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The complete nucleotide sequence of Beet black scorch virus (BBSV), a new member of the genus *Necrovirus*

Brief Report

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Summary. The complete nucleotide sequence of Beet black scorch virus (BBSV) was determined. The BBSV genome is composed of 3641 nucleotides and has similar organization with *Tobacco necrosis virus D* of 61% nucleotide identity. The 5′-proximal open reading frame (ORF) encodes a putative 23 kDa protein and a 82 kDa protein by reading-through of an amber termination codon. Three small ORFs located in the center of the genome may encode for a 4.2 kDa protein and two 7 kDa proteins. The 3′-proximal ORF encodes a 24.5 kDa protein equivalent in mass to the viral coat protein. Considering biological and molecular similarities with TNV, it is concluded that BBSV is a new member of the genus *Necrovirus*.

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Since the 1980's, Beet black scorch virus (BBSV) has been reported as a natural pathogen of sugar beet only in China. This virus has caused significant loss of both total yield and sugar contents in Ningxia, Xinjiang, Inner Mongolia and Heilongjiang Provinces in China [6]. Sugar beet plants infected by this virus showed severe symptoms of black scorch lesions on leaves and necrosis on roots. The virus is transmitted efficiently in a non-persistent manner by zoospores of *Olpidium brassicae* in the field [10]. Using mechanical inoculation, thirteen plant species in four families could be locally infected. Among them, *Chenopodium amaranticolor*, *C. quinoa*, *C. murale*, *Spinacia oleracea* and *Tetragonia expansa* showed local lesions while symptomless infections were produced on *Lactuca sativa*, *Physalis florisana* and six *Nicotinia* species [2]. Using immunosorbent electron microscopy with twenty-six different antibodies, BBSV was decorated weakly only by an antiserum to a willow tree isolate of TNV in Germany, and did not react with other TNV isolates or any other plant virus tested [3]. BBSV is an icosahedral

virus of 28 nm in diameter consisting of a single type of CP of 24.5 kDa, with a positive-strand RNA genome without 5′-cap structure and 3′-poly(A)$_n$ tail. Although only one RNA component of molecular mass of 0.95×10^6 Da was extracted from most virus preparations isolated in different parts of China, a smaller single-stranded satellite RNA of 0.45×10^6 Da was found in Xinjiang isolates [2]. Based on biological, serological morphological and RNA characteristics, BBSV was presumed to be a new *Necrovirus* [3] and, in this report, the complete nucleotide sequence of the BBSV genome was determined and compared with that of related plant viruses.

BBSV isolated in Ningxia Province was propagated in *C. amaranticolor* by mechanical inoculation in greenhouse. After five to six days post-inoculation, the inoculated leaves were harvested when local lesions appeared. Virions were purified using two cycles of sucrose density gradient centrifugation, according to Bo et al. [1]. The BBSV RNA was extracted from 50 μg purified preparation in an equal volume of extraction buffer (20 mM Tris-HCl, pH 8.0, 1% SDS, 200 mM NaCl, 5 mM EDTA) and phenol:chloroform (1:1) and precipitated by ethanol. The RNA template was polyadenylated by poly(A) polymerase (GIBCO BRL) and used for cDNA synthesis with an Universal RiboClone cDNA Synthesis System (Promega Biotech). The double-stranded cDNA was blunted with T_4 DNA polymerase before ligated into the *Sma*I site of pGEM-7Zf(+) vector and the ligated DNA transformed into competent cells of *Escherichia coli* strain DH5α. Putative recombinant plasmids were identified by Southern blotting using BBSV RNA probe labeled at the 5′-end with γ-^{32}P using T_4 polynucleotide kinase. Hybridization positive clones and the derived subclones by restriction endonuclease digestion were sequenced in both directions of insertion with a DNA autosequencer (Model 377A, Perkin Elmer). The 5′-proximal sequence was confirmed by 5′-RACE PCR carried out with two BBSV specific primers according to the method of Frohman (1988). Sequence analyses were performed using the software DNAMAN (Version 3.0, Lynnon BioSoft).

A near full-length BBSV genome sequence comprised 3627 nucleic acids was generated from seven overlapped cDNA clones amplified from BBSV RNA isolated in Ningxia Province. By RACE-PCR, another fourteen nucleic acids of 5′-AAGAAA CCTAACCA-3′ at the 5′-proximal end of BBSV genome was determined, while identical 3′-proximal ends were confirmed using three different cDNA clones. Compilation of clones sequenced indicated that the BBSV genome has a total of 3641 nucleic acids. Six coding regions were detected in the BBSV genome (Fig. 1). The first ORF (ORF1), from position 36 nt to an UAG codon at nts 645–647, encodes a 23 kDa protein (p23) and extended into the second ORF (ORF2) by read-through to encode a 82 kDa protein (p82). Three overlapping small ORFs (ORF3, ORF4, and ORF5) were located in the central region of BBSV genome, of 4.2 kDa (p4) and two 7 kDa proteins (p7a and p7b), respectively. The 3′-proximal ORF (ORF6) encodes a 24.5 kDa protein (p24) corresponding to the molecular mass of BBSV CP. The similarity of genome organizations among BBSV, TNV stain A (TNV-A) and D (TNV-D) is shown in Fig. 1.

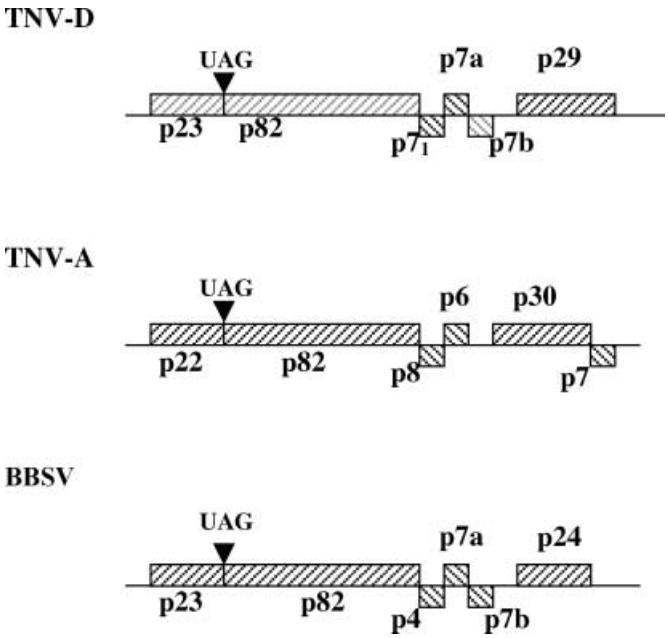

Fig. 1. Comparison of the genomic organization among TNV-A, TNV-D and BBSV. The ORFs for each virus genome are shown as boxes and the predicted proteins sizes are shown below

Table 1. Sequence identity comparison of BBSV in nucleotide and amino acids with TNV-A, TNV-D, CarMV, CRV and TCV

Virus	Nucleotide (%)			Amino acids (%)	
	Overall	Polymerase	CP	Polymerase	CP
TNV-A	45	46	49	35	41
TNV-D	61	65	51	67	42
CarMV	44	46	42	30	25
CRV	40	44	42	30	14
TCV	45	47	44	31	20

Comparing BBSV with viral sequences deposited in GenBank, varying levels of nucleotide identity were found between BBSV and TNV-A, TNV-D, *Carnation mottle virus* (CarMV), *Turnip crinkle virus* (TCV) and *Carnation ringspot virus* (CRV) (Table 1). The motif GXXXTXXXNX$_{18-50}$GDD and other characteristics of an RNA dependent RNA polymerase [9, 11] were identified in the p82 domain of BBSV. Alignment of amino acids encoded by the read-through domain of BBSV p82 and that of TNV-D also showed an identity of 71%, as compared to TNV-A (41%), CarMV (31%), TCV (37%) and CRV (31%). However, BBSV p23,

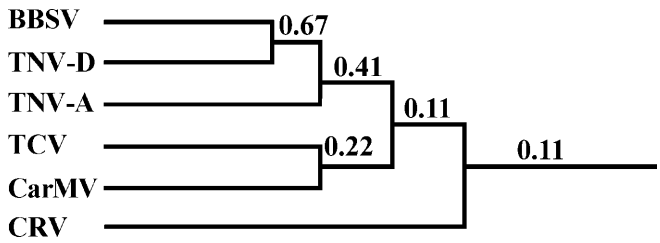

Fig. 2. Relationship of BBSV to some other plant viruses shown by homologous tree constructed by alignment of amino acid sequence of their pre-readthrough domains. The numbers beside each node indicate bootstrap values. (Generated by DNAMAN software)

the pre-readthrough domain of p82, showed higher amino acid sequence identity (58%) with that of TNV-D, but significantly lower amino acid identity with TNV-A (20%), CarMV (12%), CRV (10%) and TCV (15%). Since it was suggested previously that the pre-readthrough domain might be used as a criterion for classification of *Carmovirus* [13], the evolutionary relationships between BBSV and other plant viruses are shown in Fig. 2. These phylogenetic relationships of pre-readthrough domains, which may be required for RNA replication as the TNV-D p22 [12], showed that BBSV and TNV-D were more closely related to each other than to other viruses.

The amino acid sequence of BBSV CP was aligned with that of TNV-A and TNV-D. Compared to the molecular mass of TNV-A (30 kDa) and TNV-D (29 kDa) CPs, BBSV CP has a smaller mass (24.5 kDa) and shows identities of 41% to TNV-A and 42% to TNV-D, respectively. It has been demonstrated previously [5] that CPs of TNV-A and TNV-D have three sub-structural domains: R (random, N-terminal), A (arm, connecting R and S) and S (shell), but not P (projecting, C-terminal). These four domains are arranged similarly in the CPs of other small icosahedral plant viruses such as Cucumber necrosis virus (CNV), CarMV, Melon necrosis spot virus (MNSV) [14], TCV [4] and Tomato bushy stunt virus (TBSV) [8]. Because BBSV CP seems to lack a P domain, BBSV appears to have a virion structure more similar to TNV than *Carmo*- or *Tombusvirus*. In addition, among the three small ORFs of BBSV as with TNV-D, the two 7 kDa BBSV ORFs showed relatively high amino acid sequence identity to TNV-D p7a (58%) and p7b (50%).

According to these and other similar characteristics with TNV-D in morphology and transmission by *O. brassicae*, it is concluded that the BBSV is a new member of *Necrovirus*.

Accession numbers

The GenBank accession number of BBSV sequence reported in this paper is AF452884. Nucleotide sequence reported in this paper have been submitted to DDBJ/GenBank/EMBL database with the accession number AF452884.

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