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Molecular characterization of a novel *Betanucleorhabdovirus* infecting sugar beet in Iran

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Abstract: We report the characterization and genetic variations of a novel betanucleorhabdovirus infecting sugar beet in Iran. Pairwise comparison of the obtained betanucleorhabdovirus sequence with betanucleorhabdoviruses from GenBank showed that it shares the highest nucleotide identity with tomato betanucleorhabdovirus 2 (TBRV2) and Sambucus betanucleorhabdovirus 4. The obtained sequence contained six open reading frames (ORFs) in antigenomic sense flanked by complementary 3′ leader and 5′ trailer sequences (3'-N-P-P3-M-G-L-5'). In the phylogenetic tree, the detected isolate was clustered with the betanucleorhabdoviruses and was most closely related to TBRV2, tomato betanucleorhabdovirus 1, and Sambucus betanucleorhabdovirus 1 to 5. The genome organization, phylogenetic relationships, and sequence similarities to other betanucleorhabdoviruses suggest that the virus is a new member of the genus *Betanucleorhabdovirus*, which we propose to name beet betanucleorhabdovirus 1 (BNRV1). The virus's partial spread and host-range investigations revealed that sugar beet fields in northeast Iran were infected by BNRV1, which also infects *Chenopodium album*, *Malva neglecta*, and *Carthamus oxyacanthus* weeds. Low values of *Ks**, *Kst**, *Z**, and *Snn* indicate no substantial genetic differentiation between populations of this virus. Selection pressures on a portion of the BNRV1 *N* gene analyzed were negative, showing purifying selection was occurring. The magnitude of negative selection in the BNRV1 *N* gene was consistent with what has been reported for other betanucleorhabdoviruses.

Keywords: beet betanucleorhabdovirus 1, phylogenetic analysis, natural host range

INTRODUCTION

Sugar beet (*Beta vulgaris*) is an important crop plant mainly cultivated as a source of sugar worldwide [1] and for bioethanol and animal feed [2]. It accounts for nearly 30% of global sugar production [2] and ranks second in world sugar production after sugarcane [3,4]. The *Betanucleorhabdovirus* genus belongs to the family *Rhabdoviridae*, whose members contain a negative-sense, non-segmented, single-stranded RNA genome, ranging from 10-16 kb [5-7]. Genomes of members of the genus *Betanucleorhabdovirus* have 6-7 open reading frames (ORFs) [8-12] separated by gene junction sequences flanked by complementary 3′ leader and 5′ trailer sequences [5,13]. Their ORFs encode nucleocapsid protein (N), phosphoprotein (P), movement protein (P3), matrix protein (M), glycoprotein (G), and large protein (L, RNA-dependent RNA polymerase) [5,7,14]. The genomes of alfalfa-associated nucleorhabdovirus [11] and apple rootstock virus A [12] encode a protein of unknown function.

Members of the genus *Betanucleorhabdovirus* infect different plant species, such as *Rhododendron delavayi*, *Plectranthus aromaticus*, *Asclepias syriaca* [8], and *Bacopa monnieri* [15]. Several betanucleorhabdoviruses are transmitted by aphids in a persistent and propagative manner, and some, such as Sonchus yellow net virus and Paris yunnanensis rhabdovirus 1, are transmitted by mechanical inoculation to *Nicotiana benthamiana* [16-18]. This reads like a virus catalogue. High-throughput sequencing (HTS) of sugar beet plants in the United States has provided the first genomic sequence of beet necrotic yellow vein virus, identifying a novel *Alphanecrovirus* and putative satellite viruses [19]. Here, we report the molecular characterization, evolutionary relationships, and partial host range and distribution of a novel member of the genus *Betanucleorhabdovirus* infecting sugar beet in Iran.

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MATERIALS AND METHODS

Ethics statement

This research did not involve human participants, animals, or genetically modified organisms requiring ethical approval. All samples were collected and handled in accordance with the institutional and national guidelines, with appropriate permissions and biosafety measures.

Virus source and RNA extraction

A total of 196 sugar beet leaves with viral symptoms (curling and mild yellowing) were collected from 6 sugar beet-growing provinces (North Khorasan, Khorasan Razavi, South Khorasan, Lorestan, Hamedan, and Kermanshah) in Iran during September and October 2023. Total RNA was extracted from a pool of sugar beet leaves using the SV Total RNA Isolation Kit (Promega, Madison, USA) following the manufacturer's instructions. After determining the quantity and quality of total RNA using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), one pure RNA sample with an optical absorption ratio at 260/280 nm of approximately 2 [20] was sent to Macrogen (South Korea) for high-throughput sequencing (HTS).

HTS

The TruSeq stranded whole RNA sample preparation kit (Illumina, USA) was used to build a cDNA library, and the Illumina NovaSeq 6000 with 2×151 bp pair-ended was used for sequencing. After adapter trimming, quality control, and *de novo* assembly into contiguous consensus sequences (contigs) of at least 200 bp in length using the CLC Genomics Workbench software version 12.0.3 (CLC bio, Qiagen, CA, USA), the contig fragments were annotated in the NCBI database (GenBank) using BLASTX and BLASTN algorithms to identify sequences with possible viral origin.

Viral genome sequence validation and detection

To validate the presence of this virus in the sampled plants, reverse transcription-polymerase chain reaction (RT-PCR) was performed using primer pairs BBNRV1-F (5′-ACGAGCTCAAAGCCATCCG-3′) and BBNRV1-R (5′-TGCAGCATACAGCTTGTCCA-3′), which were designed based on HTS results (Geneious Prime 2022.1), yielding an amplicon of 1208 bp. The primer specificity was validated using the BLAST tool [https://blast.ncbi.nlm.nih.gov/Blast.cgi]. RT was performed using reverse BBNRV1-R primer and MMuLV (100 U/μL) reverse transcriptase (Takara, Japan), following the manufacturer's protocol. Amplification of cDNA was conducted by Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark) following the manufacturer's protocol. PCR conditions were as follows: 95°C for 5 min; 35 cycles at 95°C for 1 min, 54°C for 1 min, 72°C for 2 min, final step at 72°C for 15 min. The specificity of the PCR product was verified by Sanger sequencing.

Sequence analysis

Pairwise comparison of sequences with those published for betanucleorhabdoviruses was performed using the EMBOSS Needle Program [https://www.ebi.ac.uk/jdispatcher/psa/emboss_needle]. Protein coding regions of the virus were recognized with EXPASY [https://web.expasy.org/translate/] and ORF finder [https://www.ncbi.nlm.nih.gov/orffinder]. Clustal X 2.1 [21] and BioEdit [22] softwares were used to perform multiple sequence alignments. For detection of potential recombinations, analyses were carried out using RDP5 [23], and nuclear localization signals were predicted by cNLS Mapper [https://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi] [24]. Phylogenetic trees were constructed using the maximum-likelihood (ML) method and General Time Reversible (GTR+G+I) (Supplementary Table S1) [25], LG+G (Supplementary Table S2) [26], and LG+G+I+F (Supplementary Table S3) [26] models for complete genome, nucleoprotein, and large protein sequences, respectively, implemented in MEGA version 11.0.13 [27] with 1,000 bootstrap replicates.

RT-PCR, virus detection from field samples, and partial distribution and natural host range determination

Leaves of symptomatic sugar beet plants were randomly collected from major sugar beet production regions of Iran during the 2024 growing season to determine the partial distribution of BNRV1. Symptomless weed

samples were taken from sugar beet fields infected by the virus to determine the virus's natural host range. Specific detection of the virus was performed by RT-PCR using the specific primer pair (BBNRV1-F/BBNRV1-R), as described above.

Phylogenetic analysis, genetic diversity, and population differentiation

After multiple nucleotide sequences of the *N* gene of the virus isolates with Clustal X 2.1 software [21] alignment, 1,146 nucleotides were selected for analysis with BioEdit software [22]. The nucleotide identity matrix of the isolates was determined using SDTv.1.2 software [28]. The phylogenetic tree was constructed using MEGA ver. 11.0.13 [27] with the maximum likelihood method and the Tamura 3-parameter (T92) model (Supplementary Table S4) [29] with 1,000 replications. The dynamics of sequence variation were assessed by DnaSP v6.12.03 software [30] based on phylogenetic groups and geographical distribution (North Khorasan and Khorasan Razavi subpopulations). The estimated parameters include nucleotide diversity (π) [31], number of polymorphic (segregation) sites (S), Watterson's estimator (Θw) [31,32], nonsynonymous substitution rate (dN) and synonymous substitution rate (dS), fixation index (Fst), gene flow level (Nm) [33], and the subpopulation genetic differentiation statistics, *Ks*, Kst*, Z** [34] and *Snn* [35]. The permutation test obtained the P values of *Ks*, Kst*, Z*,* and *Snn* with 1,000 replicates. The Z test of negative selection was performed using the Nei and Gojobori method [36] with MEGA ver. 11.0.13 software [27].

Partial biological assay

Virus-infected sugar beet leaves were homogenized at a 1:5 (w/v) ratio in 0.1 mol/L sodium phosphate buffer (pH 7.4) and mechanically inoculated onto carborundum-dusted leaves of *B. vulgaris*, *C. album*, and *N. benthamiana* seedlings, which were then maintained in a growth chamber under a 16/8 h photoperiod at 25 °C. Specific virus detection was performed at 60 dpi by RT-PCR using specific primers (BBNRV1-F/BBNRV1-R) as before mentioned.

RESULTS

Sequence analysis

Trimmed reads were mapped onto the sugar beet genome, and unmapped reads were *de novo* assembled. One long contig was used to interrogate BLASTn and BLASTx, and the genome sequence of a plant betanucleorhabdovirus was identified. The presence of the obtained sequence was validated in the original RNA sample by RT-PCR followed by Sanger sequencing. Comparison of the BNRV1 sequence with other betanucleorhabdovirus genomes in GenBank confirmed its divergence, with nucleotide sequence identity below 75% relative to all other betanucleorhabdoviruses. Pairwise comparison of the genome sequence of the virus with betanucleorhabdoviruses from GenBank showed that it shares the highest nucleotide identity (63.5-64.8%) with tomato betanucleorhabdovirus 2 (TBRV2) (OP441765), Sambucus betanucleorhabdovirus 4 (PP711314), Sambucus betanucleorhabdovirus 1 (PP711309), tomato betanucleorhabdovirus 1 (TBRV1) (OL472119), Sambucus betanucleorhabdovirus 3 (PP711316), Sambucus betanucleorhabdovirus 2 (PP711321), and Sambucus betanucleorhabdovirus 5 (PP711313) (Supplementary Table S5). This level of nucleotide identity is below the 75% species demarcation threshold for betanucleorhabdoviruses, supporting its classification as a new species in the genus *Betanucleorhabdovirus*. We proposed the name beet betanucleorhabdovirus 1 (BNRV1, GenBank accession no. OR227650) for this virus.

Genome organization and predicted protein features of BNRV1

The near-complete genome sequence of BNRV1 is 13,548 nucleotides in length and contains six ORFs in antigenome sense (3'-N-P-P3-M-G-L-5'), flanked by complementary 3′ leader and 5′ trailer sequences (Table 1). ORF1 (N gene), located at nucleotide positions 170-1,543 (Table 1), encodes a nucleocapsid protein of 457 amino acids with a predicted molecular mass of 50,828 Da (Table 2). The nucleocapsid protein contains a cytoplasmic and nuclear localization signal (NLS) (cNLS) at aa 439-449 (Table 2). Analysis of pairwise sequence comparison showed that the nucleocapsid protein of BNRV1 shared 58.5-84.5% aa identity with Sambucus betanucleorhabdovirus 4, Sambucus

Table 1. Annotation of open reading frames (ORFs) detected in the BNRV1 genome sequence

Region	Nucleotide Position	Length (nt)
3′ leader sequence	1-169	169
ORF1/*N*	170-1543	1374
ORF2/*P*	1622-2638	1017
ORF3/*P3*	2731-3708	978
ORF4/*M*	3966-4817	852
ORF5/*G*	5034-6929	1896
ORF6/*L*	7070-13390	6321
5′ trailer sequence	13391-13548	158

betanucleorhabdovirus 1, Sambucus betanucleorhabdovirus 3, TBRV1, Sambucus betanucleorhabdovirus 5, Sambucus betanucleorhabdovirus 2, TBRV2, Bacopa monnieri virus 2 (BmV2), and datura yellow vein virus (DYVV) (Supplementary Table S5). ORF2, *P* gene at nucleotide positions 1,622-2,638 (Table 1), encodes a 338 amino acid phosphoprotein (37,344 Da) (Table 2). The phosphoprotein shared 33.0-56.0% aa identity with TBRV2, Sambucus betanucleorhabdovirus 4, TBRV1, Sambucus betanucleorhabdovirus 2, Sambucus betanucleorhabdovirus 5, Sambucus betanucleorhabdovirus 1, Sambucus betanucleorhabdovirus 3, DYVV, and BmV2 (Supplementary Table S5). ORF3, *P3* gene at nucleotide positions 2,731-3,708 (Table 1), encodes a 325 amino acid movement protein (37,029 Da) (Table 2). The movement protein shared 42.0-75.7% aa identity with Sambucus betanucleorhabdovirus 2, Sambucus betanucleorhabdovirus 4, Sambucus betanucleorhabdovirus 3, TBRV2, Sambucus betanucleorhabdovirus 5, Sambucus betanucleorhabdovirus 1, Sambucus betanucleorhabdovirus 1, TBRV1, DYVV, and BmV2 (Supplementary Table S5). ORF4, *M* gene at nucleotide positions 3966-4817 (Table 1), encodes a 283 amino acid matrix protein (31,103 Da) (Table 2). The matrix protein contains an NLS and cNLS at aa 211-230 (Table 2). The matrix protein shared 34.6-59.7% aa identity with TBRV2, Sambucus betanucleorhabdovirus 4, Sambucus betanucleorhabdovirus 1, TBRV1, Sambucus betanucleorhabdovirus 2, Sambucus betanucleorhabdovirus 3, Sambucus betanucleorhabdovirus 5, DYVV, and BmV2 (Supplementary Table S5). ORF5, *G* gene at nucleotide positions 5034-6929 (Table 1), encodes a 631 amino acid glycoprotein (71,322 Da) (Table 2). The glycoprotein contains an NLS and a cNLS at aa 587-614 (Table 2). The glycoprotein shared 50.8–67.0% aa identity with TBRV2, TBRV1, Sambucus betanucleorhabdovirus 3, Sambucus betanucleorhabdovirus 4, Sambucus betanucleorhabdovirus 5, Sambucus betanucleorhabdovirus 1, Sambucus betanucleorhabdovirus 2, DYVV, and BmV2 (Supplementary Table S5). ORF6, *L* gene at nucleotide positions 7,070-13,390 (Table 1), encodes RNA-dependent RNA polymerase (2106 amino acids (24,0584 Da) (Table 2). The RNA-dependent RNA polymerase contains an NLS and a cNLS at aa 1,639-1,663 (Table 2). The RNA-dependent RNA polymerase shared 50.3-69.9% aa identity with TBRV2, TBRV1, Sambucus betanucleorhabdovirus 1, Sambucus betanucleorhabdovirus 5, Sambucus betanucleorhabdovirus 2, Sambucus betanucleorhabdovirus 4, Sambucus betanucleorhabdovirus 3, DYVV, and BmV2 (Supplementary Table S5). The partial lengths of the 3′ leader and 5′ trailer sequences are 169 and 158 nucleotides, respectively (Table 1), and fourteen of the terminal nucleotides of their sequences are complementary. The BNRV1 genome is organized similarly to other betanucleorhabdoviruses, comprising six ORFs separated by a putative polyadenylation/transcription termination signal (5'-AUAUAAGAAAAA-3'), a putative untranscribed dinucleotide spacer (5'-CC-3'), and a putative transcription initiation motif (5'-AAC-3').

Evolutionary relationships of BNRV1

To determine whether recombination played a role in the origin of BNRV1, the genomic sequence of BNRV1

Table 2. Analysis of the BNRV1 sequence for nuclear localization signals (NLS) and cytoplasmic localization signal (cNLS)

Gene	Putative gene function	Size(aa)	Predicted NLS	cNLS mapper score
N	nucleocapsid protein	457	EPAKKRKTPVL	7
P	phosphoprotein	338	-	-
P3	movement protein	325	-	-
M	matrix protein	283	-PKKRAKSPTHIMRKKLITKK	4.5
G	glycoprotein	631	NVKGRIVTFADDELEGFTTPVRAPSAPK	3.4
L	RNA-dependent RNA polymerase	2106	DTVSIKRKRLSLF VSIKRKRLSL	8

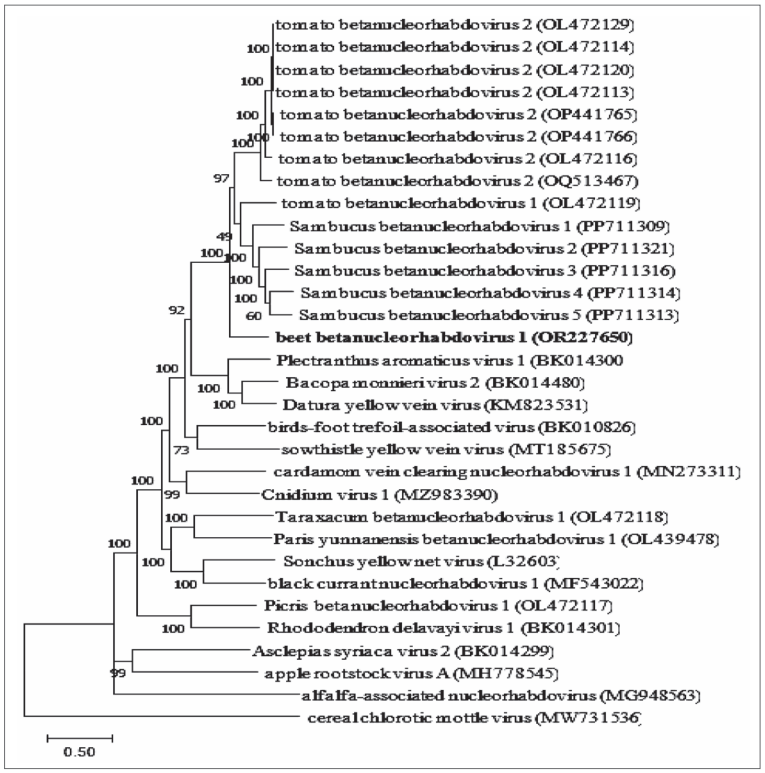

Fig. 1. Maximum likelihood tree of BNRV1 and other *Betanucleorhabdovirus* species constructed in MEGA version 11.0.13 using the General Time Reversible (GTR+G+I) model, based on complete genome sequences, with 1,000× bootstrap replications. The tree was rooted using the gammanucleorhabdovirus cereal chlorotic mottle virus sequence (MW731536).

was compared to 23 other betanucleorhabdoviruses available in GenBank. No evidence of recombination between BNRV1 and other betanucleorhabdoviruses was found. This suggests that BNRV1 diverged from other betanucleorhabdoviruses primarily through mutations rather than recombination. In the complete genome sequence-based phylogenetic tree, BNRV1 clustered with betanucleorhabdoviruses and was most closely related to TBRV2, TBRV1, and Sambucus betanucleorhabdovirus 1-5, and was distinct from other *Betanucleorhabdovirus* species (Fig. 1).

Phylogenetic trees constructed based on the aa sequences of the nucleocapsid protein, phosphoprotein, movement protein, matrix protein, glycoprotein, and large protein had a similar topology to the complete genome sequence-based phylogenetic tree (only phylogenetic trees constructed based on the nucleoprotein and large protein aa sequences are shown, Fig. 2), supporting the RDP5 analysis and the conclusion that no evidence of recombination between BNRV1 and other betanucleorhabdoviruses was found.

Distribution and natural host range

During a one-year survey (2024), samples from 14 cities belonging to six major sugar beet-cultivating provinces of Iran (Supplementary Table S6) were collected, and a portion of the BNRV1 *N* gene was amplified. The results showed that sugar beet fields in the cities of Fariman, Sefid Sang, and Torbat Jam (Khorasan Razavi Province) and in Farouj (North Khorasan Province) were infected with BNRV1 (Supplementary Table S6). The incidence of BNRV1 infection was 6.6%, 9.0%, 0.0%, 0.0%, 0.0%, and 0.0% in North Khorasan, Khorasan Razavi, South Khorasan, Lorestan, Hamedan, and Kermanshah provinces, respectively (Supplementary Table S6). Overall, the incidence of BNRV1 infection was estimated at 5.8% (5 infected samples out of 85 total samples) in the surveyed provinces (Supplementary Table S6).

To examine the partial natural host range of BNRV1, *C. album* (Chenopodiaceae), *Malva neglecta* (Malvaceae), and *Carthamus oxyacanthus* (Asteraceae), common weeds in sugar beet fields, were collected and tested for BNRV1. All were infected (Supplementary Table S7) but remained asymptomatic.

Phylogenetic and genetic analyses based on partial *N* gene sequences of the BNRV1 isolates

The aligned partial *N* gene sequences (1146 nucleotides) from 8 BNRV1 isolates (Supplementary Table S8) were used to construct a phylogenetic tree (Fig. 3) in MEGA version 11.0.13, using the maximum likelihood (ML) method with 1,000 bootstrap values. In the phylogenetic tree, the eight BNRV1 isolates clustered into two major groups, with the North Khorasan and Razavi Khorasan subpopulations showing no strong differentiation (Fig. 3). The results of genetic differentiation and gene flow between subpopulations of BNRV1 sequences are shown in Table 3.

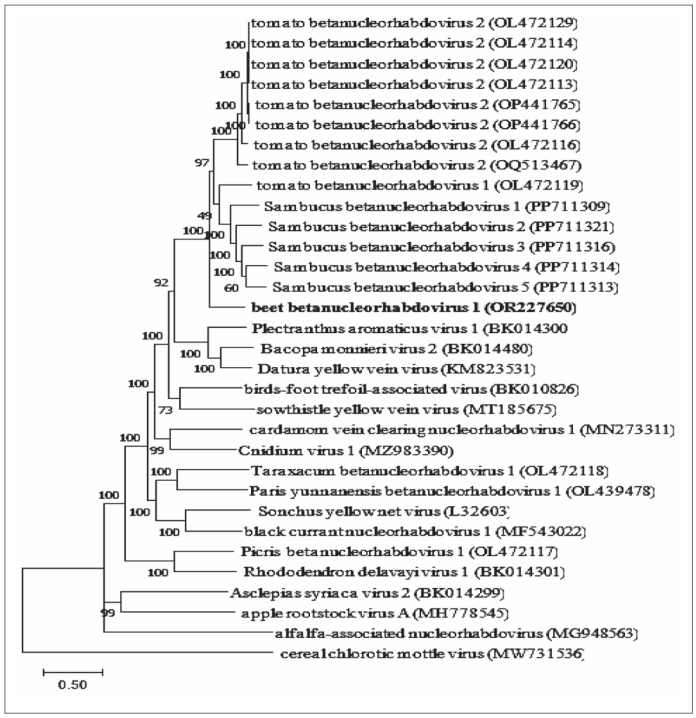

Fig. 2. Maximum likelihood trees of BNRV1 and other *Betanucleorhabdovirus* species constructed in MEGA version 11.0.13 using the LG+G model, based on the nucleoprotein aa sequences (**A**) and using LG+G+I+F model, based on the large protein aa sequences (**B**), with 1000× bootstrap replications. The tree was rooted using the gammanucleorhabdovirus cereal chlorotic mottle virus sequence (MW731536).

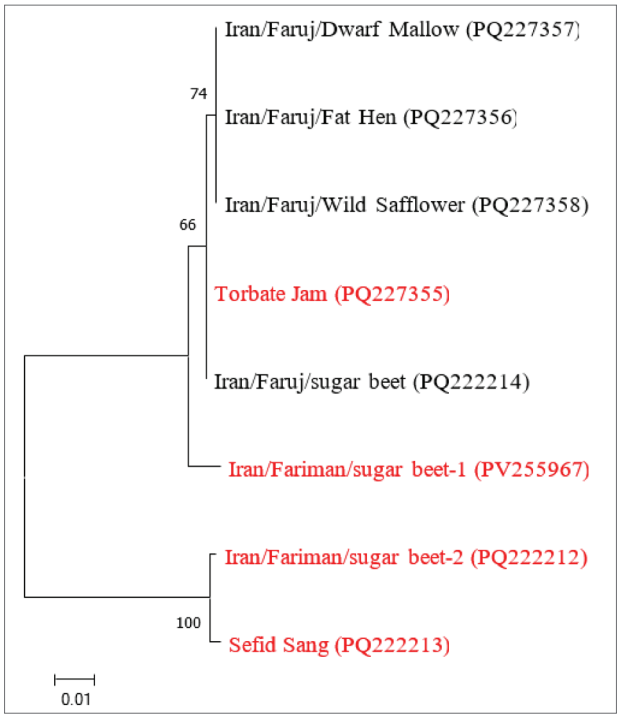

Fig. 3. Maximum likelihood phylogenetic tree drawn based on eight partial sequences of BNRV1 *N* gene (1146 nucleotides) using MEGA version 11.0.13 software by Tamura 3-Parameter (T92) model and 1000× bootstrap replications. The Khorasan Razavi subpopulation is shown in red, and the North Khorasan subpopulation is shown in black. Details of the isolated host and location are given in Supplementary Table S8.

Table 3. Estimate of genetic differentiation and gene flow between subpopulations of BNRV1 sequences

Population	*Ks**	*Kst**	*Ks**, *Kst** P	*Z**	*Z** P	*Snn*	*Snn* P	*Fst*	*Nm*
North Khorasan/ Razavi Khorasan	2.27122	0.20161	0.063ns	2.22841	0.063ns	0.6875	0.234ns	0.32104	0.53

*Ks**, *Kst**, *Ks**, *Z**, and *Snn* P values were obtained by the permutation test with 1,000 replicates; ns, not significant, *0.01<P<0.05, **0.001<P<0.01, ***P<0.001.

The identity matrix of the partial nucleotide sequences of the BNRV1 *N* gene (1,146 nucleotides) (Supplementary Table S8) was calculated using SDTv1.2 software, which showed a high nucleotide identity ranging from 90.8-100% (Fig. 4). Results of genetic diversity and selection pressure based on a 1,146-nucleotide fragment of the BNRV1 *N* gene from different isolates are presented in Table 4.

Partial biological assay

Preliminary mechanical inoculation of *B. vulgaris*, *C. album*, and *N. benthamiana* seedlings with crude sap from an infected sample was unsuccessful, as BNRV1 was not detected in any of the inoculated plants. Failure of mechanical transmission of the virus could be due to the low titer of the virus or the inability of the virus to be transmitted by mechanical means in some host plants.

DISCUSSION

BNRV1 is a novel member of the genus *Betanucleorhabdovirus* within the family *Rhabdoviridae* based on several criteria. The genome of BNRV1 contained six ORFs in the order 3'-N-P-P3-M-G-L-5' and was organized similarly to those of cnidium virus 1 [9], Paris yunnanensis rhabdovirus 1 [16], and Sambucus

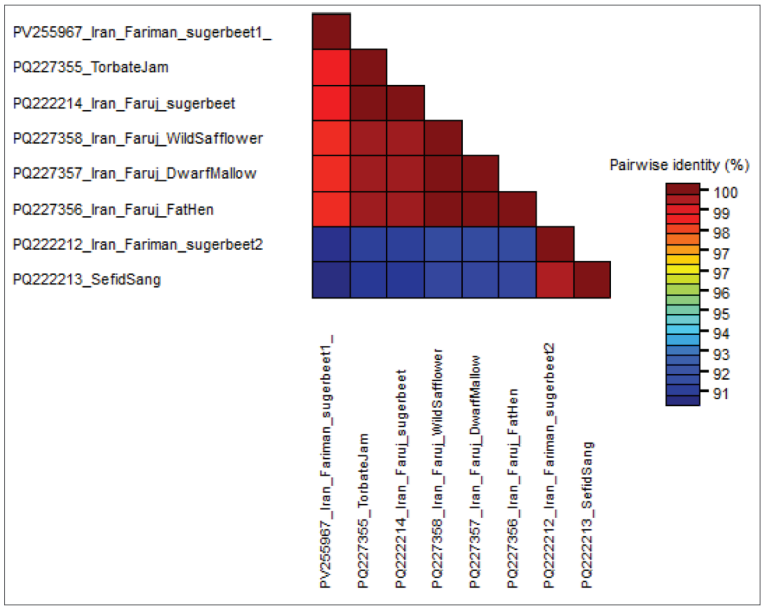

Fig. 4. Identity matrix of partial nucleotide sequences of the BNRV1 *N* gene (1146 nucleotides) using SDTv1.2 software. Blue and red colors indicate the minimum and maximum identity, respectively. Details of the isolated host and location are given in Supplementary Table S8.

betanucleorhabdovirus 1-5 [37]. As with all rhabdovirus genomes, the genome of BNRV1 has gene junction sequences and complementary 3′ leader and 5′ trailer sequences, potentially forming a panhandle structure [5,9,11-13,16]. Phylogenetic analysis showed that BNRV1 clustered with betanucleorhabdoviruses and was distinct from other *Betanucleorhabdovirus* species. Results of a previous phylogenetic study showed that Sambucus betanucleorhabdovirus 1-5 are separated from other *Betanucleorhabdovirus* species [37].

Studies of the natural host range revealed that weeds of the Chenopodiaceae, Malvaceae, and Asteraceae families were infected by BNRV1. These weeds may act as potential inoculum sources for sugar beet infection, while remaining symptomless. This observation agrees with the findings of other researchers who showed that elderberry plants infected with Sambucus betanucleorhabdovirus 1-5 were either asymptomatic or only exhibited mild leaf chlorosis [37]. In this study, sugar beet plants infected by BNRV1 showed leaf curling and mild yellowing symptoms. However, these symptoms could also be attributed to nutrient deficiency.

Fst ranges from 0 (no genetic differentiation between populations) to 1 (complete differentiation) [38]. A threshold of 0.33 is commonly applied: values >0.33 indicate limited gene flow and strong genetic differentiation, whereas values ≤0.33 indicate frequent gene flow [30,38]. In this study, the Fst value between the North Khorasan and Razavi Khorasan populations was below 0.33, indicating weak genetic differentiation, consistent with the phylogenetic tree (Fig. 3). Gene flow levels are classified as low (0.00-0.249), moderate (0.25-0.99), and high (Nm >1) [39]. In this study, the Nm value between the two subpopulations of North Khorasan and Razavi Khorasan was between 0.25 and 0.99, pointing to moderate gene flow between them. Nonsignificant *Ks**, *Kst**, *Z**, and *Snn* values indicate no substantial differentiation between the North Khorasan and Razavi Khorasan subpopulations.

To evaluate selection pressure on a portion of the *N* gene, the dN/dS ratio was calculated as 0.02446, indicating strong negative selection and its influence on BNRV1 genetic diversity and evolution. The Z-test of negative selection confirmed that the BNRV1 *N* gene is under negative selection, rejecting the null hypothesis (dN=dS) in favor of the alternative hypothesis (dN<dS) (P<0.05). A similar study on Sambucus betanucleorhabdovirus 2-5 found that all genes exhibited a higher frequency of synonymous

Table 4. Genetic diversity and selection pressure are based on part of *N* gene sequence (1146 nucleotides) of BNRV1 isolates

Geographical population	m	π(SD)	*S*	*Θw*(SD)	dN	dS	dN/dS	Z	P
North Khorasan	4	0.00131±0.00069	3	0.00143±0.00104	0.00000	0.0058	0	1.75	0.04
Razavi Khorasan	4	0.06297±0.01768	113	0.05378±0.02919	0.00621	0.25667	0.02419	11.29	0.00
All	8	0.04083±0.014	113	0.03803±0.01661	0.00407	0.16633	0.02446	10.89	0.00

m (number of sequences), π (nucleotide diversity), S (number of polymorphic sites), Θw (mutation rate calculated from segregating sites), dN (nonsynonymous substitution rate), dS (synonymous substitution rate), dN/dS (ratio of rates of non-synonymous to synonymous substitution), Z (codon-based Z-test of purifying selection (dS - dN)), P (the probability of rejecting the null hypothesis of strict-neutrality ($d_N = d_S$) in favor of the alternative hypothesis ($d_N < d_S$). Values of P less than 0.05 are considered significant at the 5% level)

than non-synonymous mutations and were subject to relatively strong negative selection pressures [37]. A portion of the *N* gene of the new virus was under relatively strong negative selection, indicating that variation in different betanucleorhabdovirus genes arises primarily through mechanisms such as mutation rather than selection pressure.

In this study, we present the molecular characterization of a novel *Betanucleorhabdovirus* infecting sugar beet in Iran, designated BNRV1. Its partial distribution and natural host range were also determined. Further research is required to identify its insect vectors and assess pathogenicity.

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Conflict of interest disclosure: The authors declare no conflicts of interest.

Data availability: All sequences of BNRV1 have been deposited in the GenBank database, National Center for Biotechnology Information, accession nos. PQ222212-PQ222214, PQ227355-PQ227358, PV255967, and OR227650. The data are accessible at https://www.ncbi.nlm.nih.gov/nuccore/

REFERENCES

1. Samuel A. Dines L. Lockhart and Wiseman's Crop Husbandry Including Grassland. Woodhead Publishing. 2023;694p.
2. Dohm JC, Minoche AE, Holtgräwe D, Capella-Gutiérrez S, Zakrzewski F, Tafer H, Rupp O, Sörensen TR, Stracke R, Reinhardt R, Goesmann A, Kraft T, Schulz B, Stadler PF, Schmidt T, Gabaldón T, Lehrach H, Weisshaar B, Himmelbauer H. The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*). Nature. 2014;505:546-9. https://doi.org/10.1038/nature12817
3. Brar NS, Dhillon BS, Saini KS, Sharma PK. Agronomy of sugar beet cultivation. Agric Rev. 2015;36:184-97. https://doi.org/10.5958/0976-0741.2015.00022.7
4. Mall AK, Srivastava S, Mulet JM, Popović V, Misra V. Sugar Beet Special Issue: Biotechnology and Breeding Techniques for Stress-Resistant Sugar Beet. Sugar Tech. 2024;26:1195-8. https://doi.org/10.1007/s12355-024-01501-1
5. Walker PJ, Freitas-Astúa J, Bejerman N, Blasdell KR, Breyta R, Dietzgen RG, Fooks AR, Kondo H, Kurath G, Kuzmin IV, Ramos-González PL, Shi M, Stone DM, Tesh RB, Tordo N, Vasilakis N, Whitfield AE, Ictv Report Consortium. ICTV Virus Taxonomy Profile: Rhabdoviridae 2022. J Gen Virol. 2022;103:001689. https://doi.org/10.1099/jgv.0.001689
6. Wu LP, Yang T, Liu HW, Postman J, Li R. Molecular characterization of a novel rhabdovirus infecting blackcurrant identified by high-throughput sequencing. Arch Virol. 2018;163:1363-6. https://doi.org/10.1007/s00705-018-3709-x
7. Dietzgen RG, Kondo H, Goodin MM, Kurath G, Vasilakis N. The family Rhabdoviridae: mono- and bipartite negative-sense RNA viruses with diverse genome organization and common evolutionary origins. Virus Res. 2017;227:158-70. https://doi.org/10.1016/j.virusres.2016.10.010
8. Bejerman N, Dietzgen RG, Debat H. Illuminating the Plant Rhabdovirus Landscape through Metatranscriptomics Data. Viruses. 2021;13(7):1304. https://doi.org/10.3390/v13071304
9. Belete MT, Igori D, Kim SE, Lee SH, Moon JS. Complete genome sequence of cnidium virus 1, a novel betanucleorhabdovirus infecting Cnidium officinale. Arch Virol. 2022;167:973-7. https://doi.org/10.1007/s00705-021-05348-9
10. Rivarez MPS, Pecman A, Bačnik K, Maksimović O, Vučurović A, Seljak G, Mehle N, Gutiérrez-Aguirre I, Ravnikar, M, Kutnjak D. In-depth study of tomato and weed viromes reveals undiscovered plant virus diversity in an agroecosystem. Microbiome. 2023;11:60. https://doi.org/10.1186/s40168-023-01500-6
11. Gaafar YZA, Richert-Poggeler KR, Maass C, Vetten HJ, Ziebell H. Characterisation of a novel nucleorhabdovirus infecting alfalfa (*Medicago sativa*). Virol J. 2019;16:55. https://doi.org/10.1186/s12985-019-1147-3
12. Baek D, Lim S, Ju HJ, Kim HR, Lee SH, Moon JS. The complete genome sequence of apple rootstock virus A, a novel nucleorhabdovirus identified in apple rootstocks. Arch Virol. 2019;164:2641-4. https://doi.org/10.1007/s00705-019-04348-0
13. Jackson AO, Dietzgen RG, Fang RX, Goodin MM, Hogenhout SA, Deng M, Bragg JN. Plant rhabdoviruses.In: Mahy BWJ; Van Regenmortel MHV, editors. Encyclopedia of Virology. Amsterdam, Netherlands: Elsevier; 2008 p. 187-96. https://doi.org/10.1016/B978-012374410-4.00492-1
14. Wang Q, Ma X, Qian S, Zhou X, Sun K, Chen X, Zhou X, Jackson AO, Li Z. Rescue of a Plant Negative-Strand RNA Virus from Cloned cDNA: Insights into Enveloped Plant Virus Movement and Morphogenesis. PLoS Pathog. 2015;11:e1005223. https://doi.org/10.1371/journal.ppat.1005223
15. Sidharthan VK, Baranwal VK. Mining of the water hyssop (*Bacopa monnieri*) transcriptome reveals genome sequences of two putative novel rhabdoviruses and a solendovirus. Arch Virol. 2021;166:1985-1990. https://doi.org/10.1007/s00705-021-05061-7
16. Hu J, Miao T, Que K, Rahman MS, Zhang L, Dong X, Ji P, Dong J. Identification, molecular characterization and phylogenetic analysis of a novel nucleorhabdovirus infecting

Paris polyphylla var. yunnanensis. Sci Rep. 2023;13:10040. https://doi.org/10.1038/s41598-023-37022-2
17. Jackson AO, Christie SR. Purification and some physiochemical properties of Sonchus yellow net virus. Virology. 1977;77:344-55. https://doi.org/10.1016/0042-6822(77)90431-7
18. Jackson AO, Wagner JDO. Procedures for plant rhabdovirus purification, polyribosome isolation, and replicase extraction. Methods Mol Biol. 1998;81:77-97. https://doi.org/10.1385/0-89603-385-6:77
19. Weiland JJ, Sharma Poudel R, Flobinus A, Cook DE, Secor GA, Bolton MD. RNAseq Analysis of Rhizomania-Infected Sugar Beet Provides the First Genome Sequence of *Beet Necrotic Yellow Vein Virus* from the USA and Identifies a Novel *Alphanecrovirus* and Putative Satellite Viruses. Viruses. 2020;12(6):626. https://doi.org/10.3390/v12060626
20. Barbas III CF, Burton DR, Scott JK, Silverman GJ. Quantitation of DNA and RNA. Cold Spring Harb Protoc. 2007;pdb.ip47. https://doi.org/10.1101/pdb.ip47
21. Aiyar A. The Use of CLUSTAL W and CLUSTAL X for Multiple Sequence Alignment. In: Misener S, Krawetz SA, editors. Bioinformatics Methods and Protocols. Totowa, NJ: Humana Press; 2000. p. 221-41. (Methods in Molecular Biology™. Vol. 132). https://doi.org/10.1385/1-59259-192-2:221
22. Hall TA. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95-98.
23. Martin DP, Varsani A, Roumagnac P, Botha, G, Maslamoney S, Schwab T, Kelz Z, Kumar V, Murrell B. RDP5: A computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. Virus Evol. 2021;7:veaa087. https://doi.org/10.1093/ve/veaa087
24. Kosugi S, Hasebe M, Tomita M, Yanagawa H. Systematic identification of yeast cell cycle-dependent nucleocytoplasmic shuttling proteins by prediction of composite motifs. Proc Natl Acad Sci U S A. 2009;106:10171-6. https://doi.org/10.1073/pnas.0900604106
25. Nei M, Kumar S. Molecular Evolution and Phylogenetics. Oxford University Press, New York. 2000.
26. Le SQ, Gascuel O. An Improved General Amino Acid Replacement Matrix. Mol Biol Evol. 2008;25:1307-20. https://doi.org/10.1093/molbev/msn067
27. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol. 2021;38:3022-7. https://doi.org/10.1093/molbev/msab120
28. Muhire BM, Varsani A, Martin DP. SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One. 2014;9:e108277. https://doi.org/10.1371/journal.pone.0108277
29. Tamura K. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Mol Biol Evol. 1992;9:678-87. https://doi.org/10.1093/oxfordjournals.molbev.a040752
30. Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sanchez-Gracia A. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol. 2017;34:3299-302. https://doi.org/10.1093/molbev/msx248
31. Nei M. Molecular evolutionary genetics. New York: Columbia University Press; 1987. 512 p.
32. Watterson GA. On the number of segregating sites in genetical models without recombination. Theor Popul Biol. 1975;7:256-76. https://doi.org/10.1016/0040-5809(75)90020-9
33. Hudson RR, Slatkin, M, Maddison WP. Estimation of levels of gene flow from DNA sequence data. Genetics. 1992b;132:583-9. https://doi.org/10.1093/genetics/132.2.583
34. Hudson RR, Boos DD, Kaplan NL. A statistical test for detecting geographic subdivision. Mol Biol Evol. 1992a; 9:138-51. https://doi.org/10.1093/oxfordjournals.molbev.a040703
35. Hudson RR. A new statistic for detecting genetic differentiation. Genetics. 2000;155:2011-4. https://doi.org/10.1093/genetics/155.4.2011
36. Nei M, Gojobori T. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol. 1986;3:418-26. https://doi.org/10.1093/oxfordjournals.molbev.a040410
37. Šafářová D, Candresse T, Veselská J, Navrátil M. Novel Betanucleorhabdoviruses Infecting Elderberry (*Sambucus nigra* L.): Genome Characterization and Genetic Variability. Pathogens. 2024;13(6):445. https://doi.org/10.3390/pathogens13060445
38. Hartl DL, Clark AG. Principles of Population Genetics. 3rd ed. Sunderland: Sinauer Associates Inc. 1997. 519 p.
39. Govindajuru RD. Variation in gene flow levels among predominantly selfpollinated plants. J Evol Biol. 1989;2:173-81. https://doi.org/10.1046/j.1420-9101.1989.2030173.x

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Maximum Likelihood fits of 24 different nucleotide substitution models for complete genome sequences of BNRV1 and other *Betanucleorhabdovirus* species

Model	BIC
GTR+G+I	713739.7398
GTR+G	714220.7502
TN93+G+I	715432.3475
HKY+G+I	715733.9421
TN93+G	716192.2956
HKY+G	716462.4717
T92+G+I	717517.0876
T92+G	718295.87
K2+G+I	721110.8102
K2+G	721894.8497
JC+G+I	730585.664
JC+G	731284.0764
GTR+I	735346.6269
TN93+I	736775.6575
HKY+I	737038.6315
T92+I	737813.857
K2+I	740723.9323
JC+I	748112.9481
GTR	750525.4034
TN93	752443.117
HKY	752607.7953
T92	752995.4288
K2	755782.1424
JC	762829.1014

Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. TR (General Time Reversible), HKY (Hasegawa-Kishino-Yano), TN93 (Tamura-Nei), T92 (Tamura 3-parameter), K2 (Kimura 2-parameter), JC (Jukes-Cantor)

Supplementary Table S2. Maximum likelihood fits of 56 different amino acid substitution models for nucleoprotein sequences of BNRV1 and other *Betanucleorhabdovirus* species

Model	BIC
LG+G	31104.59316
LG+G+F	31105.01251
LG+G+I	31114.18978
LG+G+I+F	31114.60913
rtREV+G+F	31179.04116
rtREV+G+I+F	31188.63778
WAG+G	31248.35741
WAG+G+I	31257.44624
WAG+G+I+F	31288.95162
rtREV+G	31346.7263
rtREV+G+I	31356.32292
JTT+G+F	31362.44635
JTT+G	31368.46713
JTT+G+I+F	31372.04304
JTT+G+I	31377.93217
Dayhoff+G+F	31396.79328
Dayhoff+G+I+F	31406.05765
WAG+G+F	31438.27509
Dayhoff+G	31475.55493
Dayhoff+G+I	31484.37347
cpREV+G+I+F	31559.74588
cpREV+G+F	31560.38884
cpREV+G	31604.69292
cpREV+G+I	31608.64194
mtREV24+G+F	31807.661
mtREV24+G+I+F	31817.25757
WAG+I	32308.47283
LG+I	32373.81921
WAG+I+F	32392.77336
LG+I+F	32429.72972
rtREV+I+F	32494.51797
rtREV+I	32554.09991
JTT+I	32567.72495
WAG	32587.65627
JTT+I+F	32641.47513
Dayhoff+I+F	32641.49007
LG	32645.5253
Dayhoff+I	32658.48903
WAG+F	32677.83808
mtREV24+G	32699.10718
mtREV24+G+I	32708.70381
LG+F	32709.34392
rtREV+F	32767.87973
cpREV+I	32787.80152
cpREV+I+F	32795.09512
rtREV	32809.82128
JTT	32852.81291
JTT+F	32926.1787
Dayhoff+F	32934.78954
Dayhoff	32940.06451
cpREV	33064.34113
cpREV+F	33084.99173
mtREV24+I+F	33457.50038
mtREV24+F	33696.42919
mtREV24+I	34504.79009
mtREV24	34718.2787

Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. TR (General Time Reversible), JTT (Jones-Taylor-Thornton), rtREV (General Reverse Transcriptase), cpREV (General Reversible Chloroplast), mtREV24 (General Reversible Mitochondrial).

Supplementary Table S2. Maximum likelihood fits of 56 different amino acid substitution models for nucleoprotein sequences of BNRV1 and other *Betanucleorhabdovirus* species

Model	**BIC**
LG+G	31104.59316
LG+G+F	31105.01251
LG+G+I	31114.18978
LG+G+I+F	31114.60913
rtREV+G+F	31179.04116
rtREV+G+I+F	31188.63778
WAG+G	31248.35741
WAG+G+I	31257.44624
WAG+G+I+F	31288.95162
rtREV+G	31346.7263
rtREV+G+I	31356.32292
JTT+G+F	31362.44635
JTT+G	31368.46713
JTT+G+I+F	31372.04304
JTT+G+I	31377.93217
Dayhoff+G+F	31396.79328
Dayhoff+G+I+F	31406.05765
WAG+G+F	31438.27509
Dayhoff+G	31475.55493
Dayhoff+G+I	31484.37347
cpREV+G+I+F	31559.74588
cpREV+G+F	31560.38884
cpREV+G	31604.69292
cpREV+G+I	31608.64194
mtREV24+G+F	31807.661
mtREV24+G+I+F	31817.25757
WAG+I	32308.47283
LG+I	32373.81921
WAG+I+F	32392.77336
LG+I+F	32429.72972
rtREV+I+F	32494.51797
rtREV+I	32554.09991
JTT+I	32567.72495
WAG	32587.65627
JTT+I+F	32641.47513
Dayhoff+I+F	32641.49007
LG	32645.5253
Dayhoff+I	32658.48903
WAG+F	32677.83808
mtREV24+G	32699.10718
mtREV24+G+I	32708.70381
LG+F	32709.34392
rtREV+F	32767.87973
cpREV+I	32787.80152
cpREV+I+F	32795.09512
rtREV	32809.82128
JTT	32852.81291
JTT+F	32926.1787
Dayhoff+F	32934.78954
Dayhoff	32940.06451
cpREV	33064.34113
cpREV+F	33084.99173
mtREV24+I+F	33457.50038
mtREV24+F	33696.42919
mtREV24+I	34504.79009
mtREV24	34718.2787

Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. TR (General Time Reversible), JTT (Jones-Taylor-Thornton), rtREV (General Reverse Transcriptase), cpREV (General Reversible Chloroplast), mtREV24 (General Reversible Mitochondrial).

Supplementary Table S3. Maximum Likelihood fits of 56 different amino acid substitution models for the large protein sequences of BNRV1 and other *Betanucleorhabdovirus* species

Model	BIC
LG+G+I+F	161846.8266
LG+G+F	161893.997
rtREV+G+I+F	162159.0294
rtREV+G+F	162212.2914
WAG+G+I+F	162586.5672
WAG+G+F	162649.8018
LG+G+I	162889.7348
JTT+G+I+F	162915.3338
LG+G	162933.3148
JTT+G+F	162977.0669
Dayhoff+G+I+F	163496.8779
Dayhoff+G+F	163561.561
WAG+G+I	163957.3763
cpREV+G+I+F	163998.5834
WAG+G	164022.7679
JTT+G+I	164166.6
cpREV+G+F	164166.6322
JTT+G	164224.7562
rtREV+G+I	164471.2797
rtREV+G	164526.1249
mtREV24+G+I+F	164674.1559
mtREV24+G+F	164700.1848
cpREV+G	165575.6416
cpREV+G+I	165588.9262
Dayhoff+G+I	166028.2183
Dayhoff+G	166087.444
WAG+I+F	170053.7675
mtREV24+G+I	170454.5343
mtREV24+G	170478.3354
rtREV+I+F	170625.8772
LG+I+F	170672.3718
WAG+I	171129.0525
JTT+I+F	171182.4569
LG+I	171503.492
Dayhoff+I+F	171796.9174
cpREV+I+F	172081.9383
JTT+I	172258.9986
rtREV+I	172493.7848
cpREV+I	173447.6606
Dayhoff+I	174085.6695
WAG+F	174129.2974
mtREV24+I+F	174794.9473
rtREV+F	174861.656
LG+F	175010.2334
WAG	175090.9879
JTT+F	175493.241
LG	175727.5968
Dayhoff+F	176047.1503
cpREV+F	176317.4454
JTT	176437.0655
rtREV	176561.3994
cpREV	177483.4616
Dayhoff	178165.5949
mtREV24+F	179148.4518
mtREV24+I	181616.2831
mtREV24	185846.0653

Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. TR (General Time Reversible), JTT (Jones-Taylor-Thornton), rtREV (General Reverse Transcriptase), cpREV (General Reversible Chloroplast), mtREV24 (General Reversible Mitochondrial).

Supplementary Table S4. Maximum Likelihood fits of 24 different nucleotide substitution models for the partial *N* gene sequence (1146 nucleotides) of BNRV1 isolates

Model	BIC
T92	4374.103996
T92+I	4381.052957
T92+G	4381.797481
HKY	4390.201281
TN93	4390.444855
T92+G+I	4393.409097
TN93+G	4393.567613
TN93+I	4396.932376
HKY+I	4397.042677
HKY+G	4397.603887
K2	4399.082511
TN93+G+I	4403.345002
K2+I	4406.068138
K2+G	4406.832728
HKY+G+I	4408.920443
GTR	4412.563118
K2+G+I	4417.067375
GTR+G	4417.824703
GTR+I	4424.226742
GTR+G+I	4427.599189
JC	4482.22069
JC+I	4489.651389
JC+G	4489.674157
JC+G+I	4498.797033

Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. TR (General Time Reversible), HKY (Hasegawa-Kishino-Yano), TN93 (Tamura-Nei), T92 (Tamura 3-parameter), K2 (Kimura 2-parameter), JC (Jukes-Cantor)

Supplementary Table S5. Pairwise sequence identity (%) between BNRV1 and the other most relevant betanucleorhabdoviruses

Virus	GenBank accession no.	Genome	*N*		P		P3		*M*		*G*		*L*	
			nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
tomato betanucleorhabdovirus 2	OP441765	64.8	71.6	82.1	63.1	56.0	68.2	73.8	57.0	59.7	65.0	67.0	66.3	69.9
sambucus betanucleorhabdovirus 4	PP711314	64.7	74.4	84.5	60.1	56.0	69.0	74.8	61.9	58.7	65.7	65.2	66.0	68.1
sambucus betanucleorhabdovirus 1	PP711309	64.3	72.6	84.5	57.6	53.8	68.5	73.2	61.6	58.3	65.0	63.9	65.6	69.3
tomato betanucleorhabdovirus 1	OL472119	64.2	74.7	83.4	59.9	54.5	66.8	70.5	63.7	57.7	65.1	65.6	64.8	69.9
sambucus betanucleorhabdovirus 3	PP711316	64.1	72.5	83.8	60.0	53.0	70.8	74.8	61.4	56.4	65.3	65.4	64.6	67.7
sambucus betanucleorhabdovirus 2	PP711321	63.8	74.4	82.9	59.8	54.5	69.8	75.7	60.2	57.7	64.1	62.8	65.0	68.4
sambucus betanucleorhabdovirus 5	PP711313	63.5	73.4	83.4	58.9	54.0	70.1	73.5	61.3	55.1	63.5	64.4	64.8	68.7
datura yellow vein virus	KM823531	53.9	61.0	58.5	49.2	34.8	53.8	42.4	50.6	36.3	57.1	51.4	54.9	50.3
bacopa monnieri virus 2	BK014480	53.5	61.4	61.0	51.4	33.0	50.8	42.0	51.3	34.6	56.7	50.8	55.7	50.3

Supplementary Table S6. Results of BNRV1 detection and incidence in major sugar beet production regions of Iran during the 2024 growing season

Province	Region	Fields surveyed no.	Leaf samples no. (Infected leaf samples no.)	Virus incidence (%)
North Khorasan	Shirvan	1	7(0)	0
North Khorasan	Faruj	1	8(1)	12.5
Khorasan Razavi	Fariman	1	6(2)	33.3
Khorasan Razavi	Sefid Sang	1	5(1)	20
Khorasan Razavi	Torbate Jam	1	7(1)	14.2
Khorasan Razavi	Mashhad	2	10(0)	0
Khorasan Razavi	Torbat Heydarieh	1	5(0)	0
Khorasan Razavi	Joveyn	1	6(0)	0
Khorasan Razavi	Taybad	1	5(0)	0
South Khorasan	Nimboluk	1	6(0)	0
South Khorasan	Khezri Dasht Beyaz	1	5(0)	0
Lorestan	Azna	1	5(0)	0
Hamadan	Nahavand	1	5(0)	0
Kermanshah	Kermanshah	1	5(0)	0
Total		15	85(5)	5.8

Supplementary Table S7. Natural host range of BNRV1

Family	Species	Common name
Chenopodiaceae	*Chenopodium album*	Fat Hen
Malvaceae	*Malva neglecta*	Dwarf Mallow
Asteraceae	*Carthamus oxyacanthus*	Wild Safflower

Supplementary Table S8. Characteristics of BNRV1 isolates used in this study

Isolate	Host	Location	GenBank accession no.
Iran/Fariman/sugar beet-2	Sugar beet	Iran- Khorasan Razavi-Fariman	PQ222212
Sefid Sang	Sugar beet	Iran- Khorasan Razavi- Sefid Sang	PQ222213
Torbate Jam	Sugar beet	Iran- Khorasan Razavi- Torbate Jam	PQ227355
Iran/Faruj/sugar beet	Sugar beet	Iran- North Khorasan - Faruj	PQ222214
Iran/Faruj/Fat Hen	Fat Hen	Iran- North Khorasan - Faruj	PQ227356
Iran/Faruj/Dwarf Mallow	Dwarf Mallow	Iran- North Khorasan - Faruj	PQ227357
Iran/Faruj/Wild Safflower	Wild Safflower	Iran- North Khorasan - Faruj	PQ227358
Iran/Fariman/sugar beet-1	Sugar beet	Iran- Khorasan Razavi-Fariman	PV255967