Shahid Bahonar University of Kerman

Journal of Advances in Plant Protection

JAPP 2023

Association of Iranian Plant Protection Societies

Occurrence and molecular characterization of two potyviruses on basil (*Ocimum basilicum*) in Iran

Mahbubeh Alikhany[1], SeyyedehAtefeh Hosseini[1]✉, FaezehoSadat Abtahi[2], Malik Nawaz Shuja[3]

1 Department of Plant Protection, Faculty of Agriculture, University of Birjand, Birjand, Iran.

2 Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, 38156-8- 834, Arak, Iran.

3 Department of Microbiology, Kohat University of Sciences and Technology, Kohat, Pakistan.

✉ Corresponding author: ahosseini@birjand.ac.ir

Article Info.

Article type:
Original article

Article history:
Received 29 May 2024
Received in revised form 17 Jun 2024
Accepted 19 Jun 2024
Available Online 22 Jun 2024

Keywords:
Basil,
Bean yellow mosaic virus,
PCR,
Potyvirus,
Phylogeny,
Turnip mosaic virus.

Abstract

Potyviruses cause severe diseases in different crops around the world. During March and April 2018, 90 basil and 21 weed samples were collected from basil fields in the eastern provinces of Iran. The total RNA of samples was extracted, and RT-PCR was performed using degenerative primers of potyviruses. Sequence analysis showed that positive samples were most closely related to *Turnip mosaic virus* (TuMV) or *Bean yellow mosaic virus* (BCMV). Subsequently, specific primers of TuMV were used to amplify a 980 bp amplicon in a sample of *Ocimum basilicum* and *Malva sylvestris*. Furthermore, a 950 bp amplicon was also detected in six samples of *O. basilicum* when specific BYMV primers were used for the PCR assay. All amplified PCR products were cloned and sequenced. According to CP-based phylogenetic analysis, two Iranian isolates of TuMV were placed in the Asian BR group. Five Iranian isolates of BYMV were classified in the broad bean group, and another isolate was placed in the general group. The homology matrix also showed that an isolate from India had the least nucleotide identity (80%) with the Iranian isolate, while among the Iranian isolate, SIB9 was very close to the Japanese isolate (98%). According to recombination analysis, three Iranian isolates of BYMV were recombinant. Sequence analysis of the TuMV isolate (SIB1) showed that it shared 99% nucleotide identity with the Chinese isolate. This survey is the first report of two potyviruses, including TuMV and BYMV, infecting *O. basilicum* in Iran.

Cite this article: Alikhany, M., Hosseini, S. A., Abtahi, F., & Shuja, M. N. (2024). Occurrence and molecular characterization of two potyviruses on basil (*Ocimum basilicum*) in Iran. *Journal of Advances in Plant Protection*, *1*(1), 41–50.

Publisher: Shahid Bahonar University of Kerman

Introduction

Vegetables are an essential part of a healthy human diet owing to their nutritional value. In the meantime, leafy vegetables are great sources of vitamins, dietary fibers, and minerals (Van Duyn & Pivonka, 2000). Basil plant (*Ocimum basilicum* L.) (Ryding, 1994) is an herbaceous annual plant of the family Lamiaceae (formerly called Labiatae). An approximate area of 200 ha has been devoted to the cultivation of basil plant in eastern provinces (Sistan-o-Baluchistan and Khorasan). Due to suitable climate and rich soil, basil is cultivated in these areas throughout the year. Thus, leafy vegetables play a key role in the economy of two provinces. Production of vegetables are affected by several abiotic and biotic factors, in which, potyviruses are the major cause of vegetables disease in Iran. Recently, symptoms such as mosaic, vein clearing and mild mosaic were observed in the basil fields in Iran suggesting the presence of potyviruses in Iranian fields.

The genus *Potyvirus* is one of the largest genera of plant viruses comprises over 100 species with flexuous particle morphology 700 nm long which are transmitted by aphids via non-persistent manner (Adams et al., 2005). They infect a wide range of mono-and dicotyledonous plants and cause significant losses around the world (Gibbs & Ohshima, 2010). The genome is a single-stranded, positive-sense RNA molecule of approximately 1000 nts in length with a major open reading frame (ORF) that is translated into a large polyprotein with small overlapping ORFs (Nguyen

et al., 2013). A wide range of plant species, primarily from the family Brassicaceae are infected by TuMV (Walsh & Jenner, 2002). TuMV is one of the most important virus diseases in 28 countries (Tomlinson, 1987). Coat protein (CP) is an important protein for detection and molecular characterization of potyviruses and the complete sequence of the coat protein gene has been analyzed for many isolates in the world (Chen et al., 2006; Korkmaz et al., 2007; Nguyen et al., 2013; Nicolas & Laliberte, 1992; Ohshima et al., 1996, 2010; Tomitaka & Ohshima, 2006; Wang & Pirone, 1999). Furthermore, CP is involved in aphid transmission, cell-to-cell and systemic movement and encapsidation. CP sequences have been extensively studied to assess the difference within and between potyvirus species (Tomimura et al., 2004). The genome of different isolates of TuMV from Iran, Italy, Germany, Canada, Japan, Denmark, Russia, Poland, New Zealand and the United Kingdom have been fully sequenced.

The second virus, BYMV, occurs worldwide and infects plant species of the monocotyledonous and dicotyledonous families. Many strains of BYMV have been reported in different crops, but have not been properly characterized (Barton et al., 1964; Doolittle & Jones, 1925, Hampton et al., 1992; McKern et al., 1992; Randles et al., 1980; Schroeder & Prowidenti 1966).

According to the results of various studies on basil fields, Tobacco mosaic virus (TMV; *Tobamovirus*), *Cucumber mosaic virus* (CMV; *Cucumovirus*) (Marei & Magdy, 2017), Lucerne mosaic virus (LMV; *Alfamovirus*) (Feldman & Gracia, 1970), Tomato spotted wilt virus (TSWV; *Tospovirus*) (Holcomb et al., 1999), *Pepino mosaic virus* (PepMV; Potexvirus) (Jones et al., 1980), Impatiens necrotic spot virus (INSV; Tospovirus) (Poojari & Naidu, 2013), Broad bean wilt virus (BBWV; Fabavirus) (Santz et al., 2001), and Barely stripe mosaic virus (BSMV; Fabavirus) (Marei & Magdy, 2017) were detected as main viruses naturally infecting basil in the world. To the best of our knowledge, there is no report on basil infection by the genus Potyvirus in Iran. Considering leafy vegetables as one of the most important group of vegetables in Iran, this work studied the occurrence of potyviruses in Iranian basil fields for the first time. Therefore, the full-length coat protein gene of detected potyviruses as a well-known parameter was sequenced to classify the detected potyviruses (Berger et al., 1997).

Material and Methods

Sample collection

During March and April 2018, ninety samples of basil and 21 samples of weeds showing vein clearing and mild mosaic symptoms were collected from some main cultivation regions in Sistan-o-Baluchistan and South Khorasan Provinces in Iran. Ninety symptomatic samples of basil leaves with different symptoms were transferred to the lab for testing under cool conditions.

Total RNA extraction and RT-PCR

Total RNA was extracted from fresh leaves of basil samples using an RNA Extraction Kit (Qiagen) following the manufacturer's instructions. Primary detection was carried out for all collected samples by PCR assay using degenerate primers of potyviruses (Marie-Jeanne et al., 2000) followed by using two specific primer sets of BYMV and TuMV (Bhadramurthy et al., 2011; Sanchez et al., 2003) (Table 1). For the reverse transcription (RT) reaction, 5 µl of total RNA with 2.5µl revers primer and six µl of DEPC-treated (nuclease-free) water. The mixture was subsequently incubated at 60°C for 10 min to denature the RNA, then chilled in an ice bath for one min. Four µl of RT reaction mixture containing one µl of M-MLV RT buffer and two µl of dNTPs as well as one µl of RNasin were added to the denatured RNA extract. Totally, nine µl of RT reaction was incubated at 42°C for one hour followed by incubation at 95°C for three min in an automated thermal cycler (Bioreba) to terminate the RT reaction.

For BYMV detection, the PCR reaction was carried out in a 25 µl reaction volume containing the sense and antisense primers (10 pmol each), Taq DNA polymerase, 1×PCR buffer and 10 µM of dNTPs. The PCR mixture containing 46 µl of the above components was added to the tubes containing four µl template DNA resulting in a final reaction volume of 25µl. Amplification was performed in an automated thermal cycler (Bioreba). The program for BYMV detection consisted of an initial denaturing cycle at 94°C for four min followed by 40 cycles reaction profile involving of denaturation at 94°C for 30 s, annealing at 50°C for one min and extension at 72°C for one min. A final extension step at 72°C for 10 min was included to duplicate fragments using the specific primers of BYMV. The program for TuMV detection consisted of an initial cDNA synthesis step at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 20 seconds, annealing at 55.5°C for 30 seconds, and extension at 72°C for one minute. After the PCR reaction, four µl of the PCR product was electrophoresed on a 1% agarose gel containing 10% DNA green viewer (Pars Tous Biotechnology Inc., Iran) in 0.5 x Tris-borate EDTA buffer (TBE) with 0.7 µl of

GeneRuler 100 bp DNA ladder (Fermentase). The gels were observed under UV light in a gel documentation system.

Biological properties

Leaf tissues of eight positive samples were ground in a 1% (w/v) solution of K_2HPO_4 at pH 7.5 containing 0.01% Na_2SO_3, 2% polyvinyl pyrrolidone (PVP) and 0.05% ethylene diamine tetra acetic acid (EDTA). Subsequently, extracts were mechanically inoculated on *Nicotiana tabacum* cv. Samsun. Inoculated plants were kept in a greenhouse and checked regularly and symptoms were recorded.

Cloning and sequencing

Six and two amplified PCR products of TuMV and BYMV, respectively, using specific primers were finally cloned into pTZ57R/T plasmid vector (InsTAclone^{TM} PCR Cloning Kit, Fermentas) and completely sequenced bidirectionally by Macrogen Company (South Korea).

Sequence comparisons and phylogenetic analysis

Complete nucleotide sequences of coat protein gene of BaYMV and TuMV isolates were first analyzed by the BLASTn algorithm available at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic trees were constructed using Maximum Likelihood (ML) method embedded in MEGA7 (Tamura et al., 2013) and viewed using Treeview (Page, 1996). Complete nucleotide sequences of CP gene were used to construct phylogenetic tree using MEGA7 (Tamura et al., 2013). The full-length sequences of CP genes were aligned and manually adjusted by Clustal W. Phylogenetic trees were constructed using the Maximum Parsimony method. Bootstrap re-sampling (1000 replications) was used to measure the reliability of individual nodes in each phylogenetic tree. Homology and distance matrices were analyzed by Megalign and SDTv softwares (Clewley & Arnold 1997; Muhire et al., 2014).

Recombination analysis by RDP4

Recombination analysis was carried out using Recombination Detection Program v.4.16 (RDP4) (Martin et al., 2015). Recombination events, possible parental isolate of recombination, and recombination breakpoints were analyzed by the RDP, GENECOVN, Chimaera, Maxchi, BOOTSCAN and SISCAN methods implemented in the RDP4 program with default setting (Martin et al., 2015).

Table 1. Primers used to amplify coat protein (CP) and NIb gene sequences of the Iranian BYMV and TuMV isolates.

Primer names	Primer sequences (5′-3′)	Amplified region	Virus	References
Oligo1n- F1	ATggTHTggTgYATHgARAAYgg	CP-UTR	Potyvirus	Marie-Jeanne et al. (2000)
Oligo2n-R1	TgCTgCKgCYTTCATYTg	CP-UTR	Potyvirus	Marie-Jeanne et al. (2000)
TuMV-F2	TATTTCCCATAAGCGAGAATA	CP	TuMV	Sanchez et al. (2003)
TuMV-R2	CAAGCAATCTTTGAGGATTAT	CP	TuMV	Sanchez et al. (2003)
BYMV-F3	GCACCATATAGTCAATTGAG	CP-NIb	BYMV	Bhadramurthy et al. (2011)
BYMV-R3	GACATCTCCTGCTGTGTG	CP-NIb	BYMV	Bhadramurthy et al. (2011)

Results

Potyvirus detection

Ninety samples of basil and 21 symptomatic samples of weeds from the basil fields of the eastern provinces of Iran were screened by PCR assay for the presence of potyviruses. Results revealed the potyvirus infection of eight samples (out of 111). Screening these samples by RT-PCR indicated that 760 bp fragment was amplified for one weed (*M. sylvestris*) and one basil samples using specific primers of TuMV. In addition, 950 bp fragment was amplified for six basil samples using specific primers of BYMV (Fig.1 and Table 2).

Biological properties

Symptoms of the plants were evaluated at 14-day intervals until four weeks of post-inoculation. All eight Iranian isolates in this study induced symptoms on the test plants and BYMV and TuMV were detected in inoculated plants by RT-PCR using specific primers as previously described.

Fig. 1. Symptoms observed in basil and *Malva sylvestris* samples infected with turnip mosaic virus (TuMV) or bean yellow mosaic virus (BYMV) in basil fields. Basil plants infected with TuMV showing yellow mosaic and dwarfing (A); Basil plants infected with BYMV showing mild chlorosis and dwarfing (B); *M. sylvestris* plants infected with TuMV showing mild chlorosis and dwarfing (C).

Table 2. Occurrence of potyviruses on collected samples from basil fields in different parts of Sistan-o-Baluchistan Province.

Province	Region	Host	Number of samples	Positive sample (TuMV)	Positive sample (BYMV)	Name of isolates	Recombinant
Sistan-o-Baluchistan Khorasan	Mahmod Abad	*Ocimium basilicum*	15	-	3	SIB7-SIB8-SIB9	SIB8
		Chenopodium album	2	-	-	-	-
	Abasabad	*Ocimum basilicum*	25	-	-	-	-
		Malva sylvestris	5	1	-	SIB3	-
	Esmaelabad	*Ocimium basilicum*	15	1	1	SIB1-SIB12	SIB12
		Chenopodium album	2	-	-	-	-
	Birjand	*Ocimium basilicum*	25	-	2	SIB10-SIB11	SIB11
		Chenopodium album	3	-	-	-	-
		Portulaca oleacea	3	-	-	-	-
		Amaranthus retroflexus	4	-	-	-	-
		Solanum nigrum	2	-	-	-	-
	Neyshabor	*Ocimum basilicum*	10	-	-	-	-

Genome characterization and phylogenetic analysis of BYMV

The nucleotide sequence of the CP-NIb gene of the six Iranian and selected GenBank isolates were used for multiple sequence alignment and then a phylogeny tree was constructed using MEGA7 software (Fig. 2). Accordingly, BYMV isolates were classified in five clusters of General, Broad bean, Canna, Monocot and Pea. Among these categories, the first group consisted of the Iranian isolate (SIB10) together with Japanese, Australian, Dutch, Indian and American isolates. The second category consisted of the Iranian isolates (SLB8, SIB7, SIB11, SIB12 and SIB9) together with Iraqi and Japanese isolates. The homology matrix also showed that SIB9 isolate was very similar to the Japanese isolate from *Vicia faba* and shared 98% nucleotide identity. However, the least nucleotide identity of 80% was observed with Indian isolate infecting *Glodiolus* sp. (data not shown).

Recombination analysis of BYMV by RDP4

For detection of the putative recombination signals observed by the network analysis, recombination detection analysis was performed using RDP4 software which contains various recombination detection algorithms such as RDP, GENECONY(G), Chimaera

(C), Maxchi (M), BOOTSCAN (B) and SISCAN (S). Results showed that three isolates including SIB12, SIB11 and SIB8 were recombinant (Table 3). SIB11and SIB12 were estimated to have two recombination events detected by Chimaera, Maxchi, BOOTSCAN and SISCAN. SIB8 was estimated to have one recombination event detected by Maxchi, BOOTSCAN and SISCAN.

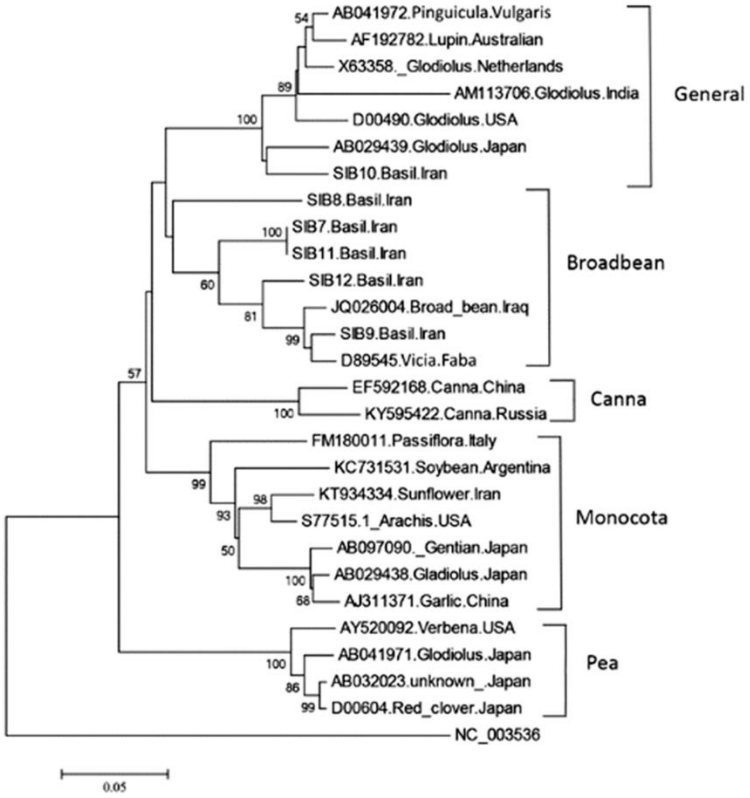

Fig. 2. Phylogenetic tree obtained from the alignment of nucleotide sequences of the CP-NIb of BYMV isolates from six Iranian isolates of Basil and selected GenBank isolates. For each sequence, the country of origin, name of isolate and accession number are indicated. Phylogenetic tree was constructed using MEGAX version 7 software and Maximum Likelihood method with 1000 bootstrap replications. *Narcissus mosaic virus* (NC_003536) is considered as outgroup.

Table 3. List of detected recombination events in Iranian basil isolates of BYMV genome.

Number	Recombinant isolate	Major parent	Minor parent	Detection methods						
				R	G	B	M	C	S	T
1	SIB12.Basil.Iran	D89545.*Vicia faba*.Japan	AB029438.Glodiolus.Japan	-	+	+	+	+	+	+
2	SIB11.Basil.Iran	SIB12.*Ocimum basilicum*.Iran	AB041971.Glodiolus.Japan	-	+	+	+	+	+	+
3	SIB8.Basil.Iran	AB029439. *Glodiolus*sp.Japan	SIB9.Basil.Iran	+	+	-	+	-	+	+
4	SIB11.Basil.Iran	SIB9.*Ocimum basilicum*.Iran	AM113706.Glodiolus.India	-	+	-	+	+	+	+
5	SIB10.Basil.Iran	AB041972.*Pinguicula vulgaris*.Japan	Unknown	-	+	-	+	+	+	+
6	SIB10.Basil.Iran	Unknown	AB029439.Glodiolus.Japan	-	-	-	+	-	-	-
7	S77515.1.Arachis.USA	KT934334.Sunflower.Iran	JQ026004.Broadbean.Iraq	+	+	-	+	+	+	+
8	SIB9.Basil.Iran	SIB12.*Ocimumbasilicum*.Iran	AB029438.Glodiolus.Japan	-	+	-	-	-	+	+
9	AB029439.Glodiolus.Japan	AM113706.*Glodiolus*sp.Indi a	Unknown	+	-	-	+	+	+	+
10	AM113706.Glodiolus.India	AB041972.*Pinguiula vulgaris*.Japan	Unknown	-	+	-	-	-	-	-

Phylogenetic analysis of TuMV Isolates

Comparison of the CP gene of two TuMV isolates with selected GenBank isolates revealed that they fitted within five major phylogenetic groups including basal B, basal BR, World B, Iranian and Asian BR (Fig. 3). The sequences obtained from the Iranian isolates (SIBI and SIB3) were closest to the isolates in the Asian group. There were 12 Iranian isolates (sequenced in

another study), two Chinese isolates and a Turkish isolate in the Asian group. In this study, none of the Iranian isolates were included in the basal B, basal BR, Iranian and World B groups. The alignment of the nucleotide sequences of the SIB1 isolate with other isolates showed the maximum nucleotide identity of 99% with the Chinese isolate (data not shown).

Recombination analysis of TUMV byRDP4

The full-length sequence of coat protein gene was determined for Iranian isolates (SIB1 and SIB3) from *O. basilicum* and *M. sylvestris*. No evidence of recombination event was found for both isolates.

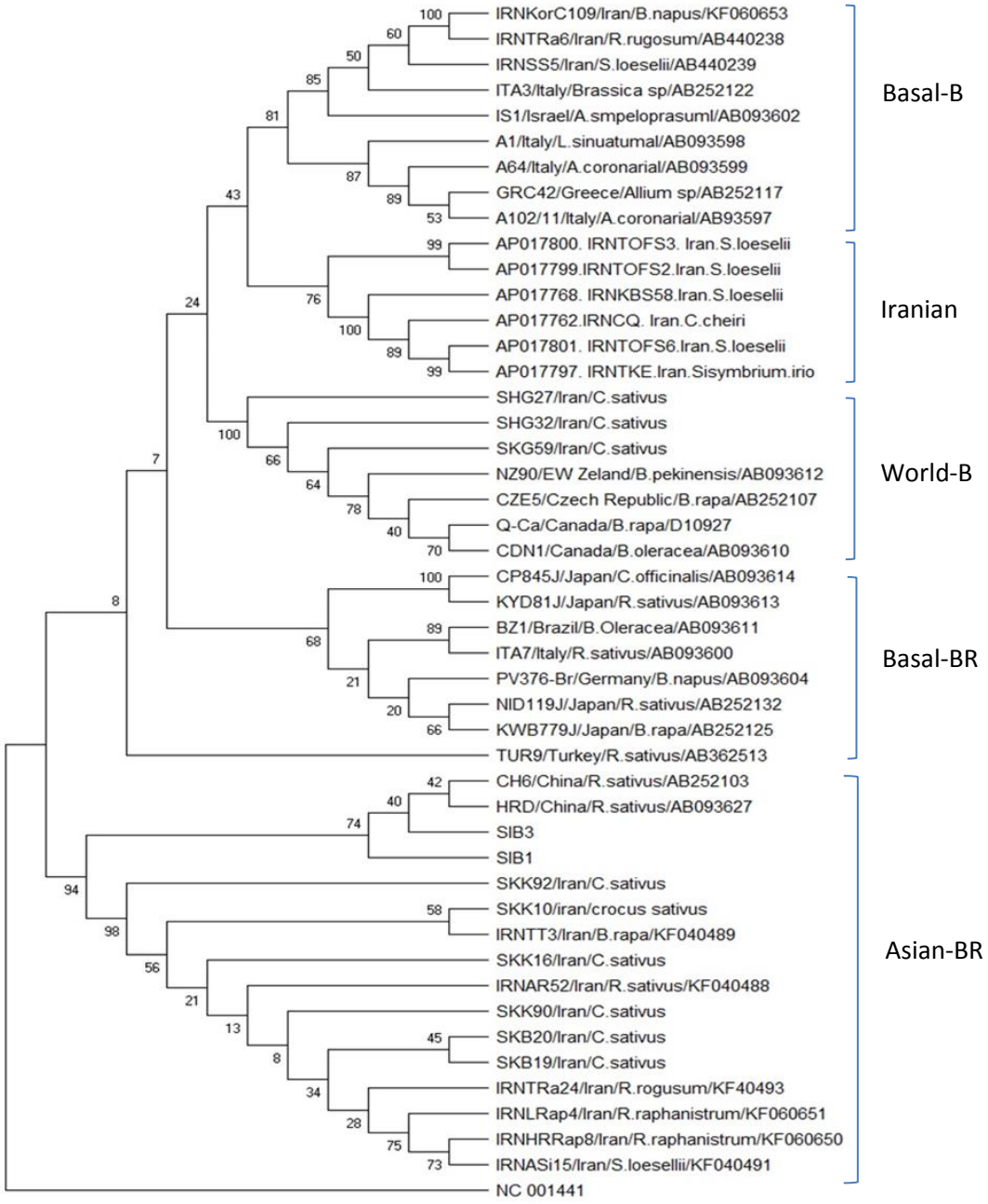

Fig. 3. Phylogenetic tree obtained from alignment of nucleotide sequences of the CP of TuMV isolates for two Iranian and selected GenBank isolates. For each sequence, the country of origin, name of isolate and accession numbers are indicated. Phylogenetic tree was constructed using MEGAX version 7 and the Maximum Likelihood tree test with 1000 bootstrap replications as the best method for phylogeny of this virus. Clover yellow vein virus (NC001441) is considered as outgroup.

Discussion

According to the literature, potyviruses infect a large number of economical crops and wild plants around the world. In this study, two economically important potyviruses including BYMV and TuMV were detected in the Iranian basil fields. RT-PCR assay carried out using degenerate and specific primers followed by sequencing of PCR amplicons. Finally, expected fragments were amplified for six basil and two basil and *M. sylvestris* samples (one basil and one weed samples) for BYMV and TuMV infection, respectively. Comparison of the resulting sequences with those in the NCBI database confirmed the presence of BYMV in basil and TuMV in the basil and weed samples. This study is the first report of the basil infection with TuMV and BYMV in Iran. Moreover, *M. sylvestris* was reported as a natural weed host of TuMV for the first time in this study. As important reservoirs, weeds act as mixing bowls for recombination events (Hosseini & Salari, 2017).

Analysis of the phylogenetic tree showed that most Iranian isolates of BYMV were classified in the Broad Bean group indicating a correlation between geographic and genotypic origins. Although this is not the case for all isolates, the coincidence of the Iranian isolate (SIB10) with the Japanese, Australian, Indian, Dutch and American isolates in Group 1 disrupts geographical distribution with phylogenetic proximity. This shows that the worldwide spread of BYMV is not possible by natural vectors (aphids) which supports the possibility of human interference and in other words its spread by seeds. Another point to note about the diversity of Iranian isolates is that BYMV has probably entered to Iran more than once in various ways leading to recombination among the virus isolates (Lovisolo et al., 2003; Spetz et al., 2003).

The alignment of BYMV nucleotide sequences of the Iranian isolates showed the highest nucleotide identity of 100% between SIB11 and SIB7 in the CP coding region. However, the SIB10 isolate shared the least identity with the SIB9 isolate. Based on multiple comparisons of the nucleotide level of BYMV with other GenBank isolates, it was found that BYMV shared 98% identity with the Japanese isolate (D89545) as reported by Wylie et al. (2008) based on part of the CP gene. This survey also indicated a high nucleotide identity between the Iranian and Japanese isolates. This observation may suggest that BYMV moved from Japan to Iran or vice versa perhaps due to human migrants who carried infected soil with seeds. Moreover, the lowest nucleotide identity of 80% was observed for the Indian isolate in General group (Kaur et al., 2013) and the highest nucleotide identity between Indian and Iranian BYMV isolates on ornamental plants and with unrelated Australian and Japanese bean isolates.

The occurrence of recombination among BYMV isolates was investigated by BOOTSCAN, RDP, GENECOV, SISCAN, MAXCHI and ChIMERA methods. In the meantime, the recombinant SIB8, SIB11 and SIB12 isolates were detected in the CP region. The recombination events with a P-value of less than $1.0 \times 10{-}6$ were selected. The most important reason for these recombination events is probably the common viral hosts and the mixed infection of some of them leading to viral adaption to the new conditions and ultimately plant infection by the virus. The parents of BYMV recombinants have been originated from the Iranian and Japanese isolates. The emergence of the new compatible isolates among the BYMV populations on *O. basilicum* in this study indicated the recombination flow in nature. It is suspected that newly recombinant isolates appear on weed reservoirs for the adaption of new hosts to new environmental conditions. Therefore, a better understanding of genetic diversity and emotional evolution of BYMV populations in weed reservoirs leads to the design of more stable control methods (Lian et al., 2013).

Molecular and phylogenetic analysis of Turnip mosaic virus in a basil field in eastern provinces of Iran confirmed infection of two samples by TuMV. The virus was not detected in some regions of the province where the samples were collected. This can be attributed to a variety of factors such as the lack of primary sources of infection, vector activity and different ecological changes. In this study, five phylogenetic groups were formed based on the coat protein sequence. The isolates in this study were classified into the Asian BR group (Tan et al., 2004). The Iranian isolates, however, in the basal B, basal BR, World B and Iranian groups were included from previous research (Farzadfar et al., 2009; Heydari et al., 2018; Yasaka et al., 2017). Surprisingly, no isolates in this study were included in these groups. The isolates of this study were placed in the same groups with other Iranian isolates including *Crocus sativus*, *Brassica rapa*, *Raphanus rogusum*, *Raphanus raphanistrum* and *S. loesellii*. This shows the common ancestors of the above-mentioned isolates and also indicates the lack of role of the host in classification. According to the results of RT-PCR and sequencing, the weed M. sylvestris was identified as the wild host of TuMV for the first time. Phylogenetic analysis showed that this isolate is classified in the Asian BR group close to another Iranian isolate because of the same geographical location despite the detection of the virus from the *M. sylvestris*.

Sistan-o-Baluchistan and Khorasan Provinces are major areas under vegetable cultivation in Iran and often offer a climate suitable for developing aphids. In this research, TuMV and BYMV were identified in some regions of these eastern areas in Iran. These potyviruses could therefore represent a new potential threat to vegetable growers, particularly basil in Iran.

Conclusion

Potyviruses are among the most economically important plant viruses, capable of causing severe diseases in various crops. In this study, we report the first detection and molecular characterization of two potyviruses, turnip mosaic virus and bean yellow mosaic virus, infecting basil (*O. basilicum*) and associated weed species in Iran. The phylogenetic and recombination analyses revealed genetic diversity among the Iranian isolates, with some BYMV isolates identified as emerging recombinants. This suggests that the viral populations are adapting to new host plants and environmental conditions in Iran. The results of this study emphasize the importance of continuous monitoring of potyviruses in vegetable crops and wild reservoirs. To secure the future of vegetable cultivation in Iran, it is crucial to incorporate, resistance against potyviruses such as BYMV and TuMV into the current breeding strategies.

Acknowledgement

We are grateful to the University of Birjand for providing the funds of operating the project named Molecular investigation of Potyviruses in vegetable in Iran. This paper is a part of our fundings.

Conflict of interest

The authors declare that there are no conflicts of interest present.

CRediT author statement

S. A. Hosseini,: Supervision, methodology, writing, reviewing & editing. M. **Alikhany**: Field and laboratory works & writing original draft preparation. **F. S. Abtahi:** Methodology & Analysing. **M. N. Shuja**: reviewing & editing.

References

Adams, M. J., Antoniw, J. F & Beaudoin, F. (2005). Overview and analysis of the polyprotein cleavage sites in the family Potyviridae. *Molecular Plant Pathology*, *6*(4), 471-487.

Barton, D. W., Schroeder, W. T., Provvidenti, R., & Mischanec, W. (1964). Clones from segregating progenies of garden pea demonstrate that resistance to BV2 and PV2 is conditioned by the same genotype. *Plant Disease Reporter*, *48*, 353-355.

Berger, P. H., Wyatt, S. D., Shiel, P. J., Silbernagel, M. J., Druffel, K., & Mink, G. I. (1997). Phylogenetic analysis of the Potyviridae with emphasis on legume-infecting potyviruses. *Archives of Virology*, *142*(10), 1979-1999.

Bhadramurthy, V., Bhat, A. I., & Biju, C. N. (2011). Occurrence and coat protein sequence-based characterisation of *Bean yellow mosaic virus* (BYMV) associated with vanilla (*Vanilla planifolia* Andrews) in India. *Archives of Phytopathology and Plant Protection*, *44*(18), 1796-1801.

Chen, C. C, Chang, C. A., & Yeh, S. D. (2006). Molecular characterization and phylogenetic analysis of *Turnip mosaic virus* collected from calla lily. *Plant Patho*logy *Bulletin*, 15, 1-8

Clewley, J. P., & Arnold, C. (1997). MEGALIGN. The multiple alignment module of LASERGENE. *Methods in Molecular Biology*, *70*, 119-29.

Doolittle, S. P., & Jones, F. R. (1925). The mosaic disease in the garden pea and other legumes. *Phytopathology*, *15*(12), 763–771.

Farzadfar, S., Tomitaka, Y., Ikematsu, M., Golnaraghi, A. R., Pourrahim, R., & Ohshima, K. (2009). Molecular characterization of *Turnip mosaic virus* isolates from Brassicaceae weeds. *European Journal of Plant Pathology*, *124*(1), 45.

Feldman, J. M., & Gracia, O. (1970). Two new natural hosts of *Alfalfa mosaic virus*. *Plant Disease Reporter*, *54*(8), 722-723.

Gibbs, A., & Ohshima, K. (2010). Potyviruses and the digital revolution. *Annual Review of Phytopathology*, *48*, 205-223.

Hampton, R. O., Shukla, D. D., & Jordan, R. L. (1992). Comparative Potyvirus host range, serology, and coat protein peptide profiles of white *lupin mosaic virus*, *Phytopathology*, *82*(5), 566-571.

Heydari, M., Hoseini, A., & Dori, R. (2018). Detection and phylogenetic analysis of *Turnip mosaic virus* on saffron (*Crocus sativus*) in Iran. *Applied Researches in Plant Protection, 7* (1), 17-28. (In Farsi).

Holcomb, G. E., Valverde, R. A., Sim, J., & Nuss, J. (1999). First report on natural occurrence of *Tomato spotted wilt Tospovirus* in basil (*Ocimum basilicum). Plant Disease*, *83*(10), 966-966.

Hosseini, S. A., & Salari, K. (2017). Detection and molecular characterization of *Potato virus S* of weed reservoirs in Iran. *Archives of Phytopathology and Plant Protection*, *50* (15-16), 828-838.

Jones, R. C., Koenig, R., & Lesemann, D. E. (1980). *Pepino mosaic virus*, a new Potexvirus from pepino (*Solanum muricatum*). *Annals of Applied Biology*, *94*(1), 61-68.

Kaur, C., Kumar, S. & Raj, S. K. (2013). New record of association of *Bean yellow mosaic virus* with mosaic disease of *Vicia faba* in India. *Indian Journal of Virology*, *24*(1), 95-96

Korkmaz, S., Onder, S., Tomitaka, Y., & Ohshima, K. (2007). First report of *Turnip mosaic virus* on Brassicaceae crops in Turkey, *Plant Pathology*, *56*(4), 719.

Lian, S., Lee, J. S., Cho, W. K., Yu, J., Kim, M. K., Choi, H. S., & Kim, K. H. (2013). Phylogenetic and recombination analysis of tomato spotted wilt virus. *Plos One*, *8*(5), e63380.

Lovisolo, O., Hull, R., & Rösler, O. (2003). Coevolution of viruses with hosts and vectors and possible paleontology. *Advances in Virus Research*, 62, 325-379.

Marei, E. M., & Magdy, M. (2017). Isolation and characterization of basil mosaic virus in Egypt. *Archives of Phytopathology and Plant Protection*, *50*(13-14), 687-699.

Marie-Jeanne, V., Ioos, R., Peyre, J., Alliot, B., & Signoret, P. (2000). Differentiation of Poaceae Potyviruses by reverse transcription-polymerase chain reaction and restriction analysis. *Journal of Phytopathology*, *148*(3), 141-151.

Martin, D. P., Murrell, B., Golden, M., Khoosal, A., & Muhire, B. (2015). RDP4: Detection and analysis of recombination patterns in virus genomes, *Virus Evolution*, *1*(1), vev003.

McKern, N. M., Mink, G. I., Barnett, O. W., Mishra, A., Whittaker, L. A., Silbernagel, M. J., & Shukla, D. D. (1992). Isolates of *bean common mosaic virus* comprising two distinct Potyviruses. *Phytopathology*, *82*(9), 923-929.

Muhire, B. M., Varsani, A., & Martin, D. P. (2014). SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS One*, *9*, e108277.

Nguyen, H. D, Tomitaka, Y, S., Duchêne, S., Vetten, H. J, Lesemann, D., & Ohshima, K. (2013). *Turnip mosaic* Potyvirus probably first spread to Eurasian brassica crops from wild orchids about 1000 years ago. *PLoS One*, *8*(2), e55336.

Nicolas, O., & Laliberté, J. F. (1992). The complete nucleotide sequence of *Turnip mosaic* Potyvirus RNA. *Journal of General Virology*, *73*(11), 2785-2793.

Ohshima, K., Akaishi, S., Kajiyama, H., Koga, R., & Gibbs A. J. (2010). Evolutionary trajectory of *Turnip mosaic virus* populations adapting to a new host. *Journal of General Virology*, *91*(3), 788-801.

Ohshima, K., Tanaka, M., & Sako., N. (1996). The complete nucleotide sequence of *Turnip mosaic virus* RNA Japanese strain. *Archives of Virology*, *141*(10), 1991-1997.

Page, R. D. (1996). Tree View: An application to display phylogenetic trees on personal computers. *Bioinformatics*, *12*(4), 357-358.

Poojari, S., & Naidu, R. A. (2013). First report of *Impatiens necrotic spot virus* (INSV) infecting basil (*Ocimum basilicum*) in the United States. *Plant Disease*, *97*(6), 850..

Randles, J. W., Davies, C., Gibbs, J. & Hatta, T. (1980). Amino acid composition of capsid protein as a taxonomic criterion for classifying the atypical S strain of *bean yellow mosaic virus*. *Australian Journal of Biological Sciences*, *33*(2), 245-254.

Ryding, O. (1994). Notes on the sweet basil and its wild relatives (Lamiaceae). *Economic Botany*, *48*(1), 65-67.

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 Research*, *94*(1), 33-43.

Santz, N. T., Chen, T. H., & Lai, P. Y. (2001). A newly discovered mosaic disease of bush busil

(*Ocimum basilicum*) in Taiwan. *Plant Pathology Bulletin*, *10*(4), 155-164.

Schroeder, W. T., & Prowidenti, R. (1966). Further evidence that *Common Pea mosaic vims* (PV2) is a strain of *Bean yellow mosaic virus* (BV2). *Plant Disease Reporter*, *50*(5), 337-340.

Spetz, C., Taboada, A. M., Darwich, S., Ramsell, J., Salazar, L. F., & Valkonen, J. P. T. (2003). Molecular resolution of a complex of Potyviruses infecting solanaceous crops at the center of origin in Peru. *Journal of General Virology*, 84(9), 2565-2578.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). Molecular evolutionary genetics analysis version 6.0. *Molecular Biology Evolution*, *30*(12), 2725-2729.

Tan, Z., Wada, Y., Chen, J., & Ohshima, K. (2004). Inter-and intralineage recombinants are common in natural populations of *Turnip mosaic virus*. *Journal of General Virology*, *85*(9), 2683-2696.

Tomimura, K., Katis. N., Jenner, C. E., Walsh, J. A, Gibbs, A. J., & Ohshima K. (2004). Comparisons of the genetic structure of populations of *Turnip mosaic virus* in West and East Eurasia, *Virology*, *330*(2), 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. *Molecular Ecology*, *15*(14), 4437-4457.

Tomlinson, J. A. (1987). Epidemiology and control of virus diseases of vegetables. *Annals of Applied Biology*, *110*(3), 661-681.

Van Duyn, M. A. S., & Pivonka, E. (2000). Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: selected literature. *Journal of the American Dietetic Association*, *100*(12), 1511-1521.

Walsh, J. A., & Jenner, C. E. (2002). *Turnip mosaic virus* and the quest for durable resistance. *Molecular Plant Pathology*, *3*(5), 289-300.

Wang, R. Y., & Pirone, T. P. (1999). Purification and characterization of *turnip mosaic virus* helper component protein. *Phytopathology*, *89*(7), 564-567.

Wylie, S. J., Coutts, B. A., Jones M. G. K., & Jones, R. A. C. (2008). Phylogenetic analysis of Bean yellow mosaic virus isolates from four continents: relationship between the seven groups found and their hosts and origins. *Plant Disease*, *92*(12), 1596-1603.

Yasaka, R., Fukagawa, H., Ikematsu, M., Soda, H., Korkmaz, S., Golnaraghi, A., & Ohshima, K. (2017). The timescale of emergence and spread of *turnip mosaic* Potyvirus. *Scientific Reports*, *7*(1), 4240.