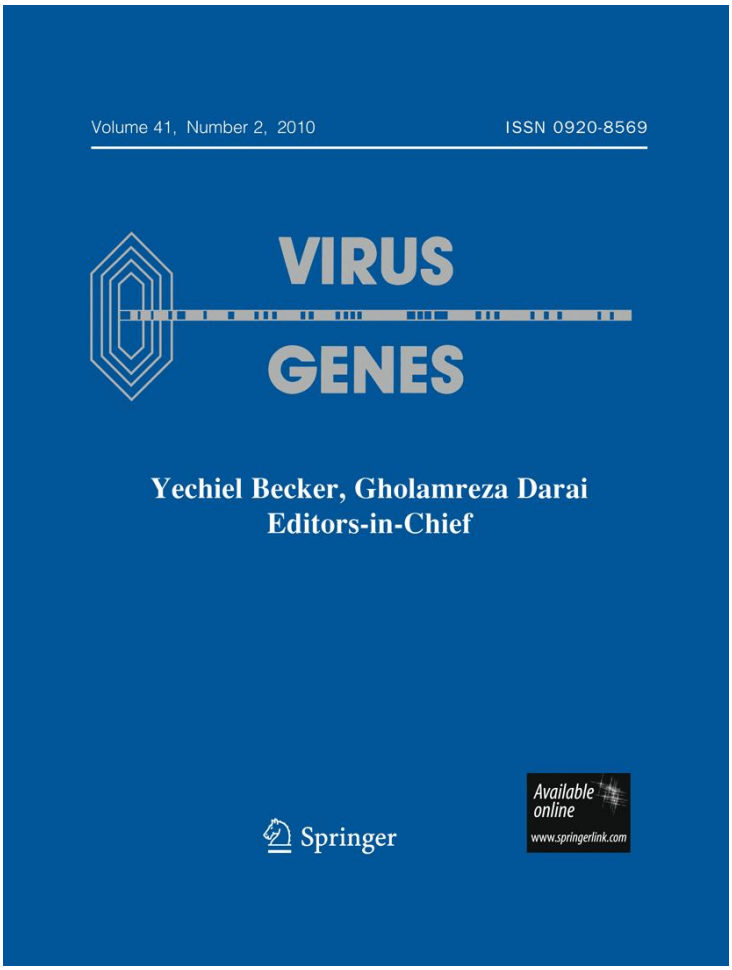
ISSN 0920-8569, Volume 41, Number 2



Virus Genes (2010) 41:282–291
DOI 10.1007/s11262-010-0514-3

Molecular variability and genetic structure of the population of *Onion yellow dwarf virus* infecting garlic in Iran

K. Baghalian · O. K. Kim · K. T. Natzuaki

Received: 23 March 2010 / Accepted: 6 July 2010 / Published online: 21 July 2010

Abstract *Onion yellow dwarf virus* (OYDV) is one of the most important viral diseases of garlic crops worldwide. This study surveyed the occurrence of OYDV in 26 garlic ecotypes collected from different regions in Iran during 2008–2009. Using an electron microscope, we detected filamentous particles with about 700–800 nm in length and 12 nm in width in five samples. These features are typical of the genus *Potyvirus*. The coat protein (CP) gene from 26 samples was PCR amplified, cloned, sequenced, and compared with the sequences available in GenBank. Phylogenetic analysis using 235 deduced amino acids of the CP gene showed that virus isolates fell into two groups, group A and group B. Members of group A were divided into two subgroups: A-I and A-II. The subgroup A-I appears to be a new subgroup comprising 17 Iranian isolates. The identity levels among the amino acid of 26 Iranian isolates ranged between 90 and 100%. The results indicated that the genetic diversity found in Iran is due to local OYDV populations rather than introduction from other geographical regions. This study is the first report about the molecular structure and geographically diverse range of OYDV populations in this country.

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Keywords *Onion yellow dwarf virus* · *Potyvirus* · Virus diversity

Introduction

Garlic (*Allium sativum* L.) is an important bulb vegetable in Iran. Garlic is also cultivated for its medicinal properties such as lowering total plasma cholesterol, reducing blood pressure, and decreasing platelet aggregation [1]. The medicinal importance of garlic is steadily increasing worldwide. Global production of garlic exceeded 13 million tons in 2003 [2]. Viral diseases of garlic cause serious losses in crop yields and lead to deterioration of crop quality. Vegetative mode of propagation in garlic tends to increase mixed infections from a large number of different viruses [3].

Viruses infecting garlic are widespread globally and affect a great number of other members of Alliaceae [4]. These viruses are categorized in three main genera including *Potyvirus*, *Carlavirus*, and various members of the recently established genus *Allexivirus* [5]. Among them, *Onion yellow dwarf virus* (OYDV), which is a member of *Potyvirus*, is one of the most important viral pathogens of garlic crops worldwide [3, 4].

In contrast to other virus genera, serology is not a very good parameter for virus differentiation among viruses of the genus *Potyvirus*, as some serological cross-reactions may cause misinterpretation of results. Furthermore, diagnostic methods based on host range and symptomatology cannot differentiate garlic viruses. This is because garlic viruses induce similar symptoms or sometimes very mild ones. They may even induce no symptoms at all [2, 6]. Recent advances in molecular biology, however, have provided new tools for detection, identification, and classification of *Allium* viruses.

In Iran, areas under garlic cultivation are estimated at 10000 ha. Despite having a high agronomical and industrial potential [1, 7], there is a shortage of garlic in the internal market. The main reason for this is the high prevalence of virus infection. This virus infection has been so extensive that in some Iranian garlic fields, viral disease incidences have reached up to 100% [8].

Considering the importance of garlic production in Iran and the significance of virus infection, virus control should be given high priority. At the same time, conservation of garlic ecotypes with the potential for medicinal use is significant. Thus, basic information such as identity and molecular characteristics of viruses will provide invaluable knowledge to help establish an efficient virus control program. The information currently available on viral infection of garlic crops in Iran is quite limited and is based on a small number of field samples and serological methods [8].

Based on the foregoing, we carried out an extensive survey on the occurrence and evolutionary history of OYDV in Iran.

To the best of our knowledge, this research provides the first report of the RT–PCR-based detection of OYDV in Iranian garlic ecotypes and their phylogenic analysis in relation to reported OYDV from other countries. In this study, we have attempted to develop an integrative approach to understanding OYDV diversification mechanisms in Iran. These findings would help manage disease and the risk of introduction of new viruses [9].

Materials and methods

Plant samples

In order to cover the range of virus distribution and diversity, the main areas of garlic cultivation from all over Iran were selected according to the information of local agricultural extension offices and also producers. Based on this information, a total of 26 garlic ecotypes were selected from areas with different climatic conditions (Fig. 1). Sampling was undertaken during the growing season of autumn 2004. One garlic field in each area was selected, and garlic clove samples were collected in four distributed sectors of 1 m^2 size inside the field, regardless of symptoms. The samples were transferred and cultivated in experimental field of Institute of Medicinal Plant, Karaj, Iran. In 2008, leaf samples were selected from each ecotype, and the samples were dried under silica gel and stored at 4°C until their use.

Electron microscopy

A total of five plant samples were used for electron microscopy. A drop of plant sap was placed onto collodion–carbon-coated copper grids and was stained with 2% phospho tungstic acid (PTA) solution. The specimens were examined with a transmission electron microscope (Hitachi-8100, Japan) at 80 kV.

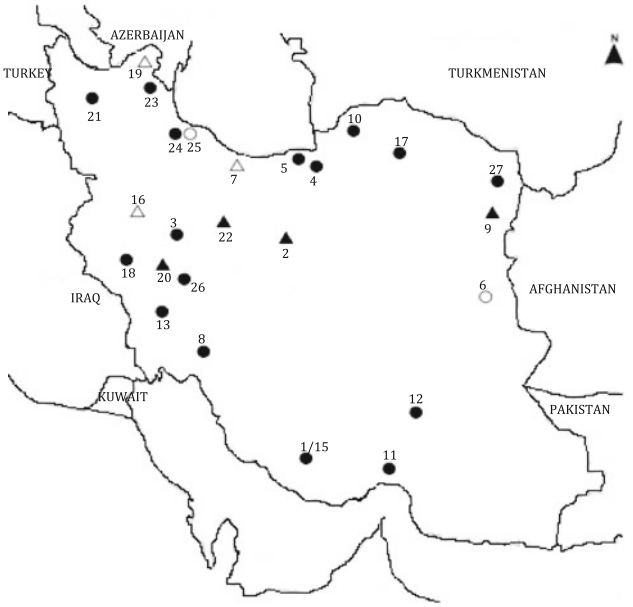

Fig. 1 Geographical origins of 26 samples used in this study (*filled circle*, *open circle*, *filled triangle*, and *open triangle* represent isolates belonging to phylogenetic groups A-I, A-II, B–I, and B-II, respectively)

Reverse-transcription PCR

PCR assays were carried out using TaKaRa Ex Taq TM kit (TAKARA BIO Inc., Japan) according to the manufacturer's instructions. Amplification was performed in a DNA Engine peltier thermal cycler (BIORAD, USA) using the primer pair OG-RT1 (F): 5′-GAAGCGCACATGCAA ATGAAG-3′ and OG-RT2 (R): 5′-CGCCACAACTAGT GGTACACC-3′ [10] to amplify part of coat protein (CP) including 3′ terminal end of RNA (290 bp). The PCR program consisted of 5 min at 94°C, 30 reaction cycles of 60 s for melting at 94°C, 60 s for annealing at 58°C, 2 min for synthesis at 72°C, followed by the final extension for 10 min at 72°C. The PCR product was separated in 1.5% agarose gel stained in a solution of ethidium bromide and photographed under UV illumination.

Nucleic acid sequencing and sequence analysis

The CP genes of OYDV from Iranian garlic samples were sequenced. The whole CP gene was amplified by RT–PCR using the primer pair OYDVVKB-F: 5′-ATAGCAGAAA CAGCTCTTA-3′ and OYDVVKB-R: 5′-GTCTCYGTA ATTCACGC-3′ [11]. These were predicted to amplify a fragment of 1.1 kb, at the 3′-UTR of the OYDV genome

covering the whole CP gene. The amplification product was separated on a 1.5% agarose gel in TAE buffer, stained in a solution of ethidium bromide, and viewed under UV illumination. The target band of expected size was excised and purified using Wizard SV Gel and PCR Clean-Up System (Promega, USA). Purified DNA products were ligated into pGEM-T Vector (pGEM-T Vector System, Promega, USA). Competent cells of *Escherichia coli* (Strain DH5α, Invitrogen, USA) were transformed by DNA-inserted plasmid according to the standard transformation procedure suggested by the company. Recombinant *E. coli* clones were identified in LB medium cultures containing IPTG and ampicillin. Plasmid DNAs were isolated and purified from four randomly selected positive colonies of each plate, using LaboPass Plasmid Mini Kit (Cosmo Genetech, South Korea) and then sequenced with a 3130/3130xl Genetic Analyzer with BigDye Terminator v3.1 Cycle Sequencing Kit (ABI Applied Biosystems, USA).

Sequence analysis

For the phylogenetic analysis, the nucleotide and deduced amino acid sequences of the CP gene of the Iranian isolates were compared to sequences of isolates originating in other countries. The related sequences retrieved from the NCBI database (Table 1).

Table 1 GenBank accession numbers and origin of OYDV isolates used in this study

Code	Isolate	Origin	Accession number
Fi1	Japan	Japan	AB000841
Fi2	Japan	Japan	AB000839
Fi3	Japan	Japan	AB000837.1
Fi4	Japan	Japan	AB000838.1
Fi5	Japan	Japan	AB000843.1
Fi6	Japan	Japan	AB000845.1
Fi7	G79	Japan	AB219833.1
Fi8	G5h	Japan	AB219834.1
Fi9	Yuhang	China	AJ510223.1
Fi10	YH1	China	Aj292231.1
Fi11	Xixia	China	AJ307033.1
Fi12	Hub	China	AJ409309.1
Fi13	YH2	China	AJ292223.1
Fi14	Yunnan	China	AJ409312.1
Fi15	OYDV-VN/L4	Vietnam	DQ925454.1
Fi16	OYDV-VN/L5	Vietnam	DQ925455.1
Fi17	js2	China	AJ909310.1
Fi18	sd: Jinxiang	China	AJ409311.1
Fi19	Rajasthan	India	EU045556

Sequences were analyzed using DNASIS software (Hitachi Software Engineering Co., Tokyo, Japan), and CP coding region was selected for further comparisons and phylogenetic analysis. Furthermore, amino acids (aa) were deduced using "CLC Sequence Viewer" program versions 6.1. Percent identity between sequences was calculated using the "MacVector" program [12]. BLAST program provided by NCBI was used to search for homologous genes. Amino acid sequences were deduced using CLC Sequence Viewer version 6.1 [13]. Sequences were aligned with ClustalW version 1.83 using the default parameters [14]. The initial tree was constructed by Neighbor-Joining using a MEGA version 4.0 program [15]. Support values at each node were calculated after 1,000 bootstrap replicates with the parsimony algorithm being used on the resampled matrix. An isolate of *Watermelon mosaic virus* (GenBank accession no. AY464948) was used as the outgroup.

Results

Electron microscope

Under electron microscope, PTA-stained leaf-dip sample showed a notably flexuous, filamentous shape about 700–800 nm in length and 12 nm in width with a morphological similarity to viruses in family *Potyviridae* (Fig. 2)

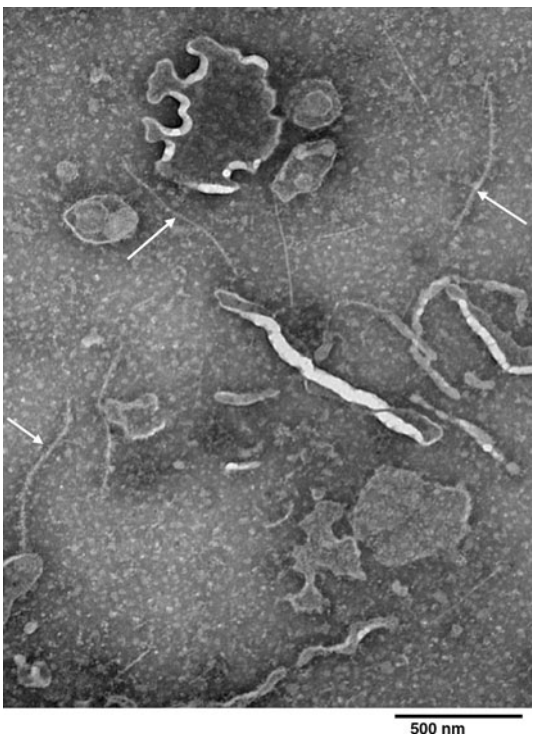

Fig. 2 Electron micrograph of purified virus particles (possible *Potyvirus* particles have been signed with *white mark*)

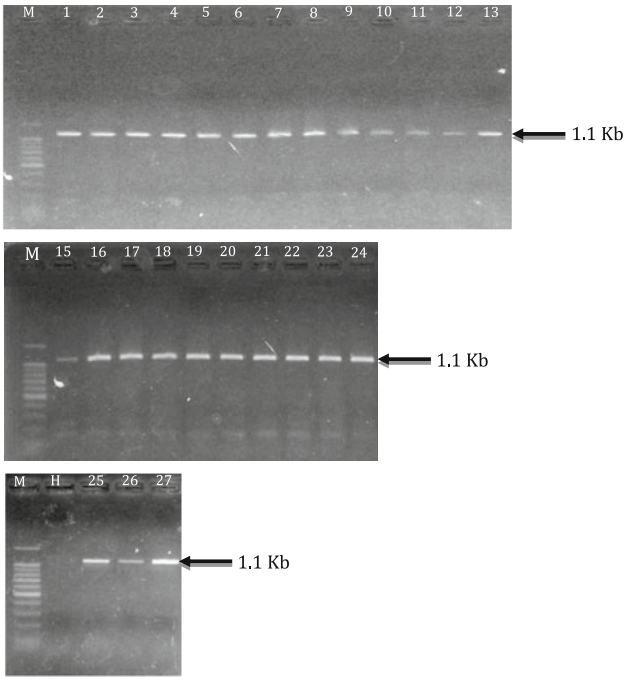

Fig. 3 Amplification of approximately 1.1-kb band including CP and 3′ UTR region in isolates 1–27; *M* 100 bp DNA ladder, *H* healthy control

[16]. Furthermore, some shorter virus particles in some samples were also observed as the results of mix infection with the viruses in other families (data not shown).

OYDV detection in the garlic ecotypes

In the RT–PCR, which was used to detect possible infection with OYDV, an expected band of 290 bp was amplified for all 26 Iranian garlic ecotypes (data not shown). When the same cDNA of these isolates was tested using different primer sets, the primer pair OYDVVKB-F and OYDVVKB-R, expected bands of approximately 1.1 kb were produced for all samples (Fig. 3).

Sequence data and identity matrix

Sequence information of 705 nt of the CP genes and 235 deduced amino acid sequences for these 26 OYDV isolates has been submitted to NCBI-GenBank with the accession numbers from GU295126 to GU295151 (Table 2). The identity of OYDV CP nucleotide sequences among the 26 Iranian isolates ranged from 79 to 99%. Since the optimal nucleotide identity threshold at CP region for species belonging to *Potyviridae* has been determined as 76% [17], all isolates studied here can confidently be assigned to one virus species. The identity levels in the amino acid of 26 Iranian isolates ranged between 90 and 100% (Table 3). Furthermore, the identity levels for amino acid (Table 4) and nucleotide (Table 5) between Iranian (Ec) and foreign isolates (Fi) ranged between 87 and 97% and between 79 and 98%, respectively.

Table 2 Locality and accession numbers of Iranian OYDV isolates used in this study

Isolate	Locality	Accession number
Ec1	Shiraz	GU295126
Ec2	Ali Abad	GU295127
Ec3	Hamedan	GU295128
Ec4	Barma	GU295129
Ec5	Behshahr	GU295130
Ec6	Birjand	GU295131
Ec7	Klardasht	GU295132
Ec8	Dezful	GU295133
Ec9	Gonabad	GU295134
Ec10	Gorgan	GU295135
Ec11	Heid Arabad	GU295136
Ec12	Khaf	GU295137
Ec13	Khoram Abad	GU295138
Ec15	Lobnani Shiraz	GU295139
Ec16	Merjanaj	GU295140
Ec17	Mooin	GU295141
Ec18	Qasre Shirin	GU295142
Ec19	Savalan	GU295143
Ec20	Shevarin	GU295144
Ec21	Tabriz	GU295145
Ec22	Tafresh	GU295146
Ec23	Talesh	GU295147
Ec24	Tarom-1	GU295148
Ec25	Tarom-2	GU295149
Ec26	Toiserkan	GU295150
Ec27	Torbat-e-jam	GU295151

The DAG triple box (Asp-Ala-Gly), which is believed to be crucial for *potyvirus* transmission by aphid [18], was found in the CP of all but four Iranian isolates, namely, Ec2, Ec9, Ec20, and Ec22.

Phylogenetic analysis

To show evolutionary history and diversification of the sequences, phylogenetic tree was generated using the deduced amino acid of 26 Iranian OYDV sequences with representative sequences present in GenBank. OYDV CP sequences formed two main groups (Fig. 4).

Nineteen of 26 Iranian isolates fell into group A together with five isolates from Japan, China, and India, which can further be divided into two subgroups I and II. The subgroup A-I included 17 Iranian isolates with a strong bootstrap value (99%) estimated from 1,000 resamplings. In subgroup A-II, two Iranian isolates from different

Table 3 Comparison of the percent nucleotide (top and right) and amino acid (bottom and left) identities among the CP sequences of OYDV isolated from Iran

	Ec1	Ec2	Ec3	Ec4	Ec5	Ec6	Ec7	Ec8	Ec9	Ec10	Ec11	Ec12	Ec13
Ec1	*	82	82	80	93	84	87	85	90	85	91	93	87
Ec2	91	*	98	87	82	81	82	80	81	82	81	82	82
Ec3	100	91	*	87	82	81	81	79	81	82	81	82	82
Ec4	98	91	98	*	80	81	80	80	80	80	80	80	81
Ec5	100	91	100	98	*	85	87	84	91	85	92	99	88
Ec6	91	91	91	91	91	*	84	84	85	87	84	85	84
Ec7	91	94	91	91	91	90	*	82	86	83	87	87	99
Ec8	98	91	98	100	98	91	91	*	84	91	84	84	83
Ec9	91	98	91	92	91	91	94	92	*	84	95	91	86
Ec10	100	91	100	98	100	91	91	98	91	*	83	85	83
Ec11	100	91	100	98	100	91	91	98	91	100	*	91	87
Ec12	100	91	100	98	100	91	91	98	91	100	100	*	88
Ec13	100	91	100	98	100	91	91	98	91	100	100	100	*
Ec15	100	91	100	91	100	91	91	98	91	100	100	100	100
Ec16	91	95	91	98	91	91	99	91	95	91	91	91	91
Ec17	100	91	100	98	100	91	91	98	91	100	100	100	100
Ec18	100	91	100	91	100	91	91	98	91	100	100	100	100
Ec19	90	94	90	91	90	90	98	91	94	90	90	90	90
Ec20	91	100	91	91	91	91	94	91	98	91	91	91	91
Ec21	100	91	100	98	100	91	91	98	91	100	100	100	100
Ec22	91	99	91	91	91	91	94	91	97	91	91	91	91
Ec23	100	91	100	98	100	93	91	98	91	100	100	100	100
Ec24	100	91	100	98	100	91	91	98	91	100	100	100	100
Ec25	91	90	91	90	91	95	89	90	91	91	91	91	91
Ec26	100	91	100	98	100	91	91	98	91	100	100	100	100
Ec27	98	91	98	100	98	91	91	100	92	98	98	98	98

	Ec15	Ec16	Ec17	Ec18	Ec19	Ec20	Ec21	Ec22	Ec23	Ec24	Ec25	Ec26	Ec27
Ec1	90	87	93	90	87	93	93	93	85	93	85	93	80
Ec2	82	82	82	81	81	82	82	82	80	82	80	82	87
Ec3	82	82	82	81	81	82	83	82	80	82	79	82	87
Ec4	81	81	80	80	80	80	80	80	80	80	80	80	97
Ec5	91	87	98	92	87	99	99	95	85	99	84	98	81
Ec6	84	84	84	85	84	85	85	84	89	85	87	84	82
Ec7	86	99	87	87	99	87	87	87	83	87	83	87	81
Ec8	84	83	85	84	83	84	84	85	99	85	97	84	80
Ec9	99	86	91	98	86	91	91	91	85	91	84	91	80
Ec10	84	83	85	84	83	85	85	84	90	85	91	85	80
Ec11	95	87	91	95	87	91	91	91	84	91	84	91	80
Ec12	91	87	98	91	87	99	99	95	85	99	84	98	81
Ec13	86	99	87	87	99	87	88	88	83	88	83	87	81
Ec15	*	86	91	98	86	91	91	91	84	91	84	91	81
Ec16	91	*	87	87	99	87	88	88	83	87	83	87	81
Ec17	100	91	*	91	87	98	98	95	85	98	85	99	81
Ec18	100	91	100	*	86	91	91	91	85	91	84	91	80
Ec19	90	99	90	90	*	87	88	88	83	87	83	87	81
Ec20	91	95	91	91	94	*	99	95	85	99	84	97	81
Ec21	100	91	100	100	90	91	*	95	85	99	85	97	82

Table 3 continued

	Ec15	Ec16	Ec17	Ec18	Ec19	Ec20	Ec21	Ec22	Ec23	Ec24	Ec25	Ec26	Ec27
Ec22	91	95	91	91	94	99	91	*	85	95	85	95	81
Ec23	100	91	100	100	90	91	100	91	*	85	97	85	80
Ec24	100	91	100	100	90	91	100	91	100	*	84	97	81
Ec25	91	90	91	91	89	90	91	90	91	91	*	85	80
Ec26	100	91	100	100	90	91	100	91	100	100	91	*	81
Ec27	98	91	98	98	91	91	98	91	98	98	90	98	*

Table 4 Comparison of the percent nucleotide identities among the CP sequences of Iranian isolates (Ec) with foreign isolates (Fi)

	Ec1	Ec2	Ec3	Ec4	Ec5	Ec6	Ec7	Ec8	Ec9	Ec10	Ec11	Ec12	Ec13
Fi1	94	82	82	80	98	84	88	85	91	85	92	98	88
Fi2	93	81	82	79	97	84	87	84	91	84	91	97	87
Fi3	89	82	82	81	88	83	86	84	87	83	86	88	86
Fi4	88	80	80	80	88	84	89	83	87	83	87	88	89
Fi5	83	80	80	81	84	81	82	83	82	82	82	84	82
Fi6	83	81	80	81	84	82	82	83	83	81	84	84	82
Fi7	87	81	80	80	87	85	88	84	87	84	86	87	88
Fi8	87	81	80	80	87	85	88	83	88	84	86	87	88
Fi9	89	81	81	81	88	85	89	85	89	85	88	88	89
Fi10	94	82	82	80	88	84	88	85	91	85	82	98	88
Fi11	89	81	81	81	88	85	89	85	89	85	88	88	89
Fi12	88	80	79	79	87	83	88	83	87	84	86	87	88
Fi13	87	81	81	81	88	83	88	84	86	84	85	88	88
Fi14	88	81	81	81	88	84	90	82	87	83	88	88	90
Fi15	87	80	80	80	87	85	89	83	88	85	87	87	89
Fi16	86	80	80	80	87	84	90	83	86	83	86	88	90
Fi17	83	81	81	80	84	83	83	82	82	82	82	84	83
Fi18	85	80	70	80	84	89	83	93	84	91	84	84	83
Fi19	84	82	82	83	85	86	84	85	84	84	82	85	84
	Ec15	Ec16	Ec17	Ec18	Ec19	Ec20	Ec21	Ec22	Ec23	Ec24	Ec25	Ec26	Ec27
Fi1	91	88	97	92	88	98	97	95	85	98	84	97	81
Fi2	91	87	97	91	87	97	87	84	84	97	84	97	80
Fi3	87	87	88	87	86	88	88	88	84	88	85	87	81
Fi4	87	89	88	87	89	88	88	88	83	88	82	88	80
Fi5	82	83	83	82	82	84	84	82	84	84	83	85	81
Fi6	84	82	84	84	82	84	84	83	83	84	82	85	81
Fi7	87	89	87	86	88	87	87	87	84	87	84	87	81
Fi8	87	88	87	86	88	87	87	87	84	87	84	87	81
Fi9	89	89	89	88	89	88	88	87	85	88	84	89	82
Fi10	91	88	97	92	88	97	97	95	85	97	84	97	81
Fi11	89	89	89	88	89	88	88	87	85	88	84	89	82
Fi12	87	89	87	86	88	87	87	87	84	87	83	87	80
Fi13	86	88	88	86	88	88	88	87	84	88	83	88	81
Fi14	87	90	88	88	90	88	88	88	82	88	83	88	81
Fi15	88	89	87	88	89	87	87	86	83	87	83	87	80
Fi16	86	90	88	87	90	87	87	86	83	87	83	88	80
Fi17	83	83	84	82	83	84	83	84	83	85	82	84	81

Table 4 continued

	Ec15	Ec16	Ec17	Ec18	Ec19	Ec20	Ec21	Ec22	Ec23	Ec24	Ec25	Ec26	Ec27
Fi18	84	83	84	84	83	84	84	84	93	85	93	84	80
Fi19	84	84	85	83	88	84	85	84	86	85	85	84	83

Table 5 Comparison of the percent amino acid identities among the CP sequences of Iranian isolates (Ec) with foreign isolates (Fi)

	Ec1	Ec2	Ec3	Ec4	Ec5	Ec6	Ec7	Ec8	Ec9	Ec10	Ec11	Ec12	Ec13
Fi1	91	99	91	92	91	92	95	92	87	91	91	91	91
Fi2	90	97	90	90	90	90	94	90	96	90	90	90	90
Fi3	92	92	92	92	92	88	91	92	93	92	92	92	92
Fi4	90	94	90	90	90	90	95	91	96	90	90	90	90
Fi5	92	91	92	93	92	91	91	93	91	92	92	92	92
Fi6	92	91	92	92	92	91	91	92	91	92	92	92	92
Fi7	90	94	90	91	90	90	94	91	95	90	90	90	90
Fi8	90	94	90	91	90	90	94	91	95	90	90	90	90
Fi9	90	95	90	91	90	91	95	91	96	90	90	90	90
Fi10	90	85	90	91	90	91	95	91	96	90	90	90	90
Fi11	91	95	91	91	91	91	95	91	95	91	91	91	91
Fi12	89	94	89	90	89	90	94	90	94	89	89	89	89
Fi13	89	94	89	89	89	89	94	89	94	89	89	89	89
Fi14	92	96	92	92	92	91	96	92	96	92	92	92	92
Fi15	88	93	88	88	88	89	92	88	93	88	88	88	88
Fi16	90	94	90	90	90	91	96	90	94	90	90	90	90
Fi17	94	97	94	94	94	90	92	94	92	94	94	94	94
Fi18	90	91	90	90	90	90	94	90	96	90	90	90	90
Fi19	94	97	94	94	94	90	92	94	92	94	94	94	94
	Ec15	Ec16	Ec17	Ec18	Ec19	Ec20	Ec21	Ec22	Ec23	Ec24	Ec25	Ec26	Ec27
Fi1	91	96	91	91	95	99	91	99	91	91	91	91	92
Fi2	90	94	90	90	94	97	90	97	90	90	89	90	90
Fi3	92	91	92	92	91	92	92	92	92	92	89	92	92
Fi4	90	96	90	90	95	94	90	94	90	90	89	90	91
Fi5	92	91	92	92	91	91	92	91	92	92	90	92	93
Fi6	92	91	92	92	90	91	92	91	92	92	90	92	92
Fi7	90	95	90	90	94	94	90	94	90	90	89	90	91
Fi8	90	95	90	90	94	94	90	94	90	90	89	90	91
Fi9	90	96	90	90	95	95	90	95	90	90	91	90	91
Fi10	90	96	90	90	95	95	90	95	90	90	91	90	91
Fi11	91	95	91	91	94	95	91	95	91	91	91	91	91
Fi12	89	95	89	89	94	94	89	94	89	89	90	89	90
Fi13	89	95	89	89	94	94	89	94	89	89	90	89	89
Fi14	92	97	92	92	96	96	92	96	92	92	90	92	92
Fi15	88	93	88	88	92	93	88	92	88	88	89	88	88
Fi16	90	97	90	90	96	94	90	94	90	90	90	90	90
Fi17	94	93	94	94	92	91	94	91	94	94	91	94	94
Fi18	90	94	90	90	94	97	90	97	90	90	89	90	90
Fi19	94	93	94	94	92	91	94	91	94	94	91	94	94

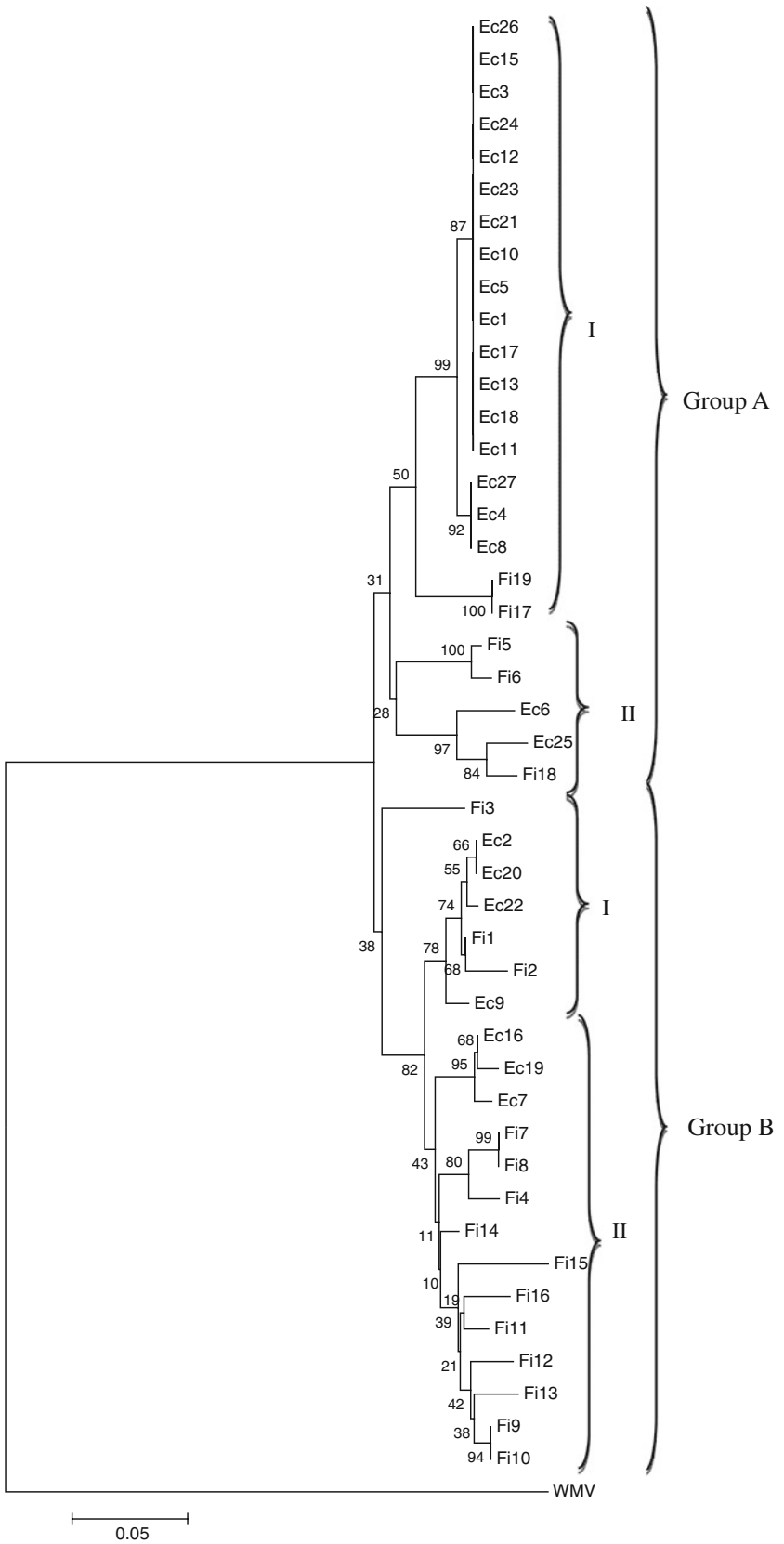

Fig. 4 Phylogenetic tree based on deduced amino acid of the CP coding region. The *scale bar* shows the number of substitutions per residue. *Numbers* at the nodes indicate the bootstrap values (%) of the cluster at the right (codes for each of the virus isolates are shown in Tables 1, 2)

geographical origins were clustered with two Japanese and one Chinese isolates.

The majority of foreign isolates used in this study (14 out of 19) fell into group B. This group was further divided into two subgroups I and II. Subgroup B–I included those foreign and four Iranian isolates, which do not include the DAG box. Just three Iranian isolates (Ec16, Ec19, and Ec7) fell into subgroup B-II, and the remaining members of this subgroup were foreign isolates irrespective of their origins.

Discussion

Onion yellow dwarf virus, a member of the genus *Potyvirus* in the family *Potyviridae*, is a flexuous, filamentous virus containing a single positive sense genomic RNA of approximately 10 kb with a 30-poly A tail [5, 19, 20]. OYDV is one of the several viruses causing mosaic diseases in commercially important *Allium* species such as garlic (*A. sativum*), onion (*A. cepa*), shallot (*A. cepa* var. *perutile*), and leek (*A. porrum*) [20]. OYDV also has been considered the most pathogenic *potyvirus* to *Allium* plants and is recognized as the major viral pathogen in garlic [3, 11, 21]. In garlic, OYDV produces different types of mosaic symptoms on leaves depending on virus isolate and plant cultivars [11] and decreases garlic bulb weight in a range from 24 to 60% [4]. The CP gene is most often used for OYDV identification and classification [11, 22].

Although occurrence of OYDV in Iranian garlic plants had been reported by serological analysis [8], there has been no information about the molecular variability and structure of OYDV isolates among Iranian garlic ecotypes. With preliminary studies under electron microscope, virus particles were detected which appeared to have the typical morphology of members in the genus *Potyvirus*. With RT–PCR, OYDV was detected in all Iranian garlic ecotypes. Furthermore, sequence analysis and phylogenetic studies were conducted throughout all geographical areas where Iranian garlic ecotypes are grown.

Iranian isolates showed a considerable range of identity (79–99%) for nucleotide sequence of CP region (Table 3), which clearly represents high diversity in the country. The lowest identity (79%) was found between Ec3 and two ecotypes originating from opposite sides of country, namely, southwest (Ec8) and northwest (Ec25). The highest identity (99%) was found between different ecotypes from different geographical origins. Geographical distance and percent identity showed that there is no population subdivision among multiple fields in Iran (Fig. 1).

Nucleotide identity between Iranian (Ec) and foreign isolates (Fi) ranged from 79 to 98%. For 18 out of 26 Iranian isolates, this range was between 79 and 92% (Table 4). This information demonstrates the divergence of Iranian isolates in comparison with other foreign isolates submitted to GenBank.

Since only a few amino acid substitutions in CP sequence can alter the biological features as well as the antigenic properties of the virus, the deduced amino acid sequence of CP coding region has been considered a useful marker for the classification of OYDV isolates. According to phylogeny analysis (Fig. 4), there are two distinct groups of OYDV isolates, group A (mostly Iranian isolates) and group B (mostly foreign isolates). This result as well as the wide range of identity between Iranian isolates (Table 3) may indicate that there are variants unique to the Iranian OYDV populations. However, clustering of isolates illustrates lack of phylo-geographical relation as isolates from different regions (Table 2) were found in the same cluster group A (Fig. 4). This is probably due to recent migration or negative constraint.

Isolates of group B were the most frequently detected isolates worldwide and included most of the Japanese, Chinese, and Vietnamese isolates. Seven Iranian isolates were also clustered in this group. Those foreign isolates (Fi1, Fi2, and Fi3) and Iranian isolates, which do not include the DAG box, fell into subgroup B–I. These isolates shared 99–100% identity with each other (Table 3) and 92–99% with foreign isolates in this subgroup (Table 5). The Iranian isolate members of this subgroup showed a wide distribution throughout the country (Fig. 1). This probably means that these isolates have been exchanged between theses origins by local farmers or traders.

At any rate, a comprehensive analysis of pathogenicity determinants and vectors present in the same surveyed area should be carried out, considering that most of the Iranian isolates had the motif responsible for aphid transmission, which is similar to other aphid-transmissible potyviruses [18, 23].

Iranian isolates of subgroup B-II shared 92–96% identity with foreign isolates of this subgroup (Table 5). Considering the high range of identity and noting that these isolates have originated from northern border (Ec7 and Ec19) and western border (Ec16) led to believe that those isolates may have been introduced from foreign sources.

The high divergence exhibited within Iranian isolates and between Iranian isolates with foreign isolates could be attributed to new introductions—possibly from other Asian countries—or from evolution of local populations. However, the fact that Iranian farmers produce garlic using local garlic ecotypes decreases the possibility of foreign source introduction. The high degree of sequence diversity and the basal position of many of the viral sequences in phylogenetic trees suggest that OYDV has been present in Iran for a considerable time.

Baghalian et al. have identified garlic clove exchanges among Iranian farmers in different geographical origins as an important cause of ecotype diversity [1, 7]. As garlic is reproduced non-sexually, exchange of cloves, which already infected by various OYDV isolates, may have also encouraged considerable distribution of isolates. This may explain lack of phylo-geographical relation between OYDV isolates and their geographical origins. Furthermore, natural recombination known in RNA viruses, such as OYDV, may enhance virus variability and evolution [24]. That is why evolutionary process that could be led by natural recombination should also be considered in Iran.

Acknowledgments The authors gratefully acknowledge the financial support of Japanese Society for Promotion of Science (JSPS) through this research program.

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