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Complete genome sequences of three bean common mosaic virus isolates identified from peanut (*Arachis hypogaea* L.)

Yeonhwa Jo[1†], Hoseong Choi[2†], Jin-Sung Hong[3], Hyunjung Chung[4], Soo Yeon Choi[4], Sang-Min Kim[4], Bong Choon Lee[4*], and Won Kyong Cho[1*]

[1]*College of Biotechnology and Bioengineering, Sungkyunkwan University, Suwon 16419, Republic of Korea*
[2]*Plant Genomics and Breeding Institute, Seoul National University, Seoul 08826, Republic of Korea*
[3]*Department of Applied Biology, Kangwon National University, Chuncheon 24341, Republic of Korea*
[4]*Crop Foundation Division, National Institute of Crop Science, Rural Development Administration, Wanju 55365, Republic of Korea*

땅콩에서 동정한 세 개 강낭콩모자이크바이러스의 전체 유전체 서열

조연화[1†] · 최호성[2†] · 홍진성[3] · 정현정[4] · 최수연[4] · 김상민[4] · 이봉춘[4*] · 조원경[1*]

[1]성균관대학교 생명공학대학, [2]서울대학교 식물유전체육종연구소, [3]강원대학교 응용생물학전공, [4]국립식량과학원 작물기초기반과

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To identify viruses infecting peanut (*Arachis hypogaea* L.) plants, we collected leaf samples from three peanut plants showing viral disease symptoms in Jeonju, Korea. After extracting total RNA from the individual plant samples, we generated three different ribosome-deleted libraries for RNA-sequencing. The three libraries were paired-end sequenced, and the raw data were used for *de novo* transcriptome assembly followed by a BLASTN search against a viral genome database. We only identified bean common mosaic virus (BCMV) infecting all three peanut plants. From the three libraries, we obtained three complete genomes of BCMV 10,049 nucleotides to 10,039 nucleotides in length encoding two open reading frames. All three BCMV complete genomes showed sequence similarity to the known BCMV isolate Habin1 identified from soybean in Korea. This result suggests that BCMV infecting peanut might originate from BCMV infecting soybean or vice versa. Taken together, we report complete genome sequences of three BCMV isolates identified from peanut in Korea.

Keywords: bean common mosaic virus, genome, peanut, virus

[†]These authors contributed equally to this work.
***For correspondence.** (B.C. Lee) E-mail: leebc21@korea.kr;
Tel.: +82-63-238-5342 /
(W.K. Cho) E-mail: wonkyong@gmail.com;
Tel.: +82-31-290-7860; Fax: +82-31-290-7870

The peanut (*Arachis hypogaea* L.) is a kind of legume plant known as groundnut. It is famous for its edible seeds that are consumed worldwide after dry roasting or boiling. In addition, peanut seeds are used as materials for peanut oil, butter, proteins, and flour. The peanut is widely cultivated in the tropical to subtropical regions. China is the largest peanut-producing country followed by India, Nigeria, and the United States according to the 2020 statistics of the Food and Agriculture Organization of the United Nations (https://www.fao.org/).

In Korea, the peanut is generally planted from the end of April to the beginning of May, and repeated cultivation in the same field is avoided to prevent diseases. Several viruses infecting peanut plants, such as bean common mosaic virus (BCMV) in the genus *Potyvirus*, peanut mottle virus (PeMoV) in the genus *Potyvirus*, peanut stripe virus in the genus *Potyvirus*, and peanut stunt virus (PSV) in the genus *Cucumovirus*, have been identified (Jo *et al*., 2020). Of them, BCMV is the prominent virus infecting peanut plants in Korea. Peanut plants infected by BCMV display yellow mosaic and stripe symptoms, resulting in smaller seeds and reduced seed yield. The known hosts for BCMV are the common bean (*Phaseolus vulgaris* L.) (Jo *et al*., 2021), soybean (*Glycine max* L.) (Jo *et al*., 2020), and peanut

(*A. hypogaea*) (Koo *et al*., 2002). BCMV is usually transmitted by aphids in a non-persistent manner and is also seed-borne.

Studies on viruses infecting peanut plants in Korea have been previously conducted (Koo *et al*., 2002). However, only RT-PCR-based approaches have been employed. In this study, we examined viruses infecting peanut plants by RNA-sequencing. For that, we collected leaves from three peanut plants showing viral disease symptoms grown in the field of the National Institute of Crop Science in Jeonju, Korea. Leaf samples were immediately frozen in liquid nitrogen. From each plant sample, total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. We deleted ribosomal RNA from the total RNA using TruSeq Stranded Total RNA with a Ribo-Zero Plant Kit (Illumina). A total of three different libraries representing the three individual peanut plants were prepared for RNA-sequencing using the TruSeq Stranded Total RNA LT Sample Prep Kit (Illumina). The three different libraries referred to as Jeonju-1 to Jeonju-3 were indexed and paired-end (2 × 101 bp) sequenced using the NovaSeq 6000 system (Macrogen). Using the raw data, *de novo* transcriptome assembly was carried out using the Trinity program with default parameters (Haas *et al*., 2013). To identify virus-associated contigs, we performed a BLASTX search using the assembled contigs against the viral protein database with E-value 1e-10 as a cutoff. In addition, we predicted open reading frames (ORFs) for the virus-associated contigs using the ORFfinder program (https://www.ncbi.nlm.nih.gov/orffinder/). Three BCMV genome sequences as well as 10 known BCMV genome sequences were aligned using MAFFT version 7 (Katoh and Standley, 2013) followed by sequence trimming using the trimAl program (Capella-Gutiérrez *et al.,* 2009). The trimmed sequences were imported into the MEGA7 program for the phylogenetic tree construction. The phylogenetic tree was generated using the MEGA7 program with the following parameters: the maximum likelihood method and 1,000 bootstrap replicates (Kumar *et al.,* 2016).

We only identified five virus-associated contigs associated with BCMV ranging from 212 bp to 10,142 bp from the three libraries. From each library, we obtained a high number of reads associated with BCMV. For example, 1,487,650 reads (Jeonju-1), 741,049 reads (Jeonju-2), and 585,105 reads (Jeonju-3) were associated with BCMV. From each library, we obtained the complete genome sequence of BCMV named BCMV isolate Jeonju-1 (10,041 nucleotides [nt]), isolate Jeonju-2 (10,049 nt), and isolate Jeonju-3 (10,039 nt) after deleting the poly (A) tail. BCMV consisted of a positive-sense single-stranded RNA genome and encoded two ORFs (Table 1). In the case of BCMV isolate Jeonju-1, ORF1 (position 120 to 9,788) encoded a polyprotein, whereas ORF2 (position 3,278 to 3,502) encoded a PIPO protein. It is known that the polyprotein precursor of BCMV is proteolytic cleaved, generating 10 mature proteins (protein 1 [P1], helper component protease [HC-Pro], protein 3

Table 1. Summary of BCMV isolate Jeonju-1 genome organization
Open reading frames (ORFs) and untranslated regions (UTRs) are indicated with respective location and size. nt, nucleotides; aa, amino acids.

Features	Location	Size	Function
5' UTR	1–119	119 nt	UTR
ORF1	120–9,788	3,222 aa	Polyprotein
Mature peptide	120–1,448	443 aa	P1 protein
Mature peptide	1,449–2,819	457 aa	HC-Pro protein
Mature peptide	2,820–3,860	347 aa	P3 protein
Mature peptide	3,861–4,016	52 aa	6K1 protein
Mature peptide	4,017–5,918	634 aa	CI protein
Mature peptide	5,919–6,077	53 aa	6K2 protein
Mature peptide	6,078–6,647	190 aa	NIa-VPg protein
Mature peptide	6,648–7,376	243 aa	NIa-Pro protein
Mature peptide	7,377–8,924	516 aa	NIb protein
Mature peptide	8,925–9,785	287 aa	coat protein
ORF2	3,278–3,502	74 aa	PIPO
3' UTR	9,789–10,041	253 nt	UTR

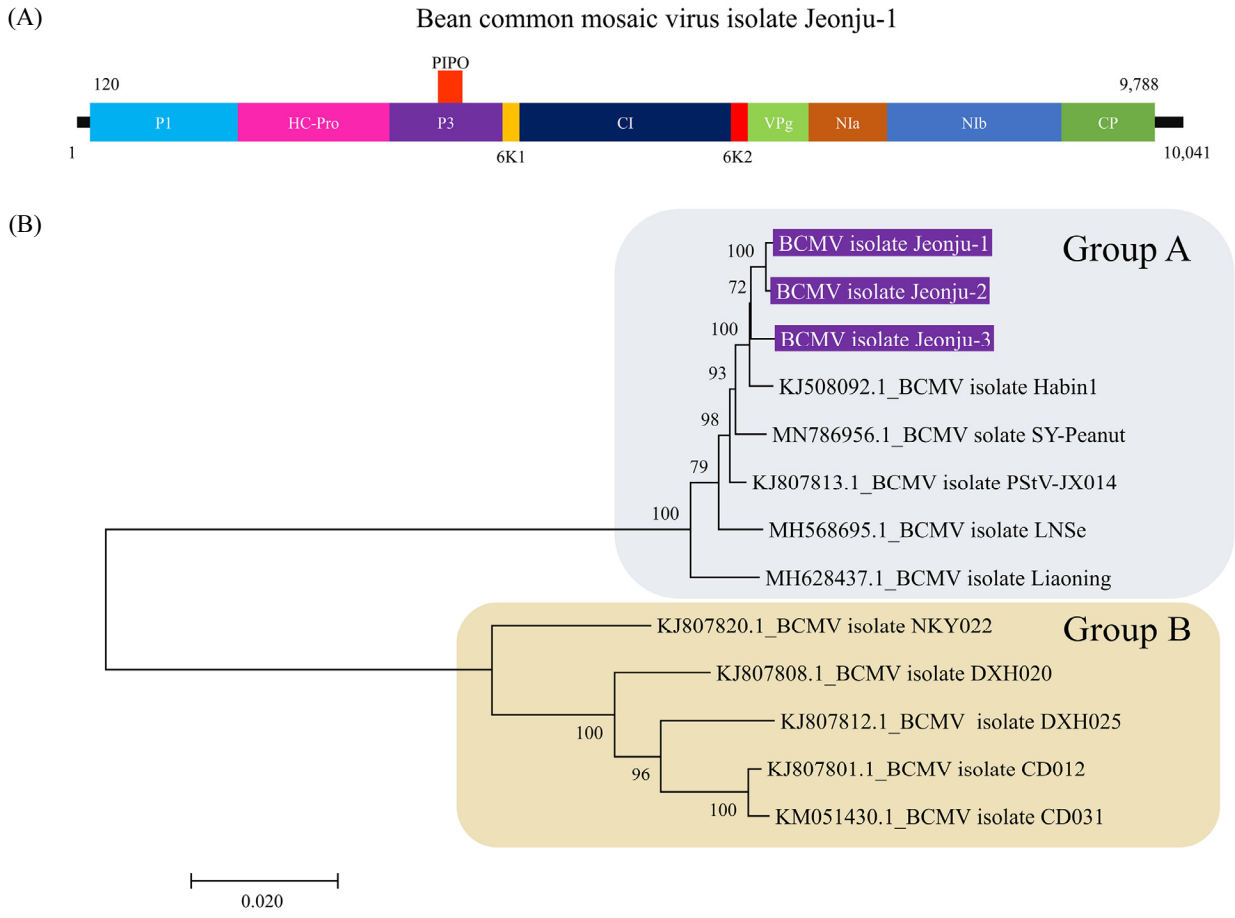

Fig. 1. Genomic organization of BCMV isolate Jeonju-1 and phylogenetic relationship of three BCMV isolates in this study with 10 known BCMV isolates. (A) Genomic organization of BCMV isolate Jeonju-1. The numbers indicate the nucleotide positions of individual ORFs in the BCMV genome. (B) Phylogenetic relationship of 13 BCMV isolates based on complete genome sequences. The phylogenetic tree was constructed using the MEGA7 program with the maximum likelihood method and 1,000 bootstrap replicates.

[P3], 6 kDa protein 1 [6K1], cylindrical inclusion body [CI], 6K2, nuclear inclusion protein a-viral genome-linked protein [NIa-VPg], NIa-Pro, nuclear inclusion protein b [NIb], and coat protein [CP]) (Fig. 1A).

We found that all three BCMV isolates in this study showed sequence similarity to the known BCMV isolate Habin1 (GenBank KJ508092) identified from soybean (*G. max* L.) in Korea with 100% coverage and 99.38% nt identity. This result suggests that the BCMV infecting peanut might originate from BCMV infecting soybean or vice versa. To elucidate the genetic relationship of the three identified BCMV isolates with known BCMV isolates, we conducted a phylogenetic analysis. The phylogenetic tree showed two distinct groups of 13 BCMV isolates (Fig. 1B). Group A contained the three isolates in this study, one isolate from Korea, and four isolates from China. The four isolates from China were identified from diverse hosts, such as peanut (SY-Peanut and Liaoning), soybean (PStV-JX014), and *Sesamum indicum* (LNse). By contrast, in Group B, all five isolates were derived from soybean in China.

Taken together, we report the complete genomes of three BCMV isolates identified from three peanut plants showing viral disease symptoms by RNA-sequencing.

Nucleotide sequence accession number

The complete genome sequences of the three BCMV isolates Jeonju-1 (ON843751), Jeonju-2 (ON843752), and Jeonju-3 (ON843753) have been deposited in GenBank with their respective accession numbers. BCMV isolate Jeonju-1 has been deposited in the Korean Agricultural Culture Collection (KACC) under the accession number CV220915-3.

적 요

땅콩 식물을 감염시키는 바이러스를 확인하기 위해, 우리는 바이러스성 질병 증상을 보이는 3개의 땅콩 식물의 잎 샘플을 한국 전주 지역에서 수집했다. 개별 식물 샘플에서 total RNA를 추출한 후, 우리는 RNA 시퀀싱을 위해 세 개의 서로 다른 리보솜 삭제 라이브러리를 생성했다. 세 개의 라이브러리가 쌍으로 배열되어 염기 서열이 분석되었다. Raw data를 이용해 *de novo* 전사체 조립을 수행하였고, 바이러스 게놈 데이터베이스에 대한 BLASTN 검색을 수행하였다. 세 개의 땅콩 식물에 감염된 강낭콩 일반 모자이크바이러스(bean common mosaic virus, BCMV) 확인하였다. 세 개의 라이브러리에서 10,049개의 뉴클레오타이드에서 10,039개의 뉴클레오타이드로 두 개의 open reading frame을 인코딩하는 완전한 게놈 BCMV 3개를 얻었다. 세 개의 BCMV 게놈은 모두 한국의 콩에서 동정된 BCMV 분리형 하얼빈 1호와 서열 유사성을 보였다. 이 결과는 BCMV가 땅콩을 감염시킨 것이 BCMV가 콩을 감염시킨 것에서 비롯되었거나 그 반대일 수 있음을 시사한다. 본 연구에서는 한국의 땅콩에서 동정된 3개의 BCMV 분리주의 완전한 게놈 서열을 보고한다.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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