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Comparison of the coat protein gene sequence of Iranian canola-infecting *Beet western yellows virus* isolates

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Abstract: *Beet western yellows virus* (BWYV), a species of the genus *Polerovirus* in the family *Luteoviridae*, is an agriculturally important virus infecting over 150 plant species in 23 dicotyledonous families worldwide. A survey of BWYV in canola fields in Golestan and Tehran provinces of Iran using indirect triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) indicated 8.3 % infection. The presence of BWYV was confirmed by amplification of the coat protein (CP) gene of the virus via running a reverse transcription-polymerase chain reaction (RT-PCR) on total RNA extracted from ELISA positive leaf tissues. DNA sequences of the BWYV coat protein (CP) gene of seven Iranian isolates were determined and compared at the nucleotide (nt) and amino acid (aa) levels with those of twelve BWYV isolates from different countries deposited in GenBank. Sequence analysis data showed that the identity of BWYV-CP at nt and aa levels among the Iranian isolates were 93.4 % to 100 % and 93.2 % to 100 %, respectively. The maximum similarity of isolates at nt and aa levels were 97.2 and 96.6 %, which occurred among two Iranian isolates (Ir 8 and Ir 100) and four isolates from France (L39967 and X13063) and England (L39973 and L39970). The recombination analysis among the nineteen isolates including seven Iranian isolates revealed that there was no distinct intra-specific recombination event among BWYV isolates. This is the first report of sequencing and analyzing of the BWYV CP gene of Iranian BWYV isolates.

Keywords: BWYV, *Polerovirus*, Genetic Diversity

Introduction

Beet western yellows virus (BWYV) is one of the most economically important and widespread viruses of *Brassicaceae* in the world (Hampton *et al.,* 1998). The virus causes yellowing and stunting in a large number of host crop plants, including sugar beet (*Beta vulgaris* L.), spinach (*Spinacia oleracea* L.), lettuce (*Lactuca sativa* L.), canola (*Brassica napus* L.) and has a wide host range including over 150 plant species belonging to 23 dicotyledonous families (Stevens *et al.,* 2005). BWYV belongs to the genus *Polerovirus* in the family *Luteoviridae* which is phloem limited and only transmitted by aphids in a circulative non-propagative manner (King *et al*., 2012). The main virus vector is the peach-potato aphid, *Myzus persicae* Sulz. (Hampton *et al*., 1998). The BWYV genome is a linear, single stranded positive sense RNA of ~ 5.7 kb, contains six open reading frames (ORFs) and with a viral protein genome-linked (VPg) at the 5`end and an OH group at the 3` end (King *et al*., 2012). ORF0

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seems to be involved in disease symptoms expression, as determined for PLRV (van der Wilk *et al.,* 1997). The ORF1 and ORF2 encode the viral replicase complex, ORF1having the helicase activity, while ORF2 contains the replicase motif, characterized by the presence of the highly conserved GDD motif. The major capsid protein of BWYV is encoded by ORF3 and corresponds to a 22.5 kDa. The minor capsid protein is a readthrough protein P74 derived from ORF3 (Wang *et al.,* 1995) and the adjacent ORF5 by translational readthrough of the ORF3 codon (Veidt *et al.,* 1992). This protein is required for efficient virus accumulation and since it intervenes in virus movement, it increases the rate of new infection foci (Brault *et al.,* 1995; Mutterer *et al.,* 1999). The product encoded by ORF4 (19 kDa) has been suggested to be a MP-like protein (Schmitz *et al.,* 1997).

The genes in the 3' half of the BWYV genome are expressed from a subgenomic RNA (sgRNA), having a high homology at the 3' end to each other, probably because they confer the properties of circulative aphid transmission and phloem specific cell to cell movement (Miller *et al.,* 1995). It has been postulated that an ORF (ORF7) at the 3' end could be present in BWYV, as a product from a sgRNA2, which has been found in PLRV (Ashoub *et al.,* 1998). *Brassica* spp. are known to be susceptible to BWYV disease (Smith and Hinckles, 1985). Although most of the BWYV reports have originated from the United States, BWYV-like symptoms have also been reported from Britain and Euroupe, as well as from the old world (e. g., Iran). Phylogenetic analysis using partial nucleotide sequences containing a 1.2 kb of the genome and covering the C-terminus of the polymerase gene, the intergenic region and the coat protein/ movement protein genes, differentiated BWYV isolates into three distinct groups (de Miranda *et al.,* 1995). Further analysis of the CP sequences of additional isolates corresponding to either their biological properties or geographical locations showed five clusters (Hauser *et al.,* 2000).

Although BWYV has been reported from different regions in Iran (Makkouk *et al.,* 2002, Shahraeen *et al*., 2003), little is known about the molecular diversity of the isolates. Therefore in the present study, the phylogenetic relationship and recombination analysis of Iranian BWYV isolates infecting canola plants were investigated based on their coat protein gene.

Materials and Methods

Sampling and virus isolates

In a survey of some Iranian canola fields conducted in April 2008 and 2009 about six-hundred leaf samples were randomly collected from three distinct regions in Golestan province and a canola field located in faculty of Agriculture of Tarbiat Modares University, Tehran. Samples were assayed for the presence of BWYV by indirect triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) using the anti-BWYV monoclonal antibody (DSMZ, Germany) following the manufacturer's instructions. Three replicates of each sample were added into three wells on the microtiter plate. Samples were considered as positive if the mean absorbance value at 405 nm for three replicates was at least twice that of the negative control (Dijkstra and de Jager, 1998). Of 600 samples collected, 50 samples were infected by BWYV. Based on region and year of collections, seven out of the 50 samples were selected for further study.

RNA isolation, RT-PCR, cloning and sequencing

RNA extraction of infected samples was carried out according to the method of de Miranda *et al.,* (1995). Primers BWYV-CP-F (5′ATGAATACGGTCGTGGGTAGAAG3′) consisted of 23 nt identical to nucleotides 3484-3506 and the reverse primer BWYV-R (5′CCAGCTATCGATGAAGAACCATTG-3′) consisted of 24 nt, the complementary to

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nucleotides 4022- 4046 were used to amplify the coat protein gene of the BWYV genome (Stevens *et al.,* 2005). In summary, 2.5µl of total plant RNA were added to 2µl of the reverse primer (10 pmol) and it was incubated at 70 °C for 5min to denature nucleic acid. The tube was immediately chilled on ice and then cDNA was synthesized by adding this mixture to 0.5 µl M-MuLV Reverse Transcriptase (5 unit/µl) (Fermentas, Lithuania), 1 µl dNTPs (1mM), 0.25 µl Riboblock-Rnasin (2 unit/µl) (Fermentas, Lithuania), 2 µl of M-MuLV Reverse Transcriptase buffer (Fermentas, Lithuania) and 1.75 µl DEPC water incubated at 42 °C for 60 min. Polymerase chain reaction (PCR) was performed using template cDNA (2.5 µl) in a 10 µl reaction mix containing 1X PCR buffer, 50 mM $MgCl_2$, 10 pmol each of reverse and forward primers and 5 unit of *Taq* DNA polymerase (Fermentas, Lithuania).

PCR amplification was performed under the following conditions: an initial denaturation at 94 ºC for 5 min, followed by 30 cycles of denaturation at 94 ºC for 30 s, annealing at 61 ºC for 30 s, and extension of 72 ˚C for 72 s and a final elongation step for 5 min at 72 ºC.

PCR products were separated by electrophoresis on 1.2 % agarose gel in 1×TBE buffer (100 mM Tris, 500 mM boric acid and 1 mM EDTA), at 100V for 40min and stained with ethidium bromide. A 1Kb GeneRuler^{TM} ladder (Fermantas, Lithuania) was used as a size marker.

The amplified DNA fragment of the predicted size (~ 562 bp) was recovered from agarose gel by silica beads DNA gel extraction kit (Fermentas, Lithuania). Three purified RT-PCR products of each isolate were ligated into the pTZ57R/T vector (Fermentas, Lithuania) following the manufacturer's instructions and transferred into *Escherichia coli* DH5α (Sambrook *et al.,* 2001). Three clones from each of the recombinant plasmids were selected for sequencing. The desired recombinant plasmids were sequenced by Macrogen (South Korea).

phylogenetic analysis

The sequencing data were assembled using Chromas ver 1.41 (Mc Carthy, 1996). Based on nucleotide sequences of the BWYV CP gene, the phylogenetic relationship between the Iranian and exotic isolates (Table 1) were assessed using Clustal X ver. 2.0.10 (Larkin *et al.,* 2007). *Beet mild yellowing virus* (BMYV) was used as an out-group in the analyses. The alignment was checked visually and edited manually with GeneDoc ver. 2.7.00 (Nicholas and Nicholas, 1997) and phylogenetic trees were drawn using neighbor-joining (NJ), maximum likelihood (ML), and bayesian methods. The tree was visualized using Mega4 software (Kumar *et al.,* 2008). A bootstrap value for each node of the generated trees was calculated using 1000 bootstrap replicates, and then the tree was viewed using Treeview software (Page, 1996). A similar procedure was used when the tree was based on deduced amino acid data.

The coat protein gene sequences of BWYV Iranian isolates and twelve exotic sequences were assessed for the occurrence of putative recombination events by two methods of SiScan (Gibbs *et al.* 2000) and RDP (Martin and Rybicki, 2000) using Recombination Detection Program version 3 (RDP3) (Martin *et al.,* 2010), with the default settings (highest acceptable probability value = 0.05).

Results & Discussion

The incidence of BWYV was determined in three canola growing regions of Golestan and Tehran provinces in Iran by TAS-ELISA and RT-PCR. Results indicated that the virus was naturally distributed in Brassica-growing areas in these provinces of Iran and was detected in 50 out of some 600 tested samples. Based on the geographical origins of sampling and year of collections, seven isolates including IR-10, IR-4, IR-8, IR-11, IR100, IR317 and IR15 were selected for further studies. An expected fragment (~ 563 bp) was obtained from each ELISA positive isolate using RT-PCR with a pair of primers

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which amplified BWYV coat protein. The amplified fragments by RT-PCR were ligated into the pTZ57RT vector (Fermentas, Lithuania) and transformed into *Escherichia coli* DH5α. The desired recombinant plasmids were sequenced. Furthermore, the sequences of twelve more BWYV isolates which were previously characterized were included in the analysis (totally 19 BWYV isolates). The GenBank accession numbers of sequences determined in this study and those of previously recorded sequences in GenBank are presented in Table 1.

The nucleotide and amino acid sequence identities between the seven Iranian isolates were 93.4-100 % and 93.2-100 % respectively. In most cases the identity between the isolates based on aa sequences was in accordance with that of nt sequences (Table 2, 3). Comparison of Iranian BWYV isolates at both nt and aa levels showed that seven sequenced isolates were divided into two distinct clusters of which isolates Ir8, Ir100, Ir11 and Ir4 were placed in a same group with two branches and the other three Iranian isolates Ir15, Ir317 and Ir10 were located in another group in which Ir15 and Ir317 were identical at both nt and aa levels (Fig. 1).

Table 1 The GenBank accession numbers and characteristics of *Beet western yellows virus* isolates/strains used in this study.

Isolate/strain	Accession No.	Host	Country of origin	Reference
BWYV1-Isolate 7	L39974	*B. oleraceae*	England	de Miranda *et al.*, 1995
BWYV2-Isolate 2	L39967	*B. napus*	France	de Miranda *et al*., 1995
BWYV1-Isolate3a2	L39969	*B. napus*	England	de Miranda *et al*., 1995
BWYV2-Isolate3b	L39970	*B. napus*	England	de Miranda *et al*., 1995
BWYV1-Isolate6	L39973	*B. oleraceae*	England	de Miranda *et al*., 1995
BWYV-CP	L40019	*B. vulgaris*	Italy	de Miranda *et al*., 1995
BWYV1-Isolate 12a	L39977	*B. vulgaris*	Italy	de Miranda *et al*., 1995
BWYV1-Isolate 5	L39986	*B. oleraceae*	England	de Miranda *et al*., 1995
BWYV-CP	L40016	*B. vulgaris*	France	de Miranda *et al*., 1995
BWYV-CP	L40020	*B. vulgaris*	Italy	de Miranda *et al*., 1995
BWYV strain USA	AF473561	*B. vulgaris*	USA	Direct submission
BWYV-FL1	X13063	*Lactuca sativa*	France	de Miranda *et al*., 1995
IR 4	JX501661	*B. napus*	Iran	This study
IR 8	JX501662	*B. napus*	Iran	This study
IR 10	JX501663	*B. napus*	Iran	This study
IR 11	JX501664	*B. napus*	Iran	This study
IR 15	JX501665	*B. napus*	Iran	This study
IR 100	JX501666	*B. napus*	Iran	This study
IR 317	JX501667	*B. napus*	Iran	This study
BMYV	NC_003491	*B. vulgaris*	USA	Guilley *et al*., 1995

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Table 2 Percent sequence identity and diversity of the first 528 nucleotides of the coat protein gene between 19 *Beet western yellows virus* isolates including seven Iranian *Beet western yellows virus* isolates. Details of the isolates are presented in Table 1.

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
1		97.5	95.1	97.9	94.9	97.7	94.9	94.7	95.1	95.3	95.5	95.5	94.0	95.1	95.7	93.4	93.2	93.6	96.0	92.2	1	Ir4.seq
2	2.3		93.8	98.3	93.6	99.4	93.6	95.8	96.2	96.4	96.8	96.4	94.9	96.0	96.6	94.5	93.8	94.5	97.2	93.6	2	Ir8.seq
3	4.7	5.9		94.9	99.8	93.6	99.8	94.1	95.3	95.1	92.8	92.8	91.3	92.8	93.0	92.6	92.2	92.1	94.5	91.7	3	Ir10.seq
4	2.1	1.7	4.9		94.7	98.1	94.7	95.1	95.8	95.7	95.8	95.5	94.0	95.1	95.7	93.4	93.4	93.6	96.6	93.0	4	Ir11.seq
5	4.9	6.1	0.2	5.1		93.4	100.0	94.0	95.1	94.9	92.6	92.6	90.9	92.6	92.8	92.4	92.1	91.9	94.3	91.5	5	Ir15.seq
6	2.1	0.6	6.1	1.9	6.3		93.4	95.7	96.0	96.2	97.0	96.6	95.1	96.2	96.8	94.3	93.8	94.5	96.6	92.8	6	Ir100.seq
7	4.9	6.1	0.2	5.1	0.0	6.3		94.0	95.1	94.9	92.6	92.6	90.9	92.6	92.8	92.4	92.1	91.9	94.3	91.5	7	Ir317.seq
8	5.5	4.3	5.7	4.9	5.9	4.5	5.9		98.5	99.1	94.7	94.3	92.8	94.3	94.5	94.7	94.3	93.4	96.0	94.1	8	L39967.seq
9	5.1	3.9	4.7	4.1	4.9	4.1	4.9	1.5		99.4	95.5	95.1	93.6	95.1	95.3	95.1	94.7	94.1	96.0	93.8	9	L39969.seq
10	4.9	3.7	4.9	4.3	5.1	3.9	5.1	1.0	0.6		95.3	94.9	93.4	94.9	95.1	95.3	94.9	94.0	96.6	94.3	10	L39970.seq
11	4.5	3.3	7.0	4.3	7.2	3.1	7.2	5.3	4.5	4.7		99.6	98.1	99.4	99.8	95.8	94.7	95.8	96.6	92.4	11	L39973.seq
12	4.5	3.7	7.0	4.7	7.2	3.5	7.2	5.8	4.9	5.1	0.4		98.1	99.4	99.8	95.8	94.3	95.5	96.6	92.0	12	L39974.seq
13	5.9	5.1	8.5	6.2	8.7	4.9	8.7	7.2	6.4	6.6	1.7	1.7		97.9	98.3	94.3	92.8	93.8	95.1	90.7	13	L39977.seq
14	4.7	3.9	7.0	4.9	7.2	3.7	7.2	5.8	4.9	5.1	0.6	0.6	1.9		99.6	95.8	94.3	95.8	96.2	92.0	14	L39986.seq
15	4.3	3.5	6.8	4.5	7.0	3.3	7.0	5.6	4.7	4.9	0.2	0.2	1.5	0.4		96.0	94.5	95.7	96.8	92.2	15	L40016.seq
16	6.8	5.6	7.2	6.8	7.4	5.8	7.4	5.6	5.1	4.9	4.1	4.1	5.5	4.1	3.9		96.6	94.9	96.0	93.4	16	L40019.seq
17	7.2	6.4	8.0	6.8	8.2	6.2	8.2	6.0	5.5	5.3	5.3	5.8	7.2	5.7	5.5	3.5		94.3	95.1	93.4	17	L40020.seq
18	6.6	5.8	7.8	6.4	8.0	5.6	8.0	6.8	6.0	6.2	4.1	4.5	6.0	4.1	4.3	5.3	5.7		94.0	92.6	18	AF473561.seq
19	4.1	2.9	5.3	3.5	5.5	3.5	5.5	4.1	4.1	3.5	3.5	3.5	4.9	3.7	3.3	4.1	5.1	6.2		93.9	19	X13063.seq
20	7.9	6.6	7.8	7.2	8.0	7.2	8.0	6.0	6.4	5.8	7.9	8.3	9.4	8.3	8.1	7.0	7.0	7.7	6.2		20	NC_003491.seq
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		

Table 3 Percent sequence identity and diversity of the deduced amino acid of ORF 3 between 19 *Beet western yellows virus* isolates including seven Iranian *Beet western yellows virus* isolates. Details of the isolates are presented in Table 1.

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
1		98.3	94.9	97.2	94.3	98.3	94.3	94.9	94.3	94.9	94.9	94.9	92.6	95.5	95.5	93.8	91.5	92.6	96.0	11.4	1	Ir4.seq
2	1.7		93.8	98.9	93.2	100.0	93.2	96.6	96.0	96.6	96.6	95.5	93.2	96.0	96.0	94.3	92.6	93.2	96.6	11.4	2	Ir8.seq
3	5.3	6.5		94.3	99.4	93.8	99.4	93.8	94.9	94.3	92.0	92.0	89.8	92.6	92.6	93.2	90.3	89.8	94.3	10.8	3	Ir10.seq
4	2.9	1.1	5.9		93.8	98.9	93.8	95.5	96.0	95.5	96.0	94.9	92.6	95.5	95.5	93.2	91.5	92.0	95.5	11.4	4	Ir11.seq
5	5.9	7.2	0.6	6.5		93.2	100.0	93.2	94.3	93.8	91.5	91.5	89.8	92.0	92.0	92.6	89.8	89.2	93.8	10.8	5	Ir15.seq
6	1.7	0.0	6.5	1.1	7.2		93.2	96.6	96.0	96.6	96.6	95.5	93.2	96.0	96.0	94.3	92.6	93.2	96.6	11.4	6	Ir100.seq
7	5.9	7.2	0.6	6.5	0.0	7.2		93.2	94.3	93.8	91.5	91.5	89.8	92.0	92.0	92.6	89.8	89.2	93.8	10.8	7	Ir317.seq
8	5.3	3.5	6.5	4.7	7.2	3.5	7.2		98.3	98.9	94.3	93.2	90.9	93.8	93.8	94.3	92.6	90.9	95.5	11.4	8	L39967.seq
9	5.9	4.1	5.3	4.1	5.9	4.1	5.9	1.7		99.4	93.8	92.6	90.3	93.2	93.2	93.8	92.0	91.5	94.9	11.4	9	L39969.seq
10	5.3	3.5	5.9	4.7	6.5	3.5	6.5	1.1	0.6		94.3	93.2	90.9	93.8	93.8	94.3	92.6	92.0	95.5	11.4	10	L39970.seq
11	5.3	3.5	8.4	4.1	9.1	3.5	9.1	5.9	6.5	5.9		98.9	96.6	99.4	99.4	95.5	91.5	93.2	95.5	11.4	11	L39973.seq
12	5.3	4.7	8.4	5.3	9.1	4.7	9.1	7.2	7.8	7.2	1.1		96.6	99.4	99.4	95.5	90.9	92.0	95.5	11.4	12	L39974.seq
13	7.8	7.2	11.0	7.8	11.0	7.2	11.0	9.7	10.4	9.7	3.5	3.5		97.2	97.2	93.2	88.6	89.8	93.2	11.4	13	L39977.seq
14	4.7	4.1	7.8	4.7	8.4	4.1	8.4	6.5	7.2	6.5	0.6	0.6	2.9		100.0	96.0	91.5	92.6	96.0	11.4	14	L39986.seq
15	4.7	4.1	7.8	4.7	8.4	4.1	8.4	6.5	7.2	6.5	0.6	0.6	2.9	0.0		96.0	91.5	92.6	96.0	11.4	15	L40016.seq
16	6.5	5.9	7.2	7.2	7.8	5.9	7.8	5.9	6.5	5.9	4.7	4.7	7.2	4.1	4.1		93.2	92.0	96.0	11.9	16	L40019.seq
17	9.1	7.8	10.4	9.1	11.0	7.8	11.0	7.8	8.4	7.8	9.1	9.7	12.4	9.1	9.1	7.2		90.3	93.2	12.5	17	L40020.seq
18	7.8	7.2	11.0	8.4	11.7	7.2	11.7	9.7	9.1	8.4	7.2	8.4	11.0	7.8	7.8	8.4	10.4		90.9	11.4	18	AF473561.seq
19	4.1	3.5	5.9	4.7	6.5	3.5	6.5	4.7	5.3	4.7	4.7	4.7	7.2	4.1	4.1	4.1	7.2	9.7		10.8	19	X13063.seq
20	330.0	344.0	330.0	344.0	330.0	344.0	330.0	344.0	344.0	344.0	377.0	360.0	360.0	360.0	360.0	330.0	315.0	377.0	344.0		20	NC_003491.seq
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		

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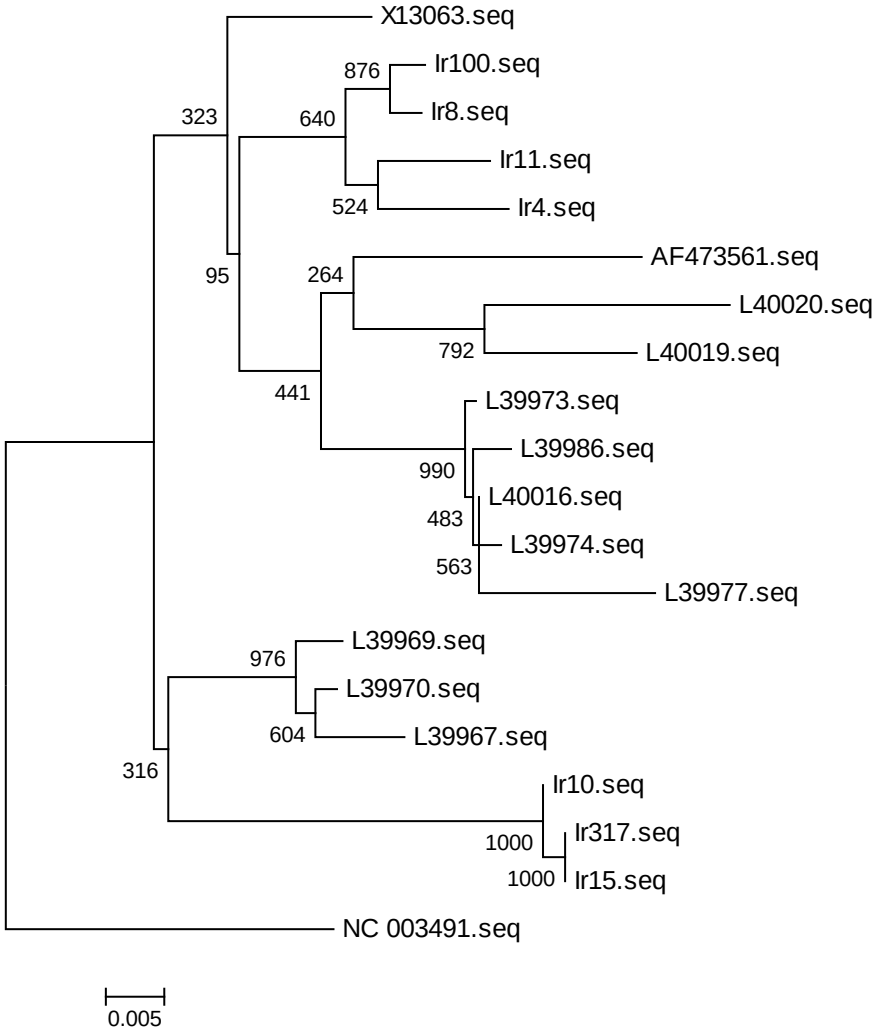

Figure 1 Phylogenetic tree reconstructed by the neighbor-joining method from the multiple alignments of 19 *Beet western yellows virus* isolates coat protein nucleic acid sequences. Bootstrap values are given on each cluster node. *Beet mild yellowing virus* another species of *Polerovirus* was used as out-group. Details of the isolates are presented in Table 1.

Therefore, sequence analysis data showed that the Ir15 and Ir317 isolates were most closely related Iranian isolates with 100 % identity, whereas they had the lowest homology with Ir100 isolate at both nt and aa levels with 93.4 % and 93.2 % identity, respectively. The Ir15 and Ir317 isolates were also most distinct from Ir8 isolate at aa level with 93.2 % identity (Tables 2 and 3). The phylogenetic tree reconstructed from the CP gene sequences for all of the 19 BWYV isolates including seven Iranian BWYV isolates showed that the Ir8 isolate was the closest to the French isolate (X13063) with 97.2 % identity that was isolated from *Lactuca sativa* and next to it the isolate Ir100 was closely related to the England isolate (L39973) with 97 % identity that was isolated from *B. oleraceae* , while Ir15, and Ir317 isolates with 90.9 % identity were the most distant to Italian isolate (L39977) that was isolated from sugar beet (*B. vulgaris*) (Table 2). These results demonstrated that the host of the

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virus could play an important role in genetic diversity of BWYV.

Comparison of deduced amino acid sequences of cp gene between Iranian and non-Iranian isolates of BWYV obtained from different host plants showed some differences. Ir8 and Ir100 isolates obtained from *Brassica napus* infected host, indicated the closest similarity to the isolates from France (X13063 and L39967) and England (L39970 and L39973) obtained from *B. napus* and *B. oleraceae*, whereas, the Iranian BWYV isolates Ir15 and IR317 were the most distant from an American isolate (AF473561) which was obtained from sugar beet (*B. vulgaris*) with 89.2 % identity (Table 3). This difference could be due to the virus host rather than geographical effects. On the other hand, sequence analysis of the CP amino acid of BMYV and BWYV isolates showed that they are grouped into five clusters according to either their host range or geographical location (Hauser *et al.,* 2000). Therefore, the effect of host range and geographical location on phylogeny and genetic diversity of BWYV isolates remains to be examined.

The results presented here are to some extent in accordance with those of de Miranda *et al.*, 1995 who showed that an Iranian BWYV isolate from Fars province and Southern European BWYV isolates are placed in the same group and are distinct from American and Northern European isolates.

Recombination analysis of the coat protein gene of BWYV with SiScan, BootScan, Geneconov, and RDP3 recombination analysis methods indicated that the recombination event had not occurred within the 19 studied BWYV isolates (data not shown). This could be due to not very long history of canola cultivation in Iran.

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Refrences

Ashoub, A., Rohde, W. and Prufer, D. 1998. In planta transcription of a second subgenomic RNA increases the complexity of the subgroup 2 luteovirus genome. Nucleic Acids Research, 26: 420-426.

Brault, V., van der Heuvel, J.F.J.M., Verbeek, M., Ziegler-Graff, V., Reutenauer, A., Herrbach, E., Garaud, J-C., Guilley, H., Richards, K. and Jonard, G. 1995. Aphid transmission of Beet western yellows luteovirus requires the minor capsid read-through protein P74. EMBO Journal, 14 (4): 650-659.

de Miranda, J. R., Stevens, M., de Bruyne, E., Smith, H. G., Bird, C., and Hull, R. 1995. Sequence comparison and classification of beet luteovirus isolates. Archives of Virology, 140: 2183-2200.

Dijkstra, J., and de Jager, C. P. 1998. Practical Plant Virology: Protocols and Exercises. Springer.

Gibbs, M. J., Armstrong, J. S., and Gibbs, A. J. 2000. Sister-scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. Bioinformatics, 16: 573-582.

Guilley, H., Richards, K. E. and Jonard, G. 1995. Nucleotide sequence of beet mild yellowing virus RNA. Archives of Virology, 140: (6), 1109-1118.

Hampton, R. O., Keller, K. E., and Baggett, J. R. 1998. Beet western yellows luteovirus in western Oregon pathosystem relationships in a vegetable-sugar beet seed production region. Plant Disease, 82: 140-148.

Hauser, S., Stevens, M., Mougel, C. Smith, G. H., Fritsch, C., Herrbach, E., and Lemaire, O. 2000. Biological, serological and molecular variability suggest three distinct Polerovirus species infecting beet or rape. Phytopathology, 90: 460-466.

King, A. M. Q., Adams, M. J., Carstens, E. B. and Lefkowitz, E. J. 2012. Virus Taxonomy. Academic Press.

Kumar, S. M., Nei, J. Dudley, and Tamura, K. 2008. MEGA: a biologist-centric software

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for evolutionary analysis of DNA and protein sequences. Briefings in Bioinformatics, 9 (4): 299-306.

Larkin, M., Blackshields G., Brown, N., Chenna, R., McGettigan, P., McWilliam, H., Valentin, F., Wallace, I., Wilm, A., Lopez R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. 2007. Clustal W and Clustal X version 2.0. Bioinformatics, 23:2947-2948.

Makkouk, K. M., Fazlali, Y., Kumari, S. G., Farzadfar, S. 2002. First report of beet western yellow virus, chickpea chlorotic dwarf virus, faba bean necrotic yellows virus and soybean dwarf virus infecting chickpea and lentil crops in Iran. Plant Pathology 51: 387.

Martin, D. and Rybicki, E. 2000. RDP: detection of recombination amongst aligned sequences. Bioinformatics, 16: 562-563.

Martin, D. P., Lemey, P., Lott, M., Moulton, V., Posada, D., and Lefeuvre, P., 2010. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics 26: 2462-2463.

McCarthy, C. 1996. Chromas, version 1.4. School of Biomolecular and Biomedical Science, Griffith University, Brisbane, Queensland.

Miller, W. A., Dinesh-Kumar, S. P., and Paul, C. P. 1995. Luteovirus gene expression. Critical Review of Plant Science, 14: 179-211.

Mutterer, J. D., Stussi-Garaud, C., Michler, P., Richard, K. E., Jonard, G., and Ziegler-Graff, V. 1999. Role of the beet western yellows readthrough protein in virus movement in *Nicotiana clevelandii*. Journal of General Virology, 80: 2771-2778.

Nicholas, K., and Nicholas, H. B. 1997. GeneDoc: a tool for editing and annotating multiple sequence alignments. Distributed by the author.

Page, R. D. M. 1996. Tree View: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences: CABIOS, 12: 357.

Sambrook, J., and Russell, D. W. 2001. Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Laboratory Press, New York.

Schmitz, J., Stussi-Garaud, C., Tacke, E., Prüfer, D., Rohde, W., and Rohfritsch, O. 1997. *In situ* localization of the putative movement protein (pr17) from potato leafroll luteovirus (PLRV) in infected and transgenic potato plants. Virology, 235: 311-322.

Shahraeen, N., Farzadfar, SH., and Lesemann, D. E. 2003. Incidence of viruses infecting winter oilseed rape (*Brassica napus* ssp. *oleifera*) in Iran. Journal of Phytopathology, 151: 614-616.

Smith, H. G., and Hinckes, J. A. 1985. Studies on Beet western yellows virus in oilseed rape (*Brassica napus* ssp. *oleifera*) and sugar beet (*Beta vulgaris*). Annual Applied Biology, 107: 473-484.

Stevens, M., Patron, N. J., and Dolby, C. A. 2005. Distribution and properties of geographically distinct isolates of sugar beet yellowing viruses. Plant Pathology, 54: 100-107.

Van der Wilk, F., Verbeek, M., Dullemans, A. M. and van den Heuvel, J. F. 1997. The genome-linked protein of Potato leafroll virus is located downstream of the putative proteinase domain of the ORF1 product. Virology, 234, 300-303.

Veidt, I., Bouzoubba, S. E., Leiser, R. M., Ziegler-Graff, V., Guilley, H., Richards, K. and Jonard, G. 1992. Synthesis of Full-length transcripts of beet western yellows virus RNA: messenger properties and biological activity in protoplast. Virology, 186: 192-200.

Wang, J. Y., Cahy, C., Gildow, F. E., and Gray, S. M. 1995. Readthrough protein associated with virions of barley yellow dwarf luteovirus and its potential role in regulating the efficiency of aphid transmission. Virology, 206: 962-964.

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مقایسه ترادف ژن پروتئین پوششی جدایه‌های ویروس زردی غربی چغندر آلوده کننده‌ی کلزا

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چکیده: ویروس زردی غربی چغندر (BWYV) از گونه‌های جنس Polerovirus و خانواده Luteoviridae یکی از ویروس‌های مهم کشاورزی می‌باشد که بیش از ۱۵۰ گونه گیاهی متعلق به ۲۳خانواده گیاهان دو لپه‌ای را در سطح دنیا آلوده می‌کند. ردیابی BWYV در مزارع کلزای استان‌های گلستان و تهران توسط آزمون الیزای غیرمستقیم ۸/۳٪ آلودگی را نشان داد. آلودگی نمونه‌های الیزا مثبت با تکثیرژن پروتئین پوششی ویروس توسط RT-PCR تأیید شد. ترادف ژن پروتئین پوششی هفت جدایه منتخب تعیین و در سطوح اسید نوکلئیک و اسید آمینه با ترادف ۱۲ جدایه از کشورهای متفاوت موجود در GenBank مقایسه شد. آنالیز مقایسه‌ای ترادف اسید نوکلئیک ژن پروتئین پوششی BWYV جدایه‌های ایرانی ۹۳/۴-۱۰۰ درصد و در سطح اسید آمینه ۹۳/۲ - ۱۰۰٪ مشابهت نشان داد. بیشترین تشابه جدایه‌های ایرانی با سایر جدایه‌ها بین جدایه‌های Ir8 و Ir100 از ایران با جدایه‌های فرانسه (X13063 وL39967) و انگلیس (L39973 و L39970) با ۹۷/۲٪ و ۹۶/۶٪ به‌ترتیب در سطح اسید نوکلئیک و اسید آمینه بود. آنالیز نوترکیبی هیچگونه رخداد نوترکیبی درون گونه‌ای را در بین ژن پروتئین پوششی BWYV نشان نداد. این اولین گزارش از تعیین ترادف، تجزیه و تحلیل بخشی از ژنوم جدایه‌های ایرانی ویروس زردی غربی چغندر است.

واژگان کلیدی: BWYV، *Polerovirus*، تنوع ژنتیکی

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