Genomic Characterization of a New Enamovirus Infecting Common Bean

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Research Article

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Abstract

A novel enamovirus was identified from bean plants with disease symptoms. Its genome of 5,781 nucleotides (nt) encodes five open reading frames. The virus and other species of the genus *Enamovirus* share identities of 50.4%-68.4% at the complete genome, and 19.9%-51.9% of P0, 24.9%-52.5% of P1, 33.4%-62.9% of P1-P2, 30.6%-81.1% of P3, 32.3%-74.2% of P3-P5 at amino acid sequence level, respectively. Phylogenetic analysis showed that the virus is most closely related to *Alfalfa enamovirus 1* and *Pea enation mosaic virus 1* in the genus *Enamovirus* within family *Solemoviridae*. These results suggest that the virus should be considered as a novel species in the genus *Enamovirus* and tentatively named as “bean enamovirus 1”.

Introduction

Common bean (*Phaseolus vulgaris* L.), also called French bean, is a herbaceous annual crop in the family Fabaceae. It is native to Mesoamerica and was introduced to China in 15th century [1]. Young pods of bean are used as vegetable, and the dry seeds are one of the major staple food in countries of Africa, Latin America, the Caribbean and Asia. According to the statistics of FAO in 2018, China is the fifth largest production of bean with the annual planting area of about 100 million hm^2 and output of near 1.3 million tons. Bean production is vulnerable to many diseases including those caused by viruses. The virus diseases are of great importance due to their effects on yield as practice of monoculture and expansion of planting areas. To date, at least 43 viruses have been reported to infect beans and cause production declination [2, 3, 4].

Genus *Enamovirus* was originally classified in family Luteoviridae but newly assigned into family Solemoviridae that also contains genera *Polemovirus*, *Polerovirus* and *Sobemovirus* [5]. The members of the genus have single-stranded RNA genome of 5.7-6.0 kb in length that encodes five open reading frames (ORF). The enamoviruses have narrow host range and are transmitted by aphids. In this study, a novel enamovirus was identified from common bean, and molecularly characterized. A survey of multiple crops showed that the virus infects several other legume crops.

Materials And Methods

During September 2020, common bean samples with symptoms of leaf crinkle and vein distortion were collected from fields in Kunming, Yunnan, China (Fig. 1A). To identify the potential pathogens, five symptomatic samples were pooled together for preparation of a total RNA sample that was subjected to high throughput sequencing (HTS). The rRNA depleted sample was used for construction of RNA-seq library for sequencing on an Illumina HiSeq X-ten platform with PE150 bp (Origingene Bio-pharm Technology Co., Ltd, Shanghai, China). The RNA-seq data were analyzed by the CLC Genomic Workbench 20.0 (QIAGEN). A total of 48,792,184 paired-end reads were obtained, and *de novo* assembly of the reads generated 138,508 contigs [>200 nucleotides (nt)]. Blastx search in the GenBank database revealed the presence of bean yellow mosaic virus (BYMV), tomato spotted wilt orthotospovirus (TSWV), alfalfa

mosaic virus (AMV) and a large contig of 5,374 nt that had the highest nt sequence identity of 72.3% with alfalfa enamovirus 1 (AEV-1, GenBank acc. no. KU297983).

To determine its complete genome sequence, 6 pairs of virus-specific primers covering the nearly genomic sequence were designed according to the contig (Supplemental Table 1). Total RNAs from the original field bean samples were extracted using TRIpure Reagent (Bioteke Corporation, Beijing, China). The 3'-end sequence were obtained by adding a poly (A) tail to its 3'-terminus using poly(A) Polymerase, followed by conducting RT-PCR using a virus-specific forward primer and a degenerated primer of viral8 for viruses with poly(A) tail. The amplification strategy, genome sequence assembling and analysis were conducted as Lan [6].

Genomic properties

The complete genome of the novel enamovirus consists of 5,781 nt (GenBank acc. no. MZ361924) and encodes five ORFs. Its genomic organization and structural is a typical of the genus *Enamovirus* (Fig. 1B). The 5' and 3' UTRs are 174 nt and 233 nt, respectively, and the intergenic region (IR) between ORFs 2 and 3 is 188 nt in size. ORF0 starts at nt 175 and ends at nt 1,086, and it encodes putative P0 protein of 303-aa residues that contains the key domain of ^{213}LPxxL^{217}, the putative F-box-like motif in the *Enamovirus* [7]. ORF1 is expressed by a ribosomal leaky scanning mechanism. It starts at nt 269 and ends at nt 2,569, encodes putative P1 protein of 766-aa residues containing the conserved domain of ^{390}H(x_{29})D(x_{67})GxSG^{591} of the S39 serine protease [8]. ORF2 is translated by a -1 ribosomal frameshift at a "putative slippery heptamer" sequence (^{2039}GGGAAAT^{2045}) within ORF1, which overlaps ORF2 at its 5' end and incorporates a fusion protein of P1-P2 of 1188-aa residues. P1-P2 was considered to be involved in virus replication in enamoviruses as it contains highly conserved motif of ^{1,085}GDD^{1,087} for the viral RNA-dependent RNA polymerase (RdRp) [8]. After a short IR, ORF3 starts at nt 4,022 and ends at nt 4,594 encodes coat protein (CP) of 190 aa. Whereas ORF5 is expressed by a putative in-frame readthrough from ORF3, producing a fusion protein of 508-aa residues (P3-P5). P3-P5 is thought to be an aphid-transmission subunit in the genus *Enamovirus* [9].

Pairwise comparisons were conducted using the complete nt sequences and the aa sequences of individual proteins between this virus and other members of the genus. The results show that the new virus shares sequence identities of 50.4% to 68.4% with enamoviruses at the nt sequence level, and 19.9%-51.9% at P0, 24.9%-52.5% at P1, 33.4%-62.9% at P1-P2, 30.6%-81.1% at P3, 32.3%-74.2% at P3-P5 at aa sequence level, respectively (Supplementary Table 2). It is worth notice that the CP is the most conserved protein among the viruses, whereas ORF0 is the most divergent one. A maximum-likelihood tree conducted using the deduced P1-P2 fusion protein sequences of this virus and the members of genera *Enamovirus*, *Polemovirus*, *Sobemovirus* and *Polerovirus* places the new virus with AEV-1 [10] and pea enation mosaic virus 1 (PEMV-1) [11] in a legume clade (Fig. 2). These data indicate that the new virus, tentatively named bean enamovirus 1 (BEnV-1), should be considered as a novel species in the genus *Enamovirus*.

To investigate the distribution and potential natural host speices of BEnV-1 in the fields, a total of 378 leaf samples was collected from different families of plants with and without symptoms in Yunnan. The results of RT-PCR showed that 5 out of 25 vetch (21.7%), 3 out of 10 alfalfa (30.0%) and 5 out of 59 common bean (8.5%) samples were infected with BEnV-1. The virus was not detected from cowpea, soybean, pea, faba bean, pepper, tomato, potato, cucurbit, cucumber and passion fruit. The data suggest that the host of BEnV-1 is limited to legume plants. The effects of the virus on legume crops need to be assessed in the future.

Declarations

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Compliance with ethical standards

Conflict of interest

As the corresponding authors, Ruhui Li and Fan Li declare no conflict of interest involving in the authors of this paper.

Ethical approval

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

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Figures

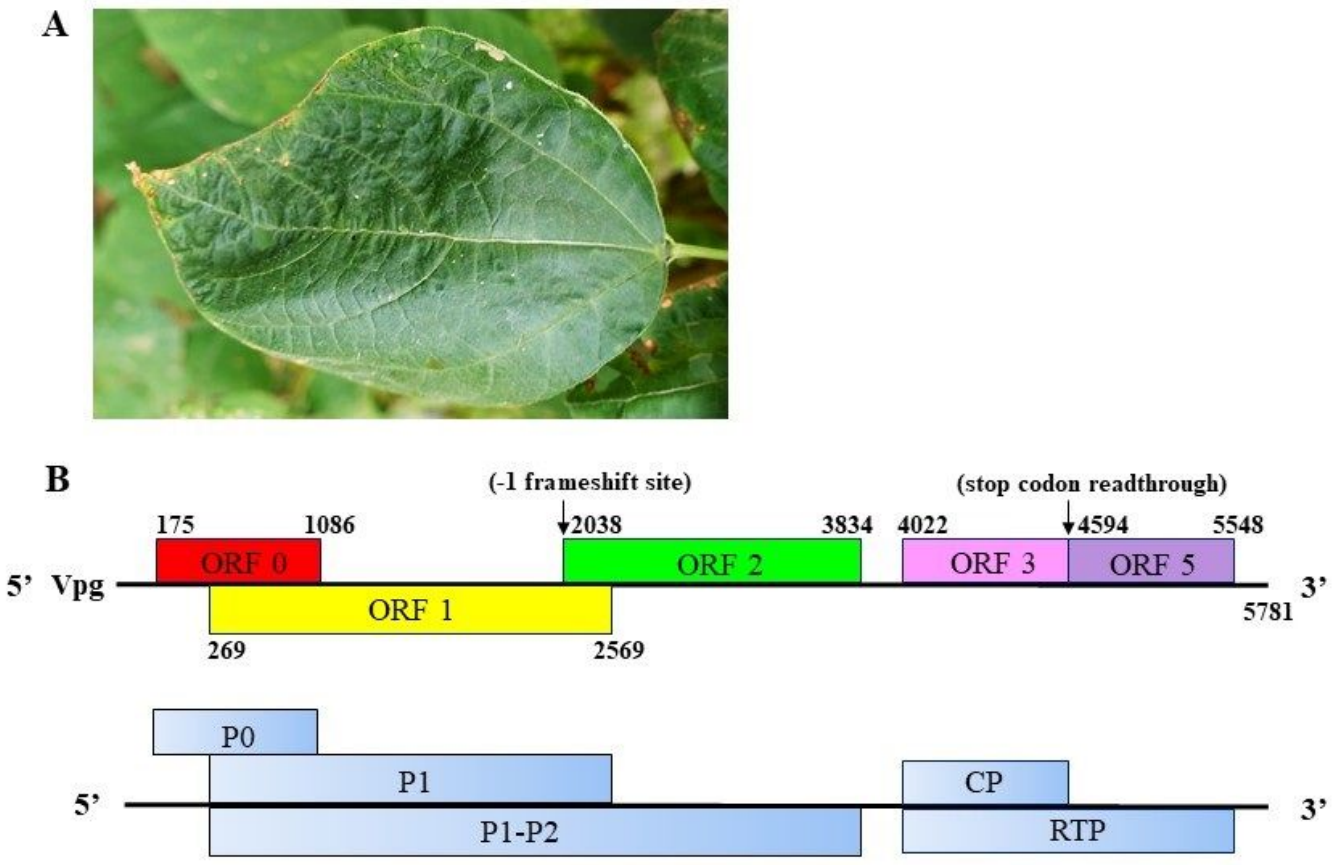

Figure 1

(A) Symptoms of the diseased common bean leaf. (B) Schematic representation of the genomic organization of bean enamovirus 1. The open reading frames (ORFs) are depicted by rectangles with different colors. The positions of start and stop points of each ORF were labeled. Translation products are depicted as blue rectangles.

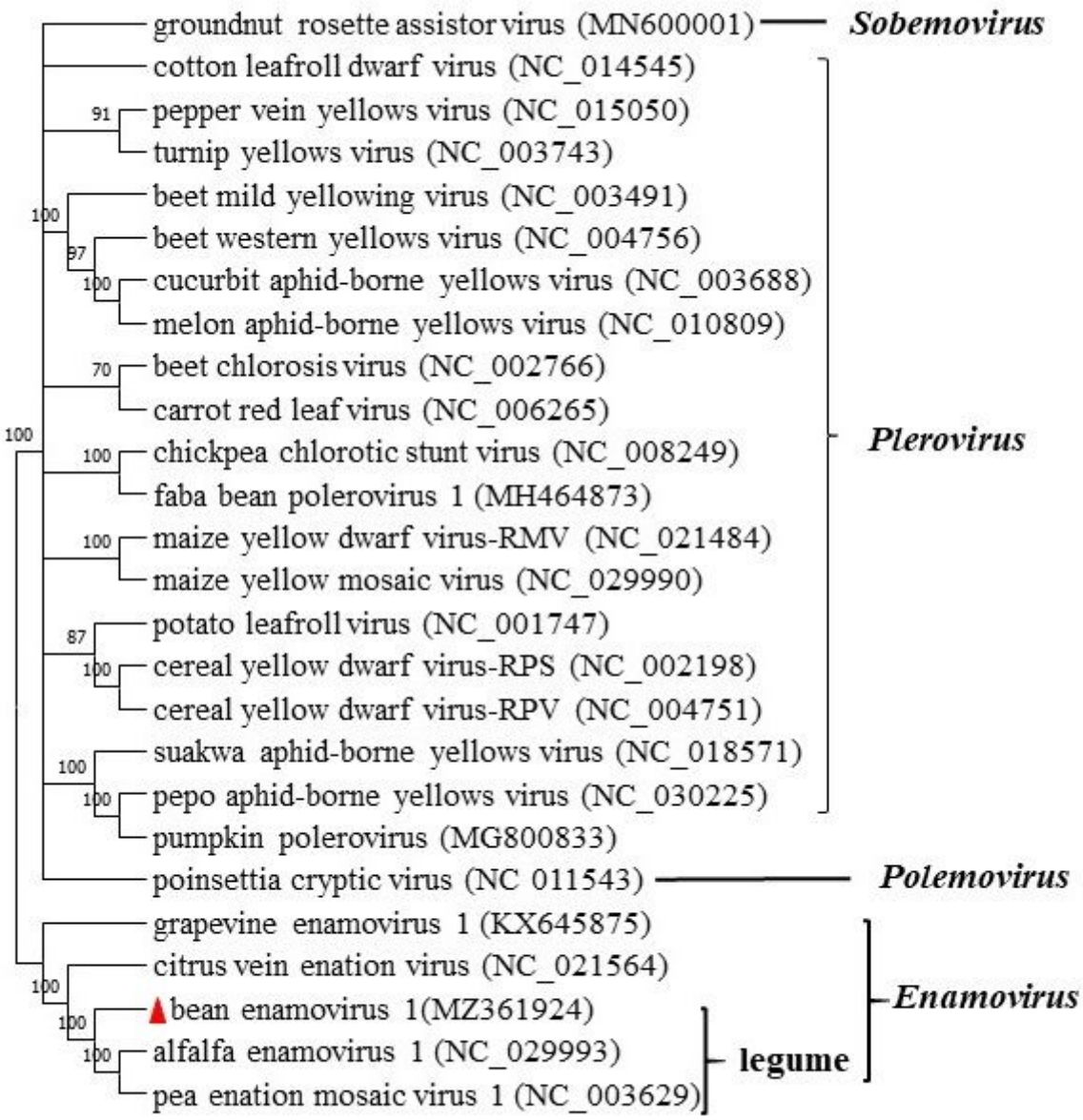

Figure 2

Maximum-likelihood tree based on the deduced fusion protein sequences of P1-P2 of BEnV-1, members of Enamovirus, Sobemovirus, Polemovirus and Polerovirus. Bootstrap analysis was applied using 1000 bootstrap replicates. A solid triangle indicates the BEnV-1 characterized in this study.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable.docx
- sequence.txt