Received: 3 July 2018 | Revised: 5 August 2018 | Accepted: 7 August 2018 | Published on: 10 October 2018

DOI: 10.1111/aab.12456

WILEY Annals of Applied Biology

RESEARCH ARTICLE

Description and genetic variation of a distinct species of *Potyvirus* infecting saffron (*Crocus sativus* L.) plants in major production regions in Iran

Shirin Parizad[1] | Akbar Dizadji[1] | Mina Koohi Habibi[1] | Stephan Winter[2] | Siamak Kalantari[3] | Shahrbanoo Movi[1] | Fernando García-Arenal[4,5] | Maria A. Ayllón[4,5]

[1]Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

[2]German Collection of Microorganisms and Cell Cultures, Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany

[3]Department of Horticultural Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

[4]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)—Instituto Nacional de Investigación Agraria y Alimentaria (INIA), Madrid, Spain

[5]Departamento Biotecnología-Biología Vegetal, E.T.S.I. Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid (UPM), Madrid, Spain

Correspondence
Akbar Dizadji, Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj 31587-77871, Iran.
Email: adizaji@ut.ac.ir
María A. Ayllón, Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)—Instituto Nacional de Investigación Agraria y Alimentaria (INIA), Campus de Montegancedo, Pozuelo de Alarcón 28223, Madrid, Spain.
Email: mariaangeles.ayllon@upm.es

Funding information
University of Tehran, Grant/Award Number: 73148924/6/12; Iran National Science Foundation, Grant/Award Number: 93021253

Saffron (*Crocus sativus* L.), a highly valuable crop famous for the unique taste, aroma, colour and medicinal properties of the stigmas, is widely cultivated in Iran. During 2011–2016, a total of 965 samples were randomly collected from six important saffron growing provinces of Iran to test for the presence of viruses. Assays revealed the presence of a distinct species of the genus *Potyvirus*, which has been tentatively named saffron latent virus (SaLV). Phylogenetic analysis of the SaLV complete genome sequence, 9,693 nucleotides (nt) excluding poly(A) tail, revealed that this virus grouped into Bean common mosaic virus subgroup in the genus *Potyvirus* with the closest relative being *Bean common mosaic necrosis virus*. A number of distinct plant species were evaluated for the host range of SaLV; *Chenopodium amaranticolor* and *Chenopodium quinoa* were found to be systemic hosts of SaLV. There were no nucleotide sites that indicated any recombination events, and the analysis of the coat protein (CP) sequence of 50 samples, collected from different geographical origins, did not show spatially structured diversity. Overall, our data shows strong negative selection pressure in CP, estimated by nucleotide sequences. The relevance of SaLV for saffron production in Iran remains to be established.

KEYWORDS

genetic diversity, new *Potyvirus*, saffron

1 | INTRODUCTION

Saffron (*Crocus sativus* L.) belongs to the *Iridaceae* family and is a triploid and sterile crop that propagates vegetatively by corm (Fernández, 2007). It has a great therapeutic potential in Alzheimer's disease because of its antiamyloidogenic properties (Papandreou et al., 2006), and it has also anticarcinogenic (Abdullaev & Espinosa-Aguirre, 2004) and antidepressant (Wang et al., 2010) properties. Its dried stigmas are a valuable spice which is extensively used for colouring and flavouring of many foods, and is currently the major use of the crop.

Saffron is intensely cultivated in different countries and the world's total saffron production is assessed to around 300 t per year (Gohari, Saeidnia, & Kourepaz Mahmoodabadi, 2013). However, saffron production is often hampered by diseases. Reports indicate that diseases in

 © 2018 Association of Applied Biologists

saffron were caused by fungi, bacteria and viruses, normally resulting in a decrease of growth and flower production (Kafi, Koocheki, Rashed, & Nassiri, 2006). The viruses reported are *Arabis mosaic virus* (ArMV, *Nepovirus*), *Cucumber mosaic virus* (CMV, *Cucumovirus*), *Narcissus mosaic virus* (NMV, *Potexvirus*), *Tobacco necrosis virus* (TNV, *Necrovirus*), *Tobacco rattle virus* (TRV, *Tobravirus*) and *Tomato spotted wilt virus* (TSWV, *Tospovirus*) (Bellardi & Pisi, 1987; Miglino, Jodlowska, & van Schadewijk, 2005; Samuitienė, Navalinskienė, & Jackevičienė, 2008). In addition, several species from the genus *Potyvirus* (family *Potyviridae*, with a linear single-stranded (+) RNA genome (Revers & García, 2015)) were also reported, in particular, *Bean yellow mosaic virus* (BYMV), *Turnip mosaic virus* (TuMV), *Iris severe mosaic virus* (ISMV) and *Iris mild mosaic virus* (IMMV) (Chen & Chen, 2000; Miglino et al., 2005; Navalinskienė & Samuitienė, 2001). Recently, RNA-seq transcriptome analysis of apparently healthy saffron plants from Jammu and Kashmir (India) revealed transcripts with 79% identity to *Soybean mosaic virus* (SMV) polyprotein (Chakraborty, 2016). Ultrastructural variations, the typical cytopathological effects described for potyviruses, were also found in *C. sativus* and *Crocus cartwrightianus* (wild and ornamental species, a putative ancestor of saffron) leaves collected from asymptomatic apparently healthy plants (Grilli Caiola & Faoro, 2011). Both the studies of Chakraborty (2016) and Grilli Caiola and Faoro (2011) suggest the presence of asymptomatic infections of potyviruses in saffron, and indicate the relevance of potyvirus infection in this crop may be severely underrated (Chakraborty, 2016).

Iran is the major saffron producer in the world, with 76% of the world's total production (Gohari et al., 2013). The main saffron production regions in Iran are located in Razavi Khorasan and South Khorasan and recently the production has expanded to Fars, Isfahan, Tehran and East Azarbaijan provinces (Amirghasemi, 2001). Despite the importance of this crop, till now there are no reports of diseases of saffron in Iran. Our preliminary study on the occurrence of viruses on apparently healthy saffron plants of Iran revealed the presence of a *Potyvirus* (Parizad et al., 2017). Subsequently, the high prevalence of this potyvirus in saffron fields of Iran was evaluated. The complete nucleotide sequence of this virus genome was obtained, which according with the species demarcation criteria of genus *Potyvirus* (Adams, Antoniw, & Fauquet, 2005) indicates it is a new species that has been tentatively named saffron latent virus (SaLV) (Parizad et al., 2017). Here we report on how SaLV was identified, its biological and molecular characterisation, its phylogenetic relationships within the genus *Potyvirus*, and its genetic diversity and structure of populations.

2 | MATERIALS AND METHODS

This article does not contain any studies with human participants or animals performed by any of the authors.

2.1 | Saffron plant materials

During the growing seasons of 2011 until 2016, several surveys were carried out in the major saffron cultivation areas of Iran, including Tehran, Isfahan, Fars, East Azarbaijan, Razavi Khorasan and South Khorasan provinces and leaf tissue samples from a total of 965 asymptomatic and apparently healthy plants were randomly collected.

2.2 | Serological assays

Collected samples were tested for the presence of viruses by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA; Clark & Adams, 1977). ELISA tests were selected to detect viruses previously reported from *Crocus* spp. including CMV, TRV, TSWV and TNV. Since specific antibody against the recently identified potyvirus, SaLV, has not yet been produced, an antigen coated-plate ELISA (ACP-ELISA) using a *Potyvirus* genus-specific antibody (Richter, Rabenstein, Proll, & Vetten, 1995) was used for general detection of this virus and other potyviruses. Positive results were subsequently verified with virus-specific DAS-ELISA, including potyviruses previously reported infecting saffron (BYMV, SMV and TuMV) as well as unreported viruses *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Cowpea aphid-borne mosaic virus*, *Lettuce mosaic virus*, *Potato virus* Y, *Tobacco etch virus* (TEV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* and *Alfalfa mosaic virus* (AMV) from *Crocus* spp. ELISA tests were performed using DSMZ antibodies (Germany).

2.3 | Host range studies

Two SaLV-infected samples from each province (Ir-Kh1 and Ir-Kh2 from Razavi Khorasan province, Ir-Kh3 and Ir-Kh4 from South Khorasan province, Ir-T1 and Ir-T2 from Tehran province, Ir-F1 and Ir-F2 from Fars province and Ir-I1 and Ir-I2 from Isfahan province) were selected for host range studies. All the plants were mechanically inoculated with extracts of SaLV-infected samples using 0.5 g of fresh leaves ground in 0.1 M phosphate buffer of pH 7 (1:10, wt/vol) containing 0.2% sodium diethyldithiocarbamate and the controls were inoculated with only buffer. The experiment was repeated three times; each time 10 replicates of each plant species were assayed along with three mock-inoculated plants of the respective species. Bio-assay hosts were mostly selected according to the host plants of potyviruses closely related to SaLV which included *Gomphrena globosa*, *Chenopodium amaranticolor*, *Chenopodium quinoa*, *Cucumis sativus*, *Phaseolus vulgaris* cv. Red Kidney, *P. vulgaris* cv. Bountiful, *Pisum sativum*, *Vigna unguiculata*, *Nicotiana tabacum* cv. White Burley, *N. tabacum* cv. Samsun, *Nicotiana glutinosa*, *Nicotiana clevelandii*, *Nicotiana debneyi*, *Nicotiana benthamiana*, *Nicotiana rustica*, *Cicer arietinum* and *Vicia faba.*

2.4 | Immunosorbent electron microscopy, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis

SaLV particles (from Ir-Kh1 sample) were decorated by the *Potyvirus* genus-specific antibody, stained with 1.6% uranyl acetate in distilled water (Milne & Luisoni, 1977) and observed with a transmission electron microscope (Zeiss E900, Oberkochen, Germany).

To estimate molecular mass (Mr) of the coat protein (CP) of SaLV, total soluble proteins were extracted from healthy (H1) and infected saffron (Ir-kh1) plants and fractionated on discontinuous 15% SDS-polyacrylamide gels (Laemmli, 1970). After electro-blotting of protein bands, the nitrocellulose membrane was blocked and incubated first with *Potyvirus* genus-specific antibody and then with alkaline phosphatase conjugated rabbit antimouse antibody. Blots were developed

by using the Sigma nitro blue tetrazolium/ 5-bromo-4-chloro-3-indolyl-phosphate (NBT/ BCIP) (Towbin, Staehelin, & Gordon, 1979). CP Mr was estimated based on $\log_{10}$ of the Mr of standard marker proteins (PageRuler Plus Prestained Protein Ladder; 10–250 kDa, 26,619, Thermo Scientific, Lithuania) and their relative movement on the gel (Fried & Crothers, 1981).

2.5 | RNA extraction, cDNA synthesis, reverse transcription polymerase chain reaction (RT-PCR) and sequencing

Total RNA was extracted from 100 mg of fresh leaf tissues of the SaLV-infected sample (Ir-kh1) using a RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. To obtain full-length sequence of the viral genome, different fragments were amplified using primer pairs covering the complete viral genome and were cloned. In the first step, three *Potyvirus* genus-specific degenerate primer pairs were used to amplify regions of the genome comprising the 3′ terminus, the CI and the HC-Pro regions (Table 1 and Figure 1). Subsequently, using newly designed virus-specific primer pairs, based on conserved or specific sequences, sequence gaps were closed (Table 1 and Figure 1). The 5′ and 3′ terminal sequences were determined by a rapid amplification of cDNA ends (RACE) protocol using a FirstChoice RLM-RACE Kit (Invitrogen, Carlsbad, CA). Reverse transcriptions using SuperScript III reverse transcriptase (Invitrogen), followed by PCR using Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific/Finnzymes; Vantaa, Finland) were done following standard conditions. All PCR fragments obtained were purified, cloned into CloneJET PCR vector (CloneJET PCR Cloning Kit; Thermo Scientific, Hudson, NY) and recombinant plasmids were subsequently

TABLE 1 Information on primers used for amplification of different parts of the SaLV genome

Primer	Sequence (5′→3′)	Conserved motif or position	Use
PV1/SP6[a]	GATTTAGGTGACACTATAG AATTTTTTTTTTTTTTTTTVN	3′ extreme end	Potyviridae oligo dT primer
PV21/T7[a]	TAATACGACTCACTATAGGGNA AYAAYAGYGGNCARCC	GNNSGQP	Potyviridae NIb gene-specific primer
CIFor[b]	GGIVVIGTIGGIWSIGGIAARTCIAC	GxVGSGKST	Potyvirus CI gene-specific primer
CIRev[b]	ACICCRTTYTCDATDATRTTIGTIGC	ATNIIENGV	Potyvirus CI gene-specific primer
HPFor[b]	TGYGAYAAYCARYTIGAYIIIAAYG	CDNQLDxN	Potyvirus HC-Pro gene-specific primer
HPRev[b]	GAICCRWAIGARTCIAIIACRTG	HVxDSY/FGS	Potyvirus HC-Pro gene-specific primer
NIb Rev	CGGTTCTGTGCATCAACTCTTTACT	8334–8358	
CI For	GGTGACTAAAGTGGATGGTCGCAC	4526–4551	
NIb2 Rev	CAACTTCATTCAACACCACTGG	7299–7320	
CI2 For	TCATATGAAAGAATGGGTGTGC	5127–5149	
CI Rev	CCTAACCTCTGAATTCTCTCACC	4743–4765	
HC For	GGCCAGCTATGATGGATGTAGC	2263–2284	
CI2 Rev	CGAAGGTGCTATAGTGATAT	3240–3259	
HC2 For	GGTGCAGTTTTTGAAATGAGCTG	2775–2797	
5 end BCMNV	GTACRCACCCGATCACTAATGGC	162–184	
Saf5′RACE inner	ATTTCYTCTTCCTCTGACSACG	1052–1073	
Saf5′RACE outer	GCCACCTCTCCACACTTCAC	1233–1252	
6K1 For	GGCAATGGTCATTGATTCTGAACG	3605–3628	
CI3 Rev	CATTCTCTTCCTGGTGGTGTAGC	4314–4336	
P1 For	TACACATCTTTCACATTTCGTG	1034–1055	
HC Rev	TCTTATCAAGTTGGTTATCACA	1716–1737	
HC3 For	GTGAAGTGTGGAGAGGTGGCA	1233–1253	
NIb For	AGTAAAGAGTTGATGCACAGAACCG	8334–8358	
3′ UTR rev	CGAAAGGTGGTAGAACCACTC	9526–9546	
Saf 3′RACE outer	ATGAATGGHTTYATGGTNTGGTG	8967–8989	
Saf 3′RACE inner	GARRATACTGARAGGCAYACTGC	9363–9385	
CP Rev	CGAAAGGTGGTAGAACCACTC	9526–9546	Diversity
CP For	ACCATACATTGCAGAGACAGC	8483–8503	Diversity
pJET1.2 forward sequencing primer	CGACTCACTATAGGGAGAGCGGC		Colony PCR
pJET1.2 reverse sequencing primer	AAGAACATCGATTTTCCATGGCAG		Colony PCR

Note. I: inosine, Y: C/T, R: G/A, W: A/T, V: A/C/G, S: C/G, D: A/G/T, N: A/T/C/G.
[a] Mackenzie et al. (1998).
[b] Ha et al. (2008).

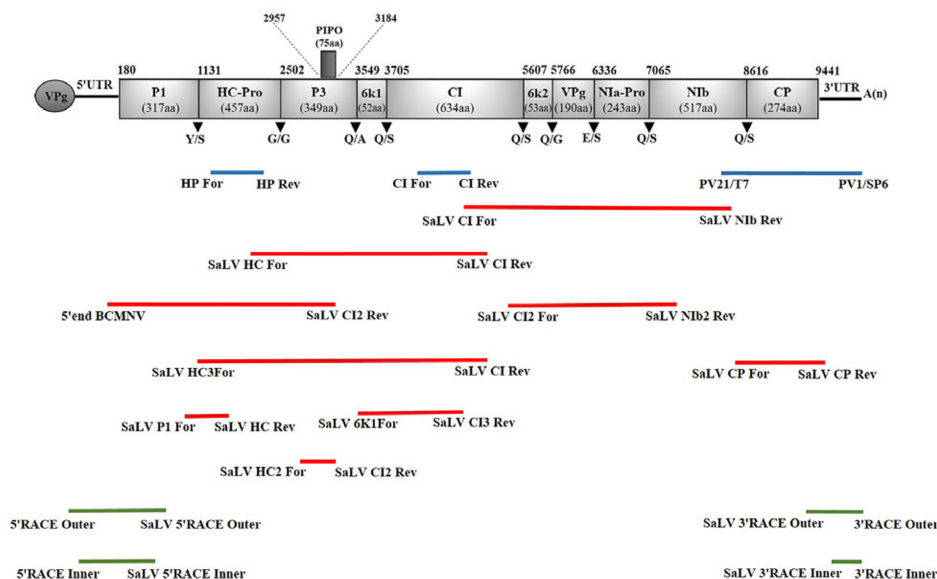

FIGURE 1 Schematic presentation of the genome structure of saffron latent virus. The different coding regions, the size of putative proteins (in aa), the conserved cleavage sites (triangles) and the position of primers used for this study are shown

sequenced using a commercial service (Macrogen, Amsterdam-Zuidoost, the Netherlands).

2.6 | Sequence analyses

The nucleotide and amino acid sequences of amplified fragments were analysed by Blastn (http://www.ncbi.nlm.nih.gov/BLAST). The complete genome of the SaLV isolate Ir-kh1 was assembled using SeqMan (DNASTAR, Madison, WI) and open reading frames (ORFs) were identified using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Alignment of the complete genome and the separate genes was conducted using VectorNTI Suite7 program (Lu & Moriyama, 2004). Full-length genome sequence of SaLV and 36 *Potyvirus* species (available at GenBank) were analysed and compared using MAFFT (Multiple Alignment using Fast Fourier Transform, version 7; Katoh, K., National Institute of Advanced Industrial Science and Technology (AIST), Japan) using default settings (Katoh & Standley, 2013). The phylogenetic analyses were performed on complete genome nucleotide and amino acid sequences using the maximum likelihood (ML) method, implemented in PhyML 3.0 (Guindon et al., 2010), applying the bootstrap phylogenic test with 1,000 replicates and *Triticum mosaic virus* as outgroup. Trees were visualised using FigTree software v.1.3.1 (Rambaut, A., the Institute of Evolutionary Biology, University of Edinburgh) using default settings (Rambaut, 2010).

For detection of possible recombinants, analyses were performed using RDP4 Beta 4.69 program (Recombination Detection Program Beta 4.69; Martin, D. P., University of Cape Town, Cape Town, South Africa) (Martin, Murrell, Golden, Khoosal, & Muhire, 2015) implementing seven different recombination detection algorithms.

2.7 | Evaluation of genetic diversity

The genetic diversity of the Iranian SaLV population was analysed on the basis of the complete CP gene nucleotide sequences of 50 SaLV isolates, obtained from five different regions of Iran (Tehran, Isfahan, Fars, Razavi Khorasan and South Khorasan provinces) considered as subpopulations. The primer set to amplify the CP region of SaLV was newly designed according to the sequence obtained from Ir-Kh1 sample (CP For/Rev, Table 1). The CP nucleotide and translated amino acid sequence of 50 SaLV isolates were multiply aligned by using muscle implemented in MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) with the default parameters (Edgar, 2004; Tamura et al., 2013). Subsequently, the genetic diversity between any pair of sequences from the same or different subpopulations was done by using Tamura–Nei model (Tamura & Nei, 1993) as implemented in MEGA6. This model was selected as the best-fitted nucleotide substitution model in MEGA6. Moreover, the rate variation among sites was modelled with a gamma distribution (shape parameter = 0.06) as a default and standard errors of each measure were estimated based on bootstrap procedure (1,000 replicates). The average number of nonsynonymous (d_N) and synonymous (d_S) nucleotide substitutions per site, and the d_N/d_S ratio as an estimate of the selection pressure on the CP were computed for the whole population and for each subpopulation using the DnaSP v.5 software (Librado, P., Universitat de Barcelona, Barcelona, Spain) (Librado & Rozas, 2009) with a mean value of 0.05. The fixation index statistic F_{ST} (Weir & Cockerham, 1984) was calculated for each pair of subpopulations using DnaSP v.5.

3 | RESULTS AND DISCUSSION

3.1 | Screening of saffron plants for the presence of viruses

Leaf tissue samples were collected from a total of randomly chosen 965 asymptomatic saffron plants for further analyses. In order to detect the viruses infecting saffron in Iran, ELISA tests were performed on 965 saffron samples, using AMV, CMV, TRV, TSWV, TNV and *Potyvirus*-specific antibodies. ELISA tests showed 703 samples to be potyvirus-positive (Table 2), while no sample was positive for AMV,

TABLE 2 Detection of saffron infecting potyviruses in saffron plants from different locations of Iran from 2011 to 2016

Location	Total of infected/collected plants (infection rate, %)
East Azarbaijan	22/30 (73)
Fars	44/55 (80)
Isfahan	105/175 (60)
Razavi Khorasan	393/520 (75)
South Khorasan	94/130 (72)
Tehran	45/55 (81)
Total	703/965 (73)

CMV, TRV, TSWV and TNV. Potyvirus-positive samples were tested by DAS-ELISA using specific antibodies against different *Potyvirus* species (see Section 2) to determine those infecting the samples. Results indicated that all potyvirus-infected samples reacted with the antibody against BCMV. This result demonstrates the presence of a *Potyvirus* serologically related to BCMV (Parizad, Dizadji, Koohi Habibi, Winter, et al., 2017), and the absence of the other 10 *Potyvirus* species in potyvirus-infected samples. SaLV belongs to the BCMV cluster within the potyviruses (see below) which may explain cross-reactivity with BCMV antisera (Mink & Silbernagel, 1992; Shukla, Lauricella, & Ward, 1992; Verma & Gupta, 2010). Therefore, surveys conducted during five consecutive years in the main regions of saffron cultivation revealed that 73% of saffron plants (703 out of 965 samples) tested by ELISA were infected with SaLV and the incidence of infection among provinces varied from 60% (Isfahan) to 81% (Tehran) (Table 2). Thus, there is a high prevalence of this potyvirus in saffron plants of Iran.

3.2 | Host range studies

Seventeen indicator plant species were mechanically inoculated with SaLV-infected samples. All inoculated plants of each assayed species remained asymptomatic even at 40 days post-inoculation (dpi), except for *C. amaranticolor* and *C. quinoa*, which developed chlorotic local lesions on leaves followed by systemic leaf distortion symptoms at 5–7 dpi; no symptoms were observed in mock-inoculated test plants (Figure 2). All virus- and mock-inoculated plants were analysed for SaLV infection by using RT-PCR. The results confirmed that only *C. amaranticolor* and *C. quinoa* were hosts of SaLV among all the analysed plant species.

3.3 | Immunosorbent electron microscopy and western blot analysis

Electron microscope observations revealed the presence of flexuous filamentous viral particles similar to the ones corresponding to genus *Potyvirus* in plants infected by SaLV (data not shown) (Chen, Chen, & Adams, 2001).

The Mr of the CP of SaLV was estimated to be 35 kDa on the basis of its electrophoretic mobility in denaturing polyacrylamide gels (Figure 3). This value is in the range of that of the CP (30–47 kDa) of

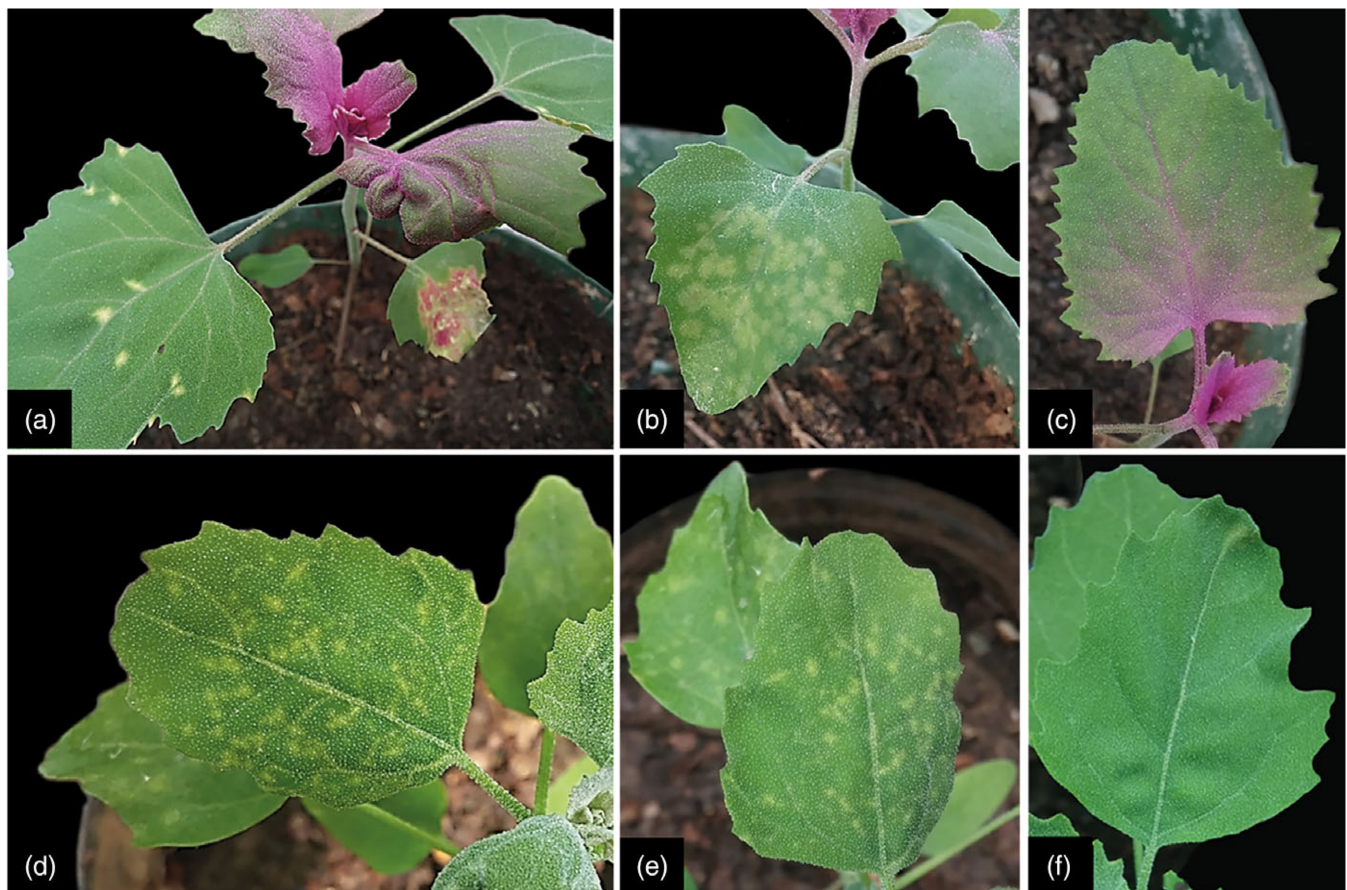

FIGURE 2 Symptoms induced by saffron latent virus in mechanically inoculated test plants. Chlorotic local lesions and systemic leaf distortion on *C. amaranticolor* (a and b) and *C. quinoa* (d and e). Mock-inoculated plants (c and f) did not show any symptoms

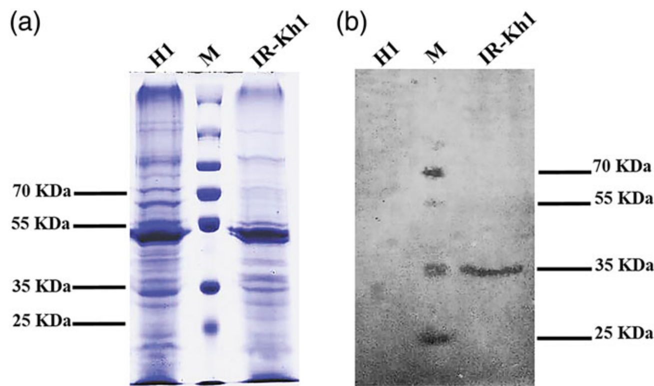

FIGURE 3 SDS-polyacrylamide gel (SDS-PAGE) (a) and western blotting (b) of total protein extracts from saffron latent virus-infected (IR-Kh1) and non-infected saffron (H1) plants, using *Potyvirus* genus-specific antibody. Difference in protein migration between SDS-PAGE and blot membrane is because of different magnification of pictures. M: PageRuler Plus Prestained Protein Ladder (10–250 kDa, 26,619, Thermo Scientific)

other *Potyvirus* species (Adams et al., 2012). As a negative control for western blot using the *Potyvirus* genus-specific antibody, total protein extracted from a healthy saffron plant was used (Figure 3).

The results indicated that the viral particles and the CP of the SaLV were similar to those described previously for other potyviruses, in agreement with the ELISA reactivity with *Potyvirus*-specific sera.

3.4 | Genome structure

The complete nucleotide sequence of the SaLV genome was determined and has been deposited in the GenBank database under accession number KY562565. The genome consists of 9,693 nucleotides (nt) excluding the poly(A) tail, and displayed a typical potyvirus organisation and comprised an ORF (nt 180–9,440) coding for a large polyprotein that may be cleaved into 10 mature functional proteins including P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP as in other potyviruses (Revers & García, 2015). Additionally, *pipo*, the second small ORF, expressing the trans-frame protein P3N-PIPO (Chung, Miller, Atkins, & Firth, 2008; Olspert, Chung, Atkins, Carr, & Firth, 2015; Rodamilans et al., 2015) was identified at positions 2,957–3,184 overlapping with the P3 coding region (Figure 1).

The 5′ untranslated region (UTR) of 179 nt, contained 60.7% A + T and had several A residues in the extreme of 5′ end. Two highly conserved blocks as reported for many potyviruses, potyboxes ‘a’ (UCAACACAACAU) and ‘b’ (CAAGCA) (Turpen, 1989) which probably play an important role in transcription initiation (Turpen, 1989; Yukawa, Sugita, Choisne, Small, & Sugiura, 2000) were also identified. In comparison to the study of Turpen (1989), the genome of SaLV had the following nucleotide sequences: potybox ‘a’ ^{22}ACAACAAAC-GAA^{33} and potybox ‘b’ ^{62}CAAGCG^{68} (the underlined nucleotide shows the difference from the reported ones). The 3′ UTR was 253 nt and contained a consensus yeast poly(A) signal sequence ^{9602}UAUGU^{9606} as in the case of many other potyviruses (Zaret & Sherman, 1982). In the 3′ UTR an AU-rich region and a TATA box-like motif, ^{9491}UAUAUAA^{9497}, were also found (Liang, Song, Tian, Li, & Fan, 2006).

The putative polyprotein had 3,086 amino acids (Mr of 353.2 kDa). The AUG start codon was located in an optimum context (CAA**AUG**GC) for translation initiation as in many potyviruses (Kong, Sohn, & Steinbiss, 1997). The analysis of the amino acid sequences revealed cleavage sites for 10 putative mature proteins (Figure 1) with many well-characterised functional motifs highly conserved in potyviruses (Adams, Antoniw, & Beaudoin, 2005; Bos, 1996; Riechmann, Laín, & García, 1992). The conserved motifs ^{269}GDSG^{272} and ^{291}FCVRG^{295} were found in the P1 region, presumably representing the serine catalytic active site responsible for the autoproteolytic activity of this protein (Adams, Antoniw, & Beaudoin, 2005). A cluster of cysteine residues ^{345}C-X_8-C-X_{13}-V-X_4-C-X_2-C^{376} was identified in the HC-Pro, which is similar to putative zinc finger metal-binding motif (C-X_8-C-X_{13}-C-X_4-C-X_2-C) (Valli, Gallo, Rodamilans, López-Moya, & García, 2018). The cysteine (C) to valine (V) replacement at the third cysteine was also found in BCMNV (Maina, Edwards, de Almeida, Ximenes, & Jones, 2016). The conserved sequence motif ^{370}KLSC^{373} and the ^{626}PTK^{628} motif are important factors for aphid transmission (Atreya & Pirone, 1993). The ^{660}C-$(X)_{72}$-H^{733} motif, responsible for the protease activity of HC-Pro, was identified, and together with ^{497}FRNK^{500} is essential in symptom expression (Gal-On, 2000) and suppression of RNA silencing (Gal-On, 2007). In the central region of HC-Pro, a ^{608}CCC^{610} motif was located, this motif was involved in the long-distance movement of TEV (Revers, Le Gall, Candresse, & Maule, 1999). NTP-binding motif ^{1260}GAVGSGKST^{1268} and the motifs ^{1349}D-E-X-H^{1352}, ^{1280}VLLLEP^{1285}, ^{1286}TRPL^{1289}, ^{1376}KVSAT^{1380}, ^{1427}LVYV^{1430}, ^{1478}VATNIIENGVTI^{1489}, ^{1522}GER-IQRLGRVGR^{1533} had been previously described as typical motifs of helicases (Adams, Antoniw, & Beaudoin, 2005; Riechmann et al., 1992) and were found in the CI protein. A conserved tyrosine residue (Y^{1924}), in the context ^{1925}MYGV^{1928}, which has been described as involved in linking VPg to potyviral RNA (Murphy, Klein, Hunt, & Shaw, 1996), was also identified in NIa-VPg. In NIa-Pro, ^{2098}H-$(X)_{34}$-D-$(X)_{69}$-C-$(X)_{15}$-H^{2219} motif was detected, and this conserved motif has been related to proteinase activity (Dougherty, Parks, Cary, Bazan, & Fletterick, 1989). In the NIb protein, putative RNA-dependent RNA polymerase motifs ^{2547}FDSS^{2550} and ^{2605}SG-X_7-NT-X_{30}-GDD^{2648}, with the conserved GDD necessary for RNA polymerase activity and NTP binding (Hong & Hunt, 1996), were also identified. In the N-terminal region of CP ORF, was found a ^{2824}DAG^{2826} motif, which is essential for the interaction with HC-Pro in insect transmission (Revers et al., 1999).

The ORF encoding the putative PIPO protein, with 228 nt, started at the conserved GA_6 motif at the 5′ end of *pipo* and ended with an UGA termination codon at positions 3,182–3,184 within the P3 coding region (Figure 1). This is consistent with the expression of PIPO as a P3N-PIPO fusion product, which occurs because of RNA polymerase slippage at a highly conserved $G_{1\text{-}2}A_{6\text{-}7}$ motif present at the 5′ end of *pipo* in potyviruses (Chung et al., 2008; Olspert et al., 2015; Rodamilans et al., 2015).

3.5 | Evolutionary relationships of SaLV

The sequence of the SaLV genome was compared with that of closely related potyviruses, showing different levels of identity at different

TABLE 3 Identity (%) in nucleotide (left) and the deduced amino acid (right) sequences between SaLV and other potyviruses in the BCMV subgroup

Virus	Entire	5′ UTR	P1	HC-Pro	P3	6K1	CI	6K2	VPg	NIa-Pro	NIb	CP	3′ UTR	PIPO
BCMNV	68/**71**	**52**	47/36	71/77	**61**/46	66/**77**	**73**/**81**	74/70	**72**/**78**	73/80	73/79	73/**76**	65	73/66
BCMV	67/68	49	50/36	68/69	58/45	68/69	72/80	72/66	**72**/75	**74**/81	72/75	71/69	62	64/45
EAPV	**69**/70	43	**53**/40	70/74	60/48	69/69	72/80	**75**/**72**	69/72	73/**84**	**74**/**80**	72/69	**72**	71/60
SMV	68/**71**	45	50/40	**72**/78	**61**/49	**75**/**77**	71/80	74/70	71/76	72/79	73/79	73/76	63	74/64
WVMV	68/70	40	49/36	**72**/78	**61**/**50**	70/71	**73**/79	72/70	69/74	72/81	**74**/**80**	**74**/70	70	72/59
WMV	67/70	50	50/**41**	**72**/**80**	60/47	72/**77**	70/78	74/**72**	69/76	**74**/79	72/**80**	72/**76**	63	**75**/**67**

Note. For each region, the highest value is indicated in bold. BCMNV: *Bean common mosaic necrosis virus*; BCMV: *Bean common mosaic virus*; EAPV: *East Asian passiflora virus*; SaLV, Saffron latent virus; SMV: *Soybean mosaic virus*; WMV: *Watermelon mosaic virus*; WVMV: *Wisteria vein mosaic virus*.

genomic regions (Table 3). Interestingly, despite that amino acid sequence identity was highest (76%) at the CP with BCMNV, SMV and WMV, no cross-reactivity was observed in ELISA tests between SaLV and polyclonal antibodies against these viruses. Overall, for the complete genome nucleotide sequence, SaLV shared the highest identity (69%) with *East Asian passiflora virus* and for the polyprotein amino acid sequence (71%) with both BCMNV and SMV (Table 3).

Recombination has been well documented as playing a role in the diversification of the *Potyvirus* genus (Gibbs & Ohshima, 2010). Analyses were performed to determine if recombination had played a role in the origin of SaLV, by comparing its genome sequence with that of 93 other potyviruses, available in GenBank. No procedure implemented in RDP provided evidence of recombination, which strongly suggests that SaLV has diversified from other potyviruses by mutation accumulation.

Since no evidence of recombination between SaLV and other potyviruses was found, phylogenetic relationships among them were reconstructed by the ML method. Previous to this analysis, the

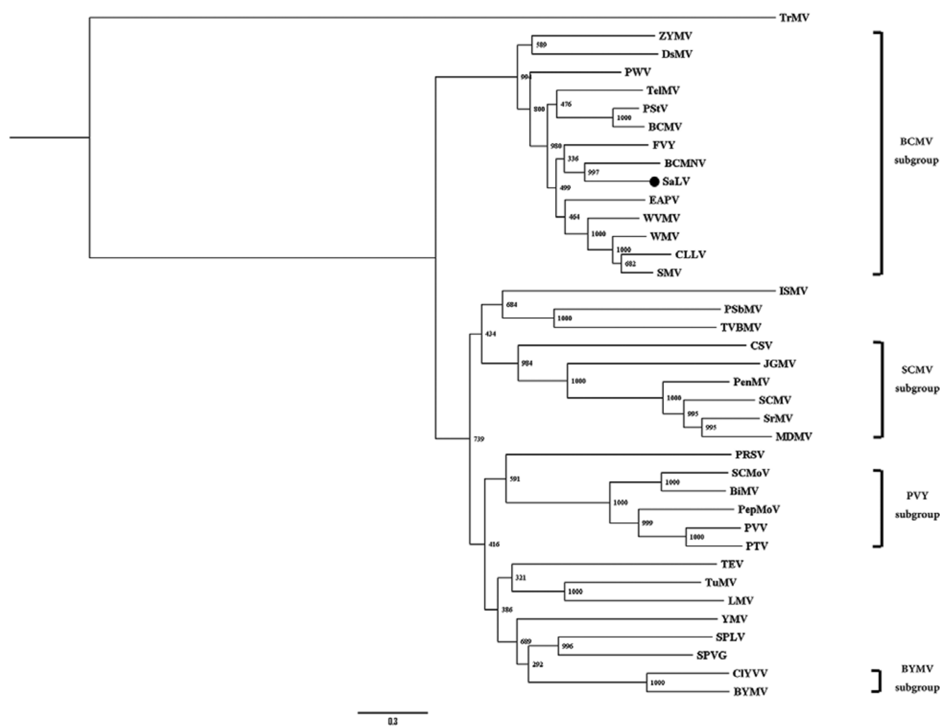

FIGURE 4 Maximum likelihood phylogenetic tree based on the complete genome nucleotide sequences of saffron latent virus (bullet) and 36 other potyviruses. The significance of nodes assessed by bootstrap (1,000 replications) is indicated. The sequence of *Triticum mosaic virus* was used as an outgroup. BCMV: *Bean common mosaic virus* (GQ219793); BCMNV: *Bean common mosaic necrosis virus* (HG792063); BYMV: *Bean yellow mosaic virus* (AY192568.1); BiMV: *Bidens mosaic virus* (NC023014); CLLV: *Calla lily latent virus* (EF105299); ClYVV: *Clover yellow vein virus* (NC003536); CSV: *Cocksfoot streak virus* (NC003742); DsMV: *Dasheen mosaic virus* (KJ786965); EAPV: *East Asian passiflora virus* (KP114137); FVY: *Fritillary virus Y* (NC010954.1); ISMV: *Iris severe mosaic virus* (KT692938.1); JGMV: *Johnsongrass mosaic virus* (NC003606); LMV: *Lettuce mosaic virus* (KJ161184); MDMV: *Maize dwarf mosaic virus* (JQ280313); PRSV: *Papaya ringspot virus* (KF734962); PWV: *Passion fruit woodiness virus* (HQ122652); PStV: *Peanut stripe virus* (PSU05771); PSbMV: *Pea seed-borne mosaic virus* (NC001671); PenMV: *Pennisetum mosaic virus* (JX070146); PepMoV: *Pepper mottle virus* (EU586127); PTV: *Peru tomato mosaic virus* (AJ437280); PVV: *Potato virus* V (NC004010); SMV: *Soybean mosaic virus* (AJ310200); SCMV: *Sugarcane mosaic virus* (KT895080); SCMoV: *Sunflower chlorotic mottle virus* (GU181200); SrMV: *Sorghum mosaic virus* (KM025042); SPLV: *Sweet potato latent virus* (KP115613.1); SPVG: *Sweet potato virus* G (KF790759); TelMV: *Telosma mosaic virus* (DQ851493); TEV: *Tobacco etch virus* (KM282189); TVBMV: *Tobacco vein banding mosaic virus* (NC009994); TrMV: *Triticum mosaic virus* (NC012799); TuMV: *Turnip mosaic virus* (AY134473); WMV: *Watermelon mosaic virus* (EU660585); WVMV: *Wisteria vein mosaic virus* (AY656816); YMV: *Yam mosaic virus* (NC004752); ZYMV: *Zucchini yellow mosaic virus* (JN183062)

TABLE 4 Population diversity of Saffron latent virus within and between different regions of Iran

Region	Fars	Isfahan	Razavi Khorasan	South Khorasan	Tehran
Fars	0.025 ± 0.008				
Isfahan	0.027 ± 0.007	0.013 ± 0.004			
Razavi Khorasan	0.034 ± 0.009	0.027 ± 0.007	0.026 ± 0.008		
South Khorasan	0.039 ± 0.011	0.033 ± 0.011	0.028 ± 0.009	0.022 ± 0.008	
Tehran	0.026 ± 0.007	0.019 ± 0.005	0.023 ± 0.006	0.029 ± 0.009	0.012 ± 0.004

Values are mean ± SEM.

general time-reversible (GTR + G + I) (Gu, Fu, & Li, 1995; Tavaré, 1986) and General matrix (LG + G + I + F) (Gu et al., 1995; Le & Gascuel, 2008) methods were identified as those best fitting the nucleotide and amino acid substitutions in multiple alignments. Phylogenetic trees were constructed from the complete nucleotide and amino acid sequences of SaLV and 36 potyviruses. Both trees showed that SaLV and BCMNV (isolate PV0413) formed a well-differentiated clade within the BCMV subgroup in the genus *Potyvirus* (Figure 4).

3.6 | Genetic diversity of the SaLV population

The analyses of the CP sequence from 50 SaLV isolates showed a low diversity both within subpopulations (i.e., isolates from a province) and between subpopulations (Table 4). In agreement with these results, the value of the fixation index F_{ST} for each pair of subpopulations was lower than 0.33, indicating a low degree of spatial structure for the Iranian SaLV population and, hence, the occurrence of gene flow between geographically distant populations. This is most likely explained by the fact that saffron corms are frequently exchanged among provinces, mainly from the main producers (Razavi Khorasan and South Khorasan provinces) to the rest. A low degree of the spatial structure has also been reported for other potyviruses, for instance, for the Iranian population of SMV (Ahangaran, Koohi Habibi, Mosahebi Mohammadi, Winter, & Garcia-Arenal, 2013), although the low spatial structure is not necessarily the rule for species of this genus (Alegria et al., 2003).

To test if this low diversity could be because of selection, the d_N/d_S ratio was estimated for the whole population (0.145) and each province subpopulation (ranged between 0.100 and 0.132) indicating that the sequence of the CP was under negative selection (Ahangaran et al., 2013; García-Arenal, Fraile, & Malpica, 2001). Negative selection on the CP can be explained by the need to maintain its various functions, primarily viral particle stability (Domier, Latorre, Steinlage, McCoppin, & Hartman, 2003).

4 | CONCLUSIONS

Here we characterised a new species of the genus *Potyvirus* infecting saffron plants in Iran. Since saffron plants do not produce seeds and are propagated vegetatively through corms, the virus can be easily dispersed during plant propagation. As natural host range of SaLV has not yet been studied exhaustively, it is not presently known if saffron is the only inoculum source of the virus or there are other reservoirs. In our study, we did not detect any evident effect of SaLV on saffron yield in the span 2011–2016. However, the fact that SaLV infections are asymptomatic does not necessarily mean that they do not affect plant development and yield, a topic that deserves closer analyses. In addition, it cannot be discarded that future changes in the cultivation systems, in the saffron varieties, or in the cultivation area may result in an enhanced virulence of the current SaLV genotypes, or drive its evolution towards higher virulence (Fraile & García-Arenal, 2016). Thus, SaLV may be a potential problem for saffron production, as no qualitative resistance in saffron plants has been identified. Our quantification of SaLV titers through ELISA in common-garden experiments (not shown) indicates large differences in virus accumulation which might hint towards the presence of quantitative resistance in saffron plants (Corwin & Kliebenstein, 2017; Kushalappa & Gunnaiah, 2013). Anyhow, even under the present scenario, it would be advisable to implement control methods such as using virus-free corms.

ACKNOWLEDGEMENTS

This research was financially supported by Iran National Science Foundation (Project No. 93021253) and also University of Tehran (Grant No. 73148924/6/12). We are grateful to Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Universidad Politécnica de Madrid, for providing sabbatical work facilities. The authors wish to thank Dr Aurora Fraile, Dr Israel Pagán and Dr Sayanta Bera (CBGP-UPM), for their valuable support and suggestions and Niayesh Shahmohammadi for all kind assistance.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Akbar Dizadji http://orcid.org/0000-0001-8496-9959

REFERENCES

Abdullaev, F. I., & Espinosa-Aguirre, J. J. (2004). Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. *Cancer Detection and Prevention*, *28*, 426–432.

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*, 471–487.

Adams, M. J., Antoniw, J. F., & Fauquet, C. M. (2005). Molecular criteria for genus and species discrimination within the family Potyviridae. *Archives of Virology*, *150*, 459–479.

Adams, M. J., Zerbini, F. M., French, R., Rabenstein, F., Stenger, D. C., & Valkonen, J. P. T. (2012). Family Potyviridae. In A. M. Q. King Adams,

M. J. Adams, E. B. Carstens, & E. J. Lefkowitz (Eds.), *Virus taxonomy: Classification and nomenclature of viruses. Ninth Report of the International Committee on Taxonomy of Viruses* (pp. 1069–1089). San Diego, CA: Elsevier Academic Press.

Ahangaran, A., Koohi Habibi, M., Mosahebi Mohammadi, G. H., Winter, S., & García-Arena, F. (2013). Analysis of *Soybean mosaic virus* genetic diversity in Iran allows the characterization of a new mutation resulting in overcoming *Rsv*4-resistance. *Journal of General Virology*, *94*, 2557–2568.

Alegria, O. M., Royer, M., Bousalem, M., Chatenet, M., Peterschmitt, M., Girard, J. C., & Rott, P. (2003). Genetic diversity in the coat protein coding region of eighty-six sugarcane mosaic virus isolates from eight countries, particularly from Cameroon and Congo. *Archives of Virology*, *148*, 357–372.

Amirghasemi, T. (2001). *Saffron, red gold of Iran*. Tehran, Iran: Nashr-Ayandegan Publication In Persian.

Atreya, C. D., & Pirone, T. P. (1993). Mutational analysis of the helper component-proteinase gene of a potyvirus: Effects of amino acid substitutions, deletions, and gene replacement on virulence and aphid transmissibility. *Proceedings of the National Academy of Sciences of the United States of America*, *90*, 11919–11923.

Bellardi, M. G., & Pisi, A. (1987). Indagini sulle virosi del *Crocus* sp. in Italia. *Informatore Fitopatologico*, *9*, 33–38.

Bos, L. (1996). Wisteria vein mosaic potyvirus. In A. Brunt, K. Crabtree, M. Dallwitz, A. Gibbs, & L. Watson (Eds.), *Viruses of plants: Descriptions and lists from the VIDE database* (pp. 1407–1409). Wallingford: CAB International.

Chakraborty, S. (2016). Transcriptome from saffron (*Crocus sativus*) plants in Jammu and Kashmir reveals abundant soybean mosaic virus transcripts and several putative pathogen bacterial and fungal genera Keywords. bioRxiv. https://doi.org/10.1101/079186

Chen, J., Chen, J., & Adams, M. J. (2001). A universal PCR primer to detect members of the Potyviridae and its use to examine the taxonomic status of several members of the family. *Archives of Virology*, *146*, 757–766.

Chen, J., & Chen, J. S. (2000). Occurrence and control of mosaic disease (Turnip mosaic virus) in saffron (*Crocus sativus*). *Zhejiang Nongye Kexue*, *3*, 132–135.

Chung, B. Y. W., Miller, W. A., Atkins, J. F., & Firth, A. E. (2008). An overlapping essential gene in the Potyviridae. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 5897–5902.

Clark, M. F., & Adams, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, *34*, 475–483.

Corwin, J. A., & Kliebenstein, D. J. (2017). Quantitative resistance: More than just perception of a pathogen. *The Plant Cell*, *29*, 655–665.

Domier, L. L., Latorre, I. J., Steinlage, T. A., McCoppin, N., & Hartman, G. L. (2003). Variability and transmission by *Aphis glycines* of North American and Asian Soybean mosaic virus isolates. *Archives of Virology*, *148*, 1925–1941.

Dougherty, W. G., Parks, T. D., Cary, S. M., Bazan, J. F., & Fletterick, R. J. (1989). Characterization of the catalytic residues of the Tobacco etch virus 49-kDa proteinase. *Virology*, *172*, 302–310.

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797.

Fernández, J. A. (2007). Genetic resources of saffron and allies (*Crocus* spp.). *Acta Horticulturae*, *739*, 167–185.

Fraile, A., & García-Arenal, F. (2016). Environment and evolution modulate plant virus pathogenesis. *Current Opinion in Virology*, *17*, 50–56.

Fried, M., & Crothers, D. M. (1981). Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. *Nucleic Acids Research*, *9*, 6505–6525.

Gal-On, A. (2000). A point mutation in the FRNK motif of the potyvirus helper component-protease gene alters symptom expression in cucurbits and elicits protection against the severe homologous virus. *Phytopathology*, *90*, 467–473.

Gal-On, A. (2007). *Zucchini yellow mosaic virus*: insect transmission and pathogenicity—The tails of two proteins. *Molecular Plant Pathology*, *8*, 139–150.

García-Arenal, F., Fraile, A., & Malpica, J. M. (2001). Variability and genetic structure of plant virus populations. *Annual Review of Phytopathology*, *39*, 157–186.

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

Gohari, A., Saeidnia, S., & Kourepaz Mahmoodabadi, M. (2013). An overview on saffron, phytochemicals, and medicinal properties. *Pharmacognosy Reviews*, *7*, 61.

Grilli Caiola, M., & Faoro, F. (2011). Latent virus infections in *Crocus sativus* and *Crocus cartwrightianus*. *Phytopathologia Mediterranea*, *50*, 175–182.

Gu, X., Fu, Y. X., & Li, W. H. (1995). Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Molecular Biology and Evolution*, *12*, 546–557.

Guindon, S., Dufayard, J., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, *59*, 307–321.

Ha, C., Coombs, S., Revill, P. A., Harding, R. M., Vu, M., & Dale, J. L. (2008). Design and application of two novel degenerate primer pairs for the detection and complete genomic characterization of potyviruses. *Archives of Virology*, *153*, 25–36.

Hong, Y., & Hunt, A. G. (1996). RNA polymerase activity catalyzed by a potyvirus-encoded RNA-dependent RNA polymerase. *Virology*, *226*, 146–151.

Kafi, M., Koocheki, A., Rashed, M. H., & Nassiri, M. (2006). *Saffron* (Crocus sativus) *production and processing*. Enfield, New Hampshire, USA: Science Publishers.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780.

Kong, P., Sohn, A., & Steinbiss, H. H. (1997). Molecular characterization of two Bulgarian maize dwarf mosaic potyviruses. *Phytopathology*, *87*, S54.

Kushalappa, A. C., & Gunnaiah, R. (2013). Metabolo-proteomics to discover plant biotic stress resistance genes. *Trends in Plant Science*, *18*, 522–531.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Le, S. Q., & Gascuel, O. (2008). An improved general amino acid replacement matrix. *Molecular Biology and Evolution*, *25*, 1307–1320.

Liang, W. X., Song, L. M., Tian, G. Z., Li, H. F., & Fan, Z. F. (2006). The genomic sequence of Wisteria vein mosaic virus and its similarities with other potyviruses. *Archives of Virology*, *151*, 2311–2319.

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452.

Lu, G., & Moriyama, E. N. (2004). Vector NTI, a balanced all-in-one sequence analysis suite. *Briefings in Bioinformatics*, *5*, 378–388.

Mackenzie, A. M., Nolan, M., Wei, K. J., Clements, M. A., Gowanlock, D., Wallace, B. J., & Gibbs, A. J. (1998). Ceratobium mosaic potyvirus: Another virus from orchids. *Archives of Virology*, *143*, 903–914.

Maina, S., Edwards, O. R., de Almeida, L., Ximenes, A., & Jones, R. A. C. (2016). First complete genome sequence of *Bean common mosaic necrosis virus* from East Timor. *Genome Announcements*, *4*, e01049–e01016.

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*, vev003.

Miglino, R., Jodlowska, A., & van Schadewijk, A. R. (2005). First report of *Narcissus mosaic virus* infecting *Crocus* spp. cultivars in the Netherlands. *Plant Disease*, *89*, 342.

Milne, R. G., & Luisoni, E. (1977). Rapid immune electron microscopy of virus preparations. *Methods in Virology*, *6*, 265–281.

Mink, G. I., & Silbernagel, M. J. (1992). Serological and biological relationships among viruses in the Bean common mosaic virus subgroup. *Archives of Virology. Supplementum*, *5*, 397–406.

Murphy, J. F., Klein, P. G., Hunt, A. G., & Shaw, J. G. (1996). Replacement of the tyrosine residue that links a Potyviral VPg to the viral RNA is lethal. *Virology*, *220*, 535–538.

Navalinskienė, M., & Samuitienė, M. (2001). Viruses affecting some bulb and corm flower crops. *Biologija*, *4*, 40–42.

Olspert, A., Chung, B. Y. W., Atkins, J. F., Carr, J. P., & Firth, A. E. (2015). Transcriptional slippage in the positive-sense RNA virus family Potyviridae. *EMBO Reports*, *16*, 995–1004.

Papandreou, M. A., Kanakis, C. D., Polissiou, M. G., Efthimiopoulos, S., Cordopatis, P., Margarity, M., & Lamari, F. N. (2006). Inhibitory activity on amyloid-β aggregation and antioxidant properties of *Crocus sativus* stigmas extract and its crocin constituents. *Journal of Agricultural and Food Chemistry*, *54*, 8762–8768.

Parizad, S., Dizadji, A., Koohi Habibi, M., Mosahebi Mohammadi, G. H., Kalantari, S., Izadpanah, F., … Winter, S. (2017). Identification and partial characterization of the virus infecting saffron (*Crocus sativus*) in Iran. *Iranian Journal of Plant Protection Science*, *47*, 263–275.

Parizad, S., Dizadji, A., Koohi Habibi, M., Winter, S., Kalantari, S., Garcia-Arenal, F., & Ayllón, M. A. (2017). Prevalence of *Saffron latent virus* (SaLV), a new Potyvirus species, in saffron (*Crocus sativus* L.) fields of Iran. *Jourrnal of Plant Pathology*, *99*, 799–818.

Rambaut, A. (2010). FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/.

Revers, F., & García, J. A. (2015). Molecular biology of potyviruses. *Advances in Virus Research*, *92*, 101–199.

Revers, F., Le Gall, O., Candresse, T., & Maule, A. J. (1999). New advances in understanding the molecular biology of plant/potyvirus interactions. *Molecular Plant-Microbe Interactions*, *12*, 367–376.

Richter, J., Rabenstein, F., Proll, E., & Vetten, H. J. (1995). Use of cross-reactive antibodies to detect members of the Potyviridae. *Journal of Phytopathology*, *143*, 459–464.

Riechmann, J. L., Laín, S., & García, J. A. (1992). Highlights and prospects of potyvirus molecular biology. *Journal of General Virology*, *73* (Pt. 1), 1–16.

Rodamilans, B., Valli, A., Mingot, A., San León, D., Baulcombe, D., López-Moya, J. J., & García, J. A. (2015). RNA polymerase slippage as a mechanism for the production of frameshift gene products in plant viruses of the Potyviridae family. *Journal of Virology*, *89*, 6965–6967.

Samuitienė, M., Navalinskienė, M., & Jackevičienė, E. (2008). *Arabis mosaic virus* on ornamental plants. *Biologija*, *54*, 264–268.

Shukla, D. D., Lauricella, R., & Ward, C. W. (1992). Serology of potyviruses: Current problems and some solutions. *Archives of Virology. Supplementum*, *5*, 57–69.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*, 512–526.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. L., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*, 2725–2729.

Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. In R. M. Miura (Ed.), *Some mathematical questions in biology—DNA sequence analysis* (pp. 57–86). Providence, Rhode Island: American Mathematical Society.

Towbin, H., Staehelin, T., & Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America*, *76*, 4350–4354.

Turpen, T. (1989). Molecular cloning of a potato virus Y genome: Nucleotide sequence homology in non-coding regions of potyviruses. *Journal of General Virology*, *70*, 1951–1960.

Valli, A. A., Gallo, A., Rodamilans, B., López-Moya, J. J., & García, J. A. (2018). The HCPro from the Potyviridae family: An enviable multitasking helper component that every virus would like to have. *Molecular Plant Pathology*, *19*, 744–763.

Verma, P., & Gupta, U. P. (2010). Immunological detection of *Bean common mosaic virus* in French bean (*Phaseolus vulgaris* L.) leaves. *Indian Journal of Microbiology*, *50*, 263–265.

Wang, Y., Han, T., Zhu, Y., Zheng, C. J., Ming, Q. L., Rahman, K., & Qin, L. P. (2010). Antidepressant properties of bioactive fractions from the extract of *Crocus sativus* L. *Journal of Natural Medicines*, *64*, 24–30.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370.

Yukawa, Y., Sugita, M., Choisne, N., Small, I., & Sugiura, M. (2000). The TATA motif, the CAA motif and the poly(T) transcription termination motif are all important for transcription re-initiation on plant tRNA genes. *The Plant Journal*, *22*, 439–447.

How to cite this article: Parizad S, Dizadji A, Koohi Habibi M, et al. Description and genetic variation of a distinct species of *Potyvirus* infecting saffron (*Crocus sativus* L.) plants in major production regions in Iran. *Ann Appl Biol*. 2018;173:233–242. https://doi.org/10.1111/aab.12456