AB219834
G79	Japan	Unknown	AB219833
2/R1/863	India	Unknown	MT731495
11159/OYDV/Meerut	India	Unknown	MT731493
2/R1/3910	India	Unknown	MT731494
MS/SW1	Australia	2010	HQ258894
SG1	Spain	2011	JX429964
SW9	Argentina	2012	KF632714
G74	China	2017	MN059599
G122	China	2017	MN059637
G118-2	China	2017	MN059631
G117-6	China	2017	MN059629
G117-2	China	2017	MN059625
G110	China	2017	MN059623
G108	China	2017	MN059622
G100	China	2017	MN059620
G92-3	China	2017	MN059617
G92-1	China	2017	MN059615
G11-2	China	2017	MN059574
G32	China	2017	MN059573
G87	China	2017	MN059612
G50-1	China	2017	MN059586
G23-2	China	2017	MN059571
G35	China	2017	MN059576
G80	China	2017	MN059604
G20	China	2017	MN059569
G13	China	2017	MN059563
G68	China	2017	MN059597
G18	China	2017	MN059568
G15-2	China	2017	MN059566
G58	China	2017	MN059592
G78	China	2017	MN059603
G119	China	2017	MN059632
G127	China	2017	MN059638
G81-2	China	2017	MN059606
G120	China	2017	MN059633
G9	China	2017	MN059560
G62	China	2017	MN059594
G42	China	2017	MN059580
G37-2	China	2017	MN059578
G11-1	China	2017	MN059561
G3	China	2017	MN059559
G83	China	2017	MN059608
G44	China	2017	MN059581
G77	China	2017	MN059602
G56	China	2017	MN059590
G84	China	2017	MN059609
G121-1	China	2017	MN059634
G89	China	2017	MN059613
G34	China	2017	MN059575
G64	China	2017	MN059596



## REFERENCES

1. Abraham, A. D., Kidanemariam, D. B. and Holton, T. A. 2019. Molecular Identification, Incidence and Phylogenetic Analysis of Seven Viruses Infecting Garlic in Ethiopia. *Eur. J. Plant Pathol.*, **155**(1): 181-191.
2. Adams, M. J., Antoniw, J. F. and Beaudoin, F. 2005. Overview and Analysis of the Polyprotein Cleavage Sites in the Family *Potyviridae*. *Mol. Plant Pathol.*, **6**(4): 471-487.
3. Arya, M., Baranwal, V. K., Ahlawat, Y. S. and Singh, L. 2006. RT-PCR Detection and Molecular Characterization of Onion Yellow Dwarf Virus Associated with Garlic and Onion. *Curr. Sci.*, **91**(9): 1230-1234.
4. Baghalian, K., Kim, O. K. and Natsuaki, K. T. 2010. Molecular Variability and Genetic Structure of the Population of Onion Yellow Dwarf Virus Infecting Garlic in Iran. *Virus Genes.*, **41**(2): 282-291.
5. Bagi, F., Stojšin, V., Budakov, D., El Swaeh, S. M. A. and Gvozdanić-Varga, J. 2012. Effect of Onion yellow dwarf virus (OYDV) on yield components of fall garlic (*Allium sativum* L.) in Serbia. *Afr. J. Agric. Res.*, **7**(15): 2386-2390.
6. Bos, L. 1976. *Onion Yellow Dwarf Virus*. CMI/AAB Descriptions of Plant Viruses., 158.
7. Celli, M. G., Torrico, A. K., Kiehr, M. and Conci, V. C. 2013. Striking Differences in the Biological and Molecular Properties of Onion and Garlic Isolates of Onion Yellow Dwarf Virus. *Arch. Virol.*, **158**(6): 1377-1382.
8. Chen, J., Adams, M. J., Zheng, H. Y. and Chen, J. P. 2003. Sequence Analysis Demonstrates that Onion Yellow Dwarf Virus Isolates from China Contain a P3 Region much Larger than other Potyviruses. *Arch. Virol.*, **148**(6): 1165-1173.
9. Chung, B. Y. W., Miller, W. A., Atkins, J. F. and Firth, A. E. 2008. An Overlapping Essential Gene in the Potyviridae. *Proc. Natl. Acad. Sci. USA.*, **105**(15): 5897-5902.
10. Da Silva, L. A., Oliveira, A. S., Melo, F. L., Ardisson-Araújo, D. M. P., Resende, F. V., Resende, R. O. and Ribeiro, B. M. 2019. A New Virus Found in Garlic Virus Complex Is a Member of Possible Novel Genus of the Family *Betaflexiviridae* (order Tymovirales). *PeerJ* **7**:e6285. <http://doi.org/10.7717/peerj.6285>
11. Dombrovsky, A., Huet, H., Chejanovsky, N. and Raccach, B. 2005. Aphid Transmission of a Potyvirus Depends on Suitability of the Helper Component and the N Terminus of the Coat Protein. *Arch. Virol.*, **150**(2): 287-298.
12. Dujovny, G., Sasaya, T., Koganezawa, H., Usugi, T., Shohara, K. and Lenardon, S. L. 2000. Molecular characterization of a new Potyvirus infecting sunflower. *Arch. Virol.*, **145**(11): 2249-2258.
13. Gallie, D. R. and Walbot, V. 1992. Identification of the motifs within the tobacco mosaic virus 5'-leader responsible for enhancing translation. *Nucl. Acid Res.*, **20**(17): 4631-4638.
14. Gawande, S. J., Chimote, K. P., Gurav, V. S. and Gopal, J. 2013. Distribution and natural incidence of onion yellow dwarf virus (OYDV) on garlic and its related *Allium* species in India. *Indian J. Hortic.*, **70**(4): 544-548.
15. Ha, C., Coombs, S., Revill, P. A., Harding, R. M., Vu, M. and Dale, J. L. 2008. Design and application of two novel degenerate primer pairs for the detection and complete genomic characterization of potyviruses. *Arch. Virol.*, **153**(1): 25-36.
16. Hasan, H. A. 2004. Generation of an infectious Beet Mosaic Virus (BtMV) Full-length Clone Based on the complete Nucleotide Sequence of a German Isolate. Hannover University., 171.
17. Huson, D. H. and Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.*, **23**(2): 254-267.
18. Kadare, G. and Haenni, A. L. 1997. Virus-encoded RNA helicases. *J. Virol.*, **71**(4): 2583-2590.
19. Katis, N. I., Maliogka, V. I. and Doivas, C. I. 2012. Viruses of the genus *Allium* in the Mediterranean region. *Adv. Virus Res.*, **84**: 163-208.
20. Kehoe, M. A., Coutts, B. A., Buirchell, B. J. and Jones, R. A. C. 2014. *Hardenbergia mosaic virus*: Crossing the barrier between native and introduced plant species. *Virus Res.*, **184**: 87-92.
21. Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: molecular

- evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.*, **35**(6): 1547-1549.
22. Lian, S., Lee, J. S., Cho, W. K., Yu, J., Kim, M. K., Choi, H. S. and Kim, K. H. 2013 Phylogenetic and recombination analysis of tomato spotted wilt virus. *PLoS ONE.*, **8**(5): e63380. doi: 10.1371/journal.pone.0063380
  23. Llave, C., Mart'inez, B., D'iaz-Ru'iz, J. R. and Opez-Abella, D. L. 2002. Amino acid substitutions within the Cys-rich domain of the tobacco etch potyvirus HC-Pro result in loss of transmissibility by aphids. *Arch. Virol.*, **147**(12): 2365-2375.
  24. L'opez-Moya, J. J., Wang, R.Y. and Pirone, T. P. 1999. Context of the coat protein DAG motif affects potyvirus transmissibility by aphids. *J. Gen. Virol.*, **80**(12): 3281-3288.
  25. Lot, H., Chovelon, V., Souche, S. and Delecalle, B. 1998. Effects of Onion Yellow Dwarf and Leek Yellow Stripe Viruses on Symptomatology and Yield Loss of Three French Garlic Cultivars. *Plant Dis.*, **82**(12):1381-1385.
  26. Mahmoud, S. Y. M., Abo-El Maaty, S. A., El-Borollosy, A. M. and Abdel-Ghaffar, M. H. 2008. Identification of onion yellow dwarf potyvirus as one of the major Viruses infecting garlic in Egypt. *Int. J. Virol.*, **4**(1): 1-13.
  27. Manglli, A., Mohammed, H. S., EL Hussein, A. A., Agosteo, G. E., Albanese, G. and Tomassoli, L. 2014. Molecular analysis of the 3' terminal region of onion yellow dwarf virus from onion in southern Italy. *Phytopathol. Mediterr.*, **53**(3): 438-450.
  28. Martin, D. P., Murrell, B., Golden, M., Khoosal, A. and Muhire, B. 2015. RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evol.*, **1**(1): vev003.
  29. Melhus, I. E., Reddy, C. S., Henderson, W. J. and Vestal, E. F. 1929. A new virus disease epidemic on onions. *Phytopathology.*, **19**(1): 73-77.
  30. Nagy, P. D. 2008. Recombination in plant RNA viruses. In *Plant Virus Evolution*; Roossinck, M. J. Ed., Springer: Berlin/Heidelberg, Germany, pp. 133-156. ISBN 978-3-540-75762-7.
  31. Plisson, C., Drucker, M., Blanc, S., German-Retana, S., Le Gall, O., Thomas, D. and Bron, P. 2003. Structural characterization of HC-Pro, a plant virus multifunctional protein. *J. Biol. Chem.*, **278**(26): 23753-23761.
  32. Riechmann, J. L., Lain, S. and Garcia, J. A. 1992. Highlights and prospects of potyvirus molecular biology. *J. Gen. Virol.*, **73**(1):1-16.
  33. Sevik, M. A. 2018. Survey of viruses infecting garlic in Samsun, Turkey. *J. Cent. Eur. Agric.*, **19**(3): 587-592.
  34. Shahraeen, D., Lesemann, E. and Ghotbi, T. 2008. Survey for viruses infecting onion, garlic and leek crops in Iran. *EPPO Bull.*, **38**(1): 131-135.
  35. Shibolet, Y. M., Haronsky, E., Leibman, D., Arazi, T., Wassenegger, M., Whitham, S. A., Gaba, V. and Gal-On, A. 2007. The conserved FRNK Box in HC-Pro, a Plant Viral suppressor of gene silencing, is required for small RNA binding and mediates symptom development. *J. Virol.*, **81**(23): 13135-13148.
  36. Takaki, F., Sano, T. and Yamashita, K. 2006. The complete nucleotide sequence of attenuated onion yellow dwarf virus: a natural potyvirus deletion mutant lacking the N-terminal 92 amino acids of HC-Pro. *Arch. Virol.*, **151**(7): 1439-1445.
  37. Urcuqui-Inchima, S., Haenni, A. L. and Bernardi, F. 2001. Potyvirus proteins: a wealth of functions. *Virus Res.*, **74**(1-2): 157-175.
  38. Valli, A., L'opez-Moya, J. J. and Garc'ia, J. A. 2007. Recombination and gene duplication in the evolutionary diversification of P1 proteins in the family Potyviridae. *J. Gen. Virol.*, **88**(3): 1016-1028.
  39. Valli, A., Garc'ia, J. A. and L'opez-Moya, J. J. 2015. *Potyviridae*. In: *Encyclopedia of Life Sciences (eLS)*. Chichester: Wiley. DOI: 10.1002/9780470015902.a0000755.pub3.
  40. Verma, R. K., Mishra, R., Petrov, N. M., Stoyanova, M., Stoev, A., Bakardjieva, N. V. and Gaur, R. K. 2015. Molecular characterization and recombination analysis of an Indian isolate of onion yellow dwarf virus. *Eur. J. Plant Pathol.*, **143**(3): 437-445.
  41. Verma, R. K., Mishra, R. and Gaur, R. K. 2016. Genetic Variability of the replicase (NIb) gene of *Papaya ringspot virus* in northern India indicates common ancestry



- with isolates from China and Taiwan. *J. Plant Pathol.*, **98**(1): 105-110.
42. Yaghmaian, H. 2015. Functional characterization of P3N-PIPO protein in the potyviral Life Cycle. Electronic Tesis and Dissertation Repository 2930. Available from <https://ir.lib.uwo.ca/etd/2930>.
43. Zheng, L., Rodoni, B. C., Gibbs, M. J. and Gibbs, A. J. 2010. A novel pair of universal primers for the detection of potyviruses. *Plant Pathol.*, **59**(2): 211-220.

## تعیین اولین ترادف نزدیک به کامل ژنوم ویروس کوتولگی زرد پیاز ( *Onion yellow dwarf virus* ) در میزبان سیر از ایران

آ. انتظاری، م. مهرور، و م. زکی عقل

### چکیده

در این مطالعه توالی ژنومی نزدیک به کامل ویروس کوتولگی زرد پیاز ( *Onion yellow dwarf virus-OYDV* ) جدا شده از سیر ( *Allium sativum* L.) برای اولین بار از ایران با استفاده از تکنولوژی توالی‌یابی نسل جدید (Next Generation Sequencing-NGS) تعیین شده است. طول کامل توالی کدکننده این جدایه ایرانی ویروس کوتولگی زرد پیاز (IR-Kh2/ MN528769)، ۱۰212 نوکلئوتید بود که پلی‌پروتئینی با ۳۴۰۳ آمینواسید را رمزگذاری می‌کند. مقایسه توالی جدایه ایرانی با سایر جدایه‌های OYDV ثبت شده در بانک ژن نشان‌دهنده شباهت 97/39-74/84 و 98-75/67 در سطح نوکلئوتیدی و آمینواسیدی بود. با توجه به نتایج آنالیز فیلوژنی بر اساس توالی کدکننده ژنومی، جدایه‌های OYDV در دو گروه مجزا قرار گرفته و جدایه ایرانی به همراه جدایه‌هایی از استرالیا (HQ258895)، اسپانیا (JX429964)، چین (MN059603 and MN059578) و هند (KJ451436) در گروه A و در زیر گروه III قرار گرفت. بعلاوه وقوع یک نوترکیبی احتمالی در ژنوم جدایه ایرانی در ناحیه ژنی اینکلوزن‌بادی هسته‌ای (Nuclear inclusion body-NIb) ردیابی شد.