



First report of Phaseolus vulgaris endornavirus 1, 2 and 3 in dry bean cultivars in Greece

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In July 2024, during a field evaluation of dry bean genotypes in Kileler (Thessaly) in Greece, plants showing virus-like symptoms were identified. Forty-seven samples were collected from individual plants representing four cultivars. To identify the viruses present, total RNA (White et al. 2008) was extracted from a composite sample including leaf material from 10 plants and subjected to high-throughput sequencing (HTS). Library preparation (Illumina TruSeq Stranded Total RNA with Ribo-Zero Plant) and sequencing were performed by Macrogen on a NovaSeq 6000 platform, generating approximately 50 million 101-nt paired-end reads. *De novo* assembly produced 2069 contigs (> 240 nt). BLASTn analysis revealed Phaseolus vulgaris endornavirus 1, 2 and 3 (PvEV-1, -2 and 3), together with other bean infecting viruses such as bean common mosaic virus (BCMV) and cucumber mosaic virus (CMV). Nearly complete endornavirus genomes were assembled, represented by a 13,888 nt long contig of PvEV-1, a 14,810 nt long contig of PvEV-2 and a 15,187-nt contig of PvEV-3. HTS results were confirmed by RT-PCR using virus-specific primers designed from the assembled contigs: PvEV-1_up (5'-CG ATTACATTTGCGTGTACTCC-3')/PvEV-1_do (5'-GGAT TGATGATAACAAGGACTGC-3'), PvEV-2_up (5'-CATC ATAGCCCACCATCTCTC-3')/PvEV-2_do (5'-GACAAAT CAACCATCATCCTCAG-3'), PvEV-3_up (5'-ACCTCCT CATCAGTTAAGCTG-3')/PvEV-3_do (5'-TATACACTGA GTACATACCCGTTG-3'), generating amplicons of 435 bp,

408 bp and 403 bp, respectively. PvEV-1 and PvEV-2 were detected in most samples (45/47 and 44/47, respectively), whereas PvEV-3 was detected in 12 samples. Moreover, one amplicon per virus was Sanger sequenced. Blast analysis showed > 99% nucleotide identity among the Greek PvEV-1 and PvEV-2 isolates (PX056667, PX056668) and isolates from Canada (MK948542.1) and Kenya (OM469937.1), respectively. The Greek PvEV-3 isolate (PX056669) shared 99.7% nt identity with a Kenyan isolate (OM100070.1). Endornaviruses are persistent, vertically transmitted viruses causing no apparent symptoms (Okada et al. 2013; Valverde et al. 2019). This is the first report of PvEV-1, PvEV-2 and PvEV-3 in Greece and further research will unveil their putative impact in different bean genotypes.

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Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

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

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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