



Complete Genomic Characterization of Two Beet Soil-Borne Virus Isolates from Turkey: Implications of Comparative Analysis of Genome Sequences

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Sugar beet (*Beta vulgaris* L.) is known as a key product for agriculture in several countries across the world. Beet soil-borne virus (BSBV) triggers substantial economic damages to sugar beet by reducing the quantity of the yield and quality of the beet sugars. We conducted the present study to report the complete genome sequences of two BSBV isolates in Turkey for the first time. The genome organization was identical to those previously established BSBV isolates. The tripartite genome of BSBV-TR1 and -TR3 comprised a 5,835-nucleotide (nt) RNA1, a 3,454-nt RNA2, and a 3,005-nt RNA3 segment. According to sequence identity analyses, Turkish isolates were most closely related to the BSBV isolate reported from Iran (97.83-98.77% nt identity). The BSBV isolates worldwide ($n = 9$) were phylogenetically classified into five (RNA-coat protein read through gene [CPRT], TGB1, and TGB2 segments), four (RNA-rep), or three (TGB3) lineages. In genetic analysis, the TGB3 revealed more genetic variability ($Pi = 0.034$) compared with other regions. Population selection analysis revealed that most of the codons were generally under negative selection or

neutral evolution in the BSBV isolates studied. However, positive selection was detected at codon 135 in the TGB1, which could be an adaptation in order to facilitate the movement and overcome the host plant resistance genes. We expect that the information on genome properties and genetic variability of BSBV, particularly in TGB3, TGB1, and CPRT genes, assist in developing effective control measures in order to prevent severe losses and make amendments in management strategies.

Keywords : beet soil-borne virus, complete genome, phylogenetic analysis, selection pressure, soil-borne virus

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Sugar beet (*Beta vulgaris* L.) is one of the widely grown crops worldwide, which is grown commercially for sugar production. On top of excellent nutritional values, it provides over 25% of the world's sugar supply (Rush et al., 2006) and can be consumed in various forms. Sugar beet is one of the most important commercial crops in Turkey, grown over 307,067 ha, with an annual production of about 18.9 million tons (Food and Agriculture Organization of the United Nations, 2018). From viral sugar beet disease, the soil-borne viruses are the most dangerous threat to Turkish sugar beet industry, specifically beet necrotic yellow vein virus (BNYVV, the genus *Benyvirus*) the causal agent of rhizomania disease, and beet soil-borne virus (BSBV) which is present in most of the sugar beet cultivation areas. It could result into significant damages to sugar beet crop annually (Kutluk Yilmaz et al., 2004, 2016). BSBV is a member of the genus *Pomovirus* (the family *Virgaviridae*)

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(Adams et al., 2009), for the first time reported in England in 1982 (Henry et al., 1986). It could cause yield reduction up to 70% depending on the virus isolate and the sugar beet variety (Koenig et al., 2000; Prillwitz and Schlösser, 1992). Like other pomoviruses, BSBV genome is tripartite, consists of three positive-sense single-stranded RNA, packaged separately in three rod-shaped particles of trimodal length (Koenig and Loss, 1997; Koenig et al., 1996, 1997). RNA-1, c. 5.8 kb, encodes 145-kDa (P145) and 204-kDa (P204) 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 being produced by readthrough of the shorter protein. RNA-2 is c. 3.5 kb, encodes 19 kDa coat protein (CP), and a putative 104 kDa read-through domain that is thought to be involved in the transmission by *Polymyxa betae* (Torrance, 2008). RNA-3 is c. 3.0 kb, encodes three movement proteins (P48, P13, and P22), organized into a triple gene block (TGB) responsible for the virus cell-to-cell movement (Crutzen et al., 2009). All three RNAs are flanked by untranslated regions. The 5'-untranslated region (UTR) of RNAs are capped and the 3'-end UTR forms a tRNA-like structure that is highly conserved with only seven nucleotide variations in the terminal 46 nucleotide (nt) (Koenig et al., 1998; Torrance et al., 2011). It is probably involved in viral functions including replication, as indicated by the varying valylatability of such extremities (Dreher, 2009; Mehrvar, 2009).

BSBV has a narrow host range in the nature restricted to *Beta vulgaris* and is mechanically transmissible to members of the *Chenopodiaceae* (Torrance, 2008). The virus is vectored by the biotrophic plasmodiophorid *Polymyxa betae* (Keskin, 1964; McGrann et al., 2009). To date, this virus has been reported from sugar beet-growing areas throughout the world from the USA, western and eastern Europe, Syria, Sweden, China, Japan, Iran, and Turkey (Borodynko et al., 2006; Farzadfar et al., 2007; Kutluk Yilmaz and Sokmen, 2010; Lesemann et al., 1989; Lindsten, 1989; Meunier et al., 2000, 2003; Mouhanna et al., 2002; Ratti et al., 2005; Verhoyen et al., 1987; Wang et al., 2008). Complete viral genome sequence data have provided new insights into the relationship among isolates from different areas, diversity and evolutionary history, and the possible origins of the virus (Moradi et al., 2016). The knowledge about genome sequence and structure in the study of sugar beet soil-borne viruses would help develop novel strategies for its control in agriculture. BSBV is known to be present in most of major sugar beet-growing areas of Turkey (Kutluk Yilmaz and Sokmen, 2010; Kutluk Yilmaz et al., 2004, 2016; Meunier et al., 2003). However,

the complete genome sequences of BSBV Turkish isolates have not yet been determined. For the first time, the current study aimed to obtain the complete sequence of the BSBV in Turkey, their taxonomic status, and phylogenetic analysis in comparison to other isolates in GenBank.

Materials and Methods

Virus isolates. The soil samples were collected in 2009-2010 from sugar beet fields in Tokat province in Turkey and stored at 4°C. The sampling was performed in the symptomatic fields showing irregular patches of light green or yellow plants. Sugar beet (*B. vulgaris* cultivar Cadyx) was used as a bait plant. After one month, bait plants were harvested and their root sap was tested for the presence of the BSBV, BNYVV, beet virus Q (BVQ), and *P. betae* employing multiplex reverse transcription polymerase chain reaction (RT-PCR) developed by Meunier et al. (2003). All the samples were tested for the presence of BSBV utilizing RT-PCR with primers by Weiland et al. (2007). The plant's rootlets were washed and ground in 0.01 M sodium phosphate buffer, pH 7.0. The extract was also mechanically inoculated in the upper side of *Chenopodium quinoa* leaves, a local lesion host of BSBV. BSBV was purified through three passages of single-lesion isolation on the leaves of *C. quinoa*.

Extraction of total RNA, PCR amplification, cloning, and sequencing. Total RNA was extracted from sugar beet roots using the SV Total RNA Extraction Kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. The Expand Reverse Transcriptase kit (Roche, Mannheim, Germany), which includes a high-fidelity, thermostable polymerase, was used to generate complete cDNA fragments from each RNA using specific primers described by Mehrvar (2009). For this, 8.5 µl of diethyl pyrocarbonate (DEPC)-treated water was mixed with 1 µl of antisense primer and 1 µl of purified RNA, heated at 65°C for 10 min and chilled on ice. The mixture was added to 9.5 µl of mixed buffer (including 0.5 µl of DEPC-treated water, 4 µl of reverse transcriptase [RT] buffer, 2 µl of DTT, 2 µl of dNTPs, and 1 µl RT enzyme) and incubated at 43°C for 60 min.

PCR was done using long expand PCR kit (Roche) as follows: 2.5 µl of RT product was added to 47.5 µl of mixed buffer (38.9 µl of DEPC-treated water, 4 µl buffer 1, 1.75 µl dNTPs, 0.8 µl of each forward or reverse primers, and 0.75 µl of long expand polymerase). The PCR thermal profile was 95°C for 2 min (1 cycle), followed by 10 cycles in 94°C for 10 s, 55°C for 10 s, 68°C for 2 min, 28 cycles

in 94°C for 15 s, 55°C for 30 s, 68°C for 2 min+20 s cycle elongation for each successive cycle and finally 1 cycle in 68°C for 7 min. The long expand PCR products were separated by electrophoresis on 1% agarose gel containing 0.5 µg/ml DNA Green Viewer and visualized under UV irradiation. The PCR products of the expected size were extracted and purified from the agarose gel using the Qiaquick Gel Extraction Kit (Qiagen, Hilden, Germany). The purified products were ligated into the pGEM-T vector (Promega) according to the manufacturer's procedure. Ligation products were transferred into the *Escherichia coli* strain JM109. The positive clones were confirmed by colony-PCR using M13-forward and reverse primers and the virus-specific primers. Plasmid DNA from recombinant clones was purified using a Qiaquick Plasmid Miniprep Kit (Qiagen) and then was sequenced in both directions (Macrogen Inc., Seoul, Korea) by primer walking sequencing method. It is also worth noting that, the different sets of primers for sequencing of the full-length genome of BSBV were designed according to an Iranian BSBV sequence in the database (Nyshabour isolate) (Crutzen et al., 2009).

Sequence alignment and phylogenetic analysis. The complete sequences of 9 BSBV isolates (two from this study and seven from GenBank) (Table 1) were analyzed to determine the phylogeny, genotype composition, and possible recombination events. The sequence comparisons were carried out using the BLAST algorithm (BLASTn and BLASTp) at the National Center for Biotechnology Information (NCBI). Open reading frames (ORFs) were identified using the NCBI ORF Finder program ([http://](http://www.bioinformatics.org/sms2/orf_find.html)

www.bioinformatics.org/sms2/orf_find.html). Gene translation and prediction of deduced proteins were performed using ExPASy (<http://web.expasy.org/translate/>). Motif searches were conducted in PROSITE (<http://www.expasy.ch/>), and CDD (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) databases. RNA pseudoknot structure was predicted using the DotKnot program (Sperschneider and Datta, 2010). The nucleic acid sequences of Rep, coat protein read through (CPRT), TGB1, TGB2, and TGB3 genes were aligned by CLUSTALW using the IUB DNA weight matrix, whereas amino acid sequences were aligned by MUSCLE implemented in Geneious Prime version v. 2019.1.3 (Biomatters Ltd., Auckland, New Zealand). Comparison of nucleotide and amino acid sequence identities were carried out using the Geneious Prime 2019.1.3 (Biomatters Ltd.). Maximum-likelihood phylogenetic trees for each of the BSBV genes were constructed based on the best models in MEGAX (Kumar et al., 2018) with 1,000 bootstrap replicates. Genetic distances were estimated using the Kimura-2 parameter model in MEGAX and standard deviations were calculated by bootstrapping with 1,000 replicates. The nucleotide diversity (Pi) value, nonsynonymous substitution rate (dN), and synonymous substitution rate (dS) were calculated using the DnaSP6 (Rozas et al., 2017). Possible codons subjected to positive selection were identified utilizing three different codon-based maximum-likelihood algorithms, single likelihood ancestor counting, fixed effects likelihood (FEL), and mixed effects model of evolution (MEME) within the HYPHY software package as implemented in Datamonkey server (<http://www.datamonkey.org>) (Kosakovsky Pond and Frost, 2005) with

Table 1. Characteristics of beet soil-borne virus isolates used in this study

Isolate	Accession no.			Host	Country	Reference
	RNA1	RNA2	RNA3			
TR1	MN087411	MN087412	MN087413	Beta vulgaris	Turkey	This study
TR3	MN087414	MN087415	MN087416	Beta vulgaris	Turkey	This study
IR-Neyshabur	FN386612	FN386613	FN386614	Beta vulgaris	Iran	Crutzen et al. (2009)
JKI 1825732	MK731956	MK731955	MK731954	Beta vulgaris	Germany	Gaafar and Ziebell (unpublished)
PL	FJ971717	FJ971718	FJ971719	Beta vulgaris	Poland	Borodynko et al. (2009)
XJ	EF545139	EF545141	EF545143	Beta vulgaris	China: Xinjiang	Wang et al. (2008)
IM	EF545138	EF545140	EF545142	Beta vulgaris	China: Inner Mongolia	Wang et al. (2008)
Brazilian	MH106714	MH106715	MH106716	Beta vulgaris	Brazil	Camelo-Garcia et al. (2019)
Ahlum	Z97873	U64512	Z66493	Beta vulgaris	Germany	Koenig and Loss (1997), Koenig et al. (1996, 1997)

UTR, untranslated region; CPRT, coat protein read through; TGB, triple gene block.



Fig. 1. Necrotic local lesion symptoms on *Chenopodium quinoa* leaves inoculated with sap from beet soil-borne virus (BSBV)-TR1 infected plants.

significance levels set at $P = 0.1$. Recombination analysis was conducted using different algorithms implemented in the RDP4 v.4.16 (Martin et al., 2015).

Results

Virus detection by RT-PCR. Three out of four soil samples tested were positive for BSBV. The combination of BSBV with either BNYVV or BVQ was found in two samples. The characteristic symptoms of BSBV were also developed on inoculated leaves of *C. quinoa*. Necrotic local lesions were observed on inoculated leaves of *C. quinoa* seven days following the inoculation (Fig. 1). After retro-inoculation to *C. quinoa*, RT-PCR tests of the inoculated plants showing local lesions confirmed the presence of BSBV. Five plants were inoculated, and all the plants demonstrated the symptoms of virus infection.

Genomic features of Turkish BSBV isolates. The complete genomes of isolates, TR1 (GenBank accession nos. MN087411, MN087412, and MN087413 for RNA1, RNA2, and RNA3, respectively) and TR3 (GenBank accession nos. MN087414, MN087415, and MN087416 for RNAs 1, 2, and 3, respectively) were determined. The genome organization and base composition of both isolates were similar to other BSBV isolates (Borodynko et al., 2009; Camelo-Garcia et al., 2019; Crutzen et al., 2009; Koenig and Loss, 1997; Koenig et al., 1996, 1997; Wang et al., 2008).

RNA1 of BSBV-TR1 and -TR3 consisted of 5,835 nt and contained the putative viral replicase domain with conserved motifs methyltransferase (MetT), helicase (Hel) and RNA-dependent RNA polymerase (RdRP). A large ORF starts at AUG (130-132) and is interrupted at position 3,940 by a stop codon UAA which terminates the first coding

region (ORF1). It then continues in-frame for an additional 1,521 nt until it reaches another stop codon UAA at nt 5,464, which terminates the putative readthrough ORF2. An extra termination codon UAA was located at nt position 3,397-3,399 in TR3 isolate. On the other hand, the 5' and 3' context of the first stop codon UAA in Turkish isolates (CAATAACGGTGTGGGTCG, stop codon underlined) is identical to those of BSBV RNA1, confers leakiness on stop codon UAA. The typical MetT, hel, and RdRP motifs (Koonin and Dolja, 1993) were recognized in the N- and C-terminal parts of the putative 145 kDa protein and in the readthrough portion of ~204 kDa protein, respectively. In addition, blocks of highly conserved sequences were found in outside these motifs.

RNA2 of BSBV-TR1 and -TR3 consisted of 3,454 nt. The 19 kDa CP and a putative 104 kDa read-through domain were identified in RNA2. Several pomovirus-conserved motifs were identified in the CP sequences of both isolates; for example, we observed the conserved residues N₃₄WRE₃₇ in the N-terminal, the R₆₇F₆₈ and F₁₅₄E₁₅₅ motifs, which are typical for the coat proteins of rod-shaped plant viruses (Koonin and Dolja, 1993), in the central and carboxylic regions, and a conserved sequence E₁₂₁DSALNVAHQ_{L131} in the carboxylic end of CP.

The CP gene of BSBV RNA2, is terminated by stop codon UAG (position 875-877). It is followed in-frame by a 2,298 nt until it reaches a UAA stop codon at position 3176, which terminates the coat protein readthrough protein of BSBV. The context CAATCA flanking in the downstream of the leaky UAG codon conform to the optimal leaky UAG context (CAR±YYA) proposed by Skuzeski et al. (1991). The previously identified domains A, B, and C, and the conserved regions D, E, and F (Koenig et al., 1997), were found in the readthrough protein of BSBV.

RNA3 of BSBV-TR1 and -TR3 consisted of 3,005 nt

and contained a typical TGB. The three partially overlapping ORFs of TGB extend from 405-1,687, 1,675-2,025, and 1,889-2,461 nt, and have coding potential for proteins with molecular masses of 48, 13, and 22 kDa, respectively. In the central and C-terminal regions of 48 kDa protein, the highly conserved motifs A₁₇₄GSVT-GVPGSGKTTLLRK₁₉₁, N₁₉₉SVVILANERHKIRFT₂₁₄, V₂₃₆LLIDEYT₂₄₃, V₂₆₂VLFGDRAQ₂₇₀, Y₂₈₈SSVKSRRF₂₉₆, I₃₅₁ECSLVKDVQGNEYDSVSLFI₃₇₁, and D₃₈₁PELRS-VAFTRHRK₃₉₄ were found which were corresponding to the typical helicase motifs I, IA, II, III, VI, V, and VI, respectively, described by Koonin and Dolja (1993).

The highly conserved motifs M₁VRTNEIGARPKNKY-WP₁₆, S₄₁GDNIHKFSNGG₅₂, R₆₉AYNGSSSNN₇₈, Y₉₅AY-VQYRKPQCTVTCRGE₁₁₃, were identified in the second TGB-encoded protein.

The 5'-UTR of both isolates RNA1s contained the starting sequence GUA (U)_n, in agreement with the 5' ends of most *Pomovirus* RNAs (Koenig and Loss, 1997; Koenig et al., 1996, 1997; Mehrvar, 2009). In addition, RNAs-2 and -3 begin with GGU(A)₂(U)₄ and GGUA(U)₉, respectively. A series of hairpin structures were detected in the 5' ends of BSBV RNAs, as reported for BVQ (Koenig et al., 1998). The 3'-UTR of BSBV RNAs in both Turkish isolates contained the tRNA-like structures consisting of pseudoknot PKA, PKB, and PKC and stem-loops II-V, as reported by Koenig et al. (1998) for other BSBV and BVQ isolates. It

has also been shown that these hairpins, in the 5' and 3' end of RNAs, are of great importance for replication, symptom production (Hellendoorn et al., 1997), and efficient translation and translational enhancement (Leathers et al., 1993; Simon and Miller, 2013).

Sequence comparison and genetic variability. The comparison of the Turkish BSBV genome sequences to the seven other isolates revealed 96.38-98.64% for RNA1, 95.02-98.37% for RNA2, and 95.77-98.76% for RNA3 identity at the nucleotide level, and 97.18-98.93% (RNA1), 95.38-98.38% (RNA2), and 95.22-99.18% (RNA3) identity at the amino acid level. TR1 and TR3 are more similar to the Iranian BSBV isolate (with the nt identity from 97.83 to 98.77%). In a separate comparison, RNAs 1, 2, and 3 of BSBV-TR1 and -TR3 shared 99.90%, 98.44%, and 98.87% nucleotide sequence identities, respectively. The most conserved second TGB protein of TR1 shared 100% amino acid sequence identity with that of TR3.

The average identities of the nine BSBV genomes were 97.38% (96.38-99.89) for RNA1, 97.05% (95.02-98.81) for RNA2, and 96.92% (95.70-98.86%) for RNA3 at the nucleotide level, and 98.14% (97.18-99.83) (RNA1), 96.89% (94.62-98.92) (RNA2), and 96.74% (94.95-99.18) (RNA3) at the amino acid level, respectively (Table 2). The nucleotide diversity (Pi) of all the three RNAs among the nine isolates was below 8% (Supplementary Fig. 1). RNAs

Table 2. Pairwise sequence identities of nine beet soil-borne virus isolates at both the nucleotide and amino acid levels, either at genome scale or at individual gene scale

	Size (nt)	Size (aa)	Percent nucleotide identity (average) (%)	Percent amino acid identity (average) (%)
Genomic segment				
RNA1	5,835	-	96.38-99.89 (97.38)	97.18-99.83 (98.14)
RNA2	3,454	-	95.02-98.81 (97.05)	94.62-98.92 (96.89)
RNA3	3,005	-	95.7-98.86 (96.92)	94.95-99.18 (96.74)
Genomic region				
5'-UTR, RNA1	129	-	82.94-100 (92.66)	-
Rep, RNA1	5,337	1,778	96.12-99.88 (97.40)	97.18-99.83 (98.14)
3'-UTR, RNA1	394	-	94.40-100 (97.13)	-
5'-UTR, RNA2	382	-	94.50-99.47 (97.66)	-
CP	495	164	96.76-99.79 (98.23)	98.78-100 (99.59)
CPRT, RNA2	2,796	931	94.49-98.71 (96.89)	94.62-98.92 (96.89)
3'-UTR, RNA2	276	-	95.28-99.63 (97.77)	-
5'-UTR, RNA3	404	-	92.59-99.00 (95.93)	-
TGB1, RNA3	1,281	427	95.16-99.14 (96.97)	96.25-99.06 (97.65)
TGB2, RNA3	384	116	96.83-99.13 (97.79)	97.79-100 (97.67)
TGB3, RNA3	573	190	94.93-99.30 (96.68)	91.05-98.94 (94.13)
TGB, RNA3	2,057	733	95.52-99.02 (97.00)	94.95-99.18 (96.74)
3'-UTR, RNA3	544	-	95.58-99.26 (97.37)	-

1 and 2 of BSBV genomes were rather conserved. However, certain nonsynonymous mutations were found, most of which preserved the chemical properties of encoded amino acids.

The central parts of the MetT, Var, and RdRp coding regions on RNA1, the coding region of the CPRT domain on RNA2, the 5'- and 3'-UTRs of RNA3, and the 5' end of TGB1 and 3' end of TGB3 genes peaked at the variability of about 5%. Among TGBs, only the 5' part of the TGB1 depicted more variability (more than 6%) (Supplementary Fig. 1). This finding is in line with that of other studies (Borodynko et al., 2009; Koenig et al., 2000; Wang et al., 2008). Consistent with previous studies (Borodynko et al., 2009; Wang et al., 2008), our results revealed that TGB3 (Pi = 0.034) was a more variable gene region than TGB1 and TGB2. Sequence diversity in the 5'-UTRs and 3'-UTRs of RNA1 and RNA3 were relatively higher than those of RNA2 (Supplementary Table 1). The values of genetic dis-

tances between the pairs of isolates for all RNA1, RNA2, and RNA3 coding regions ranged from 0.001 to 0.040, 0.013 to 0.056, and 0.010 to 0.047, respectively. The overall mean value of genetic distance among coding regions of all the BSBV isolates was 0.027 ± 0.001 (RNA1), 0.031 ± 0.002 (RNA2), and 0.031 ± 0.002 (RNA3), indicating low genetic diversity.

Pairwise sequence identities were analyzed at both the nucleotide and amino acid levels, at individual gene scale (Table 2), which confirmed the genetic variation results. The genetic variability of three genomic components together accounted for a total of 224 mutations identified by a single nucleotide polymorphism (SNP) analysis algorithm implemented in the Geneious Prime 2019.1.3 (Biomatters). Positions of SNPs in the genome were in replicase (113 SNPs), CPRT (65 SNPs), TGB1 (26 SNPs), TGB2 (5 SNPs), and TGB3 gene (15 SNPs). Altogether, these results indicated that the accumulation of mutations

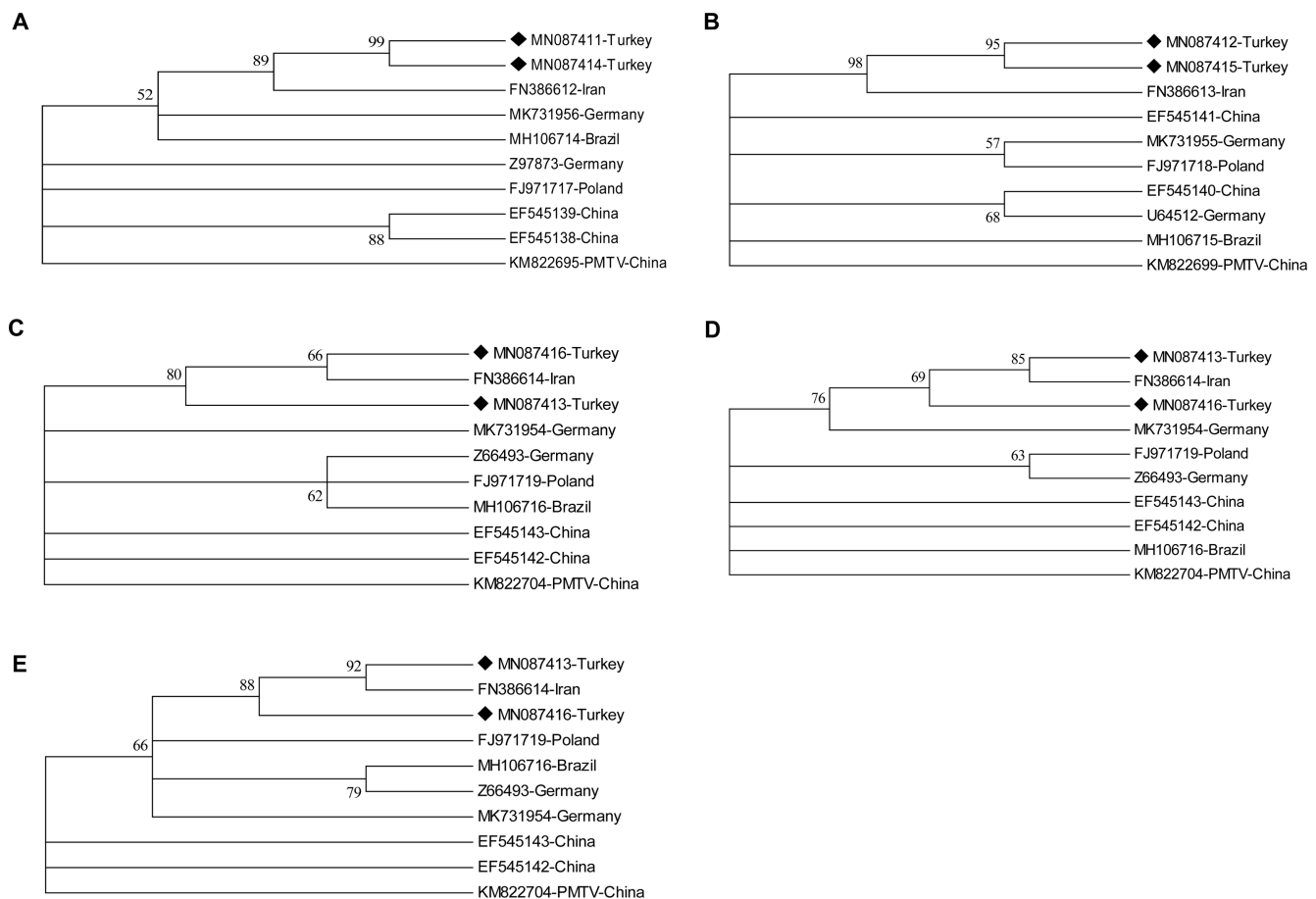


Fig. 2. Phylogenetic relationships among sequences of RNA-rep (A), RNA-CPRT (B), TGB1 (C), TGB2 (D), and TGB3 (E) genomic components of beet soil-borne virus (BSBV) inferred by MEGAX using the maximum likelihood method, with 1,000 bootstrap replications and with a 50% bootstrap threshold score. Isolates were indicated in the tree by accession number and geographical origin of collection. Turkish BSBV isolates generated from this study were marked. Potato mop-top virus was used as an outgroup.

Table 3. Analysis of genetic diversity and selection pressure acting on different cistrons of beet soil-borne virus

Genomic region	Pi	dN	dS	dN/dS	No. of negatively selected sites	No. of positively selected sites	
						FEL	MEME
Rep	0.02634	0.00901	0.10056	0.08959	159	0	9
CPRT	0.03093	0.01381	0.09076	0.15215	76	0	4
TGB1, RNA3	0.03085	0.01075	0.10321	0.10415	31	1	1
TGB2, RNA3	0.02239	0.00983	0.06632	0.14822	8	0	0
TGB3, RNA3	0.03400	0.02624	0.06216	0.42213	10	0	2

FEL, fixed effects likelihood; MEME, mixed effects model of evolution.

was not evenly distributed among the five genomic regions of the BSBVs that were analyzed. It is also worth noting that no recombination signals were found in the genomic components of BSBV isolates.

Phylogenetic relationships among the nine aligned sequences of RNA-rep, RNA-CPRT, and RNA-TGB genomic components of the BSBV were inferred employing the maximum likelihood method from multiple sequence alignments (Fig. 2). The phylogenetic tree based on the complete RNA-rep sequences showed four lineages. Phylogenetic trees based on complete nucleotide sequences of the larger CPRT, RNA-TGB1, and TGB2 showed five lineages. The tree based on the complete RNA-TGB3 sequences represented three lineages. Phylogenetic analyses of BSBV isolates based on each of the genomes revealed that the TR1, TR3, and Iranian BSBV isolate belong to one cluster.

Selection pressure analysis. To assess the variation in selection pressure on each cistron, the dN/dS ratios (ω) were also calculated separately for each gene (Table 3). The results revealed that the global selection pressure (dN/dS) for all the BSBV cistrons was less than 1, indicating negative or purifying selection pressure for amino acid conservation. As shown in Table 3, the dN/dS ratio ranged from 0.02409 to 0.42213, suggesting different constraints on sequence change depending on the virus gene. The strongest purifying selection belonged to the RdRP, supported by the smallest ω value (0.08959), while the weakest purifying selection was in the TGB3 protein, with the largest ω value (0.42213). Using HyPhy implemented in Datamonkey server, one amino acid at position 135 of TGB1 was detected by FEL and MEME method. No amino acid under positive selection was detected in other cistrons.

Discussion

Soil-borne viruses represent a group of particularly important pathogens, which cause severe yield losses in sugar

beet crops. BSBV is commonly found in the fields where BVQ and BNYVV are also present (Meunier et al., 2003). Kutluk Yilmaz and Sokmen (2010) confirmed that the virus is widespread in sugar beet-growing areas of Turkey, including Tokat.

In this study, we determined the first complete nucleotide sequences of the three genomic components of two Turkish BSBV isolates. The putative proteins of Turkish BSBV isolates contained the conserved motifs with known functions for other pomoviruses.

Understanding the distribution of genetic diversity among individuals, populations, and gene pools are crucial for the efficient management of germplasm collections and breeding programs (Borodynko et al., 2009). According to prior studies, BSBV isolates are highly conserved regarding sequence variation and evolutionary divergence (Borodynko et al., 2009; Wang et al., 2008). Our sequence analysis confirmed that all BSBV isolates identified to date are closely related to each other and relatively conserved, particularly in the case of RNA1 (Pi = 0.02513).

The order of genetic variation of individual genes, from greatest to least, was as follows: TGB3, TGB1, CPRT, Rep, and TGB2 (Table 3). Despite the low mutation rate in BSBV genome sequences, genetic diversity and population genetic analysis at the genomic level could provide useful information regarding the evolutionary features of the BSBV genome.

The genome similarities between the Turkish isolates and those from other parts of the world were above 95%. According to sequence identity analyses, Turkish isolates were most closely related to the BSBV isolates reported from Iran (97.83% to 98.77% nt identity). The overall variability observed in BSBV worldwide suggested high genetic stability that could be explained by the ecological bottleneck imposed by vectors and host plants which reduce the genetic variation of the virus population (Borodynko et al., 2009). The replicase and CPRT cistrons accumulated the largest number of mutations whereas the TGB2 region harbored the lowest number of mutations. Our findings

also revealed that the purifying selective pressure was not evenly distributed across the BSBV coding regions and distinct constraints affect different parts of the genomes. Most of the codons were under negative selection or neutral evolution; however, positive selection was detected at codon 135 in the TGB1 genomic region, which reflects the molecular adaptation. BSBV RNA-TGB encodes a movement module, the TGB, and this codon may be critical in the function of TGB1 in cell-to-cell and possibly long-distance movement. On the other hand, molecular adaptation owing to positive selection has been described in several plant viruses; for instance, the amino acid change of C to Y at codon 118 of tomato spotted wilt tospovirus (TSWV) NSm movement protein resulted in breaking the resistance conferred by the tomato Sw-5 gene (López et al., 2011). Therefore, the positive selection at codon 135 could be an adaptation to facilitate the movement and to overcome host plant resistance gene. Nevertheless, the significance of positively selected codon in the host virus-vector interactions need to be further investigated. The phylogenetic analysis showed a lack of clustering by geographical origin, which emphasizes the importance of the exchange and use of virus-free plant material in preventing the dissemination of this virus. Sugar beet, the only identified natural host of BSBV, is frequently a symptomless host, and the actual effects of BSBV on sugar beet are unknown although it has been shown that it is infectious in *C. quinoa* using full-length infectious clone from Iran (Crutzen et al., 2009). The two newly characterized BSBV isolates from Turkey confirmed this observation. BSBV can persist in soil within the long-lived resting spores of the plasmodiophorid vectors which allow the virus to infect sugar beet for over ten years after the last crop. Accordingly, it is impossible to get rid of it even with long rotation (Mehrvar, 2009) and thus, controlling it is dependent on the development of resistant cultivars (Tamada and Kondo, 2013). On the other hand, the introduction of viruses to new areas and continuous use of resistant cultivars may lead to the emergence of new variants and resistance-breaking virus strains via amino acid changes in pathogenicity-related viral genomes. Moreover, BSBV was found in combination with either BNYVV and/or BVQ, suggesting the potential for the evolution of new strains and resistance-breaking variants through recombination. The sequence characteristics and the observed genetic diversity reported in this study would be useful in the breeding of resistant varieties and developing effective control strategies. However, to understand the global molecular epidemiology and geodynamics of BSBV, there is a need for a larger number of complete genome sequences of BSBV isolates from several countries.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (<http://www.ppjonline.org/>).

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