African Journal of Biotechnology Vol. 11(80), pp. 14553-14560, 4 October, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2121
ISSN 1684-5315 ©2012 Academic Journals

Full Length Research Paper

Identification of *Turnip mosaic virus* isolated from canola in northeast area of Iran

Sabokkhiz M. A.[1]*, Jafarpour B.[1], Shahriari Ahmadi F.[2] and Tarighi S.[1]

[1]Department of Plant Pathology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
[2]Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

Accepted 15 August, 2012

During March and April of 2011, 436 samples showing viral disease symptoms were collected from canola fields in the Khorasan Razavi province. The samples were tested by double-antibody sandwich (DAS)-enzyme linked immunosorbent assay (ELISA) for the presence of *Turnip mosaic virus* (TuMV). Among the 436 samples, 117 samples were found to be infected with TuMV. One of the infected samples from Govareshk region (TuMV-IRN GSK) was selected for biological purification. Total RNA of this isolate were extracted and reverse transcriptase (RT)-PCR was performed with specific primers according to the coat protein gene. PCR products (986 bp) was first purified and then directly sequenced. Phylogenetic analyses based on ClustalW multiple alignments with previously reported 33 isolates indicated 88 to 98% similarity in nucleotide and 94 to 99% in amino acid levels among isolates. TuMV-IRN GSK represented the highest identity to another Iranian isolate (IRN TRa6). Phylogenetic tree clustered all sequences into four groups and IRN GSK fell into the basal-B group. Nucleotide and amino acid distances between IRN GSK and other isolates in the basal-B group showed that this isolate was closely related to another Iranian isolate IRN TRa6, and distinct from other isolates in the basal-B group. These results indicate that TuMV is a common pathogen of canola crops in the Khorasan Razavi province.

Key words: *Turnip mosaic virus* (TuMV), canola, reverse-transcription polymerase chain reaction (RT-PCR), coat protein gene, sequence analysis.

INTRODUCTION

Nowadays, oilseed rape or canola (*Brassica napus* L.), a member of the Brassicaceae family (*Brassicaceae* Burnett, syn. *Crusiferae* Juss.) is the world's third-leading source of both vegetable oil and oil extraction meal, behind oil palm and soybean (Vollmann and Rajcan, 2010). In Iran, the areas of oilseed rape production are going to be more distributed, due to an increasing demand for rapeseed oil. This increased production has led to prevalence of infectious diseases. *Turnip mosaic virus* (TuMV) is an important member of the *Potyvirus* genus, in the *Potyviridae* family. Wide host range of this virus can infect several plant families including Brassicaceae. TuMV could be transmitted to healthy plants by aphids in a non-persistent manner. The most important vectors have been proposed to be *Myzus persicae*, *Brevicoryne brassicae*, *Lipaphis erysimi*, *Aphis craccivora* and *Aphis gossypii* (Sako, 1980; Wang and Pirone, 1999; Dombrovsky et al., 2005; Ghorbani et al., 2007; Haj Kassem and Walsh, 2008). The CP gene is a variable region in potyvirus genomes and has been widely accepted for classification of different isolates in the genus (Sanches et al., 2003). The complete sequences of Iranian, Italian, German, Canadian, Japanese, Danish, Russian, Polish, New Zealand and UK isolates have been determined and numerous partial sequences, especially of the coat protein (CP) and 3′-UTR, have also been obtained (Nicolas and Laliberte, 1992; Ohshima et al., 1996; Jenner et al., 2000; Kozubek et al., 2007; Chen et al., 2006; Farzadfar et al., 2005; Jiang et al., 2010; Ohshima et al., 2010; Wang et al., 2009; Tomitaka and Ohshima, 2006).

*Corresponding author. E-mail: mo_sa734@stu-mail.um.ac.ir. Tel: +98-511-8795620. Fax: +98-511-8787430.

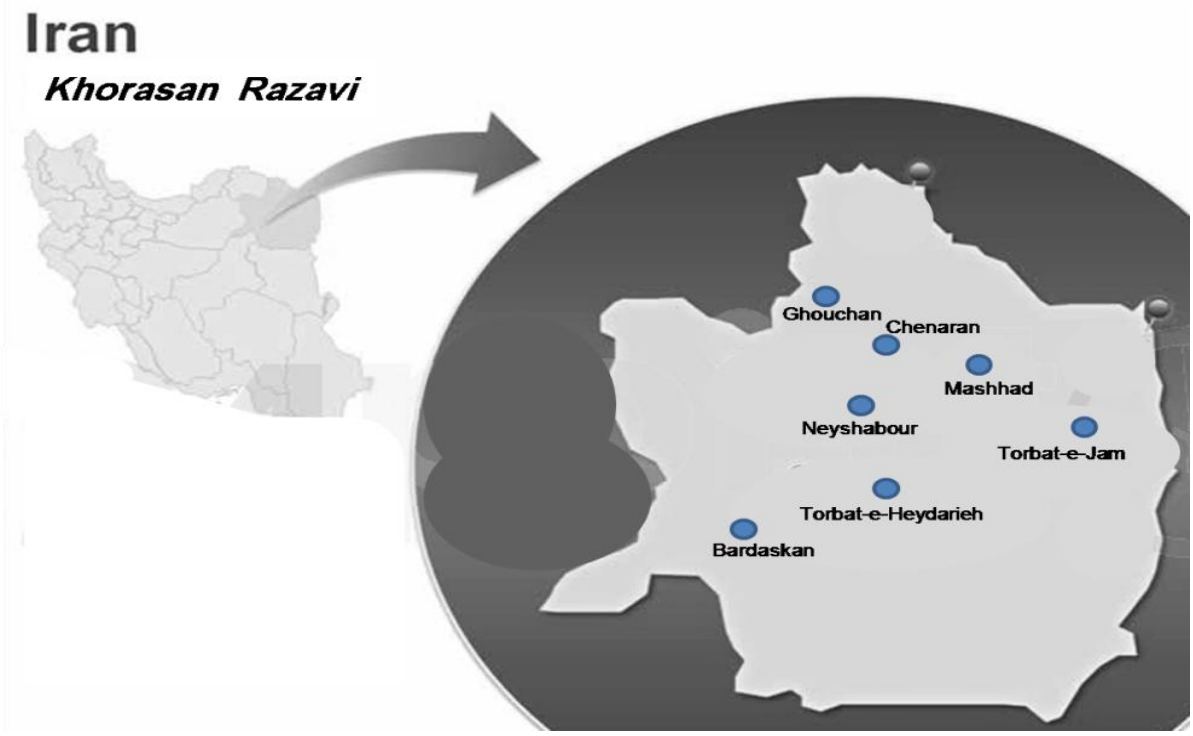

Figure 1. Map of sampled regions in Khorasan Razavi province.

Previous studies (Ohshima et al., 2002, 2007; Tomimura et al., 2003, 2004; Farzadfar et al., 2009) have shown that TuMV isolates had been biologically classified into four host-infecting types; host-type [(B)]: isolates that infected Brassica plants latently and occasionally and did not infect Raphanus plants; host-type [B]: isolates that infected many Brassica plants systemically with mosaic on uninoculated leaves, but did not infect Raphanus plants; host-type [B(R)]: isolates that infected many Brassica plants systemically, with mosaic symptoms on uninoculated leaves, but infected Raphanus plants latently and occasionally; and host-type [BR]: isolates that infected both Brassica and Raphanus plants systemically, with mosaic symptoms on uninoculated leaves. Moreover, phylogenetic analysis using different virus isolates collected from around the world has revealed four main TuMV genogroups called basal-B (Brassica), basal-BR (Brassica/Raphanus), Asian-BR and world-B. The basal-B cluster of (B) or B host-type isolates was the most variable, was not monophyletic and came from both nonbrassicas and brassicas. The isolates in the basal-BR and Asian-BR groups and Asian isolates in the world-B group seem to be more adapted to host plants such as Brassica and Raphanus than those in the ancestral basal-B group. Most, but not all, of the European isolates belonging to the world-B group are B host types, for instance UK 1 is of B host type and belongs to the world-B group, and, although many Brassica plants are susceptible to it, it rarely infects *Raphanus sativus* systemically but asymptomatically. TuMV has been reported already from different geographic region of Iran on stock plants (*Matthiola* sp.), oilseed rape (*Brassica napus*), *Cheianthus cheiri, Chrysanthemum* sp., *Impatiens balsamina, Petunia hybrida* and *Zinnia elegans* and four brassicaceae weed hosts (Bahar et al., 1985; Farzadfar et al., 2004, 2009; Ghorbani et al., 2007; Shahraeen et al., 2002, 2003; Zahedi Tabarestani et al., 2010).

In Khorasan Razavi province which is one of the most important agricultural areas of Iran, there is no evidence of the presence and distribution of this virus. In this study, we clarified distribution of TuMV in canola crops of Khorasan Razavi province. Furthermore, complete coat protein gene of an Iranian isolate of TuMV (IRN GSK) was sequenced and compared with others to provide better understanding of their relationship with other isolates of TuMV.

MATERIALS AND METHODS

Sample collection

During March and April of 2011, a total of 436 infected canola plants having viral infection symptoms including: mosaic, leaf malformation, vein clearing, chlorosis and stunting were collected from 39 canola fields throughout different regions (Figure 1 and Table 1) of the Khorasan Razavi province (northeast of Iran). Each sample was collected from a different plant and immediately bagged and transported in cold boxes. Collected samples were stored at 4°C for further analysis.

Table 1. Number of fields, samples collected and infected samples.

Location (regions of Khorasan Razavi province)	Number of fields	Number of samples infected/ collected
Mashhad	7	33/132
Chenaran	7	12/56
Ghouchan	6	12/38
Bardaskan	6	0/73
Torbat-e-Heydarieh	4	17/37
Torbat-e-Jam	4	18/40
Neyshabour	5	25/60

Enzyme-linked immunosorbent assay (ELISA)

Collected samples were tested for the presence of TuMV by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as described previously by Clark and Adams (1977), using specific polyclonal antibodies purchased from DSMZ Inc. Braunschweig, Germany. Briefly, microtitre plates were coated with 1:1000 TuMV-IgG in carbonate buffer (pH 9.6). Leaves were ground in plastic bags by phosphate buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing 0.5 ml/l of Tween 20 and 20 g/L of polyvinyl pyrrolidone (1:5 wt/vol), and leaf extracts were then added to the plates in duplicate wells and incubated overnight at 4°C. The presence of TuMV in the samples was detected by TuMV-specific antibody conjugated to alkaline phosphatase using p-nitrophenyl phosphate (pNPP) substrate. Optical density (OD) at 405 nm was measured by ELISA Reader (Stat Fax 2100, AWARNESS Tech. Inc., USA) 45 min to 1 h after incubation with the substrate pNPP (1 mg/ml, pH 9.8). Tissue samples from healthy and infected plants grown in greenhouse were used as negative and positive controls, respectively. Infection of regenerated plants was determined according to the formula; R = X + 2SD where, which X is the average OD of negative controls and SD is the standard deviation of this mean relative to its individual wells.

Collection of aphid colonies

During the sampling of canola plants for the detection of virus, some colonies of aphids were also collected from canola fields of Khorasan Razavi province. After the preparation of microscopic slides of aphids, these were identified using identification key (Blackman and Eastop, 2000).

Virus culture and biological purification

Three host species, *Chenopodium quinoa*, *Chenopodium amaranticolor* and *Brassica rapa* were used in the inoculations, the first two hosts for biological purification and the last one for virus preservation. One of the infected sample from Govareshk of Mashhad (IRN GSK) was ground in 100 mM potassium phosphate buffer, pH 7.2 and rubbed on carborundum dusted young leaves. For biological purification, one of the local lesions appearing on *C. quinoa* or *C. amaranticolor* was punched out and used to inoculate fresh *C. quinoa* plants to purify each isolate from a possible mixed infection. All inoculated plants were kept for at least four weeks in a greenhouse at 24°C.

RNA extraction

Two weeks after inoculation, the total RNA was extracted from upper leaf tissues of TuMV-infected *Brassica rapa* using the RNeasy Plant Mini Kit (Qiagen, Valensia CA. USA) according to the manufacturers' instruction.

Reverse transcription and PCR amplification

The Govareshk isolate were subjected to RT-PCR using TuMV specific primers (forward primer TuMVF 8705-8726: 5'-CAAGCAATCTTTGAGGATTAT-3' and reverse primer TuMVR 9690-9669: 5'-TATTTCCCATAAGCGAGAATA-3') (Sanchez et al., 2003), obtaining the expected amplification product of 986 bp from all samples. Reverse transcription and PCR amplification was performed using AccuPower RT PreMix Kit and AccuPower PCR PreMix (Bioneer Inc. Korea), respectively according to the manufacturers' instructions. For cDNA synthesis, 1 µg of total RNA and 10 pmol of reverse primer was mixed in a sterile tube and incubated at 70°C for 5 min and immediately placed on ice. The incubated mixture was transferred to an AccuPower RT PreMix tube and then the reaction was filled up with DEPC-DW to a final volume of 20 µl, vortex and briefly spin down. The cDNA synthesis reaction was performed for 60 min at 42°C followed by 5 min at 94°C. PCR was conducted using the Biometra (*T-personal*) thermocycler. The program consisted of a denaturation step of 4 min at 95°C, 35 cycles of 20 s at 94°C, 30 s at 55.5°C and 1 min at 72°C, and a final elongation step of 72°C for 5 min. The 986 bp amplified fragment included 54 bp of the 3′end of NIb gene, the whole CP gene and 65 bp of the 3′UTR. After the PCR reaction, 6 µl of PCR product was electrophoreses on a 1% agarose gel containing 10% DNA green viewer (Pars Tous Biotechnology Inc. Iran) in 1x Tris- borate EDTA buffer (TBE) with 0.7 µl of GeneRuler 100 bp Plus DNA ladder (Fermentase). Gels were observed with UV gel documentation.

Sequence determination and phylogenetic analyses

To confirm the detection achieved by RT-PCR, the expected product of approximately 986 bp for IRN GSK isolate was sequenced. PCR fragments (986 bp bands) were purified using the AccuPrep®Gel Purification Kit (Bioneer Inc., Korea) from agarose gel according to manufacturers' protocol. Sequences from both strands of the purified PCR product were obtained by the custom sequencing service of Bioneer Inc., Korea. Coat protein gene nucleotide and deduced amino acid sequences were analyzed using the ClustalW2 (Thompson et al., 1994) with default parameters. All positions containing gaps and missing data were eliminated. There were a total of 864 (nucleotide) and 288 positions (amino acids) in the final dataset. The nucleotide sequences of coat protein gene of the other 33 isolates in Table 2 was deposited at GenBank (NCBI). Phylogenetic analyses of the cDNA sequences were conducted using MEGA software v. 5.0 (Tamura et al., 2011). Trees were calculated from the coat protein gene sequences of the

Table 2. Scores of similarity of TuMV-GSK in nucleotide and amino acid levels with other 33 published isolates based on ClustalW2.

Isolate name/location/accession no.	Score in nucleotide (864 nt) level (%)	Score in amino acid (288 aa) level (%)
IRN Tra6/Iran/AB440238	98.0	99.0
IRN SS5/Iran/AB440239	94.0	98.0
TANX2/China/EU734433	89.0	95.0
TUR9/Turkey/AB362513	89.0	96.0
CAR51/Poland/HQ637383	89.0	95.0
GBR98/UK/EU861593	88.0	94.0
ESC8/Taiwan/AY395796	90.0	98.0
IS1/Israel/AB093602	92.0	98.0
CZE19/Czeck/AB188976	93.0	99.0
CZE10/Czeck/AB188974	93.0	99.0
ITA5/Italy/AB189012	93.0	98.0
ITA6/Italy/AB189013	92.0	98.0
ITA4/Italy/AB189011	92.0	97.0
ESP5/Spain/EF061749	91.0	97.0
A1/Italy/AB093598	91.0	96.0
A102/11/Italy/AB093597	91.0	97.0
ITA3/Italy/AB252122	93.0	98.0
GRC42/Greece/AB189006	91.0	98.0
DNK2/Denmark/AB252108	90.0	95.0
RUS2/Russia/AB093607	89.0	95.0
PV0104/Germany/AB093603	89.0	95.0
CP845J/Japan/AB093614	89.0	95.0
CHZJ23/China/AB180018	88.0	95.0
KWB779J/Japan/AB252125	89.0	94.0
KYD81J/Japan/AB093613	88.0	95.0
GFD462J/Japan/AB252115	89.0	95.0
CAR37A/Poland/DQ648591	89.0	95.0
ESP4/Spain/EF061748	90.0	96.0
HBLB/China/AY912098	89.0	95.0
NZ290/New Zealand/AB093612	89.0	95.0
BZ1/Brazil/AB093611	88.0	94.0
HRD/China/AB093627	88.0	94.0
CH6/China/AB252103	88.0	94.0

34 isolates. The maximum likelihood (ML) and neighbor joining (NJ) methods was used to generate the initial tree. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The *Plum pox virus* (PPV) *CP*gene (DQ456243) was used as outgroup for comparison.

RESULTS

Detection of TuMV in canola samples by ELISA

Totally, 117 samples (26.8%) of the symptomatic canola samples collected from 39 fields surveyed in March and April of 2011 had positive response to TuMV-specific antibody in DAS-ELISA. Number of TuMV infected samples from fields of different regions is summarized in Table 1.

Aphid identification

Three species of aphid colonies from canola fields: *Lipaphis erysimi* Kaltenbach, *Brevicoryne brassicae* Linnaeus and *Myzus persicae* Sulzer were identified. These species are the most important vectors of TuMV in canola fields that disperse TuMV and other canola viruses in Crusiferae fields.

Host range and symptoms

The results of the infectivity tests on *C. quinoa* was clear necrotic spots occurring on inoculated leaves from 7 to

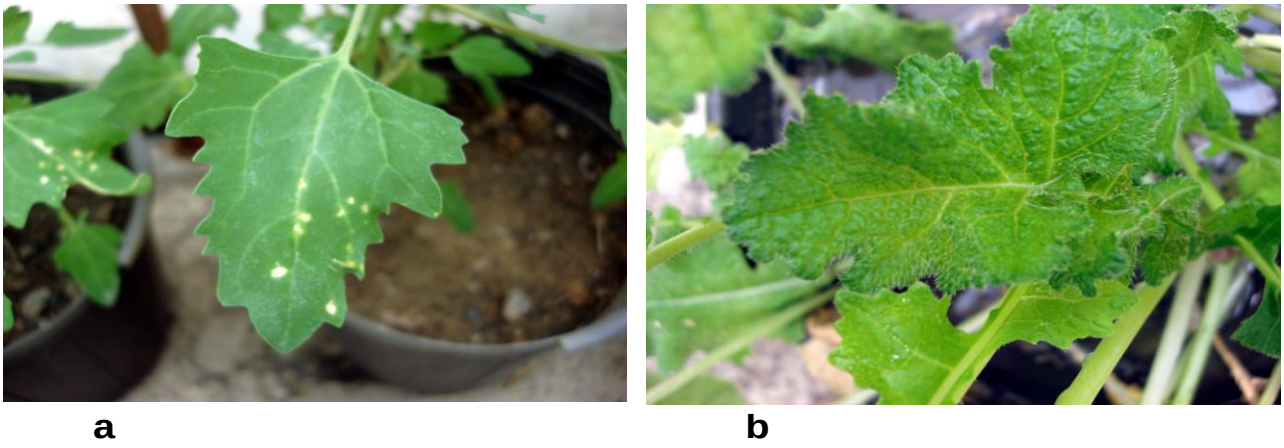

Figure 2. Symptom development on the leaves of: (a) *C. quinoa* showing necrotic local lesion, and (b) *B. rapa* exhibiting systemic malformation.

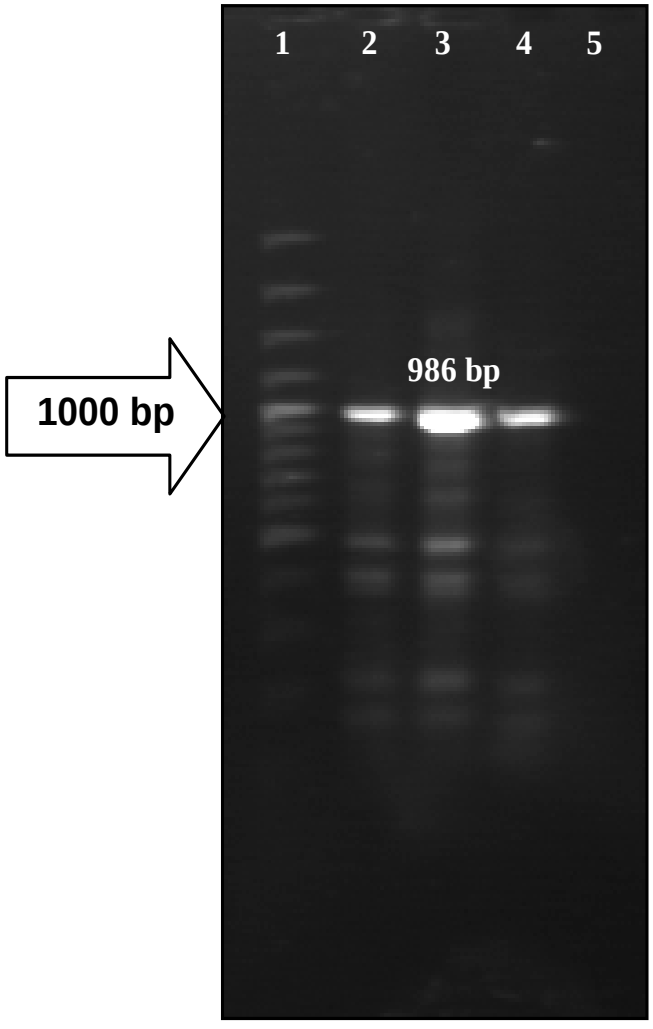

Figure 3. Detection of TuMV-IRN GSK isolate by RT-PCR. A 986 bp fragment was amplified from TuMV infected *B. rapa.* Lane 1: GeneRuler 100 bp Plus DNA ladder (Fermentase). Lanes 2, 3 and 4: TuMV infected of *B. rapa*; lane 5: healthy *B. rapa*.

15 days after inoculation (Figure 2a) and systemic malformation in *B. rapa* (Figure 2b). The TuMV antiserum reacted positively with TuMV infected *B. rapa* and did not react with healthy plants in DAS-ELISA.

RT-PCR and sequencing

An expected DNA fragment of approximately 986 bp was amplified by RT-PCR from total RNA extracted from the infected samples using specific primers (Figure 3). No amplicons were observed in similar preparations from healthy plants. The nucleotide sequence of the IRN GSK amplified fragment was determined. The CP sequence of IRN GSK was deposited in GenBank with the accession no. JQ073722.

Phylogenetic relationships

Table 2 shows scores of similarity of IRN GSK in nucleotide and amino acid levels with other 33 isolates. The relationships of these isolates were investigated by maximum likelihood (ML) and neighbor joining (NJ) methods and the ML tree is shown in Figure 4. All the trees partitioned most of the sequences into the same four consistent groups: basal-B, basal-BR, Asian-BR and world-B, as reported previously (Ohshima et al., 2007; Farzadfar et al., 2009). The basal-B group was a sister group to all others in the ML and NJ phylogenies and was supported by high bootstrap values. IRN GSK isolate fell into the basal-B group, confined to B-infecting host type isolates from Iran, Taiwan, Czeck Republic, Spain, Greece, Israel and Italy. We calculated the nucleotide and amino acid distances between the other isolates in the basal-B group using Kimura 2-parameter (Kimura, 1980) and Dayhoff matrix based model (Schwarz and Dayhoff, 1979) (Table 3). Table 3 estimates evolutionary

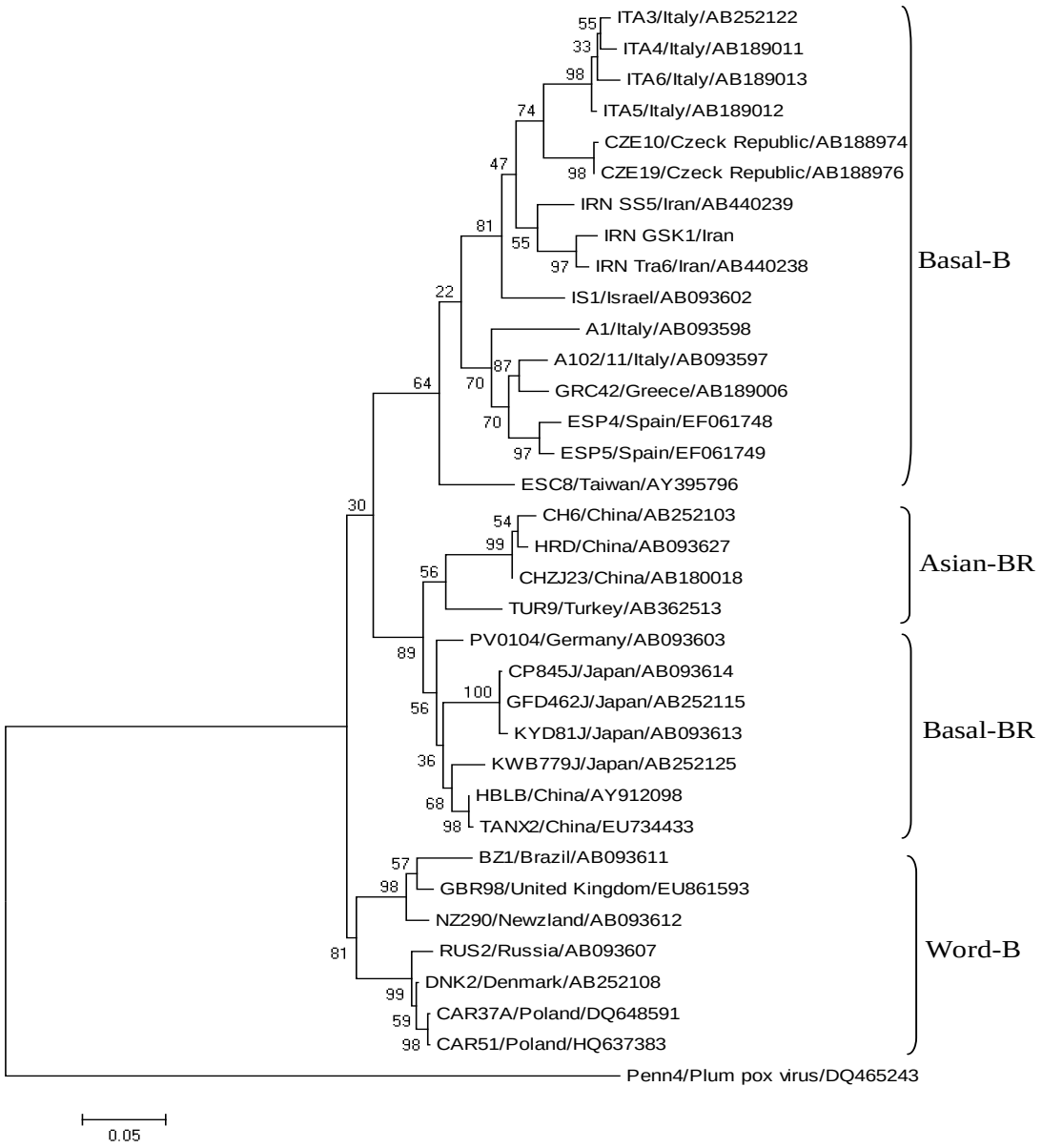

Figure 4. A maximum likelihood (ML) phylogenetic tree showing the relationship among IRN GSK isolate of TuMV with the other isolates. The tree was constructed from the complete nucleotide sequences of CP gene of all TuMV isolates. The tree comprises 34 TuMV isolates, including the Iranian isolate of this study (IRN GSK), and 33 isolates available in GeneBank. Numbers at nodes indicate percentage bootstrap scores. Bootstrap values are shown above the branch lines. Outgroup: *Plum pox potyvirus* isolate Penn4.

divergence between sequences. The number of base substitutions per site from between sequences is shown. The analysis involved 16 nucleotide and 16 amino acid sequences. Evolutionary analyses were conducted in MEGA5. The distances showed that the IRN GSK isolate was most closely related to another Iranian isolate IRN TRa6, and distinct from the other Iranian isolate IRN SS5 and other isolates in the basal-B group. Interestingly, nucleotide distance between IRN GSK and IRN SS5 is more than that of Czeck Republic isolates (CZE10 and CZE19), but not in amino acid distance.

DISCUSSION

TuMV infections occur in a variety of plants worldwide. It is a highly variable potyvirus with various biological and serological criteria. In this study, the incidence and distribution of TuMV was studied in different canola fields of Khorasan Razavi province. The samples were collected from 436 symptomatic plants in which 117 samples (26.8%) were infected with TuMV as observed in positive reactions with specific TuMV antisera. Therefore, we proposed the presence of other viruses in the

Table 3. Comparison of nucleotide and amino acid distances between IRN GSK isolate of *Turnip mosaic virus* (TuMV) and the other exotic isolates belonging to basal-B group[a].

Isolate	IRN GSK	IRN TRa6	IRN SS5	A102/11	GRC42	ESP4	ESP5	ESC8	A1	CZE10	CZE19	IS1	ITA3	ITA4	ITA5	ITA6
IRN GSK	*	0.0032	0.0097	0.0229	0.0163	0.0328	0.0262	0.0163	0.0295	0.0065	0.0065	0.0163	0.0097	0.0196	0.0097	0.0130
IRN TRa6	0.0201	*	0.0065	0.0196	0.0130	0.0295	0.0229	0.0130	0.0262	0.0032	0.0032	0.0130	0.0065	0.0163	0.0065	0.0097
IRN SS5	0.0548	0.0510	*	0.0262	0.0196	0.0295	0.0229	0.0130	0.0262	0.0032	0.0032	0.0195	0.0130	0.0163	0.0130	0.0162
A102/11	0.0915	0.0901	0.0887	*	0.0098	0.0261	0.0196	0.0196	0.0295	0.0229	0.0229	0.0262	0.0196	0.0297	0.0196	0.0229
GRC42	0.0901	0.0915	0.0955	0.0347	*	0.0163	0.0097	0.0131	0.0196	0.0163	0.0163	0.0262	0.0196	0.0297	0.0196	0.0229
ESP4	0.1025	0.1011	0.0913	0.0548	0.0535	*	0.0065	0.0163	0.0229	0.0262	0.0262	0.0362	0.0361	0.0463	0.0361	0.0394
ESP5	0.0930	0.0943	0.0820	0.0473	0.0485	0.0212	*	0.0098	0.0163	0.0196	0.0196	0.0295	0.0295	0.0397	0.0295	0.0328
ESC8	0.1073	0.1059	0.0904	0.0864	0.0947	0.0932	0.0920	*	0.0229	0.0098	0.0098	0.0261	0.0195	0.0296	0.0195	0.0228
A1	0.0967	0.1037	0.0966	0.0877	0.0809	0.0833	0.0768	0.1096	*	0.0229	0.0229	0.0328	0.0328	0.0430	0.0328	0.0294
CZE10	0.0730	0.0743	0.0757	0.1039	0.0997	0.1052	0.0956	0.1027	0.1078	*	0.0000	0.0163	0.0097	0.0196	0.0097	0.0130
CZE19	0.0730	0.0743	0.0757	0.1039	0.0997	0.1052	0.0956	0.1027	0.1078	0.0023	*	0.0163	0.0097	0.0196	0.0097	0.0130
IS1	0.0867	0.0853	0.0706	0.0900	0.0969	0.0982	0.0998	0.1001	0.1036	0.0798	0.0798	*	0.0130	0.0229	0.0130	0.0163
ITA3	0.0744	0.0678	0.0758	0.1010	0.1038	0.1037	0.0969	0.1171	0.1148	0.0677	0.0651	0.0799	*	0.0098	0.0000	0.0032
ITA4	0.0863	0.0809	0.0850	0.1119	0.1148	0.1147	0.1078	0.1225	0.1231	0.0701	0.0675	0.0905	0.0152	*	0.0098	0.0131
ITA5	0.0731	0.0691	0.0812	0.1094	0.1094	0.1093	0.1025	0.1171	0.1177	0.0651	0.0625	0.0826	0.0141	0.0176	*	0.0032
ITA6	0.0810	0.0743	0.0852	0.1079	0.1136	0.1121	0.1052	0.1141	0.1121	0.0703	0.0676	0.0852	0.0213	0.0248	0.0188	*

[a], The nucleotide (below diagonal) and amino acid (above diagonal) distances were assessed using Kimura two-parameter (Kimura, 1980) and Dayhoff matrix based model (Schwarz and Dayhoff, 1979). Isolates IRN TRa6 and IRN SS5 were collected in IRAN, isolates ITA3, ITA4, ITA5, ITA6, Al and A102/11 were collected in Italy, isolates CZE10 and CZE19 were collected in Czeck Republic, isolates ESP4 and ESP5 were collected in Spain, isolates IS1,GRC42 and ESC8 were collected in Israel, Greece and Taiwan, respectively.

canola fields of Khorasan Razavi. Several viruses such as *Cauliflower mosaic virus* (CaMV), *Beet western yellows virus* (BWYV), *Turnip crinkle virus* (TCV), *Turnip yellow mosaic virus* (TYMV), *Tomato spotted wilt virus* (TSWV), *Cucumber mosaic virus* (CMV) and *Radish mosaic virus* (RaMV) have been already reported from Iran (Shahraeen et al., 2002, 2003; Farzadfar et al., 2007; Zahedi Tabarestani et al., 2010).

We also found three aphid species with high population in canola fields including *L. erysimi*, *B. brassicae* and *M. persicae*, and they are known as main vectors of TuMV (Sako, 1980; Wang and Pirone, 1999; Dombrovsky et al., 2005; Ghorbani et al., 2007). These species can transmit other canola viruses like CaMV and BWYV as well. With regards to high population of aphids in all canola fields and the presence of viral symptoms, aphid management could be a strategy for disease control.

Based on disease severity, Govareshk isolate (IRN GSK) was selected for biological and molecular tests. Biological purification, RNA extraction and RT-PCR were performed to amplify 986 bp fragment including 54 bp of the 3′end of NIb gene, the whole CP gene and 65 bp of the3′UTR. Since the CP gene is a variable region in *Potyvirus* genomes and has been accepted for classification of this genus (Sanchez et al., 2003), so the complete nucleotide sequence of CP gene from IRN GSK was identified and deposited in Genebank (GenBank accession no. JQ073722). Sequence data of TuMV isolates are available in GenBank which are mostly collected from cruciferous crops including canola.

The CP gene comparison of different strains of TuMV indicated 88 to 98% similarity in nucleotides and 94 to 99% in amino acids (Table 2). The Govareshk isolate (IRN GSK) displayed the lowest (88%) nucleotide sequence identity with isolates from UK (GBR98), Japan

(KYD81J), Brazil (BZ1), three isolate from China (CHZJ23, CH6 and HRD) and the highest (99%) one,Iranian isolate (IRN TRa6). Amino acid sequence alignment of IRN-GSK CP gene showed 94% similarity with isolates from UK (GBR98), Japan (KWB779J), Brazil (BZ1) two strains from China (HRD, CH6) and 99%

homology with IRN TRa6, CZE19 and CZE10.

Our IRN GSK isolate like Eurasia and south Europe isolates were classified in basal-B group (Ohshima et al., 2002; Tomimura et al., 2004; Farzadfar et al., 2009) (Figure 4). Therefore, we concluded that the TuMV is a common canola virus disease in Khorasan Razavi which has been distributed in all parts of province. Various strategies could be use for controlling virus diseases such as: Breeding of new plant cultivars with natural or genetical engineering, treatment with insecticides in early stages of TuMV-infected crops, eradication of weeds which serve as infectious reservoirs and several other methods (Coutts and Jones, 2000).

ACKNOWLEDGEMENT

We especially thank Dr. Mojtaba Hosseini, Department of Plant Protection in Ferdowsi University of Mashhad, Iran, for identification of aphid species and useful comments in preparing the primary draft.

REFERENCES

Bahar M, Danesh D, Dehghan M (1985). Turnip mosaic virus in stock plant. Iran. J. Plant. Pathol. 21:11.

Blackman RL, Eastop VF (2000). Aphids on the World's Crops: An Identification and Information Guide, 2nd Ed. John Wiley and Sons press. p. 466.

Chen CC, Chang CA, Yeh SD (2006). Molecular characterization and phylogenetic analysis of Turnip mosaic virus collected from calla lily. Plant Pathol. Bull. 15:1-8.

Clark MF, Adams AM (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. J. Gen. Virol. 34:475-483.

Coutts BA, Jones RAC (2000). Viruses infecting canola (*Brassica napus*) in south-west Australia: incidence, distribution, spread, and infection reservoir in wild radish (*Raphanus raphinistrum*). Aust. J. Agric. Res. 51:925–36

Dombrovsky A, Huet H, Chejanovsky N, Raccah B. (2005). Aphid transmission of a potyvirus depends on suitability of the helper component and the N terminus of the coat protein. Arch. Virol. 150:287–298.

Farzadfar S, Ahoonmanesh A, Mosahebi GH, Pourrahim R, Golnaraghi AR (2007). Occurrence and distribution of Cauliflower mosaic virus on cruciferous plants in Iran. Plant Pathol. J. 6:22-29.

Farzadfar S, Ohshima K, Pourrahim R, Golnaraghi AR. Jalali S, Ahoonmanesh A (2004). Occurrence of Turnip mosaic virus on ornamental crops in Iran. Plant Pathol. 54:261.

Farzadfar S, Ohshima K, Pourrahim R, Golnaraghi A, Sajedi S, Ahoonmanesh A (2005). Reservoir Weed Hosts for Turnip mosaic virus in Iran. Plant Dis. 89: 339. (was not cited in the article)

Farzadfar S, Tomitaka Y, Ikematsu M, Golnaraghi AR, Pourrahim R, Ohshima K (2009). Molecular characterisation of Turnip mosaic virus isolates from Brassicaceae weeds. Eur. J. Plant. Pathol. 124:45–55.

Felsenstein J (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evol. 39:783-791.

Ghorbani S, Shahraeen N, Dehghanyar H, Sahandi A, Pourrahim R (2007). Serological identification and Purification of Turnip Mosaic Virus. (TuMV) in the oil seed rape. Iran. J. Biol. 20:61-71.

Haj Kassem AA, Walsh J.A (2008). Characterising Resistance to Turnip mosaic virus (TuMV) in Turnip (Brassica rapa rapa). Arab J. Plant. Prot. 26:168-172.

Jenner CE, Sanchez F, Nettleship SB, Foster GD, Ponz F, Walsh, JA (2000). The cylindrical inclusion gene of Turnip mosaic virus encodes a pathogenic determinant to the Brassica resistance gene TuRB01. Mol. Plant-Microbe Interact. 13:1102-1108.

Jiang Y, Wang JH, Yang H, Xu MY, Yuan S, Sun W, Xu WL, Xi DH, Lin, HH (2010). Identification and sequence analysis of turnip mosaic virus infection on cruciferous crops in southwest of china. J. Plant. Pathol. 92:241-244.

Kimura M (1980). A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol.16:111-120.

Kozubek E, Irzykowski W, Lehmann P (2007). Genetic and molecular variability of a Turnip mosaic virus population from horseradish (Cochlearia armoracia L.). J. Appl. Genet. 48:295-306.

Nicolas O, Laliberte JF (1992). The complete nucletoide sequence of turnip mosaic potyvirus RNA. J. Gen. Virol. 73:2785-2793.

Ohshima K, Akaishi S, Kajiyama H, Koga R, Gibbs AJ (2010). Evolutionary trajectory of turnip mosaic virus populations adapting to a new host. J. Gen. Virol. 91:788-801.

Ohshima K, Tanaka M, Sako N (1996): The complete nucleotide sequence of turnip mosaic virus RNA Japanese strain. Arch. Virol. 141:1991-1997.

Ohshima K, Tomitaka Y, Wood JT, Minematsu Y, Kajiyama H, Tomimura K. Gibbs AJ (2007). Patterns of recombination in turnip mosaic virus genomic sequences indicate hotspots of recombination. J. Gen. Virol. 88:298-315.

Ohshima K, Yamaguchi Y, Hirota R, Hamamoto T, Tomimura K, Tan Z (2002). Molecular evolution of Turnip mosaic virus; evidence of host adaptation, genetic recombination and geographical spread. J. Gen. Virol. 83:1511–1521.

Sako N (1980). Loss of aphid-transmissibility of turnip mosaic virus. Phytopathology 70:647-649.

Sanchez F., Wang X., Jenner C. E., Walsh J. A., Ponz, F (2003). Strains of Turnip mosaic potyvirus as defined by the molecular analysis of the coat protein gene of the virus. Virus Res. 94:33- 43.

Schwarz R, Dayhoff M (1979). Matrices for detecting distant relationships. In Dayhoff M, editor, Atlas of protein sequences, National Biomedical Research Foundation. pp. 353-358.

Shahraeen N, Farzadfar S, Lesemann DE (2003). Incidence of viruses infecting winter oilseed rape (Brassica napus ssp. oleifera) in Iran. J. Phytopathol. 151:614-616

Shahraeen N, Farzadfar S, Kamran R, Naseri B (2002). Report on incidence of viral diseases of oilseed rape in Iran. J. Plant Pests. Dis. 69:189-191.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. 28:2731-2739.

Tomimura K, Gibbs AJ, Jenner CE, Walsh JA, Ohshima, K (2003). The phylogeny of Turnip mosaic virus; comparisons of thirty-eight genomic sequences reveal a Eurasian origin and a recent 'emergence' in East Asia. Mol. Ecol. 12:2099-2111.

Tomimura K, Spak J, Katis N, Jenner CE, Walsh JA, Gibbs, AJ (2004). Comparisons of the genetic structure of populations of Turnip mosaic virus in west and East Eurasia. Virol. 330:408-423.

Tomitaka Y, Ohshima, K (2006). A phylogeographical study of the Turnip mosaic virus population in East Asia reveals an 'emergent' lineage in Japan. Mol.Ecol.15: 4437- 4457.

Vollmann J, Rajcan I (2010). Oil Crops: Handbook of Plant Breeding, Springer press 4:91-125.

Wang HY, Liu JL, Gao R, Chen J, Shao, YH, Li XD (2009). Complete genomic sequence analyses of Turnip mosaic virus basal-BR isolates from China. Virus Genes. 38:421-428.

Wang RY, Pirone TP (1999). Purification and characterization of turnip mosaic virus helper component protein. Phytopathology 89:564-567.

Zahedi Tabarestani A, Shamsbakhsh M, Safaei, N (2010). Distribution of three important aphid borne canola viruses in Golestan province. Iran. J. Plant Prot. Sci. 41:251-259.