First Agroinoculation System Development of *Sweet Potato Symptomless Virus* on *Nicotiana Benthamiana*

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Research Article

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Abstract

Sweet potato symptomless virus 1 (SPSMV-1) is a single-stranded circular DNA virus, belonging to genus Mastrevirus, family Geminiviridae. It was first identified in the Korean peninsula in 2012 in asymptomatic sweet potato plants. Although SPSMV-1 cannot induce any significant symptoms in sweet potato plants, its co-infection with different sweet potato viruses is so prevalent that it can pose a threat to sweet potato production in Korea by the formation of a new virus through recombination events. The complete genome sequence of SPSMV-1 Korean isolate was obtained through rolling circle amplification (RCA) from sweet potato samples collected in the field (Suwon, Korea). 1.1-mer SPSMV-1 infectious clone was constructed, cloned into the plant expression vector pCAMBIA1303 and transformed into Nicotiana benthamiana with the support of the three most commonly used agrobacteria strains, GV3101, LBA4404 and EHA105. No differences were observed between the mock and inoculated groups; however, polymerase chain reaction (PCR) results showed that the virus can enter the cells and travel from the infected area to the roots, stems, and newly produced leaves. The LBA4404 Agrobacterium strain showed the highest ability to transfer the SPSMV-1 genome to N. benthamiana. Through strand-specific amplification using virion-sense- and complementary-sense- specific primer sets, we confirmed virus replication in N. benthamiana samples. This is the first study on the infectious clone construction of SPSMV-1; hence, our research contributes toward gaining a better insight into virus-plant interactions and the biological significance of this virus in infected plants.

Introduction

Sweet potato (Ipomea batatas L.) belonging to the family Convolvulaceae is one of the most important food crops worldwide because of its high levels of vitamins A and C, iron, potassium, fiber, and other nutrients (Kim J, 2017). Over the last four decades, sweet potato production has increased continuously, reaching approximately 92 million metric tons in 2018. However, recently, with the spread of several sweet potato viruses, including the Sweet potato symptomless virus 1 (SPSMV-1), the productivity of this cultivar has fallen significantly (Loebenstein, 2015). SPSMV-1 is a circular single-stranded DNA virus, belonging to the genus Mastrevirus, family Geminiviridae, and was first reported in Peru in 2009 (Mengji Cao, 2017, Muhire B, 2013, Kreuze JF, 2009). To date, the virus has been detected in some countries in the Americas, East Africa and Northeast Asia (Mbanzibwa DR, 2014, Souza CA, 2018, Kwak HR, 2014). As the name suggests, although SPSMV-1 infects sweet potato, it does not induce symptoms. The genome of SPSMV-1 is encapsidated in a twinned icosahedral capsid, 2599–2602 nucleotides in size, consisting of five overlapping transcribed open reading frames (ORFs) and two non-transcribed regions called the intergenic regions (Qi Qiao, 2020). The large intergenic region (LIR) is the location of the stem loop structure, the origin of replication and transcription start site, whereas the short intergenic region is the location of both the complementary strand replication origin and transcription termination sites. The LIR contains an abnormal nonanucleotide sequence TAAGATTCC, which is different from that of most viruses of the family Geminiviridae (Mengji Cao, 2017, Borah BK, 2016). The virion-sense strand encodes the coat protein (CP, gene V1) that encapsidates the virion-sense ssDNA, and the movement protein (MP,
gene V2) that is involved in cell-to-cell movement. The complementary sense encodes three genes, C1, C2, and C3. The replication-associated protein (Rep) expressed from 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 the C1 ORF, binds to the plant homolog of the retinoblastoma protein to regulate cell-cycle progression and support viral DNA replication (F. Murilo Zerbini, 2017). The C3 ORF is completely embedded within the C1 ORF and appears in all eight of the other dicot-infecting mastreviruses and maize streak virus (MSV) at a similar position (Mengji Cao, 2017), however, its function has not yet been determined.

The international trade of agricultural products has led to the introduction of many new plant viruses in Korea. The SPSMV-1 was first identified in the Korean peninsula in 2012 in asymptomatic sweet potato samples (Hae-Ryun Kwak, 2014). Since then, SPSMV-1 has been reported to infect sweet potato plants in many regions of Korea. Although SPSMV-1 does not induce significant symptoms in sweet potato plants, co-infection with different disease-inducing sweet potato viruses is so prevalent that it can pose a threat to sweet potato production in Korea by the formation of new viruses through recombination (C. A. Souza, 2018). In this study, an isolated SPSMV-1 Korean infectious clone was constructed and agro-inoculation assays with different Agrobacterium strains were performed on the model plant Nicotinana benthamiana to confirm about the function of the infectious clone and, later can be used to look at the interaction between SPSMV-1 and its host plant.

**Materials And Methods**

**Samples and virus sequence collection**

Three asymptomatic sweet potato samples (Fig. 1a) were collected from the three different fields in Gunpo, Gyeonggi, South Korea, and complete sequences of SPSMV-1 isolates from China (KY565235.1, MG603669.1, MG603671.1, MG603668.1, MK802081.1, MG603672.1, MG603670.1), USA (KY565232.1), Uruguay (KY565234.1), Brazil (MH375686.1, MG680260.1), Taiwan (KY565233.1, KY565236.1, KY565237.1), and Kenya (KY565231.1) were collected from NCBI GenBank (https://www.ncbi.nlm.nih.gov/nucleotide). Additionally, nucleotide sequences of eleven mastreviruses for phylogenetic tree construction were also harvested to elucidate the genomic characteristics of the SPSMV-1 Korean isolate.

**Total DNA extraction, rolling-circle amplification (RCA) and viral genome sequencing**

Total genomic DNA was isolated from sweet potato tissues using the STE method (Shepherd LD, 2011). Samples were ground with mortar and pestle in liquid nitrogen, followed by the addition of lysis buffer containing 470 µL STE buffer (0.4 M sucrose, 20 mM Tris-HCl, 20 mM EDTA), 30 µL of 20% SDS, 200uL 8 M LiCl, 1 µL of 2-mercaptoethanol, and 100 µg of polyvinylpyrrolidone. The sample and lysis buffer were vortexed to mix well and incubated at 60 °C for 45 min. To separate the DNA from the other components,
the same amount (700 µL) of chloroform:isoamyl alcohol (24:1) was added and the mixture was centrifuged at 13,000 rpm, 4 °C for 15 min. DNA was precipitated with isopropanol, washed with cool 70% ethanol, air-dried, and dissolved in 1X TE buffer. DNA samples were used for SPSMV-1 detection and viral DNA was amplified through RCA using a Templiphi™ Kit (GE Healthcare Life Sciences, Uppsala, Sweden) following the supplier’s protocol. After confirming the presence of SPSMV-1, the RCA products were digested with several restriction enzymes and used as template for PCR to get the full-length sequence of virus. The 2.6 kb DNA band was ligated into the pGEM-T Easy vector (Promega, Madison, WI, USA) using the TA cloning technique according to the manufacturer’s instructions. A plasmid containing the full-length SPSMV-1 genome was sequenced by Macrogen (Seoul, South Korea) and submitted to the NCBI GenBank database.

**Phylogenetic tree analysis**

The multiple alignment of all sequences of SPSMV-1 isolates and 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; Maize streak virus-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; Wheat dwarf virus-WDV - AJ783960.1) was conducted using the ClustalW tool of MEGA X software (Kumar S, 2018). A phylogenetic tree was constructed with previously aligned sequences using the neighbor-joining method with a bootstrap value of 1000. The Rep A, movement protein, coat protein and both two intergenic regions were compared to figure out the relation of SPSMV-1 Korean isolate with other mastreviruses using BLAST (basic local alignment search tool, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Construction of the infectious clone of the SPSMV-1 Korean isolate**

To generate the infectious clone of the SPSMV-1 Korean isolate, primer sets were designed based on their sequences. Two partial genomes (0.3-mer and 0.8-mer) containing restriction sites at the edge were amplified using primer sets (Table 1) and ligated into the pGEM-T Easy vector (Promega) using the TA cloning technique according to the manufacturer’s instructions. The DNA fragments were sequenced (Macrogen) and cut using specific enzymes. To produce the infectious clone 1.1-mer, two partial genomes 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, South Korea) and cross-checked by digestion with three restriction enzymes, *XbaI*, *KpnI* and *HindIII* (TaKaRa, Shiga, Japan). The plasmids were then transformed into three different *Agrobacterium* strains: GV3101, LBA4404 and EHA105 (Fig. 2). Accomplished infectious clones were confirmed by both enzyme digestion and colony polymerase chain reaction (PCR) with the SPSMV-1 detection primer sets (Table 1).
### Table 1

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5’-3’)</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPSMV-1-IC1-F</td>
<td>TCTAGAGATGTATTTTGTAGAGGGGAGGTAA</td>
<td>Infectious clone construction</td>
</tr>
<tr>
<td></td>
<td>XbaI</td>
<td></td>
</tr>
<tr>
<td>SPSMV-1-IC1-R</td>
<td>GGTACCCCCCTGGTTGAACA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KpnI</td>
<td></td>
</tr>
<tr>
<td>SPSMV-1-IC2-F</td>
<td>GGTACCGTGATTTTGATGACGAT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KpnI</td>
<td></td>
</tr>
<tr>
<td>SPSMV-1-IC2-R</td>
<td>AAGCTTCAGTCATGCTATCCTTCTGATA AGA TA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HindIII</td>
<td></td>
</tr>
<tr>
<td>Detection-F</td>
<td>GATGTATTTTGTAGAGGGGAGGTAA</td>
<td>Detection by conventional PCR</td>
</tr>
<tr>
<td>Detection-R</td>
<td>CCCTGGGTTGAACA</td>
<td></td>
</tr>
<tr>
<td>SPSMV-F</td>
<td>TCGAGGTGTACCAGGTATAGTTC</td>
<td>qRT-PCR detection</td>
</tr>
<tr>
<td>SPSMV-R</td>
<td>GTCTAAGGGGAGTACCTTAAC</td>
<td></td>
</tr>
<tr>
<td>EF1α-F</td>
<td>GCTGTCAAGTTTGCTGAGATCT</td>
<td>Housekeeping gene</td>
</tr>
<tr>
<td>EF1α-R</td>
<td>GAATCATCTTAACCATACCAGCAT</td>
<td></td>
</tr>
<tr>
<td>OVS-TAG</td>
<td>AGTTTAAGAACCTTCCCAGCATGTCTACAGGGGGAAGG</td>
<td>Detection by strand-specific PCR</td>
</tr>
<tr>
<td>OCS-TAG</td>
<td>AGTTTAAGAACCTTCCCAGCATGTCTACAGGGGGAAGG</td>
<td></td>
</tr>
<tr>
<td>TAG</td>
<td>AGTTTAAGAACCTTCCCAGCATGTCTACAGGGGGAAGG</td>
<td></td>
</tr>
<tr>
<td>OVS</td>
<td>ATGTCTACGGGGGGAAGG</td>
<td></td>
</tr>
<tr>
<td>OCS</td>
<td>ACAACAGGCACCTTTATTTTGGTA</td>
<td></td>
</tr>
<tr>
<td>SPSMV-F</td>
<td>GGAAGTGCAACTTGATAAGG</td>
<td>Abutting primers to get full-length</td>
</tr>
<tr>
<td>SPSMV-R</td>
<td>TGGCCAATTGACATCTTTGTAC</td>
<td></td>
</tr>
</tbody>
</table>

### Agro-inoculation with the SPSMV-1 Korean isolate

*Nicotiana benthamiana* seeds were planted in sterilized soil and cultivated in a growth chamber at the Sungkyunkwan University, Suwon, Korea. Total 18 four-week-old *N. benthamiana* of similar size were selected and classified as mock-inoculated, GV3101, LBA4404, and EHA105. *Agrobacterium* strains were cultured in Luria broth (LB) in the presence of the pCAMBIA1303- selective antibiotic, kanamycin (50
mg/L) and other strain-specific selective antibiotics (50 mg/L) at 28 °C with agitation for 30 h (until the OD value at 600nm reached 0.8 -1.0). Inoculation was performed to the stem once using the pin-pricking method with 1 mL agrobacteria culture. Four weeks after inoculation, photos were taken in each group and samples from young leaves, stems, and roots were collected for the downstream analysis.

**Total DNA extraction and PCR detection**

After 4 weeks of inoculation, samples from young leaves, stems, and roots were collected from mock and SPSMV-1-inoculated *N. benthamiana* plants. Genomic DNA was isolated from the tissues using the STE method. To detect virus presence inside samples, PCR was conducted with specific primers for SPSMV-1 (Table 1) using the following conditions: preheating at 94 °C (3 min), followed 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 product was then separated on a 1% agarose gel by electrophoresis and sequenced by Macrogen.

**Viral DNA quantification by real-time PCR**

Virus titer of SPSMV-1 was evaluated in collected sweet potato leaves and in four-week-old-inoculated *N.benthamiana*. Total DNA was extracted using DNeasy Plant mini kit (QIAGEN, Hilden, Germany). The primer set used for SPSMV qualification was designed based on sequence of RepA protein (Table 1). The qrt-PCR experiment was set up with three replicates per sample. The SYBR® Green PCR Master mix (TAKARA, Japan) was added to the qrt-PCR reaction with 1 µg of DNA solution in a volume of 20ul according to the manufacturer’s protocol. The reaction and the detection of the fluorescent signal were performed with a Rotor Gene Q thermocycle (QIAGEN). EF1α was used as internal control data normalization (Table 1). The data analysis was carried out using the 2(-ΔΔCt) method (Kenneth J.Livaka, 2001) to determine relative expression levels.

**Strand-specific PCR**

Strand-specific amplification was performed by modifying the method introduced by Rodríguez-Negrete et al.(Rodríguez-Negrete, 2014) and Kil et al.(Kil E., 2016) 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. Products from step 1 were purified using the QIAquick PCR Purification Kit (Qiagen). In the second step, 2 µL of the first-strand reaction product was mixed with 10 µL of 2X 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, and reacted for one cycle 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**
To check the presence of virus by PCR, primer set for SPSMV-1 detection was used (Table 1). An amplicon of the expected 870 nucleotides product was obtained from all five sweet potato samples (Fig. 1b). PCR products were sequenced and showed 89.93% similarity with SPSMV-1 Chinese isolate (accession number KY565235) (Fig. 1c). The full-length SPSMV-1 genome from leaf sample was amplified by RCA using a TempliPhi DNA Amplification Kit (GE Healthcare Life Sciences). The RCA product was digested with a variety of digestion enzymes but no target band of approximately 2.6kb appeared when being electrophoresed in a 1.0% agarose gel. Hence, PCR was conducted with abutting primers (Table 1) to get a full-length amplification of the virus genome. The amplified products were analyzed in a 1% agarose gel showing the target band of 2.6 kb in all three sweet potato samples (Fig. 1d), these were then cloned into the pGEM T-easy vector (Promega) and sequenced. Result from BLAST analysis revealed 99.61% nucleotide identity with SPSMV Chinese Isolate (acc. no. KY565235). The full-length sequence of SPSMV Korean Isolate was submitted to GenBank (MF148248). qrt-PCR analysis was conducted to evaluate virus titer inside collected leaf samples indicating that SPSMV-1 presented at a small amount in host plants (Fig. 1e)

**Sequence analysis of the complete genome of the SPSMV-1 Korean isolate**

The complete sequence of the SPSMV-1 Korean isolate obtained via PCR revealed a nucleotide length of 2599 and showed the typical genome arrangement of a mastrevirus (Fig. 3a). A phylogenetic tree in Fig. 3b showed the relationship between SPSMV-1 sequences from different regions and some mastrevirus sequences. For better insight into the taxonomic relationship of this virus, amino acid sequences of proteins encoded by the ORFs, and the nucleotide sequences of intergenic sequences of SPSMV-1 were compared with those of other geminiviruses. In general, SPSMV-1 isolate Korea showed the closest relation with SPSMV-1 isolate China compared to other isolates and SPSMV-1 exhibited the high similarity with other dicot-infecting mastreviruses. Interestingly, there are no viruses which shared a part of gene with both large intergenic region and short intergenic region of SPSMV-1(Table 2). The large intergenic region of all SPSMV-1 isolates was compared at the nucleotide level and all of them contains an unusual nonanucleotide sequence TAAGATT↓CC which is different from other geminiviruses(Fig. 3c).
Table 2
List of mastreviruses sharing gene similarity with SPSMV-1 Korean isolate.

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

SPSMV-1 Korean isolate infectious clone showed infection in *N. benthamiana*

Because the choice of the *Agrobacterium* strain used for agro-inoculation process can remarkably affect the efficiency and expression of virus proteins in the plants, in this study, three different *Agrobacterium* strains were used: GV3101, LBA4404, and EHA105. Four weeks after agro-inoculation, *N. benthamiana* plants showed no symptoms in either mock or SPSMV-1-inoculated plants. We did not observe any differences among the three *N. benthamiana* groups inoculated with three different *Agrobacterium* strains (Fig. 4a). Three organs (young leaves, stems, and roots) of SPSMV-1-inoculated *N. benthamiana* plants were harvested and analyzed using PCR to investigate viral replication ability and systemic...
movement. A quantitative real-time PCR assay was also used to evaluate the distribution of virus titers in *N.benthamiana*. Results revealed that 4 weeks after inoculation the virus was detected in all three organs, and the LBA4404 *Agrobacterium* strain showed the highest SPSMV-1 genome transfer ability (Fig. 4b, c). Strand-specific amplification using virion-sense- and complementary-sense specific primer sets was used to prove the replication ability of virus. If geminiviral DNA was replicated and ssDNA converted into double-stranded DNA (dsDNA) intermediates by a rolling-circle replication mechanism, amplification would be confirmed through PCR 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) existed in the infected samples, indicating that the viral genomes had replicated in *N. benthamiana* plants (Fig. 5b). However, our attempt to identify the virus using Southern blotting failed, indicating that the virus titer was maintained at a very low level (data not shown).

**Discussion**

To our knowledge, no study has been conducted to date to construct an infectious clone of SPSMV-1; hence, our study contributes toward gaining a better understanding of the virus - plant interactions and the biological significance of this virus in infected plants. In this study, we identified the whole genome of SPSMV-1 Korean isolate revealing the typical genome structure of a mastrevirus. SPSMV-1 infectious clone was introduced into *N. benthamiana* plants using an *Agrobacterium*-mediated inoculation method, which is the most used method for plant genetic engineering because of its relatively high efficiency. Up until now, there is no report about the succeed of agro-inoculation system in mastervirus. In this study, three commonly used *Agrobacterium tumefaciens* strains, GV3101, EHA105, and LBA4404 consisting of SPSMV-containing pCAMBIA1303 were evaluated using conventional PCR analysis to determine which *Agrobacterium* strain is the best for SPSMV-1 gene transfer to *N. benthamiana*. Four weeks after inoculation, we observed that SPSMV-1 could be detected in young leaves, stems, and roots of *N. benthamiana* plants; however, the replication ability of the virus was severely limited. In addition, LBA4404 was the most effective *Agrobacterium* for SPSMV-1 gene transfer. Therefore, *A. tumefaciens* strain LBA4404 could be an appropriate strain for efficient viral gene transfer to *N. benthamiana* plants. The same result was recorded in studies by Bakhsh an Ozcan (Allah Bakhsh, 2017), Japelaghi et al. (Heidari Japelaghi et al., 2018), and Liu et al. (Zong-Zhi Liu, 2003), who found that LBA4404 is a better choice than EHA105, GV2260, C58C1, GV3101 and AGL1 for genetic transformation of foreign genes in *Nicotiana tabacum* L. (Table 3). To our knowledge, a successful transformation process requires host (plant) genome and *Agrobacterium* strain compatibility, which is influenced by several factors including the type of the chromosomal background of the *Agrobacterium* strains, different opines, mechanism of transfer and integration of T-DNA, and T-DNA copy number containing the gene of interest (Sheetal Yadav, 2014). Therefore, the choice of *Agrobacterium* strain used for the plant transformation process can remarkably affect the transformation efficiency and expression of foreign proteins (Sheetal Yadav, 2014). However, the detailed mechanism by which LBA4404 favors SPSMV-1 genome transfer to *N. benthamiana* plants in this study needs to be further analyzed.
Table 3
List of Agrobacterium strains for genetic transformation in Nicotiana species

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Tested Agrobacterium strain</th>
<th>Effective strain</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotiana benthamiana</td>
<td>HA105, LBA4404, AGL0 and AGL1 (Mateusz Wydro, 2006)</td>
<td>EHA105 and AGL0</td>
<td>2006</td>
</tr>
<tr>
<td>Nicotiana tabacum</td>
<td>GV2260, LBA4404, AGL1, EHA105, and C58C1 (Allah Bakhsh, 2017)</td>
<td>LBA4404</td>
<td>2013</td>
</tr>
<tr>
<td>Nicotiana benthamiana</td>
<td>GV3101 (Jana Ordon, 2019)</td>
<td>GV3101</td>
<td>2019</td>
</tr>
<tr>
<td>Nicotiana tabacum</td>
<td>EHA105, GV3101, and LBA4404 (Heidari Japelaghi et al., 2018)</td>
<td>LBA4404</td>
<td>2018</td>
</tr>
<tr>
<td>Nicotiana benthamiana</td>
<td>GV3101, LBA4404, C58C1, At06, At10, At77 and A4 (Moneim Shamloul, 2014)</td>
<td>GV3101</td>
<td>2014</td>
</tr>
<tr>
<td>Nicotiana benthamiana</td>
<td>EHA 105 (Pengguo Xia, 2020)</td>
<td>EHA105</td>
<td>2020</td>
</tr>
<tr>
<td>Nicotiana tabacum</td>
<td>LBA 4404 (Zong-Zhi Liu, 2003)</td>
<td>LBA 4404</td>
<td>2003</td>
</tr>
</tbody>
</table>

Our effort to infect SPSMV-1 to sweet potato plants using agro-inoculation method failed (there was no virus detected—data not shown) indicating that the agro-inoculation system didn't work well on sweet potato plants. This is understandable because for virus infection to sweet potato plants, graft transmission is well known as the most effective strategy (Mwanga Robert, 2013, Kreuze et al., 2020).

In our research, infectious clones of SPSMV-1 were generated using different Agrobacterium strains; however, virus replication remained very low, even in the sweet potato samples collected in the field. Nucleotide sequence analysis revealed that the SPSMV-1 Korean isolate contains an abnormal nonanucleotide sequence and a stem-loop region of LIR, similar to other reported isolates, and comparable to other geminiviruses. We hypothesize that this could be the reason for the limitations in virus replication. Therefore, creating some mutations in these areas is necessary to comprehensively evaluate virus replication and determine whether it is asymptomatic owing to the nature of the virus or because a small number of viruses cannot induce sufficient response.

Declarations

Acknowledgement

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Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Contributions**

Material preparation, data collection and analysis were performed by PTH, HSB, and EJK. The first draft of the manuscript was written by PTH and HSB. TTBV, AL, EJK and SL made comments and improved the manuscript. All authors read and approved the manuscript.

**Conflicts of interest**

There is no conflict of interest related to this submission.

**References**


1497–1505.

**Figures**

**Figure 1**

![Image](image_url)
**Figure 1**

Virus detection of sweet potato symptomless virus 1 (SPSMV-1) in Korea.

a) Sweet potatoes infected by SPSMV-1 did not show any symptoms.

b) DNA was extracted from sweet potatoes which were infected by SPSMV-1 and detected by PCR. L, Leaves; S, Stem; R, Roots. The number, (1)-(3), means the different field where we collected sweet potatoes.

c) Sequencing result show the detected virus in all 3 sweet potato samples show high similarity with *Sweet potato symptomless virus 1* Chinese isolate (Accession number: KY565235)

d) PCR amplification of the collected sweet potato samples using SPSMV-1 specific primers to get complete sequence. Lane 1-3 means three sweet potato samples.

e) Real-time PCR analysis indicated SPSMV-1 titer in sweet potato leaves from the fields. The bar graphs indicate the mean ± SD (n=5). The statistical comparison was performed by the unpaired t-test: *p<0.05, ***p < 0.001, ****p<0.0001.

**Figure 2**

![Diagram of experimental setup for virus detection](image-url)
Construction scheme for an infectious clone of the sweet potato symptomless virus 1 (SPSMV-1) Korean isolate.

**Figure 3**

a) Schematic diagram of two intergenic regions and five open reading frames.

b) Neighbor-joining phylogenetic tree of the complete genomic sequences of sweet potato symptomless virus 1 and other mastreviruses, constructed using 1000 bootstrap replicates (MEGA X).

c) The large intergenic region (LIR) with an unusual nonanucleotide sequence (TAAGATT↓CC) in all SPSMV-1 isolates

Sequence analysis of the complete genome of the sweet potato symptomless virus 1 (SPSMV-1) Korean isolate.

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Figure 4

Results of the infection test.

a) Symptoms of sweet potato symptomless virus 1 (SPSMV-1) Korean isolate infection in *Nicotiana benthamiana* plants after agro-inoculation with three different *Agrobacterium* strains by Korean isolate of SPSMV-1.

b) Detection of SPSMV-1 DNA in *N. benthamiana* plants after agro-inoculation with three different *Agrobacterium* strains by PCR using an SPSMV-specific primer set.

c) SPSMV-1 titer in different organs of *N. benthamiana* plants inoculated with three *Agrobacterium* strains. The bar graphs indicate the mean ± SD (n=5). The statistical comparison was performed by the unpaired t-test: *p* < 0.05, **p** < 0.001, ***p** < 0.0001.
Figure 5

Strand-specific amplification of sweet potato symptomless virus 1 (SPSMV-1)-infected *Nicotiana benthamiana* plants.

a) Illustration of strand-specific 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.