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Research Article

Novel viruses of the genus *Amalgavirus* from crown imperial *Fritillaria imperialis* transcriptome

Sajad Astaraki and Masoud Shams-bakhsh*

Plant Pathology Department, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

Abstract: In an *in silico* investigation, genome sequences of three RNA viruses were identified in the crown imperial *Fritillaria imperialis* L. transcriptome dataset. Sequence comparison and phylogenetic analyses revealed that these three novel viruses belong to the genus *Amalgavirus*. They were tentatively named Crown imperial amalgavirus (CIAV), Iranian amalgavirus (IrAV), and Koohrang amalgavirus (KAV). The RNA-dependent RNA polymerases (RdRps) of CIAV, IrAV, and KAV showed 69.53%, 42.26%, and 37.46% amino acid sequence identities with the homologous RdRp of the most closely related virus, respectively, suggesting that they are novel viruses. Also, the conserved motifs of RdRp were detected in the RdRp of each CIAV, IrAV, and KAV. Genomes of both CIAV and IrAV were complete and contained two overlapping Open Reading Frames (ORFs). A + 1 programmed ribosomal frameshifting (PRF) motif, which matches the conserved amalgaviruses consensus sequence UUU_CGN was found at the ORF1/ORF2 boundary of CIAV and IrAV. The current study reports three novel viruses for the first time from crown imperial, and these findings enrich our understanding of new plant dsRNA virus species, which may also be helpful for the study of amalgaviruses.

Keywords: *Amalgavirus*; Bioinformatics; +1 programmed ribosomal frameshifting Phylogenetic analyses

Introduction

Crown imperial *Fritillaria imperialis* L. is a valuable ornamental and medicinal plant of the lily family (Liliaceae) in the Liliales order and the monocotyledonous subgroup (Ghahreman *et al.*, 1999). Distribution of species of this genus *Fritillaria* in the temperate regions of the Northern Hemisphere is mainly in the Mediterranean regions and the eastern parts of the Balkans, Iran, Iraq, Kurdistan, Russia, Lebanon, China, Japan, Italy, and Spain (Rix, 2001). The genus *Fritillaria* is a perennial herbaceous plant that includes around 140 species. So far, 19 species of this genus have been reported from Iran, and according to available sources, Iran could be one of the leading centers of genetic diversity of the genus (Rechinger, 1963; Mozaffarian, 1992; Moradi *et al.*, 2023). Crown imperial is economically important as an ornamental plant in the horticulture, flower industry, and traditional medicine. Despite the aesthetic value of this species and its medicinal properties, its stem has analgesic properties. In

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* Corresponding author: shamsbakhsh@modares.ac.ir
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traditional medicine, *F. imperialis* is used as a wound accelerator for domestic animals and as a food flavoring agent. Also, in Iranian traditional medicine, the bulbs of this plant are used to treat toothache complications and reduce labor pain (Li *et al.*, 2001).

Fritillaria is exposed to virus diseases like other plants, and many reports show that viruses can affect bulbs of *Fritillaria*; for example, *Fritillary mosaic virus*, a species in the genus *Potyvirus*, causes mosaic symptoms in *F. thunbergii* (Wei *et al.*, 2005). *Fritillary virus Y* (FVY) is mixed with two distinct potyviruses in *Thunberg fritillary* plants (Chen *et al.*, 2006). Analysis of plant viruses by deep sequencing of small RNAs revealed FVY, *Iris mottle virus*, *Thunberg fritillary mosaic virus*, *Hop yellows virus*, and *Apple stem groove virus* from the genus *Fritillaria* plants (Chen *et al.*, 2022). However, despite the wide range of tools and data available for *Fritillaria* and the importance of this species in Iran and the world, no virus that naturally infects *Fritillaria* has been described from Iran.

Nowadays, several high throughput sequencing studies are carried out in all fields of plant science because total RNA extracted from plants contains viral genomes. As a result, many plant RNA-seq datasets contain virus sequences that can be identified by comprehensive bioinformatics analysis. So far, several complete or nearly complete virus genome sequences have been discovered among transcriptome derived from plant samples (Park *et al.*, 2017; Kim *et al.*, 2018; Lee *et al.*, 2019; Zhan *et al.*, 2019; Huo *et al.*, 2022). Given this important fact that Iran supports a great share of biodiversity and floristic endemism for *Fritillaria* spp. (Liliaceae). The genus *Fritillaria* is a precious part of this botanical richness, with 19 species, of which 10 are endemic to the country (Kiani *et al.*, 2017). Although several viral pathogens have been reported on ornamental plants from Iran (Farzadfar *et al.*, 2013; Bayat *et al.*, 2018; Jami *et al.*, 2021; Ghotbi *et al.*, 2022). So far, there have been no reports of viral contamination of crown imperial plants in Iran. Considering the danger of extinction of *F. imperialis* species and the fact that this species is endemic in Iran, any research on the viral diseases of this plant seems necessary to manage and protect genetic resources from these valuable reserves. The current study analyzed the *F. imperialis* transcriptome dataset and identified three novel viruses that are members of the genera *Amalgavirus*.

Materials and Methods

Integrated analysis of *F. imperialis* transcriptome dataset

For the virome study of Iranian *F. imperialis*, an RNA-seq dataset related to Iranian *F. imperialis* was employed (Ahmadi-Teshniz *et al.*, 2022). 19.3 Giga bases of paired-end reads were downloaded from the Sequence Read Archive of the National Center for Biotechnology Information (NCBI) (BioProject accession number PRJNA416712). After converting to FASTQ and FASTA file formats using the SRA Toolkit program (Leinonen *et al.*, 2010), the raw sequencing reads were trimmed and, adaptors were removed, then high-quality reads were collected. *De novo* sequence assembly was performed using the SPAdes Genome Assembler (with the parameter "--rna") (version 3.10.0; http://spades.bioinf. spbau.ru).

Data mining to the identification of contig-related viruses

To identify virus-associated contigs in the assembled transcriptomes, the contigs were blasted at the NCBI nucleotide BLAST database (Blast N and Blast X). ORFs of viral genome sequence extracted from transcriptome data were predicted based on Blast X searches of the NCBI protein database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and ORF finder analysis (https://www.ncbi.nlm.nih.gov/orffinder). Secondary structure prediction of proteins was done using PSIPRED (version 3.3; http:// bioinf.cs.ucl.ac.uk/psipred) (McGuffin *et al.*, 2000). Also, the RNA-seq reads were mapped to virus-associated contigs to examine sequencing depths (expression level) and to identify sequence variations using Geneious Prime 2019.1.3 and CLC Genomics Workbench 20.0 software.

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Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignments were performed based on the RdRp sequence using the MUSCLE tool in the Geneious Prime version 2019.1.3. Phylogenetic analysis was performed by the Neighbor-joining method with 1000 bootstrap replicates using the MEGA software (http://www.megasoftware.net) (Kumar *et al.*, 2018). The matrix of the pairwise identities among viral isolates was determined using the SIAS tool (Sequence Identity and Similarity) (http://imed.med.ucm.es/Tools/sias.html).

Results

Identification of three RNA viruses in the Crown imperial plant using next-generation sequencing data mining

A total of 58,057,752 clean reads were obtained after adapter, quality, and length trimming. Reads *De novo* assembled into contigs and then blasted against virus reference database in the GenBank. The results of Blast N, using the obtained contigs as queries, suggested that three kinds of RNA viruses exist in the crown imperial transcriptome. After Blast N analysis, Blast X search in the NCBI non-redundant protein database confirmed that contig 19456, contig 10820, and contig 5334 were related to plant amalgaviruses. The sequences described here were tentatively named and were deposited in the NCBI database with the accession numbers including Crown imperial amalgavirus (CIAV) (contig 19456, accession number OQ632918), Iranian amalgavirus (IrAV) (contig 10820, accession number OQ632919), and partial contig 5334, accession number OQ632920 was named Koohrang amalgavirus (KAV).

Genome characterization of viruses

Two overlapping ORFs were predicted in the genome sequences of contigs 19456, and 10820 (Fig. 1 a, b). The RNA genome of contig 19456 was 3,389 nucleotides (nt) in length. ORF1 (nt 116–1,300) followed a short 5′-UTR of 115nt and was identified to encode a 394-aa protein (Fig. 1a). Contig 10820 had a 3362 nt in length with 122 primary nucleotides as 5′-UTR, from nt 123 to 1277 was related to ORF1 that encodes a 384 aa protein (Fig. 1b). Structure prediction of protein using PSIPRED revealed that ORF1 of contigs 19456 and 10820 mainly composed of long α-helical regions and coils (Fig. 2 a, b).

The second predicted protein was an ORF1 + 2 fusion product that requires a + 1 PRF for proper translation, a common mechanism in amalgaviruses. The predicted ORF2 of contigs 19456 and 10820 started at the nucleotide positions 961 and 959, respectively, the first base after the + 1 PRF site. To identify the PRF motif, after converting the DNA to RNA sequence by comparing the PRF + 1 motif sequences in detected contigs at current research with other amalgaviruses genomes, results showed that the conserved consensus sequence was UUU_CGN (Fig. 3).

The +1 PRF in the ORF1+2 region produces a 1,059 aa and 1,063 aa fusion protein in contigs 19456 and 10820, respectively. +1 PRF motif (UUU_CGN) was identified at positions 960–965, and 958-963 in contigs 19456 and 10820, respectively (Fig. 1 a, b). The putative fusion protein (RdRp) showed around 69.53% and 42.26% at aa sequence identity in contigs 19456 and 10820, respectively, with the fusion protein of amalgaviruses, including LAV1-YN (Table 1). Also, after Blast X search at NCBI, contig 5334 (partial sequence) revealed that it was close to the RdRp protein of amalgaviruses and had 37.64% amino acid identity with LAV1-YN. Although Contig 5334 did not contain PRF + 1 motif, after protein sequence alignment, along with contigs 10820 and 19456, contained specific conserved RdRp motifs compared to other known amalgaviruses (Fig. 4). The transcript reads were mapped against the reference genome of the identified viruses to determine the expression level of the virus genome. Results showed that CIAV has a higher expression level than IrAV (Fig. 5). Also, there were 15 and 23 single nucleotide variation (SNV) sites in the CIAV and IrAV, respectively. Most of the detected SNV in both viruses occurred in the RdRp region (Fig. 5 a, b and Tables 2, 3).

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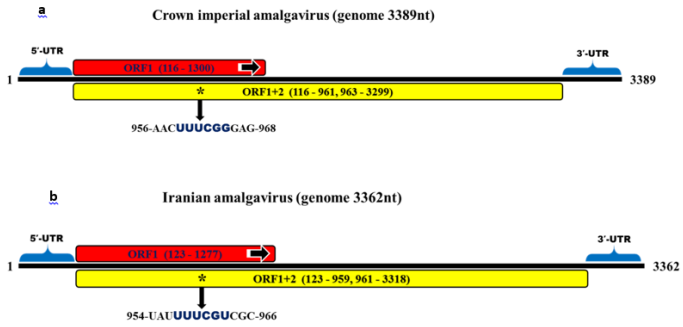

Figure 1 a) Genome organization of Crown imperial amalgavirus (CIAV). CIAV encodes two proteins: a 394 aa protein from ORF1 and a 1059 aa protein from the ORF1 + 2 fusion ORF. The CIAV + 1 PRF region (positions 956–968) is shown below the schematic of the genome structure. The + 1 PRF event at the consensus motif (UUUCGG, marked in boldface) skips the C nucleotide at position 963.
b) Genome organization of Iranian amalgavirus (IrAV). IrAV encodes two proteins: a 384 aa protein from ORF1 and a 1063 aa protein from the ORF1 + 2 fusion ORF. The IrAV + 1 PRF region (positions 954–966) is shown below the schematic of the genome structure. The + 1 PRF event at the consensus motif (UUUCGU, marked in boldface) skips the C nucleotide at position 961.

Figure 2 Secondary structure of ORF1, Crown imperial amalgavirus (a) and Iranian amalgavirus (b). The pink and gray colored sequences are α-helical and coils regions, respectively.

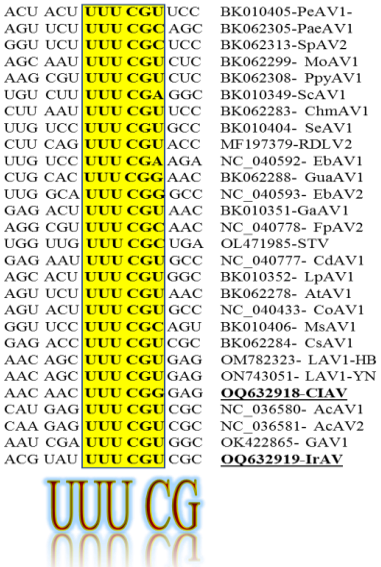

Figure 3 The putative + 1 programmed ribosomal frameshifting (PRF) motif amalgaviruses. Site matching the +1 PRF motif nucleotide UUU_CG are highlighted in bold words.

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Table 1 Identities among the RdRp (ORF1 + 2) sequences of Iranian amalgavirus (IrAV), Crown imperial amalgavirus (CIAV), Koohrang amalgavirus (KAV), and their related amalgaviruses.
1. Accession numbers of RNA segments encoding the RNA-dependent RNA polymerase (RdRp).
2. Amino acid sequence identities in a format of identical residues/aligned length (% identity).

Acronym	Full name	Accession[1]	Amino acid identity[2]		
			IrAV	CIAV	KAV
RDLV2	Rubber dandelion latent virus 2	MF197379	423/1056 (39.72)	441/1096 (40.24)	320/1046 (30.59)
AtAV1	Amaranthus tuberculatus amalgavirus 1	BK062278	436/1068 (40.82)	423/1093 (38.70)	314/1049 (29.93)
EbAV2	Erigeron breviscapus amalgavirus 1	NC_040593	438/1072 (40.86)	428/1059 (39.09)	320/1054 (30.36)
LAV1	Lily amalgavirus 1	ON743051	456/1079 (42.26)	760/1093 (69.53)	399/1060 (37.64)
CsAV1	Cannabis sativa amalgavirus 1	BK062284	441/1071 (41.18)	480/1093 (43.92)	353/1057 (33.40)
SpAV2	Spinacia oleracea amalgavirus 2	BK062313	414/1065 (38.87)	445/1100 (40.45)	322/1054 (30.55)
ChmAV1	Chionochloa macra amalgavirus 1	BK062283	404/1086 (37.20)	410/1105 (37.10)	288/1071 (26.89)
ScAV1	Secale cereale amalgavirus 1	BK010349	426/1081 (39.41)	425/1100 (38.64)	313/1066 (29.36)
EbAV1	Erigeron breviscapus amalgavirus 2	NC_040592	429/1068 (40.17)	442/1094 (40.40)	316/1049 (30.12)
AcAV2	Allium cepa amalgavirus 2	NC_036581	447/1070 (41.78)	527/1102 (47.82)	361/1065 (33.90)
STV	Southern tomato virus	OL471985	420/1075 (39.07)	419/1097 (38.20)	306/1063 (28.79)
GaAV1	Gevuina avellana amalgavirus 1	BK010351	434/1084 (40.04)	448/1105 (40.54)	320/1071 (29.88)
AcAV1	Allium cepa amalgavirus 1	NC_036580	440/1070 (41.12)	546/1094 (49.91)	350/1057 (33.11)
FpAV2	Festuca pratensis amalgavirus 2	NC_040778	398/1065 (37.37)	418/1096 (38.14)	293/1054 (27.80)
GAV1	Gymnadenia amalgavirus 1	OK422865	414/1065 (37.87)	406/1100 (36.91)	290/1058 (27.41)
SeAV1	Salicornia europaea amalgavirus 1	BK010404	357/1070 (33.36)	365/1093 (33.39)	253/1050 (24.10)
MoAV1	Melilotus officinalis amalgavirus 1	BK062299	424/1065 (39.81)	465/1103 (42.16)	338/1058 (31.95)
CdAV1	Cleome droserifolia amalgavirus 1	NC_040777	422/1082 (39)	417/1103 (37.81)	302/1070 (28.22)
MsAV1	Medicago sativa amalgavirus 1	BK010406	410/1076 (38.10)	397/1102 (36.03)	290/1058 (27.41)
PpyAV1	Paris polyphylla amalgavirus 1	BK062308	421/1075 (39.16)	441/1100 (40.09)	310/1061 (29.22)
PaeAV1	Phelipanche aegyptiaca amalgavirus 1	BK062305	407/1065 (38.22)	435/1098 (39.62)	313/1051 (29.78)
STV	Southern tomato virus	OL471995	420/1075 (39.07)	419/1097 (38.20)	306/1063 (28.79)
PeAV1	Phalaenopsis equestris amalgavirus	BK010405	439/1067 (41.14)	455/1104 (41.21)	333/1059 (31.44)
LpAV1	Lolium perenne amalgavirus 1	BK010352	408/1065 (38.31)	431/1093 (39.43)	300/1051 (28.54)

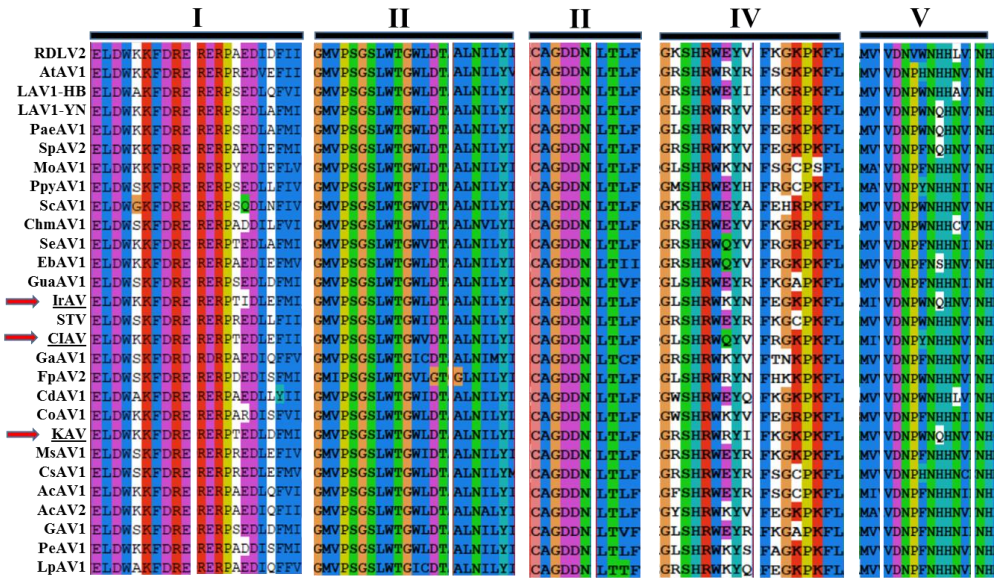

Figure 4 The sequence of RdRps in the family *Amalgaviridae*. Amino acid alignment between conserved RNA-dependent RNA polymerases (RdRp) motifs of Iranian amalgavirus (IrAV) (contig 10820), Crown imperial amalgavirus (CIAV) (contig 19456), Koohrang amalgavirus (KAV) (contig 5334), and the selected amalgaviruses.

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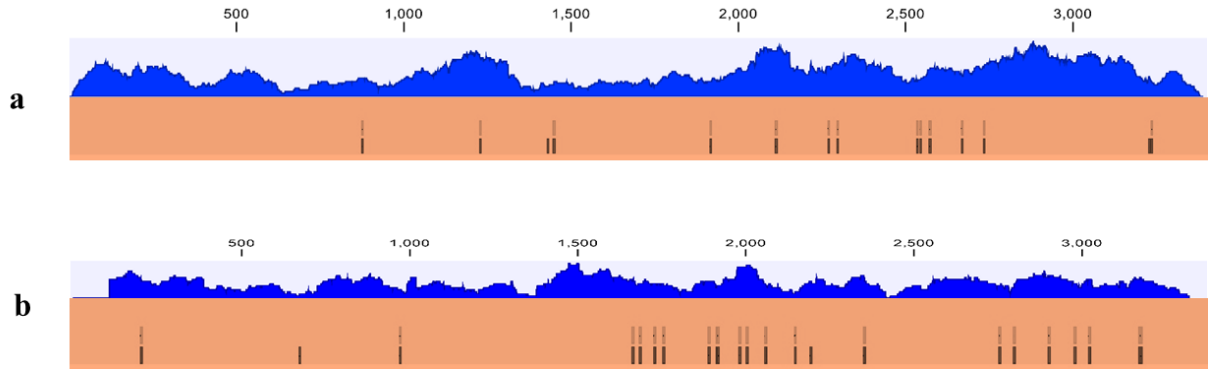

Figure 5 Maps of viral transcripts reads. The total number of reads (534 reads) assigned Crown imperial amalgavirus (a) and Iranian amalgavirus (376 reads) (b) identified in the Fritillaria transcriptome and in the below (bar) identified SNVs along the genome.

Table 2 Identified SNVs along the genome of Crown imperial amalgavirus (contig 19456).

Reference Position	Reference	Allele	Coverage	Frequency
871	G	A	10	20.00
1213	T	C	11	36.36
1408	C	T	4	100
1426	A	G	4	50.00
1880	T	C	7	42.85
2070	C	T	9	33.33
2222	G	A	9	33.33
2249	C	T	7	42.85
2480	T	A	9	33.33
2489	A	G	7	71.42
2516	C	T	7	28.57
2609	C	T	9	33.33
2672	T	C	7	28.57
3152	A	G	7	85.71
3159	G	A	7	71.42

Phylogenetic relationship analysis

To determine the taxonomic position (phylogenetic relationship) of the identified viruses and further comparisons with amalgaviruses, sequences of different amalgaviruses retrieved from NCBI and multiple sequence alignments were performed based on the RdRp sequence. A Neighbor-joining tree based on putative fusion protein (RdRp or ORF1 + 2) was inferred (Fig. 6). Results indicated that CIAV and KAV were in a group with approved and putative amalgaviruses. Importantly, IrAV forms a distinct clade among other amalgaviruses.

Table 3 Identified SNVs along the genome of Iranian amalgavirus (contig 10820).

Reference Position	Reference	Allele	Coverage	Frequency
191	C	T	12	16.66
654	T	A	4	50.00
947	A	G	7	28.57
1626	T	C	13	23.07
1647	A	G	10	20.00
1689	C	T	9	22.22
1716	A	G	9	22.22
1848	G	A	8	25.00
1872	C	T	10	20.00
1875	A	G	8	37.50
1938	G	A	11	45.45
1959	C	T	11	27.27
2013	C	T	17	17.64
2100	C	T	7	28.57
2145	T	C	4	75.00
2301	T	C	8	25.00
2697	T	C	10	70.00
2739	T	C	9	22.22
2841	T	C	11	36.36
2916	C	T	12	41.66
2958	C	T	10	50.00
3105	T	C	8	37.50
3109	G	A	7	42.85

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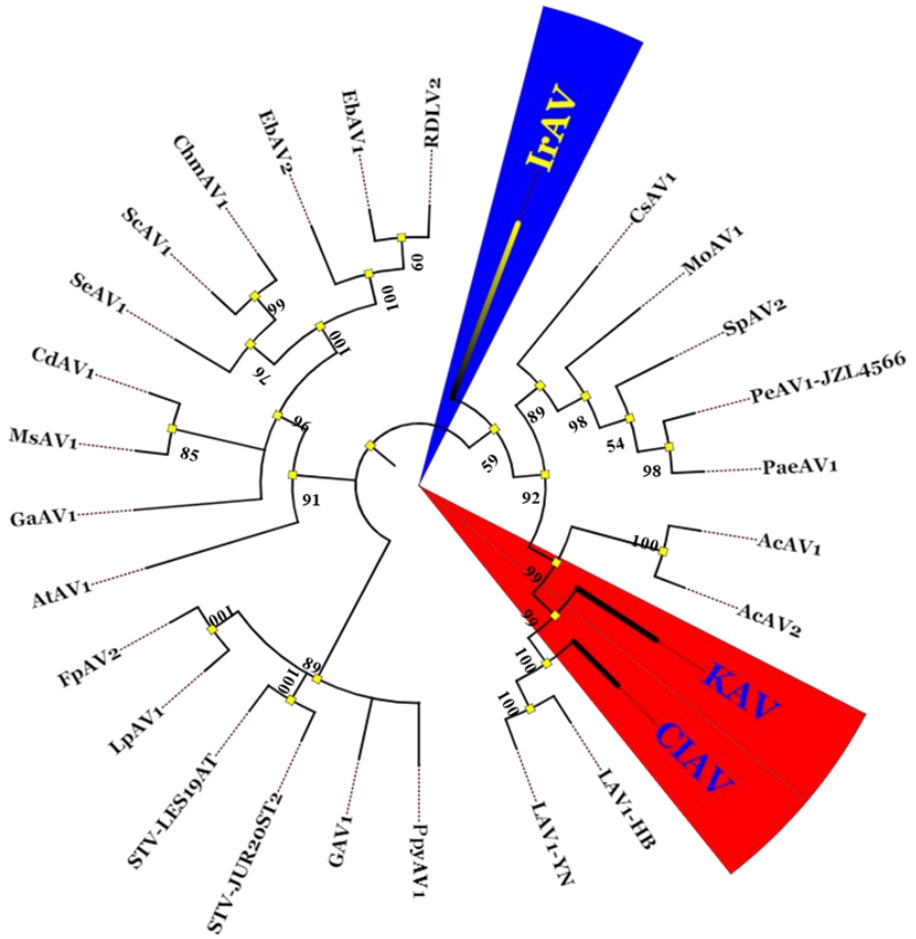

Figure 6 Phylogenetic relationships between amalgaviruses from Crown imperial (marked in red and blue) and other related viruses was reconstructed by the Neighbor-joining method using the RdRp sequence. Bootstrap values were calculated from 1000 replicates.

Discussion

Next-generation sequencing with bioinformatics approaches has many advantages in plant virology and has revolutionized the plant virome study. Also, it has created new opportunities for discovering and diagnosing new plant viruses (Massart *et al.*, 2017). In the present study, three contigs associated with *Amalgavirus* were identified in the transcriptome of the *F. imperialis* plants from Iran. Contigs were related to plant amalgaviruses, including *Allium cepa amalgavirus 1*, *Allium cepa amalgavirus 2*, *Lily amalgavirus 1* (LAV-1), *Festuca pratensis amalgavirus 2* (Nibert *et al.*, 2016; Huo *et al.*, 2022).

Amalgaviruses are double-stranded RNA viruses described as an amalgam between viruses of the *Totiviridae* and *Partitiviridae* families with a single genomic RNA of approximately 3.4 kb in length that has two partially overlapping open reading frames (Martin *et al.*, 2011; Krupovic *et al.*, 2015). First, ORF (ORF1) has been proposed to code for a protein with the function of a nucleocapsid protein (Krupovic *et al.*, 2015). The second ORF (ORF1+2) codes for a fusion protein of ORF1 and ORF2, involved in viral RNA genome replication and is known as RdRp. ORF1+2 is expressed using a +1 programmed ribosomal frameshift (PRF) mechanism (Depierreux *et al.*, 2016; Nibert *et al.*, 2016). After aligning the

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RdRp sequence of contig-related viruses with other amalgaviruses at the amino acid (aa) level, the analysis showed that contigs 10820, 19456, and 5334 harbored conserved RdRp motifs that results were consistent with funding of previously published (Zhan *et al.,* 2019). Notably, contigs 19456 and 10820 contained RdRp motifs (UUU_CGN) similar to that present in other amalgaviruses (Nibert *et al.*, 2016). A consensus sequence "UUU_CGN" of the +1 PRF motif has been found in the Influenza A virus (Firth *et al.,* 2012), and it has also been reported in amalgaviruses (Nibert *et al.,* 2016). It is highlighted that the secondary structural analysis of ORF1 proteins of contigs 19456, and 10820 disclosed that, mainly composed of long α-helical regions and coils, as also has been observed in the other amalgavirus ORF1 proteins (Sabanadzovic *et al.,* 2010; Krupovic *et al.,* 2015).

The RdRp sequence identity threshold at aa level for assigning amalgaviruses to different species is less than 65–70% (Nibert *et al.*, 2016), indicating that contigs 10820, 19456, and 5334 are novel amalgavirus species. Furthermore, protein sequence identity between the contigs 19456, and 10820 RdRps was 41.82%, indicating that these two viruses could be considered different species. The evidence showed that these three contigs were related to amalgaviruses. In the case of SNP discovery, results showed that contigs 19456, and 10820 had different trend variations. According to other studies, if there is variation between viral contigs in transcriptomic data, several variants for each virus seem to exist in the infected sample (Jo *et al.,* 2015; Park *et al.,* 2017).

Phylogenetic analysis showed that CIAV and KAV were in a group with approved and putative amalgaviruses, among which LAV1 was the most closely related virus (Huo *et al.*, 2022). However, in the case of IrAV, it forms a distinct clade among known amalgaviruses due to low sequence homology with other amalgaviruses in the RdRp protein. These results showed that with a higher probability, the viruses identified in the present study could be new species of the *Amalgavirus* genus. Due to the high difference in the identity of IrAV with other known amalgaviruses, it is possible that in the future, by discovering more viruses from this group, they will form a new genus. Further studies are required to biologically characterize IrAV, CIAV, and KAV and gain a better understanding of amalgaviruses.

Conclusion

In conclusion, most of the discovered amalgaviruses have been obtained through bioinformatics analysis of the transcriptomic dataset. Until now, most of the reported amalgaviruses in plants have been asymptomatic. However, the absence of virus symptoms in the plant does not mean that the virus does not affect the internal activities of the plant. It has been shown that the tomato plants infected by the Southern tomato virus, an *Amalgavirus*, had a different expression of micro-RNAs that could modify the several responses of the host to different conditions (Elvira-González *et al.*, 2020). Therefore, these viruses could be considered deceptive or insidious, with hidden killing and destruction power. To the best of our knowledge, this is the first report of *Amalgavirus* from Iran and *F. imperialis* in the world. Sequence similarity and phylogenetic analyses showed that CIAV, IrAV, and KAV are new virus species belonging to the *Amalgavirus* genus. The findings of our study provide useful information for the plant virome of *F. imperialis* and may help investigate the evolution and molecular biology of amalgaviruses.

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Author Contributions

The authors contributed to the study conception and design. The computations and analysis were performed by Sajad Astaraki. The first draft of the manuscript was written by Sajad Astaraki.

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Masoud Shams-bakhsh supervised the project and revised the manuscript. The authors read and approved the final manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Statements and Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ویروس‌های جدید جنس *Amalgavirus* از ترانسکریپتوم لاله واژگون (*Fritillaria imperialis* L.)

سجاد آستركی و مسعود شمس بخش*

گروه بیماری‌شناسی گیاهی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران.
پست الکترونیکي نویسنده مسئول مکاتبه: shamsbakhsh@modares.ac.ir
دریافت: ۱ مرداد ۱٤۰۲؛ پذیرش: ۱۲ مهر ۱٤۰۲

چکیده: در یک مطالعه درون رایانه‌ای، توالی ژنومی از سه ویروس با ژنوم RNA در داده‌های ترانسکریپتوم لاله واژگون *Fritillaria imperialis* شناسایی شد. مقایسه توالی و تجزیه‌وتحلیل فیلوژنتیک نشان داد که این سه ویروس جدید متعلق به جنس *Amalgavirus* هستند. نام‌های Crown imperial amalgavirus (CIAV)، Koohrang amalgavirus (KAV) و Iranian amalgavirus (IrAV) برای ویروس‌های جدید پیشنهاد شد. توالی پروتئینی RNA-dependent RNA polymerases (RdRp)، CIAV، IrAV و KAV به‌ترتیب ۶۹/۵۳ ، ۴۲/۲۶ و ۳۷/۴۶ درصد یکسانی را با RdRp همولوگ نزدیک‌ترین ویروس نشان دادند که حاکی از این است که این ویروس‌ها جدید هستند. همچنین توالی حفاظت شده (موتیف) مربوط به RdRp در این سه ویروس شناسایی شد. توالی ژنومی کامل CIAV و IrAV به‌دست آمد و بررسی‌ها نشان داد که دارای دو چارچوب خوانش به‌صورت هم‌پوشان می‌باشند. توالی حفاظت شده (UUU_CGN) مربوط به عملکرد Ribosomal frameshifting (PRF) در توالی CIAV و IrAV شناسایی شد. مطالعه حاضر سه ویروس جدید را برای اولین بار از لاله واژگون گزارش می‌کند و این یافته‌ها درک ما را از گونه‌های ویروس dsRNA گیاهی جدید، که ممکن است برای مطالعه *Amalgavirus* نیز مفید باشد، غنی می‌سازد.

واژگان کلیدی: *Amalgavirus*، بیوانفورماتیک، programmed ribosomal frameshifting +1، آنالیز فیلوژنتیکی

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