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Complete genome sequences of three isolates of *Bean yellow mosaic virus* identified from freesia (*Freesia refracta* Klatt.)

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프리지아에서 동정한 콩황화모자이크바이러스 세 개 분리주의 전체 유전체 서열

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We collected leaf samples of freesia displaying mild yellow mosaic symptoms from two freesia cultivars referred to as Pinksia-1 and Pinksia-2 to identify viruses infecting freesia (*Freesia refracta* Klatt.). By RNA-sequencing and bioinformatic analyses, we identified three complete genomes of *Bean yellow mosaic virus* (BYMV) in the genus *Potyvirus* belonging to the family *Potyviridae*. The genome size of all three BYMV isolates was 9,528 nucleotides, and the nucleotide identity of the three BYMV genomes ranged from 99.90% to 99.91%. All three isolates in this study were closely associated with the known BYMV isolate Fr from freesia in Korea. The genome of BYMV was composed of positive-sense single-stranded RNA and encoded a large polyprotein, which was further self-cleaved into 10 different mature viral proteins and the pretty interesting potyviridae ORF (PIPO) protein. Taken together, we report the complete genomes of three BYMV isolates identified from freesia in Korea by RNA-sequencing.

Keywords: *Bean yellow mosaic virus*, freesia, genome, RNA-sequencing, virus

Bean yellow mosaic virus (BYMV) is a member of the genus *Potyvirus* in the family *Potyviridae* (Nakamura *et al.*, 1996). BYMV infects a wide range of legume hosts, such as alfalfa, beans, and clovers, as well as non-legume hosts, such as gladiolus and freesia (Wylie and Jones, 2009). Infection of BYMV leads to yellow to green mosaic and mottle symptoms in the infected leaves. In addition, leaf distortion, wrinkling, and stunted growth symptoms can be observed in BYMV-infected plants. BYMV is usually transmitted by the diverse aphids that can spread it effectively. However, seed transmission of BYMV in beans has not been reported (Swenson *et al.*, 1964).

Freesia (*Freesia refracta* Klatt.) is an herbaceous perennial plant in the family *Iridaceae* famous for its fragrant funnel-shaped flowers. As freesia is usually clonally propagated by bulbs, several pathogens, including viruses, are transmitted by bulbs. To date, the most common viruses infecting freesia are BYMV and freesia mosaic virus (FMV) in the genus *Potyvirus* and cucumber mosaic virus (CMV) in the genus *Cucumovirus*. A previous study identified a BYMV isolate BYMV-Fr from a freesia cultivar in Korea (Bellardi and Bertaccini, 1989).

In May 2021, we collected leaf samples displaying mild yellow mosaic symptoms from two freesia cultivars referred to as Pinksia-1 and Pinksia-2 grown in Chungcheong Nam Do Agricultural Research & Extension. To identify viruses infecting

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freesia, total RNA was extracted from the freesia samples using an RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Using the total RNA, we prepared two libraries for RNA-sequencing using TruSeq Stranded Total RNA (Illumina). We then conducted paired-end (2 × 101 bp) sequencing using the NovaSeq 6000 system (Macrogen). We *de novo* assembled the raw data (17,733 and 34,746 contigs for library Pinksia-1 and library Pinksia-2, respectively) using the Trinity program version v2.13.2 with default parameters (Haas *et al.*, 2013). The assembled contigs were subjected to a BLASTX search with E-value 1e-10 as a cutoff against the viral protein database (https://www.ncbi.nlm.nih.gov/genome/viruses/). The virus-associated contigs were again subjected to a BLASTX search against a non-redundant protein database to remove host-derived sequences. The obtained viral contigs were subjected to a BLASTN search against a viral genome database to assign their taxonomy. Using the ORFfinder program (https://www.ncbi.nlm.nih.gov/orffinder/), we predicted open reading frames (ORFs) in the identified viral genomes. For phylogenetic analyses, we used the top 10 known BYMV genomes that showed strong sequence similarity to the three BYMV isolates in this study. The BYMV genome sequences were aligned using MAFFT version 7 (Katoh and Standley, 2013), after which the aligned sequences were trimmed using the trimAl program (Capella-Gutiérrez *et al.*, 2009). The trimmed sequences were subsequently subjected to the MEGA7 program for the construction of a phylogenetic tree using the maximum likelihood method with 1,000 bootstrap replicates (Kumar *et al.*, 2016).

From two different RNA-sequencing results, we identified two and one BYMV genomes from the Pinksia-1 and Pinksia-2 cultivars, respectively. We named the BYMV isolates Pinksia-1, Pinksia-11, and Pinksia-2 according to the cultivar name. The genome size of all three BYMV isolates was 9,528 nucleotides (nt). The sequence coverages of three BYMV isolates ranged from 12,666 (Pinksia-2) to 35,945 (Pinksia-1 and Pinksia-11). The GC contents of three BYMV isolates ranged from 40.3% (Pinksia-11 and Pinksia-2) to 40.4% (Pinksia-1). The nt identity of the three BYMV genomes ranged from 99.90% to 99.91%. The genome of BYMV was composed of positive-sense single-stranded RNA. Based on the complete genome of BYMV isolate Pinksia-1 isolate, BYMV encoded a large polyprotein (position 188 to 9,357 nt), which was further self-cleaved into 10 different mature viral proteins: protein 1 (P1) (284 aa), helper component protease (HC-Pro) (457 aa), protein 3 (P3) (348 aa), 6 kDa protein 1 (6K1) (53 aa), cylindrical inclusion body (CI) (635 aa), 6K2 (53 aa), nuclear inclusion protein a-viral genome-linked protein (NIa-VPg) (191 aa), NIa-Pro (243 aa), nuclear inclusion protein b (NIb) (519 aa), and coat protein (CP) (273 aa) (Table 1 and Fig. 1A) (Wylie and Jones, 2009). In addition, BYMV had a small ORF called pretty interesting potyviridae ORF (PIPO) (position 2,877 to 3,113)

Table 1. Detailed information of viral proteins encoded by BYMV isolate Pinksia-1

Name	Position	Size	Features
5' UTR	1–187		Untranslated region
Polyprotein	188–9357	3056 aa	Coding DNA sequence
P1 protein	187–1038	284 aa	Mature peptide
HC-Pro protein	1039–2409	457 aa	Mature peptide
P3 protein	2410–3453	348 aa	Mature peptide
6K1 protein	3454–3612	53 aa	Mature peptide
CI protein	3613–5517	635 aa	Mature peptide
6K2 protein	5518–5676	53 aa	Mature peptide
NIa-VPg protein	5677–6249	191 aa	Mature peptide
NIa-Pro protein	6250–6978	243 aa	Mature peptide
NIb protein	6979–8535	519 aa	Mature peptide
Coat protein	8536–9354	273 aa	Mature peptide
PIPO	2877–3113	78 aa	Coding DNA sequence
3' UTR	9357–9528		Untranslated region

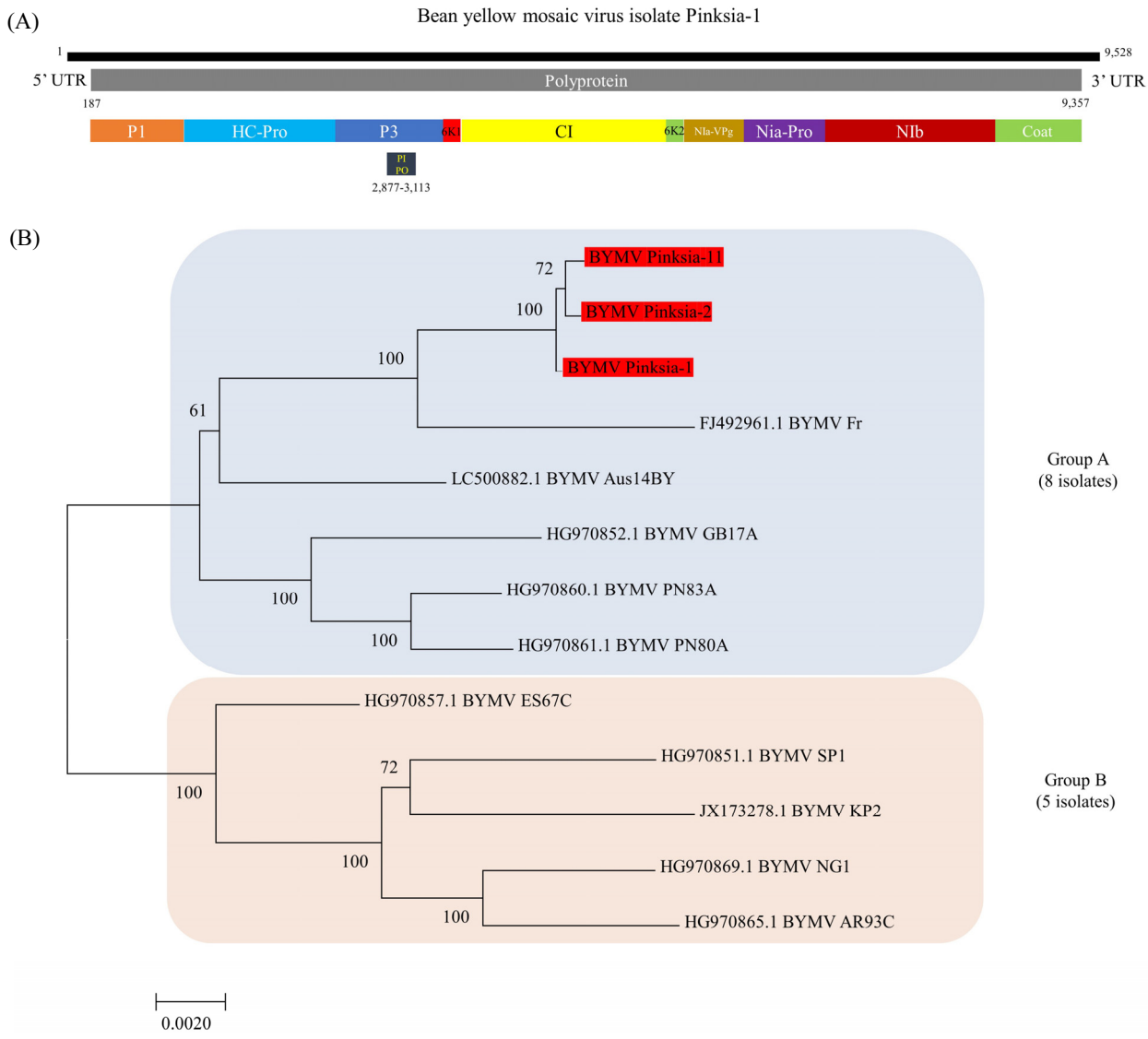

Fig. 1. Genomic organization and phylogenetic relationship of three BYMV isolates in this study with known 10 BYMV isolates. (A) Genomic organization of BYMV isolate Pinksia-1. (B) Phylogenetic relationship of 13 BYMV isolates based on complete genome sequences. The phylogenetic tree was constructed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

(78 aa). PIPO does not have its own translation initiation codon and is encoded in an alternative reading frame (White, 2015).

We conducted a BLASTN search using three BYMV complete genomes against the nt database in National Center for Biotechnology Information (NCBI). The BLAST results showed that all three BYMV isolates were closely related to BYMV isolate Fr identified from freesia in Korea with 100% coverage and 98.71% to 98.78% nt identity. To reveal the phylogenetic relationship of the three identified BYMV genomes, a phylogenetic tree was constructed using the 13 BYMV complete genome sequences including the three BYMV isolates in this study. The phylogenetic tree displayed two groups of BYMV isolates (Fig. 1B). Group A contained the three isolates in this study, an isolate (Fr) from freesia in Korea (Choi *et al*., 2013), an isolate (Aus14BY) from *Lens culinaris* in Australia, and three isolates (GB17A, PN83A, and PN80A) from *Lupinus angustifolius* in Australia. Group B included five isolates: four isolates, ES67C, SP1, NG1, and AR93C, from *Lens culinaris* in Australia and an isolate (KP2) from *Diuris magnifica* in Australia.

Taken together, we report the complete genomes of three BYMV isolates identified from freesia in Korea by RNA-sequencing.

Nucleotide sequence accession number

The complete genome sequences of the three BYMV isolates have been deposited in GenBank under the accession numbers ON462013, ON462014, and ON505756. BYMV isolates Pinksia-1 and Pinksia-2 have been deposited in the Korean Agricultural Culture Collection (KACC) under the accession number CV220915-1 and CV220915-2, respectively.

적 요

황색 모자이크 병징을 보이는 프리지아(*Freesia refracta* Klatt.) 두 품종 Pinksia-1와 Pinksia-2으로부터 잎 샘플을 채취하였다. RNA-sequencing과 생물정보학 분석을 통해 포티비리대과 포티바이러스속의 콩황화모자이크바이러스(BYMV) 3개 유전체를 동정하였다. 3개 BYMV 유전체 크기는 9,528 nucleotides (nt)이며, 세 개 BYMV 유전체 서열의 경우 99.90%에서 99.91%의 동일성을 보여주었다. 본 연구에서 동정된 BYMV 유전체의 경우 모두 한국의 프리지아에서 동정된 Fr 분리주와 매우 가까운 것으로 밝혀졌다. BYMV유전체는 단일 가닥의 positive RNA로 구성되어 있으며 하나의 큰 폴리프로틴을 만들며, 이 폴리프로틴은 총 10개의 서로 다른 바이러스 단백질로 잘려진다. 또한 BYMV유전체는 PIPO단백질을 만든다. 본 연구에서는 RNA-sequencing을 통해 한국 프리지아에서 동정된 세 개의 BYMV 유전체 정보를 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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