Construction of an Agroinfectious Clone of a Korean Isolate of Sweet Potato Symptomless Virus 1 and Comparison of Its Infectivity According to Agrobacterium tumefaciens Strains in Nicotiana benthamiana

Phuong T. Ho†1, Hee-Seong Byun†2, Thuy T. B. Vo†1, Aamir Lal3,4, Sukchan Lee*1, and Eui-Joon Kil*3,4

1Department of Integrative Biotechnology, Sungkyunkwan University, Suwon 16419, Korea
2Crop Protection Division, National Institute of Agricultural Sciences, Rural Development Administration, Wanju 55365, Korea
3Department of Plant Medicinals, Andong National University, Andong 36729, Korea
4Agricultural Science and Technology Research Institute, Andong National University, Andong 36729, Korea

(Received on December 31, 2022; Revised on April 7, 2023; Accepted on April 18, 2023)

Sweet potato symptomless virus 1 (SPSMV-1) is a single-stranded circular DNA virus, belonging to the genus Mastrevirus (family Geminiviridae) that was first identified on sweet potato plants in South Korea in 2012. Although SPSMV-1 does not induce distinct symptoms in sweet potato plants, its co-infection with different sweet potato viruses is highly prevalent, and thus threatens sweet potato production in South Korea. In this study, the complete genome sequence of a Korean isolate of SPSMV-1 was obtained by Sanger sequencing of polymerase chain reaction (PCR) amplicons from sweet potato plants collected in the field (Suwon). An infectious clone of SPSMV-1 (1.1-mer) was constructed, cloned into the plant expression vector pCAMBIA1303, and agro-inoculated into Nicotiana benthamiana using three Agrobacterium tumefaciens strains (GV3101, LBA4404, and EHA105). Although no visual differences were observed between the mock and infected groups, SPSMV-1 accumulation was detected in the roots, stems, and newly produced leaves through PCR. The A. tumefaciens strain LBA4404 was the most effective at transferring the SPSMV-1 genome to N. benthamiana. We confirmed the viral replication in N. benthamiana samples through strand-specific amplification using virion-sense- and complementary-sense-specific primer sets.

Keywords: agro-inoculation, geminivirus, mastrevirus, sweet potato, sweet potato symptomless virus 1

Sweet potato (Ipomoea batatas L.) is among the most important food crops worldwide as it contains high levels of vitamins A and C, iron, potassium, fiber, and other nutrients (Kim et al., 2017). Sweet potato production has increased continuously over the last four decades, reaching approximately 105 million metric tons worldwide in 2016 (Food and Agriculture Organization of the United Nations, 2016). However, with the recent spread of several sweet potato viruses, including the sweet potato symptomless virus 1 (SPSMV-1), the productivity of sweet potato has decreased significantly (Loebenstein, 2015). SPSMV-1 was first reported in Peru in 2009 (Cao et al., 2017; Kreuze et al., 2009; Muhire et al., 2013). Since then, the virus has been reported in several countries in...
the Americas, East Africa, Europe, and Northeast Asia including USA, Uruguay, Brazil, Kenya, Spain, Taiwan, and China (Kwak et al., 2014; Mbanzibwa et al., 2014; Souza et al., 2018). SPSMV-1 was first identified in sweet potatoes in the Korean peninsula in 2012 (Kwak et al., 2014). Since then, SPSMV-1 has been reported to have infected sweet potato plants in many regions of South Korea.

SPSMV-1 is a circular single-stranded DNA (ssDNA) virus belonging to the genus Mastrevirus (family Geminiviridae). The SPSMV-1 genome is 2,599-2,602 nucleotides in size, consisting of five overlapping transcribed open reading frames (ORFs) and two non-transcribed regions called the intergenic regions, and is encapsidated in a twinned icosahedral capsid (Qiao et al., 2020). The large intergenic region (LIR) contains the stem-loop structure, the origin of replication, and transcription start sites, whereas the short intergenic region (SIR) contains the complementary strand replication origin and transcription termination sites (Fondong, 2013; Hayes et al., 1988). LIR contains an abnormal nonanucleotide sequence, TAAGATTTC, which differs from that of most viruses of the family Geminiviridae (Borah et al., 2016; Cao et al., 2017). The virion-sense strand encodes the coat protein (gene \( V1 \)), which encapsidates the virion-sense ssDNA, and the movement protein (gene \( V2 \)), which is involved in cell-to-cell movement. The complementary sense encodes three genes: \( C1 \), \( C2 \), and \( C3 \). The replication-associated protein (Rep), expressed in genes \( C1 \) and \( C2 \) by transcript splicing, initiates rolling-circle replication by introducing a nick into the nonanucleotide sequence in the virion-sense strand. RepA, the protein product of ORF \( C1 \), binds to a plant homolog of the retinoblastoma protein to regulate cell cycle progression and support viral DNA replication (Zerbini et al., 2017). ORF \( C3 \) is completely embedded within the ORF \( C1 \) and appears in all eight other dicot-infecting mastreviruses and in the maize streak virus (MSV) at a similar position (Cao et al., 2017). However, its function remains unclear.

Although SPSMV-1 does not induce major symptoms in sweet potato plants, co-infection with different disease-inducing sweet potato viruses is highly prevalent, and threatens sweet potato production (Souza et al., 2018). In this study, an isolated SPSMV-1 Korean infectious clone was constructed, and agro-inoculation assays with different Agrobacterium tumefaciens strains were performed on the model plant Nicotiana benthamiana to confirm the function of the infectious clone and, later, investigate the interaction between SPSMV-1 and its host plant.

Materials and Methods

Samples and virus sequence collection. Sweet potato samples were randomly collected from a field in Suwon, Gyeonggi, South Korea during a 2014 survey. Complete sequences of SPSMV-1 isolates from China (KY565235.1, MG603669.1, MG603671.1, MG603668.1, MK802081.1, MG603672.1, and MG603670.1), the USA (KY565232.1), Uruguay (KY565234.1), Brazil (MH375686.1 and MG680260.1), Taiwan (KY565233.1, KY565236.1, and KY565237.1), and Kenya (KY565231.1) were retrieved from NCBI GenBank (https://www.ncbi.nlm.nih.gov/nucleotide). Additionally, the nucleotide sequences of 11 mastreviruses were selected for phylogenetic tree construction to elucidate the genomic characteristics of the Korean SPSMV-1 isolate.

Total DNA extraction, rolling-circle amplification, polymerase chain reaction, and viral genome sequencing. Total genomic DNA was isolated from sweet potato tissues using the STE method (Azad and Nematadeh, 2013). Leaf, root, and stem samples were ground using a mortar and pestle in liquid nitrogen. Subsequently, 100 mg of the obtained sample powder was transferred to 1.5 ml micro-centrifuge tubes followed by the addition of lysis buffer containing 470 µl STE buffer (0.4 M sucrose, 20 mM Tris-HCl, and 20 mM EDTA), 30 µl of 20% sodium dodecyl sulfate, 200 µl 8 M LiCl, 1 µl of 2-mercaptoethanol, and 100 mg of polyvinylpyrrolidone 40 K. The sample and lysis buffer were vortexed to mix well and incubated at 60°C for 45 min. To separate the DNA from other cellular components, 1 volume (700 µl) of chloroform: isoamyl alcohol (24:1) was added, and the mixture was centrifuged at 13,000 rpm at 4°C for 15 min. The DNA was then precipitated with isopropanol, washed with cool 70% ethanol, air-dried, and dissolved in 50 µl of 1× TE buffer. Viral DNA was amplified via rolling circle amplification (RCA) using a Templifi Kit (GE Healthcare Life Sciences, Piscataway, NJ, USA) following the manufacturer’s introduction. The RCA products were used as polymerase chain reaction (PCR) templates to obtain full-length sequences of the virus. A 2.6 kb DNA amplicon was ligated into the pGEM-T Easy vector (Promega, Madison, WI, USA) via TA cloning according to the manufacturer’s instructions. A plasmid containing the full-length SPSMV-1 genome was sequenced by Macrogen (Seoul, Korea) and submitted to GenBank.
**Phylogenetic tree analysis.** Multiple alignment of all sequences of the SPSMV-1 isolates and the 11 mastreviruses (chickpea chlorosis virus [CpCV, GU256530.1]; chickpea chlorotic dwarf virus [CpCDV, AM849097.1]; chickpea redleaf virus [CpRLV, GU256532.1]; eragrostis streak virus [ESV, EU244915.1]; MSV [Y00514.1]; maize striate mosaic virus [MSMV, MF167297.1]; oak dwarf virus [ODV, AM296025.1]; panicum streak virus [PanSV, L39638.1]; sugarcane streak virus [SCSV, M82918.1]; switchgrass mosaic-associated virus [SgMaV, KF806701.1]; tobacco yellow dwarf virus [TYDV, M81103.1]; and wheat dwarf virus [WDV, AJ783960.1]) was conducted using ClustalW tool of MEGA X software (Kumar et al., 2018). A phylogenetic tree was constructed with previously aligned sequences using the neighbor-joining method with a bootstrap value of 1,000. The Rep A, movement protein, coat protein and intergenic regions were compared to identify the relationship of the Korean SPSMV-1 isolate with other mastreviruses using basic local alignment search tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Construction of the infectious clone of the Korean SPSMV-1 isolate.** An infectious clone of the Korean SPSMV-1 isolate was obtained by constructing a partial tandem repeat of the full-length viral DNA as previously described (Urbino et al., 2008; Vo et al., 2022) (Fig. 1). Specific primer sets were designed with ends containing restriction sites to detect two fragments through PCR amplification (SPSMV-1-IC1-F/SPSMV-1-IC1-R and SPSMV-1-IC2-F/SPSMV-1-IC2-R) (Table 1). Each partial fragment of SPSMV-1 was ligated into the pGEM-T Easy vector (Promega) via TA cloning and sequenced for confirmation. The cloned constructs were digested with the following restriction enzymes: IC1 (XbaI and KpnI) and IC2 (KpnI and HindIII) (Takara, Shiga, Japan). To produce the infectious clone 1.1-mer, two partial fragments were introduced into the pCAMBIA1303 vector (Abcam, Cambridge, UK) and transformed into competent *Escherichia coli* strain DH5α using the heat shock method. The transformed plasmids were extracted from *E. coli* using the AccuPrep Nano-Plus Plasmid Mini Extraction Kit (Bioneer, Daejeon, Korea) and cross-checked by digestion with the three restriction enzymes, XbaI, KpnI, and HindIII (The plasmids were

---

**Fig. 1.** Schematic of the construction of an infectious clone of the Korean sweet potato symptomless virus 1 (SPSMV-1) isolate.
then transformed into three different *A. tumefaciens* strains: GV3101, LBA4404, and EHA105 (Fig. 1). Infectious clo-

nes were confirmed by both enzyme digestion and colony 
PCR using the 2× AccuPower PCR Master Mix (Bioneer) 
with the primers for IC1 and IC2 SPSMV-1 (Table 1).

*n.* 

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5′-3′)</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPSMV-F-full</td>
<td>GGAAGTGCAACTTGATAAGGC</td>
<td>Abutting primers to get a</td>
</tr>
<tr>
<td>SPSMV-R-full</td>
<td>TGGCCAAATGACATCTTGTAC</td>
<td>full-length genome</td>
</tr>
<tr>
<td>SPSMV-1-IC1-F</td>
<td>TCTAGAGATGTATTTGTAGAGGGAAGTAA</td>
<td>Infectious clone construction</td>
</tr>
<tr>
<td>SPSMV-1-IC1-R</td>
<td>GTTACCCCCCTGGGTTGAACA</td>
<td></td>
</tr>
<tr>
<td>SPSMV-1-IC2-F</td>
<td>GTTACCGTGATTTTGATGACGT</td>
<td></td>
</tr>
<tr>
<td>SPSMV-1-IC2-R</td>
<td>AAGCTTCAGCTATGTATCTTTGATA AGA TA</td>
<td>Detection by conventional   PCR</td>
</tr>
<tr>
<td>SPSMV-1-Detection-F</td>
<td>GATGTATTTTGATAGGGGGAGGTAA</td>
<td></td>
</tr>
<tr>
<td>SPSMV-1-Detection-R</td>
<td>CCTTGGGGTGGAACA</td>
<td>Internal normalization</td>
</tr>
<tr>
<td>SPSMV-1-F</td>
<td>TCGAGGTGATCCAGGATAGTTC</td>
<td>qPCR detection</td>
</tr>
<tr>
<td>SPSMV-1-R</td>
<td>GTCTAAGGGCGGATACCCTAAC</td>
<td></td>
</tr>
<tr>
<td>EF1α-F</td>
<td>GCTGTCAAGTTTGTAGATCT</td>
<td></td>
</tr>
<tr>
<td>EF1α-R</td>
<td>GAATCATCTAATAACATACAGCAT</td>
<td></td>
</tr>
<tr>
<td>OVS-TAG</td>
<td>AGTTTAAAGAACCCCTTCCCGCATGTCAGGAGGGGTAAGC</td>
<td>Detection by strand-specific PCR</td>
</tr>
<tr>
<td>OCS-TAG</td>
<td>AGTTTAAAGAACCCCTTCCCGCAACAGGCACTTTATTTGTAGT</td>
<td></td>
</tr>
<tr>
<td>TAG</td>
<td>AGTTTAAAGAACCCCTTCCCGC</td>
<td></td>
</tr>
<tr>
<td>OVS</td>
<td>ATGTCTACGAGGGGTAAGC</td>
<td></td>
</tr>
<tr>
<td>OCS</td>
<td>ACAACAGGCACTTTATTTGTAGT</td>
<td></td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction; qPCR, quantitative real-time PCR.

**Agro-inoculation with the Korean SPSMV-1 isolate.** *N. benthamiana* seeds were planted in sterilized soil and cul-
tivated in a growth chamber at the Sungkyunkwan University, Suwon, South Korea. Four-week-old *N. benthamiana*
plants of similar sizes were selected and divided into mock, 
GV3101, LBA4404, and EHA105. *A. tumefaciens* strains 
were cultured in Luria broth media in the presence of the 
PCambIA1303- selective antibiotic, kanamycin (50 mg/ l), and other strain-specific selective antibiotics (50 mg/ l) (for GV3101: rifampicin and gentamicin; LBA4404: rif-
ampicin; EHA105: rifampicin and streptomycin) at 28°C 
with agitation for 30 h until the OD value at 600 nm rea-
ched 0.8-1.0. The main apical shoot tips were inoculated 
with 0.5 ml of agrobacterial culture using the pin-pricking 
method. Plants in the mock group were inoculated with 
*A. tumefaciens* containing the empty pCAMBIA1303 plas-
mid. Photographs were taken for each group four weeks 
after inoculation, and samples from newly produced leaves, 
stems, and roots were collected for downstream analysis.

**Total DNA extraction and PCR detection.** Four weeks 
after inoculation, samples from young leaves, stems, and 
roots were collected from the mock- and SPSMV-1-inocu-
lated *N. benthamiana* plants. Genomic DNA was isolated 
from the tissues using the STE method described above. 
To detect the presence of the virus inside the samples, PCR 
was conducted with the detection primers SPSMV-1-Dete-
ction-F/SPSMV-1-Detection-R (Table 1). The mixture 
was prepared using 10 µl of 1× AccuPower PCR Mas-
termix (Bioneer), 1 µl of template DNA, 1 µl each of the 
forward and reverse primers (10 pM), and distilled water to 
reach a total volume of 20 µl. The conditions for the PCR 
reaction were as follows: preheating at 94°C (3 min), fol-
lowed by 35 cycles of 30 s at 94°C, 30 s at 58°C, 1 min at 
72°C, and a final extension at 72°C (5 min). The amplified 
DNA was visualized on a 1% agarose gel by electrophore-
sis, and sequenced by Macrogen.

**Viral DNA quantification by quantitative real-time PCR.** Viral accumulation of SPSMV-1 was evaluated in 
sweet potato samples (young leaves, stems, and roots) and 
four-week-old-inoculated *N. benthamiana*. Total DNA was 
extracted using a DNeasy Plant mini kit (Qiagen, Hilden, 
Germany) and 1 µg of DNA extracted from leaves was 
amplified via quantitative real-time PCR (qPCR) using the
primer set for the RepA protein (SPSMV-1-F/SPSMV-1-R) (Table 1). The SYBR Green PCR Master mix (Takara) in a final volume of 20 μl was used according to the manufacturer’s protocol. The qPCR experiment was performed in triplicate per sample. The reaction and the detection of the fluorescent signals were performed using a Rotor Gene Q thermocycle (Qiagen). EF1α was used for the internal normalization (Table 1). The data analysis was performed using the \(2^{-\Delta\Delta Ct}\) method (Livak and Schmittgen, 2001) to determine relative expression levels. An unpaired Student’s t-test was used to determine significant differences with the data expressed as mean ± standard error, and statistical significance was defined at \(P < 0.05\).

**Strand-specific PCR.** Strand-specific amplification was performed according to the method described by Rodríguez-Negrete et al. (2014) and (Kil et al., 2018) with modification using virion-sense- and complementary-sense-specific primer sets (Table 1). First, extension reactions of single-stranded viral templates with T4 DNA polymerase (Takara) and viral-specific primers (OCS-TAG or OVS-TAG) were performed for strand-specific amplification. The products from this step were purified using the QIAquick PCR Purification Kit (Qiagen). Subsequently, 2 μl of the first-strand reaction product was mixed with 10 μl of 2× AccuPower PCR Master Mix (Bioneer), 1 μl of 10 pM specific primers (TAG, OVS or OCS), and 6 μl of nuclease-free water, following the manufacturer’s protocol. PCR cycling consisted of an initial denaturation step at 95°C for 30 s, 40 cycles of 95°C for 10 s, 60°C for 15 s and 72°C for 20 s in a T100 thermal cycler (Bio-Rad, Hercules, CA, USA).

**Results**

**Virus detection in sweet potato plants.** In 2014, three symptomless sweet potato samples were collected on a farm in Suwon, South Korea, during a survey for sweepo-virus detection analysis. Total DNA was extracted and subjected to PCR amplification using SPSMV-1-Detection-F/SPSMV-1-Detection-R (Table 1). An amplicon of the expected 830 nucleotides product was obtained from three of the three sweet potato samples and was sent for sequencing (Fig. 2A). BLAST analysis indicated that the

---

**Fig. 2.** Detection of sweet potato symptomless virus 1 (SPSMV-1). (A) Detection of SPSMV-1 by polymerase chain reaction (PCR) in leaves (L), stem (S) and roots (R) of sweet potato samples collected from a field in South Korea. Lane +, positive control with plasmid DNA containing partial SPSMV-1 genomic DNA; Lane -, negative control with no DNA template; S1-S3 refer to the different sweet potato samples. (B) Sequencing results showing the detected virus in 3 sweet potato samples with high similarity to the Chinese SPSMV-1 isolate (accession number: KY565235). (C) PCR amplification of the collected sweet potato samples using SPSMV-1 specific primers to obtain complete sequence. Lanes S1-S3 refer to three sweet potato samples. (D) Quantitative real-time PCR analysis indicating SPSMV-1 titer in sweet potato leaves from the fields. Mock plants show virus-free sweet potato samples. The bar graphs indicate the mean± standard deviation (n = 5). The statistical comparison was performed with the unpaired t-test: *P < 0.05.
detected viral sequence shared 99.61% similarity with the Chinese SPSMV-1 isolate (KY565235) (Fig. 2B). When PCR was performed directly from the genomic DNA, an amplification product of sufficient concentration could not be obtained, so RCA was performed and then PCR was performed using the RCA product with abutting primers (Table 1) in PCR for full-length amplification of the viral genome. The amplified products were analyzed on a 1% agarose gel, and a target band of 2.6 kb in all three sweet potato samples (Fig. 2C) was cloned into the pGEM-T Easy vector (Promega) and sequenced. BLAST analysis revealed a 99.92% nucleotide identity with the Chinese SPSMV isolate (KY565235). The complete sequence of the Korean SPSMV-1 isolate was deposited in the Gen-

Fig. 3. Sequence analysis of the complete genome of the Korean sweet potato symptomless virus 1 (SPSMV-1) isolate. (A) Schematic diagram of two intergenic regions and five open reading frames. (B) Neighbor-joining phylogenetic tree of the complete genomic sequences of SPSMV-1 and other mastreviruses, constructed using 1,000 bootstrap replicates (MEGA X). (C) The large intergenic region (LIR) with an unusual nonanucleotide sequence (TAAGATT↓CC) in all SPSMV-1 isolates.
Construction of Agroinfectious Clone of SPSMV-1 Korean Isolate

Bank (MF148248). qPCR revealed that the relative titer of infected samples was similar (Fig. 2D). Compared to the case where PCR was performed by artificially increasing the amount of virus genome through RCA, the concentration of the amplification product in the previous PCR and the qPCR results showed a similar low level of amplification for each sample (Fig. 2).

**Sequence analysis of the complete genome of the Korean SPSMV-1 isolate.** The complete sequence of the Korean SPSMV-1 isolate obtained by PCR was 2,599 nucleotides long. The number and arrangement of ORFs and intergenic regions were identical to those of other SPSMV-1 isolates, with five ORFs encoding C1, C2, C3, V1, and V2 (Fig. 3A). The phylogenetic tree shown in Fig. 3B shows the relationship between SPSMV-1 sequences of different origins and mastrevirus sequences. The amino acid sequences of the proteins encoded by the ORFs and the nucleotide sequences of the intergenic sequences of SPSMV-1 were compared with those of other geminiviruses to obtain a better insight into the taxonomic relationship of this virus. The Korean SPSMV-1 isolate was found to be most closely related to the Chinese SPSMV-1 isolate. It was confirmed that the ORF sequences for Rep, movement protein, and coat protein showed a certain level of similarity with other dicot-infecting mastreviruses. Interestingly, however, the nucleotide sequences of the LIR and SIR of SPSMV-1 did not show any similarity with any other previously reported sequences of viruses (Table 2). The LIR of all SPSMV-1 isolates was compared at the nucleotide level, and all were found to contain an unusual nonanucleotide sequence TAAGATT↓CC, in contrast to other geminiviruses, which possess the sequence TAAT-ATTAC (Fig. 3C). This means that the IRs of SPSMV-1 have unique characteristics different from those of existing geminiviruses.

**Infectivity test of infectious SPSMV-1 clone in N. benthamiana.** Because the choice of *A. tumefaciens* strain used for the agro-inoculation process can substantially affect the efficiency and expression of viral proteins in plants, we used the three most commonly used *A. tumefaciens* strains: GV3101, LBA4404, and EHA105. Four weeks after agro-inoculation, *N. benthamiana* plants showed no visual symptoms in either the mock- or SPSMV-1-inoculated plants. No differences were observed among the three *N. benthamiana* plants inoculated with the three *A. tumefaciens* strains (Fig. 4A). Three organs (newly produced leaves, stems, and roots) of the SPSMV-1-inoculated *N. benthamiana* plants were harvested and analyzed using PCR to investigate their viral replication ability and systemic movement. qPCR was used to evaluate the relative viral titers in *N. benthamiana*. The results revealed that the virus was present in all three organs 4 weeks after inoculation.

---

**Table 2. List of mastreviruses sharing open reading frame (ORF) sequence similarities with the Korean sweet potato symptomless virus 1 (SPSMV-1) isolate**

<table>
<thead>
<tr>
<th>ORF</th>
<th>Virus (similarity (%))</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication-associated protein (Rep)</td>
<td><em>Chickpea chlorosis virus</em> (54.04%)</td>
<td>KC172700</td>
</tr>
<tr>
<td></td>
<td><em>Chickpea redleaf virus</em> (52.75%)</td>
<td>NC014739</td>
</tr>
<tr>
<td></td>
<td><em>Chickpea chlorotic dwarf virus</em> (54.55%)</td>
<td>MN178605</td>
</tr>
<tr>
<td></td>
<td><em>Chickpea yellow dwarf virus</em> (53.61%)</td>
<td>AIW42780</td>
</tr>
<tr>
<td>Large intergenic region (LIR)</td>
<td>No match with previously reported virus</td>
<td></td>
</tr>
<tr>
<td>Movement protein (MP)</td>
<td><em>Chickpea chlorotic dwarf virus</em> (61.11%)</td>
<td>KY817246</td>
</tr>
<tr>
<td></td>
<td><em>Chickpea redleaf virus</em> (84.38%)</td>
<td>NC014739</td>
</tr>
<tr>
<td></td>
<td><em>Wheat dwarf virus</em> (45.12%)</td>
<td>KJ473699</td>
</tr>
<tr>
<td>Coat protein (CP)</td>
<td><em>Chickpea chlorotic dwarf virus</em> (45.31%)</td>
<td>KC172680</td>
</tr>
<tr>
<td></td>
<td><em>Chickpea yellows virus</em> (48.67%)</td>
<td>NC038478</td>
</tr>
<tr>
<td></td>
<td><em>Chickpea chlorosis virus</em> (51.03%)</td>
<td>JN989424</td>
</tr>
<tr>
<td></td>
<td><em>Tobacco yellow dwarf virus</em> (49.03%)</td>
<td>JN989446</td>
</tr>
<tr>
<td></td>
<td><em>Chickpea yellow dwarf virus</em> (50.65%)</td>
<td>NC025475</td>
</tr>
<tr>
<td></td>
<td><em>Chickpea redleaf virus</em> (46.64%)</td>
<td>MK940528.1</td>
</tr>
<tr>
<td>Short intergenic region (SIR)</td>
<td>No match with previously reported virus</td>
<td></td>
</tr>
</tbody>
</table>

*Nucleotide sequence similarity with the Korean SPSMV-1 isolate (MF148248.1).*
Fig. 4. Results of the agro-inoculation test conducted using three *Agrobacterium* strains vectored with the Korean sweet potato symptomless virus 1 (SPSMV-1) isolate. (A) Appearance of mock *Nicotiana benthamiana* and infected plants. (B) Detection of SPSMV-1 DNA in *N. benthamiana* by polymerase chain reaction (+, positive control; –, negative control; number 1-5 indicates infected samples). (C) SPSMV-1 titer in leaf, stem and root of *N. benthamiana*. The bar graphs indicate the mean ± standard deviation (n = 5). The statistical comparison was performed with the unpaired *t*-test: *P* < 0.05, **P** < 0.01, ***P*** < 0.001, ****P*** < 0.0001, ns: not significant.

Fig. 5. Strand-specific amplification of sweet potato symptomless virus 1 (SPSMV-1)-infected *Nicotiana benthamiana* plants. (A) Illustration of strand-specific polymerase chain reaction (PCR). (B) Strand-specific amplification with root samples (1 and 2) from SPSMV-1-inoculated *N. benthamiana* plants using virion-sense- and complementary-sense-specific primer sets.
Construction of Agroinfectious Clone of SPSMV-1 Korean Isolate

and the LBA4404 A. tumefaciens strain showed the highest SPSMV-1 genome transfer ability (Fig. 4B and C). Strand-specific amplification using virion-sense- and complementary-sense specific primer sets was performed to confirm the replication ability of the virus. The assumption that geminiviral DNA replicated and ssDNA was converted into double-stranded DNA (dsDNA) intermediates by a rolling-circle replication mechanism could be confirmed by PCR amplification using OCS and OVS primers and reactions using TAG and OCS or OVS primers (Fig. 5A). Our data showed that dsDNA and two ssDNA molecules (virion and complementary senses) were present in the infected samples, indicating that the viral genomes had replicated in the N. benthamiana plants (Fig. 5B). However, the virus could not be identified through Southern blotting, suggesting that the viral titer was very low (data not shown).

Discussion

In this study, we identified the whole genome of the Korean SPSMV-1 isolate and constructed an infectious clone, which will contribute to a better understanding of virus-plant interactions and the biological significance of this virus in infected plants. The SPSMV-1 isolate exhibited the typical genome structure (number and location of ORFs) of a mastrevirus. The SPSMV-1 infectious clone was introduced into N. benthamiana plants using Agrobacterium-mediated inoculation, the most commonly used method for plant genetic engineering, owing to its relatively high efficiency. Three widely used A. tumefaciens strains, GV3101, EHA105, and LBA4404, consisting of SPSMV-containing pCAMBIA1303, were evaluated using conventional PCR to determine which A. tumefaciens strain was the best for viral gene transfer to N. benthamiana. Four weeks after inoculation, SPSMV-1 was detected in the young leaves, stems, and roots of N. benthamiana plants. We found that LBA4404 was the most effective strain for SPSMV-1 gene transfer. Therefore, A. tumefaciens strain LBA4404 could be an appropriate strain for efficient viral gene transfer of SPSMV-1 in N. benthamiana plants. Similar result was reported by Bakhsh et al. (2017), who found that LBA4404 was a better choice than EHA105, GV2260, C58C1, GV3101, or AGL1 for the genetic transformation of foreign genes in N. tabacum L. A successful transformation process requires host (plant) genome and A. tumefaciens strain compatibility, which is influenced by several factors, including the type of chromosomal background of A. tumefaciens strains, different opines, mechanism of transfer and integration of T-DNA, and T-DNA copy number containing the gene of interest (Yadav et al., 2014). Therefore, the choice of A. tumefaciens strain for plant transformation can substantially affect the transformation efficiency and expression of foreign proteins (Yadav et al., 2014). However, the detailed mechanism by which LBA4404 favors SPSMV-1 genome transfer to N. benthamiana plants requires further analysis.

The mastrevirus SPSMV-1 has been reported to infect sweet potato plants in several countries worldwide, and a high infection rate has been reported in South Korea (Kwak et al., 2014). Although SPSMV-1 is an asymptomatic mastrevirus and does not appear to play an important pathological role, it may pose a threat due to the occurrence of synergistic interactions among viruses infecting sweet potatoes (Cuellar et al., 2015; Untiveros et al., 2007). In our study, nucleotide sequence analysis revealed that the Korean SPSMV-1 isolate contains an abnormal nonanucleotide sequence and a longer stem-loop region in the LIR, similar to other reported isolates, when compared to other geminiviruses. We hypothesized that this could explain the pathological characteristics of SPSMV-1 (asymptomatic infection and low viral accumulation). Creating mutations in these regions of the genome and constructing their infectious clones are necessary to comprehensively evaluate viral replication and determine whether the plants are asymptomatic owing to the nature of the virus or because a low viral accumulation is insufficient to induce a response in the further studies. This study will help in constructing suitable mutant infectious clones.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

This work was supported by a grant (project code no. Z-1543086-2017-21-01) from the Animal and Plant Quarantine Agency of South Korea.

References


Borah, B. K., Zarreen, F., Baruah, G. and Dasgupta, I. 2016. Insights into the control of geminivirus promoters. Virology 263


