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Research Article

Incidence and molecular characterization of *Beet virus Q* in sugar beet production areas of Iran based on coat protein gene

Zohreh Moradi[1*] and Mohsen Mehrvar[2]

1. Department of Plant Pathology, Faculty of Crop Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.
2. Department of Plant Pathology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

Abstract: *Beet virus Q* (BVQ) is a soilborne pomovirus (family *Virgaviridae*) associated with rhizomania syndrome in sugar beet. In the present study, BVQ was investigated in sugar beet farms of 12 provinces in Iran by RT-PCR. Thirty-five out of 214 root samples resulted in a positive reaction to BVQ (16.3%). Moreover, 501-bp- long fragments of the coat protein gene of 11 Iranian isolates were purified, cloned, and sequenced. Phylogenetic analysis using the 501 bp fragment of 17 BVQ isolates (11 from this study and six retrieved from GenBank) showed that all isolates clustered in two main groups. Iranian isolates belonged to group I alongside isolates from France (AJ810289) and Germany (AJ223597). Iranian isolates shared 98.80–100% nucleotide sequence identity (98.19–100% amino acid identity) and 97.21–99.60% nucleotide sequence identity (96.99–99.40% amino acid identity) with the corresponding sequence of six other BVQ isolates available in the GenBank. Iranian isolates displayed the highest nucleotide and amino acid sequence identities of 98.80-99.60% and 98.19-99.40%, respectively, with the French isolate FP71 (AJ810289). To our knowledge, this is the first molecular characterization of BVQ in Iran. This information can be used in plant breeding to obtain virus-resistant plants.

Keywords: Soilborne virus, Sugar beet, RT-PCR, *Beet virus Q*

Introduction

Sugar beet *Beta vulgaris* L. is affected by several soilborne viruses such as *Beet necrotic yellow vein virus* (BNYVV; *Benyvirus*; *Benyviridae*), *Beet soilborne virus* (BSBV), *Beet virus Q* (BVQ) (*Pomovirus*; *Virgaviridae*), which are transmitted by the plasmodiophorid vector *Polymyxa betae*, and finally *Beet black scorch virus* (BBSV; *Betanecrovirus*; *Tombusviridae*) along with its satellite which is vectored by zoospores of the chytrid *Olpidium brassicae* (King *et al*., 2012). These soilborne viruses are the biggest threat to the Iranian sugar beet industry and cause significant damages to the sugar beet crop annually (Farzadfar *et al*., 2007; Mehrvar 2009). BVQ was first reported as one of the two BSBV serotypes (Lesemann *et al*., 1989; Barbarossa *et al*., 1992) and then described as a separate member of the genus *Pomovirus* (Koenig *et al*., 1998). BVQ has a tripartite, positive-sense single-stranded RNA genome encapsidated separately in rod-shaped particles. RNA-1, c. 6 kb, encodes 149-KDa (P149) and 207-KDa (P207) proteins which contain the motifs characteristic of proteins involved in viral RNA replication. These two proteins are N-terminally overlapping in the same reading frame, the longer one produced by readthrough of the shorter protein. The P149 contains

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* Corresponding author: z.moradi@sanru.ac.ir
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methyltransferase and helicase motifs, while the RT domain (P207) includes the GDD RNA-dependent RNA polymerase (RdRp) motif. RNA-2 is c. 2.9 kb, encodes the coat protein (19 kD), the coat protein readthrough (RT) (85 KD), P9 (9 kDa), and P18 (18 kDa) proteins (Koenig *et al*., 1998). In other words, the RT coding sequence of BVQ is followed by two additional ORFs for 9 and 18 kDa proteins. It is also worth noting that the sequence coding for the RT domain of BVQ RNA2 is shorter than other pomoviruses such as BSBV (Crutzen *et al*., 2009). RNA-3 is c. 2.5 kb, encodes three-movement proteins (P48, P13, P20), organized into a triple gene block (TGB) responsible for the virus cell-to-cell movement. The 5' UTR of RNAs are capped, and the 3'-end UTR forms a tRNA-like structure that contains an anticodon for valine (Koenig *et al*., 1998; Mehrvar 2009). BVQ was first detected in Iran by serological and reverse transcription-polymerase chain reaction (RT-PCR) methods (Farzadfar *et al*., 2005), and no sequence data is available for the Iranian isolates.

This virus was commonly associated with BSBV and BNYVV in the field (Mehrvar 2009; Meunier *et al*., 2003; Erkan and Kutluk Yilmaz, 2017). Sugar beet in Iran is under high pressure of rhizomania-associated viruses. To date, the only method to manage these viruses has been the use of sugar beet virus-tolerant cultivars that are not available for some viruses. The objective of this study was to molecularly characterize the BVQ distribution in the major sugar beet cultivation areas in Iran and determine the evolutionary relationship of Iranian isolates with those of other BVQ isolates from other countries.

Materials and Methods

Virus isolates

During the 2009-2015 growing seasons, more than 40 fields showing rhizomania-like and yellowing symptoms on sugar beet leaves were investigated for the presence of BVQ. In addition, 214 samples of sugar beet roots were collected from different parts of 12 provinces (Table 1).

Table 1 Occurrence of *Beet virus Q* in different sugar beet fields in the provinces surveyed.

Province	Region	No. of infected / collected samples
Razavi Khorasan	Mashhad	1/9
	Sabzevar	1/8
	Chenaran	1/21
	Quchan	0/2
	Neyshabur	3/12
	Fariman	3/17
	Robat Sefid	0/1
	Sarakhs (Eslam Qaleh)	0/1
	Torbat-e Jam	1/11
	Torbat-e Heydarieh	1/6
	Jolgeh Rokh	5/24
North Khorasan	Shirvan	0/8
	Bojnord	0/6
	Naqab	0/1
Fars	Marvdasht	0/3
	Beyza	0/3
	Zarqan	0/2
	Ramjerd	0/2
Ilam	Shirvan e Chardavol	0/3
Semnan	Shahrud	0/3
Hamadan	Malayer	1/3
	Nahavand	1/2
	Firuzan	0/2
	Asadabad	0/3
West Azerbaijan	Urmia	0/2
	Bukan	1/3
	Miandoab	2/4
	Salmas	1/2
	Mohammadyar	0/1
	Khoy	0/2
East Azerbaijan	Azarshahr	2/4
Ardabil	Parsabad	1/11
	Aghalam	0/1
	5 Km before the city from Miane	0/1
Kermanshah	Eslamabad-e Gharb	2/5
	Bisotun	2/5
	Abasabad	0/1
	Rostam Abad	1/1
	Kangavar	0/2
	Sirduleh	0/1
	Kermanshah	2/5
Kerman	Bardsir	0/5
Qazvin	Buin Zahra	2/4
	pour Yousufian Lob Field	1/1
Total		35/214

RNA extraction, RT-PCR, cloning, and sequencing

According to the manufacturer's protocol, total RNA was extracted from sugar beet rootlets

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using the SV Total RNA Isolation Kit (Promega, Madison, WI, USA).

The BVQ infection of samples was tested by RT-PCR using the primer pair 5′-CCATGGTTGATCCAAGATATGA-3′ and 5′-CTATGAGCCGGTCCACTTCAAT-3′ designed to specifically amplify the entire CP gene (501-bp) within the BVQ RNA2. The reverse transcription (RT) reaction was performed: 2 µl of reverse primer (10 pmol) and 2 µl of RNA sample were added to 6.5 µl of DEPC-treated water. The mixture was incubated at 65 °C for 10 minutes and chilled for 5 min on ice. Then, 3.5 µl of DEPC treated water, 4 µl of 5X MMLV RT buffer, 2 µl of dNTPs mix (10 mm), and 0.25 µl of MMLV (200 U/µl) reverse transcriptase (Promega) was added to the mixture. The RT reaction was carried out at 42 °C for 60 minutes. PCR was performed in a total volume of 25 µl as follows: 3 µl of cDNA, 13 µl of DEPC-treated water, 5 µl of 5X GoTaq polymerase buffer, 1.25 µl of MgCl2 (25 mM), 1 µl of each forward and reverse primers (10 pmol), 0.5 µl of a dNTPs mix (10 mM), and 0.25 µl of GoTaq polymerase (5 U/µl) (Promega, Madison, USA). The cycle parameters in the PCR program were as follows: 95 °C for 3 min; 35 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min; a final extension at 72 °C for 7 min. The PCR products were analyzed on 1% agarose gel, and DNA was then eluted from the agarose gel using the Qiaquick Gel Extraction Kit (Qiagen, Hilden, Germany). According to the manufacturer's procedure, the purified products were ligated into the pGEM-T vector (Promega, WI, USA). Ligation products were transferred into the *Escherichia coli* strain JM109. The positive clones were confirmed by colony-PCR using M13-forward and reverse primers. Recombinant plasmids were purified using a Qiaquick Plasmid Miniprep Kit (Qiagen, Hilden, Germany) and sequenced in both directions (Macrogen Inc. Seoul, South Korea).

Multiple sequence alignment and phylogenetic analysis

The multiple sequence alignments of nucleotide and deduced amino acid sequences of the CP coding region from 17 BVQ isolates, including 11 Iranian isolates from this study and six isolates from other countries (available in GenBank; Table 1), were generated by Clustal W assembled in the MEGAX package (Kumar *et al*., 2018). The pairwise nucleotide and amino acid sequence identity scores were represented as color-coded blocks using SDT v.1.2 software (Muhire *et al*., 2014). Phylogenetic trees for coat protein genes of all isolates were constructed by the maximum likelihood (ML) method using MEGAX (Fig. 1). *Potato mop-top virus*-P1 (GenBank accession no. GQ503252) was used as an outgroup. Tree branches were bootstrapped with 1,000 replications. The genetic variability of the CP gene for the BVQ population was identified by a single nucleotide polymorphism (SNP) analysis algorithm implemented in the Geneious Prime v. 2019.1.3 (Biomatters).

Results

Using specific primers, the presence of BVQ was confirmed in 35 sugar beets out of 214 tested samples collected from different parts of Iran (Table 1). The PCR amplicon (~501 bp in length) was obtained, which generated 166 amino acids. Of 35 positive samples, 11 isolates were selected based on their geographical locations for cloning and sequencing. Complete CP nucleotide sequence of 11 BVQ isolates from different areas of Iran was determined and deposited into the GenBank database under accession numbers MW798253 to MW798263. The CP gene was 501 nucleotides in length for all Iranian BVQ isolates. The CP sequence of these 11 Iranian BVQ isolates and six isolates from around the world (available in GenBank; Table 2) was compared to provide insights into the genetic diversity and phylogenic relationships of BVQ populations in the world. No significant variation was detected for the CP nucleotide sequence of Iranian BVQ isolates. Among all Iranian isolates, there were seven mutations at nucleotide positions of 28, 69, 260, 261, 345, 422, and 425 of the BVQ CP gene. However, there were only four changes in CP amino acid sequences of 11 Iranian isolates, including positions 10, 87, 141, and 142. In

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comparison of all 17 BVQ isolates, there were 16 mutations at nucleotide positions 28, 30, 39, 69, 93, 102, 141, 234, 260, 261, 306, 343, 345, 422, 425, and 429 of the CP gene. Nevertheless, in the CP amino acid sequence of the 17 BVQ isolates, seven changes were detected. These results showed that most nucleotide substitutions were silent, and the CP gene is conserved, which may be due to constraints imposed by vector or host plant factors. Sequence analysis of the CP gene for the BVQ population (including 17 isolates) identified two single nucleotide polymorphisms (SNPs). An SNP C→A (35.30% variant frequency) occurred in codon position 28 and represented the only polymorphism that led to a change in the encoded amino acid from proline (P) to threonine (T). In addition, a synonymous SNP T→C (35.30% variant frequency) occurred in codon position 102, which did not result in a change of amino acid in the protein.

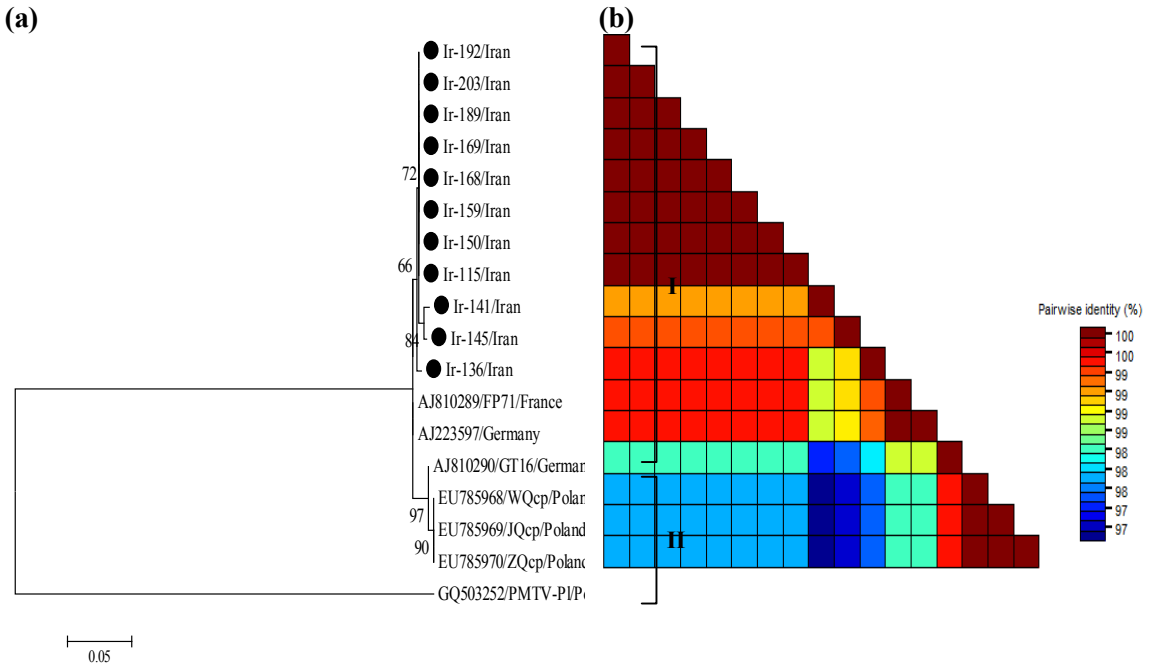

Figure 1 (a) Maximum likelihood phylogenetic tree of the 17 *Beet virus Q* isolates based on coat protein nucleotide sequences. Nodal support was evaluated by 1,000 bootstrap replications. Multiple sequence alignments were generated by ClustalW program assembled in the MEGAX package. Iranian isolates obtained in this study are without GenBank codes, in marked, but other sequences are indicated in the tree by accession numbers/isolate name/geographical origin of the collection. *Potato mop-top virus* (GQ503252) served as an outgroup (b) Nucleotide sequence identity matrix of the 17 *Beet virus Q* isolates.

Phylogenetic tree based on the nucleotide sequences of the coat protein showed that all isolates could be placed into two main groups I and II. Iranian isolates fell into group I, with isolates from France (FP71, AJ810289) and Germany (AJ223597). One German isolate (GT16, AJ810290) and three Polish isolates (WQcp, JQcp, and ZQcp) were placed in group II (Fig. 1). A phylogenetic tree based on CP amino acid sequences of the identical isolates revealed clustering into two phylogroups (Fig. 2). The identity of CP nucleotide (nt) and amino acid (aa) sequences of all BVQ isolates ranged from 97.21-100% and 96.99-100%, respectively. Iranian isolates shared 98.80–100% and 98.19–100% nt and aa sequence identity, respectively, and 97.21–99.6% and 96.99–99.40% nt and aa sequence identity with the corresponding sequence of the six other BVQ isolates obtained from GenBank. The Iranian isolates displayed the highest nt and

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aa sequence identities of 98.8-99.6% and 98.19-99.40%, respectively, with the French isolate FP71 (AJ810289). In addition, Iranian isolates shared the lowest nt sequence identities of 97.21–98.0% with three Polish isolates, while they shared the lowest aa sequence identities of 96.99–98.19% with a German isolate (AJ223597).

Table 2 Beet virus Q isolates used in this study.

Accession number	Isolate	Host	Origin	Reference
MW798253	Ir-115	Sugar beet	Miandoab, West Azerbaijan, Iran	This study
MW798254	Ir-136	Sugar beet	Parsabad-Moghan, Ardabil, Iran	This study
MW798255	Ir-141	Sugar beet	Eslamabad-e Gharb, Kermanshah, Iran	This study
MW798256	Ir-145	Sugar beet	Rostamabad, Kermanshah, Iran	This study
MW798257	Ir-150	Sugar beet	Central District, Kermanshah, Iran	This study
MW798258	Ir-159	Sugar beet	Azarshahr and Malekan, East Azerbaijan, Iran	This study
MW798259	Ir-168	Sugar beet	Qazvin, Iran	This study
MW798260	Ir-169	Sugar beet	Buin Zahra, Qazvin, Iran	This study
MW798261	Ir-189	Sugar beet	Jolgeh Rokh District-1, Razavi Khorasan, Iran	This study
MW798262	Ir-192	Sugar beet	Jolgeh Rokh District-2, Razavi Khorasan, Iran	This study
MW798263	Ir-203	Sugar beet	Jolgeh Rokh-Nazarabad, Razavi Khorasan, Iran	This study
AJ810290	GT16	Sugar beet	Thurnhof, Germany	Lennefors *et al*., 2005
AJ810289	FP71	Sugar beet	Pithiviers, France	Lennefors *et al*., 2005
EU785968	WQcp	Sugar beet	Poland	Borodynko *et al*., 2009b
EU785969	JQcp	Sugar beet	Poland	Borodynko *et al*., 2009b
EU785970	ZQcp	Sugar beet	Poland	Borodynko *et al*., 2009b
AJ223597	-	Sugar beet	Braunschweig, Germany	Koenig *et al*., 1998

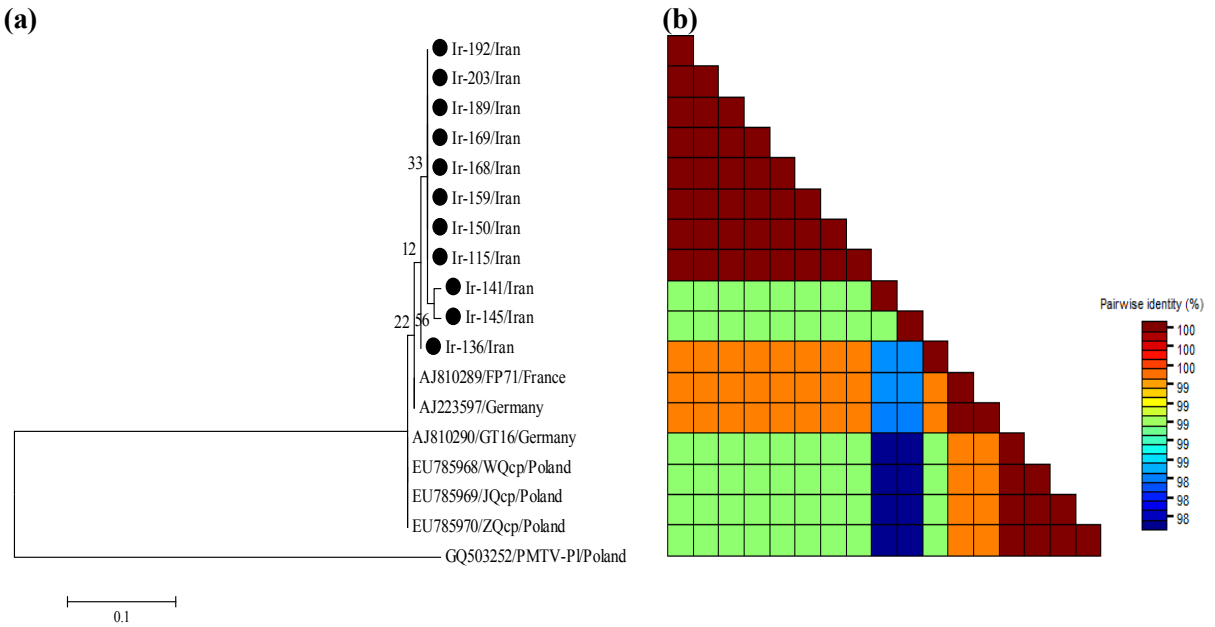

Figure 2 (a) Maximum likelihood phylogenetic tree of the 17 *Beet virus Q* isolates based on coat protein amino acid sequences (identified by their GenBank accession numbers/isolate name/ geographical origin of the figure). Iranian isolates obtained in this study are marked. Phylogenic tree was constructed using MEGAX with 1000 bootstrap replications. *Potato mop-top virus* (GQ503252) served as an outgroup. (b) The graphical representation of pairwise identities is based on SDT MUSCLE alignment.

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Discussion

Soilborne sugar beet viruses have been reported as widely distributed in the country and accompanied mainly by others (Farzadfar *et al*., 2007; Mehrvar 2009). In this work, RT-PCR results showed a relatively low prevalence (16.3%) of BVQ in the sugar beet-growing regions of Iran. The BVQ infection was found in Razavi Khorasan, Hamadan, West Azerbaijan, East Azerbaijan, Ardabil, Kermanshah, and Qazvin provinces. In contrast, BVQ was not detected in North Khorasan, Fars, Semnan, Ilam, and Kerman.

Information on the diversity of the viral CP helps to understand how the virus has adapted to hosts and vectors, which can effectively find the best disease management strategies. In this study, the nucleotide sequence of the CP gene of 11 BVQ isolates was presented for the first time in Iran. Comparison of Iranian with other GenBank sequences revealed a high level of nucleotide identity. Genetic bottlenecks have been shown in some plant virus populations during virus movement and transmission from plant to plant by vectors (French and Stenger, 2003; Ali *et al*., 2006; Borodynko *et al*., 2009a). Hence, the low variability observed in BVQ isolates could be explained, at least in part, by the genetic bottlenecks imposed by *P. betae*. According to the previous studies (Erkan and Kutluk Yilmaz, 2017) and this research (data not shown), BVQ has never been reported to occur alone and has always been in co-infections with other soilborne viruses as BNYVV, BSBV, or BBSV. Along these lines, studies on different interactions between each virus species infecting sugar beet are needed. The results of this study can help in breeding genetically virus-resistant plants, as well as the development of improved diagnostic and disease management strategies.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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بررسی وقوع و تعیین خصوصیات مولکولی ویروس کیو چغندر در مناطق تولید چغندرقند ایران براساس ژن پروتئین پوششی

زهره مرادی[1*] و محسن مهرور[2]

۱- گروه گیاه‌پزشکی، دانشکده علوم زراعی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ایران.
۲- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه فردوسی مشهد، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: z.moradi@sanru.ac.ir
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چکیده: ویروس کیو چغندر (BVQ)، یک پوموویروس خاکزاد (از خانواده *Virgaviridae*) است که با سندرم رایزومانیا در چغندرقند ارتباط دارد. در مطالعه حاضر، حضور BVQ با استفاده از آزمون RT-PCR در مزارع چغندرقند مربوط به ۱۲ استان ایران بررسی شد. ۳۵ نمونه از ۲۱۴ نمونه ریشه‌ای نسبت به BVQ واکنش مثبت نشان دادند (۱۶/۳ درصد). علاوه بر این، قطعه ۵۰۱ جفت‌بازی ژن کدکننده پروتئین پوششی مربوط به ۱۱ جدایه ایرانی پس از خالص‌سازی، کلون و توالی‌یابی گردید. آنالیز فیلوژنتیک با استفاده از ۵۰۱ نوکلئوتید مربوط به ۱۷ جدایه BVQ (شامل ۱۱ جدایه حاصل از این بررسی و شش جدایه برگرفته از بانک ژن) نشان داد که همه جدایه‌ها در دو گروه (I و II) دسته‌بندی می‌شوند. جدایه‌های ایرانی به‌همراه جدایه فرانسوی (AJ810289) و آلمانی (AJ223597) در گروه I قرار گرفتند. تشابه نوکلئوتیدی جدایه‌های ایرانی با یک‌دیگر ۹۸/۸۰-۱۰۰ درصد (۹۸/۱۹–۱۰۰ درصد تشابه آمینواسیدی)، و با شش جدایه دیگر موجود در بانک ژن ۹۷/۲۱-۹۹/۶۰ درصد (۹۶/۹۹-۹۹/۴۰ درصد تشابه آمینواسیدی) بود. جدایه‌های ایرانی دارای بیش‌ترین تشابه نوکلئوتیدی (۹۸/۸۰-۹۹/۶۰ درصد) و آمینواسیدی (۹۸/۱۹-۹۹/۴۰ درصد) با جدایه فرانسوی FP71 (AJ810289) بودند. این اولین گزارش از تعیین خصوصیات مولکولی BVQ در ایران است. از نتایج حاصل از این تحقیق می‌توان در اصلاح ژنتیکی برای تولید گیاهان مقاوم به ویروس استفاده نمود.

واژگان کلیدی: ویروس خاکزاد، چغندرقند، RT-PCR، ویروس کیو چغندر

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