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SHORT COMMUNICATION

Evidence for seed transmission of sweet potato symptomless virus 1 in sweet potato (*Ipomoea batatas*)

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Abstract

Sweet potato symptomless virus 1 (SPSMV-1) is a member of genus *Mastrevirus*, in the family *Geminiviridae*. SPSMV-1 was detected from sweet potato (*Ipomoea batatas*) seedlings by small-RNA deep-sequencing. PCR analysis indicated that SPSMV-1 was detected in 12 out of 114 individual sweet potato seedlings from seeds collected from a SPSMV-1-infected sweet potato plant, and additionally, detected in whole seeds, seed coats attached with endosperm, and in embryos. The complete genome of the virus was subsequently cloned by using a polymerase chain reaction (PCR) method with back-to-back (full) primer pairs from sweet potato seedlings and seeds. In summary, the results suggest that SPSMV-1 may be a seed-borne virus and a seed transmissible virus in sweet potato. To our knowledge, this is the first evidence for seed transmission of SPSMV-1, and seed transmission under natural conditions for any mastrevirus as well.

Keywords *Sweet potato symptomless virus 1* · Seed transmission · Sweet potato · Small-RNA deep-sequencing

Sweet potato symptomless virus 1 (SPSMV-1) with a circular single-stranded DNA genome of approximate 2.6 kb, belongs to the genus *Mastrevirus* of the family *Geminiviridae*. In 2009, the virus was initially identified in sweet potato (*Ipomoea batatas*) by using small-RNA deep-sequencing (SRDS) (Kreuze et al. 2009). In 2015, SPSMV-1 was detected from China (Wang et al. 2015). At present, the virus has been detected from some countries and regions in the Americas, East Africa and Northeast Asia (Kreuze et al. 2009; Mbanzibwa et al. 2014; Kwak et al. 2014; Cao et al. 2017; Souza et al. 2018). SPSMV-1 has a genomic organization containing five open reading frames (ORFs). Two ORFs, V1 and V2, are located in the viral-sense strand, and three ORFs, namely C1, C1:C2 (C1 and C2 spliced) and C3, are located in the complementary-sense strand (Cao et al. 2017). The large intergenic region (LIR) is located between V2 and C1:C2, containing an unusual nonanucleotide sequence TAAGATTCC (Cao et al. 2017).

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About 20% of plant viruses are seed transmitted (Wang and Maule 1994). Seed transmission plays an important role in the epidemiology of viruses. At present, the biological properties of SPSMV-1 including its natural transmission are unclear. Although sweet potato is produced by vegetative propagation using stem cuttings and storage roots, seeds have always been used for breeding and exchange of germplasm. The goal of this research was to provide evidence that SPSMV-1 is seed-borne and can be transmitted through sweet potato seed which could serve as a primary source of inoculum in the field for SPSMV-1 transmission.

In 2016, approximately 500 seeds were harvested from an SPSMV-1-infected sweet potato plant in an experimental field in Hainan Province, in southern China. A total of 150 harvested seeds were surface sterilized with 3% sodium hypochlorite, washed with sterile water, and then potted separately. The sown seeds were maintained in an insect-free greenhouse wherein an insecticide was sprayed regularly. Among the 150 seeds sown in the growth test, 114 germinated. After the appearance of the third true leaf, leaf tissues were collected for nucleic acid extraction.

Equal amounts (100 mg) of fresh leaves randomly sampled from 20 sweet potato seedlings were pooled and grounded into a fine powder in liquid nitrogen using a mortar and a pestle. The total RNA was extracted using the TRIzol method

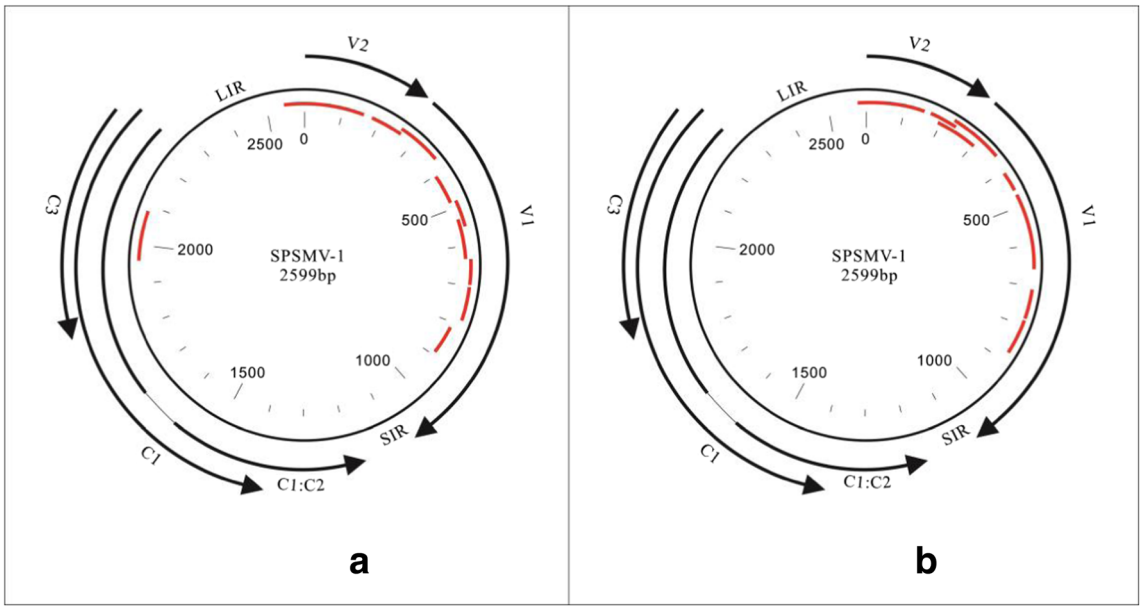

Fig. 1 Position and distribution of SPSMV-1 small RNA contigs (red lines) from the pooled samples ZC-7-1-1 (**a**) and ZC-7-1-3 (**b**) on SPSMV-1 genome (cloned from sweet potato seedlings in this study). LIR indicates large intergenic region, and SIR indicates small intergenic region

following the manufacturer's instructions (Sangon, Shanghai, China). The quantity and quality of the pooled extracted RNA above were measured using the Agilent 2100 Bioanalyzer and agarose gel electrophoresis, and three pooled RNA samples (ZC-7-1-1, ZC-7-1-2, and ZC-7-1-3) each isolated from 20 seedlings were sent to Allwegene Tech. (Beijing, China) for small RNA sequencing. Briefly, the steps for small RNA sequencing included acrylamide gel purification of RNA bands corresponding to the size range of 18–30 nucleotides (nts), ligation with a single-stranded 3′ adapter and a bar-coded 5′ adapter, and complementary DNA synthesis and amplification by PCR to generate a DNA colony template library for Illumina sequencing. Short read assembler Velvet 1.2 was applied for the small RNA reads assembly according to the program instructions. Briefly, the steps for small RNA sequence analysis included inputting reads of 18–30 nt into the Velvet assembler for the assembly of contiguous sequences (contigs) from overlapping short sequences, testing various lengths of overlap to modulate specificity and to remove errors using Velvet, and using the assembled contigs as search queries in the National Center for Biotechnology Information (NCBI) database by BLAST.

Small-RNA deep-sequencing (SRDS) on the three pooled RNA (ZC-7-1-1, ZC-7-1-2, and ZC-7-1-3) extracted from the sweet potato seedlings resulted in 19,294,410, 21,525,568, and 22,046,312 reads, respectively. The read sizes ranged from 18 to 30 nt, though the majority of the reads were 24 nt. Assembling the 18- to 30-nt-long reads with Velvet resulted in contigs longer than 50 nt, and the longest contig assembled was 399 nt, 514 nt and 460 nt long for ZC-7-1-1, ZC-7-1-2 and ZC-7-1-3 samples, respectively. The contigs were mapped against the NCBI nucleotide database using the BLAST tool. Sequence analysis showed that SPSMV-1 was detected in the ZC-7-1-1 and in the ZC-7-1-3 pooled RNA samples but not in the ZC-7-1-2 sample. The coverage of the SPSMV-1 genome sequence was 38.2% and 33.8% for ZC-7-1-1 and ZC-7-1-3, respectively (Fig. 1).

Additionally, total DNA for PCR detection was extracted from 16 whole dry seed bulks (three whole dry seeds per bulk) and 114 individual seedlings using the Universal Genomic

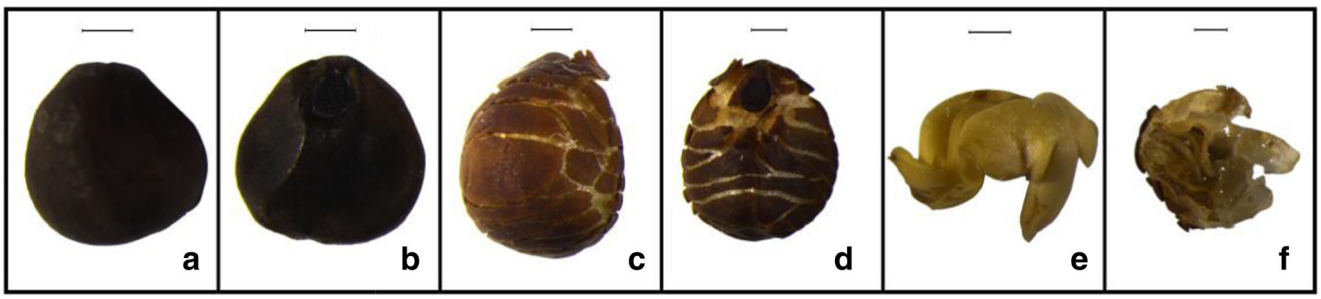

Fig. 2 Sweet potato seed and its dissected tissues: a whole dry seed (**a**, **b**), a swelled seed soaked by sterile water (**c**, **d**), embryo (**e**), seed coat attached with the endosperm (partial) (**f**). Scale bars: 1 mm

Table 1 Details of specific primers used in this study

Primer[a]	Nucleotide position[b]	Sequence from 5′ to 3’	Expected product size (bp)
SPSMV-F2 (s)	58–81	AAGTCGTCGTCCGATAGTCGTTTAC	785
SPSMV-R2 (a)	818–842	TCTGACCCGGTTTACTAATATCCAC	
SPSMV-F6 (s)	1755–1779	TGGGGGAGCAGTTGTAGGGAATAGC	2599
SPSMV-R6 (a)	1740–1764	TGCTCCCCCATATGTTTCCCCATTC	
SPSMV-BF3 (s)	806–830	ACGTCGAATGGTGTGGATATTGGTA	2599
SPSMV-BR3 (a)	793–818	CACCATTCGACGTCAGAATCGTCTTC	

[a] Primer, ‘(s)’ indicates sense primer, ‘(a)’ indicates anti-sense primer

[b] Nucleotide position corresponding to the complete genome sequence of isolate Z01019 of SPSMV-1 from the GenBank database (accession number KY565235) for primers SPSMV-F2, SPSMV-R2, SPSMV-F6 and SPSMV-R6, and to MG603672 for primers SPSMV-BF3 and SPSMV-BR3

DNA Extraction Kit (TaKaRa, Dalian, China). Four seeds were pooled to accumulate sufficient DNA from the seed coats and internal parts of the seeds. Fifty-three pooled seed samples were surface sterilized with sodium hypochlorite, and soaked in sterile water for 8 h until the seed coat softened. The seeds were blotted dry, and then the embryos and seed coats with part of the endosperm (Fig. 2) were separated with sterilized scalpel under stereoscope (Leica, MDG33, Singapore) for DNA extraction using the forementioned kit. Furthermore, to obtain the complete genome sequence of SPSMV-1 from sweet potato seeds, 48 surface sterilized whole seeds were bulked into 16 pools (3 seeds for one pool) and subjected to DNA extraction using the MiniBEST Plant Genomic DNA Extraction Kit (TaKaRa, Dalian, China).

A specific primer pair (SPSMV-F2 and SPSMV-R2; Table 1) was used to determine the presence of SPSMV-1 in whole seeds, seed coats with parts of the endosperm, embryos, and seedlings (Fig. 3). The amplicons were 785 bp in length and consisted of the partial coat protein gene and partial movement protein gene of SPSMV-1. Furthermore, the back-to-back (full) primer pairs SPSMV-F6 and SPSMV-R6 (Table 1), SPSMV-BF3 and SPSMV-BR3 (Table 1) were used to amplify the complete genome sequence from 20 seedlings and 16 seed pools (3 seeds for one pool), respectively. The PCR reaction above included a final volume of 2 μl of 10× PCR buffer and a final concentration of 0.2 mM deoxyribonucleotide triphosphates, 2 mM $MgCl_2$, and 0.5 μM each primer in a total reaction volume of 20 μl. PCR amplification

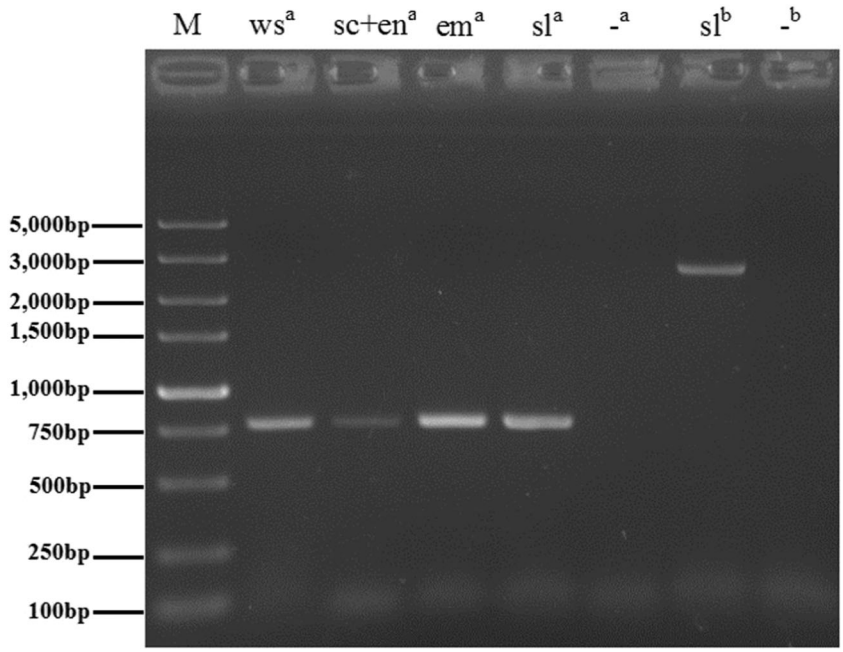

Fig. 3 Agarose gel electrophoresis of PCR products from DNA extracts of representative samples including sweet potato whole seeds, seed coats with parts of the endosperm, embryos and seedlings, amplified with sweet potato symptomless virus 1-specific primer pair (SPSMV-F2 and SPSMV-R2) or the back-to-back primer pair (SPSMV-F6 and SPSMV-R6). ‘ws’ indicates a bulk of whole seeds, ‘sc + en’ indicates a bulk of seed coats with parts of the endosperm, ‘em’ indicates a bulk of embryos, ‘sl’ indicates an individual seedling, ‘-’ indicates negative control, ‘M’ indicates DL5000 DNA marker (TaKaRa, Dalian, China). [a]Sample was amplified with the primer pair (SPSMV-F2 and SPSMV-R2) and [b]sample was amplified with the back-to-back primer pair (SPSMV-F6 and SPSMV-R6)

Table 2 Detection rates of sweet potato symptomless virus 1 (SPSMV-1) by PCR [a] in sweet potato seeds and seedlings

Type of samples tested	No. of samples tested	No. positive	Detection rate/range (% min–max)[b]
Seed bulks (three whole dry seeds per bulk)	16	3	6.25–18.75%
Bulks of seed coats + endosperm (seed coats + endosperm from four seeds per bulk)	53	7	3.30–13.21%
Bulks of embryos (embryos from four seeds per bulk)	53	7	3.30–13.21%
Individual seedlings	114	12	10.53%

[a] The specific primer pair (SPSMV-F2 and SPSMV-R2) was used in PCR detection

[b] The possible minimum to maximum infection rates based on the results of SPSMV-1 infection rates of bulks with No. seeds

was performed in a DNA thermal cycler (BioRad, USA) programmed for 3 min at 94 °C for the initial denaturation and 35 cycles, each consisting of 30 s at 94 °C, 30 s at T_m, and a 1-min extension per 1000-bp product at 72 °C, followed by a final extension for 10 min at 72 °C. Partial PCR products representing different tissues were cloned and sequenced to verify the identity of the amplified fragments. The resultant sequences were compared to known SPSMV-1 sequences in the NCBI database.

SPSMV-1 was detected in the three out of 16 whole dry seed bulks (three dry whole seeds per bulk) by PCR, indicating that the seed-borne infection rate was 6.25–18.75% (Table 2). When the sweet potato seeds were soaked in sterile water to soften the seed coat, the embryo could be separated from the seed coat and the endosperm. However, the endosperm could not be separated from seed coat. PCR analysis of 53 bulks of the embryos (embryos from four seeds per bulk) showed that 7 bulks were SPSMV-1-positive (Table 2). In all cases, when the bulk of the embryos is SPSMV-1-positive, that of the seed coats with parts of the endosperm of the same seeds are also positive.

Using the back-to-back (full) primer pair (SPSMV-F6 and SPSMV-R6; Table 1), the predicted product size was amplified from the nucleic acid extracts of four individual sweet potato seedlings (20 seedlings tested). Analysis of the four sequences cloned revealed the sequence of 2599 bp which encoded five open-reading frames (ORFs V1, V2, C1, C2, C3; Fig. 1). The large intergenic region between the V2 and the C1:C2 contained an unusual nonanucleotide sequence TAAGATTCC. Moreover, the sequence had over 97% identity with the complete genome sequence of other SPSMV-1 isolates in GenBank. The results indicated that the sequence cloned from the seedlings in this study was the complete genome sequence of SPSMV-1. As these four sequences showed 100% identity among them, only one sequence was deposited in the GenBank database with accession No. MG603672. Furthermore, using the back-to-back (full) primer pair (SPSMV-BF3 and SPSMV-BR3; Table 1), the PCR product of the predicted size (~2.6 kb) was amplified from 4 out of 16 whole seed pools DNA tested (3 seeds per pool). Two of four PCR products were cloned and sequenced. Sequence analysis and comparison indicated that sequences of both PCR products were 100% identical and had a 97.3% nucleotide sequence identity to MG603672. Additionally, SPSMV-1 was detected in 12 of the 114 individual seedlings by PCR, indicating that the virus is seed-transmitted in sweet potato at a rate of 10.53% (Table 2). A multiple sequence alignment showed that all partial genome fragments amplified from whole seeds, seed coats with parts of the endosperm, embryos, and seedlings were 100% identical in sequence to the SPSMV-1-infected plant from which seeds were collected and also, were 100% identical to the corresponding sequence of SPSMV-1 complete genome obtained from the seedlings.

Among the family *Geminiviridae*, *Sweet potato leaf curl virus* (SPLCV), belonging to the genus *Begomovirus*, can be transmitted via sweet potato seed and was the first reported seed-transmissible geminivirus (Kim et al. 2015). In the genus *Begomovirus*, members of the species *Tomato yellow leaf curl virus* (TYLCV), *Mung bean yellow mosaic virus* (MBYMV), *Tomato leaf curl New Delhi virus* (ToLCNDV), and *Dolichos yellow mosaic virus* (DoYMV) can also be seed-transmitted (Kil et al. 2016, 2017, 2018; Kothandaraman et al. 2016; Sangeetha et al. 2018; Suruthi et al. 2018). In addition, members of the species *Beet curly top virus* from the genus *Curtovirus* and *Beet curly top Iran virus* (BCTIV) from the genus *Becurtovirus* are seed-transmissible geminiviruses (Anabestani et al. 2017). In the genus *Mastrevirus*, maize streak virus (MSV) has been experimentally transmitted in maize through vascular puncture of the seeds (Redinbaugh 2003). In this study, PCR analysis showed that SPSMV-1 was located in sweet potato seeds (particularly in the embryos), and PCR together with SRDS detected SPSMV-1 in the seedlings. In most systems, virus infection of embryos is essential for seed transmission (Johansen et al. 1994). Although the mode of transmission was not fully characterized in this study, the results suggest that SPSMV-1 may be seed borne and can be transmitted from seed to seedling via the embryo. To our knowledge, this is the first evidence for seed transmission of SPSMV-1, and seed transmission under natural conditions for any mastrevirus as well.

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Compliance with ethical standards

Conflict of interest Dr. Qi Qiao declares that he has no conflict of interest. Dr. ZhenChen Zhang declares that he has no conflict of interest. Miss XiaoLi Zhao declares that she has no conflict of interest. Dr. YongJiang Wang declares that he has no conflict of interest. Dr. Shuang Wang declares that she has no conflict of interest. Dr. YanHong Qin declares that she has no conflict of interest. Mr. DeSheng Zhang declares that he has no conflict of interest. Miss YuTing Tian declares that she has no conflict of interest. Dr. Fumei Zhao declares that she has no conflict of interest.

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

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