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Molecular characterization of sweet potato leaf curl virus (SPLCV) isolates from Korea: phylogenetic relationship and recombination analysis

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Summary. – The complete DNA genome of sweet potato leaf curl virus (SPLCV) from samples obtained from eight regions was amplified by PCR and characterized in this study. The DNA genome of one group (SPLCV Korea group 1) consisted of 2828 nucleotides and that of the second group (SPLCV Korea group 2) consisted of 2829 nucleotides. Sequence comparisons showed that the genome sequences of SPLCV Korea isolates were closely related to those of SPLCV Brazil isolates (FJ969834, FJ969835, and FJ969836), SPLCV Japan isolate (AB433788), and SPLCV USA isolate (AF104036) with nucleotide sequence identity values ranging from 96-98%. Analysis of the phylogenetic relationship of SPLCV Korea isolates with other begomoviruses revealed that the majority of SPLCV Korea isolates were clustered with SPLCV Brazil isolates (FJ969834, FJ969835, and FJ969836). Recombination analysis results revealed three recombinations among SPLCV Korea isolates, SPLCV isolates from Brazil and Japan, and ipomoea yellow vein virus (IYVV) Italy isolate.

Keywords: begomovirus; Korea; molecular phylogeny; recombination; sweet potato leaf curl virus (SPLCV)

Introduction

Geminiviruses (the family *Geminiviridae*) are a group of plant pathogenic viruses of small twin-shaped isomeric particles with a single-stranded DNA genome (Van Regenmortel *et al.*, 1997; Hanley-Bowdoin *et al.*, 1999). Members of this group cause severe diseases in a wide variety of species, some of which are of profound agricultural importance (Rybicki *et al.*, 2000). The geminiviruses include four genera, *Begomovirus, Curtovirus, Topocuvirus*, and *Mastrevirus,* based on genome organization, insect vector species and host range (Jeske, 2009).

Begomoviruses are transmitted by whitefly (*Bemisia tabaci* Genn.) and have either monopartite (DNA-A) or bipartite (DNA-A and DNA-B) genome. In the bipartite viruses, the genes required for viral replication and encapsidation are encoded by DNA-A and those required for movement, occur on DNA-B. In the monopartite viruses, all of these genes are present in the DNA-A component (Maruthi *et al.*, 2007). Recently, a single-stranded DNA component designated as DNA-β has been shown to be associated with some monopartite begomoviruses. This circular single-stranded DNA of approximately 1.3–1.4 kb with some sequence homology with genomic components of nanoviruses, encodes a replication-associated protein (Saunders *et al.*, 2002; Mansoor *et al.*, 2003).

Sweet potato (*Ipomoea batatas*, the family *Convolvulaceae*) is the seventh most important food crop in global produc-

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Abbreviations: IYVV = ipomoea yellow vein virus**;** REn = replication enhancer protein; Rep = replication-associated protein; SPLCV = sweet potato leaf curl virus; SPLCGV = sweet potato leaf curl Georgia virus; SPLCCaV = sweet potato leaf curl Canary virus; SPLCLaV = sweet potato leaf curl Lanzarote virus; SPLCESV = sweet potato leaf curl Spain virus; SPGVaV = sweet potato golden vein-associated virus; SPMaV = sweet potato mosaic-associated virus; TbLCV = tobacco leaf curl virus; TrAP = transcriptional activator protein; TYLCV = tomato yellow leaf curl virus

tion. The plant originated in South America, but today is cultivated in most tropical, subtropical and temperate regions around the world (O´Brien, 1972; Lozano *et al.*, 2009). Despite their importance, viral diseases have been the least studied and least well-understood of all diseases affecting these crops (Clark *et al.*, 1988), although several sweet potato viruses have recently been characterized. Today, more than 15 viruses have been shown to infect the crop, and most of them are transmitted by aphids or whiteflies (Fuentes *et al.*, 1996; Banks *et al.*, 1999; Valverde *et al.*, 2004).

Begomoviruses infect *Ipomoea* plant species worldwide and have been identified in Japan, Israel, Peru, Italy, Spain, China, Taiwan, Korea, Kenya, and USA (Chung *et al.*, 1985; Osaki and Inouye, 1991; Banks *et al.*, 1999; Lotrakul *et al.*, 2003; Fuentes and Salazar, 2003; Briddon *et al.*, 2006; Miano *et al.*, 2006; Simmons *et al.*, 2009). Several begomovirus species (SPLCV, IYVV, SPLCGV, SPLCCaV, SPLCLaV, SPLCESV, SPGVaV, and SPMaV) have been reported to infect *Ipomoea* species and their genomes have been fully sequenced (Lotrakul *et al.*, 1998; Banks *et al.*, 1999; Lotrakul *et al.*, 2003; Lozano *et al.*, 2009; Paprotka *et al.*, 2010). SPLCV begomoviruses have been previously isolated from sweet potato plants in the USA, Asia and Africa, and from *Ipomoea indica* in Europe (Lotrakul *et al.*, 2003; Luan *et al.*, 2007; Miano *et al.*, 2008). Begomoviruses are likely to be present in many regions where the sweet potato is grown but knowledge about their prevalence and distribution remain elusive (Lozano *et al.*, 2009). The symptoms induced by *Ipomoea*-infecting begomoviruses depend on the host type and generally includes leaf curling and/or yellow veins; also asymptomatic infections are not uncommon (Banks *et al.*, 1999; Clark and Hoy, 2006; Valverde *et al.*, 2007).

During the years 2002–2004, some sweet potato plants showed leaf curling, stunting and vein yellowing symptoms in several locations in Korea. Herein, we provide the first detailed description of several SPLCV isolates taken from those sweet potatoes in Korea. The complete genome sequences of these SPLCV Korea isolates were analyzed and characterized in comparison with other known SPLCVs. These sequences were also subjected to recombination analysis to gain insight into the possible origins of SPLCV sequence variations.

Materials and Methods

Sample collection and genomic DNA extraction. Sweet potato samples showing leaf curling, vein yellowing, and stunting symptoms were collected from sweet potato plants in different locations in Korea during the years 2002–2004 (Fig. 1c). Virus-infected leaf tissues were maintained at -70°C until DNA extraction. Total genomic DNA of the sweet potato leaf tissues was extracted via the method of Dellaporta (Dellaporta *et al.*, 1983).

PCR and DNA sequencing. The presence of SPLCV in the samples was determined by using specific primer sets used for the detection of the SPLCV ORF C1 to ORF V2 genes and the SPLCV full genome was amplified by genomic PCR. The virus sense primer and complementary sense primer sets were designed from an alignment of the conserved sequences of DNA-A component of the SPLCV and the IYVV (primers of virus sense: 2315–2335, 1970–1987, 1496–1513, 1232–1254, 631–650, and 146–168 bp; primers of complementary sense: 2821–2803, 2371–2352, 2001–1981, 1733–1716, 1055–1036, and 720–699 bp). All PCRs were done by using a Thermal cycler PTC-100 (MJ Research Co.) machine, and all reactions were preformed under the following general PCR conditions: 3 mins at 95°C for pre-denaturation, thermal cycling for 35 cycles (30 secs at 94°C, 30 secs at 55°C, and 30 secs at 72°C), 10 mins at 72°C for final extension and stored at 8°C. Reactions were carried out in volumes of 20 µl, each containing 1 µl of the template genomic DNA, 5 pmoles of each primer, 2.5 mmol/l dNTPs, 5x SuperTaq buffer and 1 unit of SuperTaq DNA polymerase (SuperBio Co.). PCR products were visualized on 1% agarose gels containing ethidium bromide (EtBr). In an effort to confirm the products identities, bands were eluted, cloned into the pGEM-T easy plasmid vector (Promega Co.) and transformed into *E. coli* (DH5α strain). For each plasmid construct, two bacterial colonies were randomly selected and sequenced. Cloned constructs were sequenced by a commercial sequencing service (Macrogen Co.). The M13 primer sets (each 5 pmoles/µl) used for sequencing were as described in the pGEM-T easy vector manual. Sequencing was conducted using a 3730XL DNA analyzer (Applied Biosystems Inc.) with ethanol precipitation following the BigDye terminator v3.1 cycling reaction (Cycle Sequencing Kit, 4337454, Applied Biosystems Inc.). The resultant sequences were compared to known sequences in the databases using the BLAST algorithm in the NCBI database.

Genome organization and sequence comparison. The open reading frames (ORFs) and predicted amino acid sequences of the SPLCV Korea isolates were determined with the Vector NTI 10 program (Invitrogen Co.). The nucleotide and amino acid sequences were compared with those of other selected begomovirus sequences available in the GenBank database with the aid of the Vector NTI 10 and MEGA 4.0 programs.

Phylogenetic relationship analysis. Sequences were aligned and partially sequenced via the MUSCLE program (Edgar, 2004). Data were analyzed with MrBayes3.0. Using MrBayes 3.0 Bayesian software, Four Metropolises coupled with Markov Chain Monte Carlo (MCMC) chains were run, and halted when the standard divergence of split frequencies was less than 0.01 (Ronquist and Huelsenbeck, 2003). The SPLCV full genome sequences were analyzed over 10 million generations and four were sampled every 100 generations and the first 25% burn-in cycles were discarded prior to the construction of the consensus tree. Consensus trees were visualized using the MEGA 4.0 program (Tamura *et al.*, 2007). The accession numbers and assigned abbreviations of these begomoviruses are provided in Table 1.

Table 1. Lists of SPLCV and other begomoviruses for phylogenetic and recombination analysis

Acronym	GenBank (No.)	Location	Length (bp)	Host Plant
Sweet potato leaf curl virus (SPLCV)				
HM754634 SPLCV Korea	HM754634	Korea Chungju	2829	*Ipomoea batatas*
HM754635 SPLCV Korea	HM754635	Korea Yeojoo 1	2829	*Ipomoea batatas*
HM754636 SPLCV Korea	HM754636	Korea Nonsan	2829	*Ipomoea batatas*
HM754637 SPLCV Korea	HM754637	Korea Yeojoo 2	2828	*Ipomoea batatas*
HM754638 SPLCV Korea	HM754638	Korea Haenam 2	2829	*Ipomoea batatas*
HM754639 SPLCV Korea	HM754639	Korea Haenam 3	2829	*Ipomoea batatas*
HM754640 SPLCV Korea	HM754640	Korea Haenam 4	2829	*Ipomoea batatas*
HM754641 SPLCV Korea	HM754641	Korea Haenam 1	2828	*Ipomoea batatas*
FJ176701 SPLCV China	FJ176701	China Jiangsu	2828	*Ipomoea batatas*
DQ512731 SPLCV China	DQ512731	China Liaoning	2771	*Ipomoea batatas*
EU267799 SPLCV China	EU267799	China	2799	*Ipomoea purpurea*
AB433786 SPLCV Japan	AB433786	Japan Miyazaki	2844	*Ipomoea batatas*
AB433787 SPLCV Japan	AB433787	Japan Kumamoto	2829	*Ipomoea batatas*
AB433788 SPLCV Japan	AB433788	Japan Kyoto	2828	*Ipomoea batatas*
FN432356 SPLCV India	FN432356	India West Bengal	2823	*Ipomoea batatas*
AF326775 SPLCV USA	AF326775	USA Georgia	2773	*Ipomoea batatas*
AF104036 SPLCV USA	AF104036	USA Louisiana	2828	*Ipomoea batatas*
DQ644562 SPLCV Puerto Rico	DQ644562	Puerto Rico	2828	*Ipomoea batatas*
FJ969832 SPLCV Brazil	FJ969832	Brazil Fortaleza	2841	*Ipomoea batatas*
FJ969833 SPLCV Brazil	FJ969833	Brazil Tavares	2845	*Ipomoea batatas*
FJ969834 SPLCV Brazil	FJ969834	Brazil Esteio	2829	*Ipomoea batatas*
FJ969835 SPLCV Brazil	FJ969835	Brazil Machado Asis	2841	*Ipomoea batatas*
FJ969836 SPLCV Brazil	FJ969836	Brazil Porto Alegre	2829	*Ipomoea batatas*
EF456744 SPLCV Spain	EF456744	Spain Canary lands	2829	*Ipomoea batatas*
EU856366 SPLCV Spain	EU856366	Spain Canary lands	2828	*Ipomoea batatas*
Other begomoviruses				
FJ969831 SPMaV Brazil	FJ969831	Brazil Souza	2803	*Ipomoea batatas*
FJ969830 SPGVaV Brazil	FJ969830	Brazil Brazilia	2834	*Ipomoea batatas*
AJ586885 IYVV Italy	AJ586885	Italy Sicily	2830	*Ipomoea indica*
NC_013022 IYVV Spain	NC_013022	Spain Malaga	2791	*Ipomoea indica*
GQ477135 HYVV Korea	GQ477135	Korea Daegu	2763	*Lonicera japonica*
HM130912 TYLCV Korea	HM130912	Korea Masan	2774	*Lycopersicon esculentum*
HM164550 TbLCV Korea	HM164550	Korea Sunchang	2763	*Lycopersicon esculentum*
AF379637 BCTV USA	AF379637	USA California	3038	*Beta vulgaris*
NC_003825 TPCTV USA	NC_003825	USA Florida	2861	*Solanum nigrum*
AF329885 MSV Kenya	AF329885	Kenya	2687	*Zea mays*

Recombination analysis. Recombination analysis of SPLCV Korea isolates was conducted using the Recombination Detection Program Version 3.0 (RDP3) (Martin *et al.*, 2010). The RDP3 program is a window-based program that detects and analyzes recombination signals in a set of aligned DNA sequences. Begomoviruses and other geminiviruses (*Curtovirus*, *Topocuvirus*, and *Mastrevirus*) were used for recombination analyses and the full sequence recombination analysis was taken into account. RDP3 was used to identify possible recombinants and parental sequences among a number of sequences. RDP3 uses seven different automated methods; namely RDP, GENECONV, MAXIMUM CHI SQUARE, BOOTSCAN, CHIMERA, SISTER SCAN, and 3 Seq. A highest acceptable probability value of $P = 0.01$ was employed.

Results and Discussion

Genome characterization

During the years 2002–2004, leaf curling, stunting, and vein yellowing symptoms were observed in sweet potato plants cultivated in several Korean provinces (Gyeonggi-

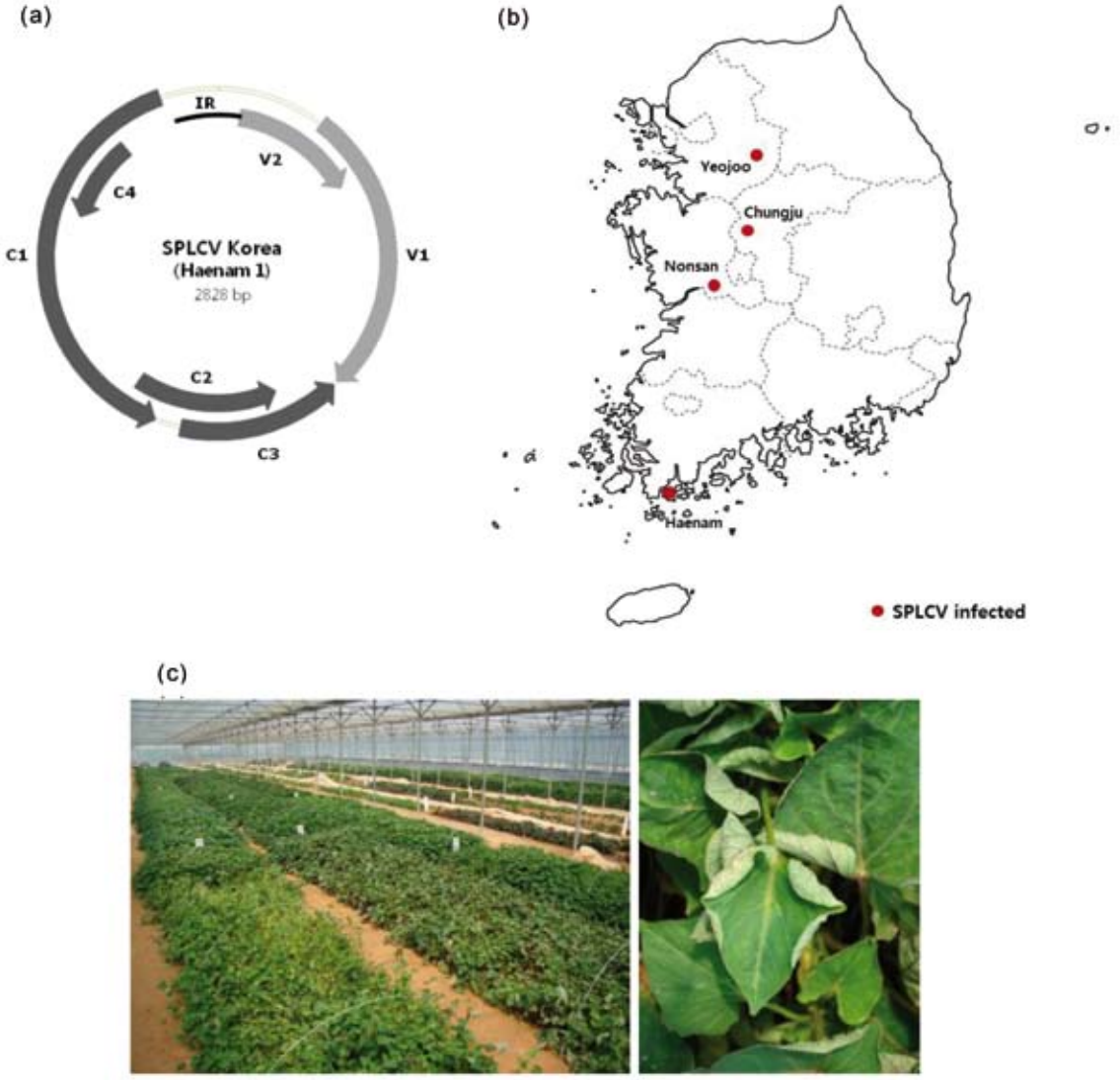

Fig. 1

Genome organization of SPLCV Korea isolate and sample collection sites

Genome composition of the SPLCV Korean isolate ('Haenam 1' isolate; HM754641) DNA-A component (a), SPLCV infected sweet potato sample collection sites in Korea (b), and typical leaf roll symptoms of SPLCV infected sweet potato leaves (c).

do, Chungcheong-do, and Jeolla-do). As SPLCV is the sole member of the geminiviruses known to infect potatoes in Korea, sweet potatoes exhibiting the aforementioned symptoms were collected and analyzed for the possible presence of SPLCV (Fig. 1c). As a result, SPLCV infections were identified in a number of samples obtained from different regions (Haenam 4 locations, Yeojoo 2 locations, Chungju 1 location, and Nonsan 1 location). Full genome sequences of each SPLCV isolate were acquired through PCR using the primer sets targeting the conserved regions of SPLCV and IYVV available from the NCBI database (Fig. 1b).

All of the SPLCV Korea isolates could be divided into two groups based on the presence/absence of a single nucleotide insertion in the intergenic region (IR), thus making the genome size of the 'Korea group 1' 2828 bp (SPLCV Korea group 1; HM754641 and HM754637) and that of the 'Korea group 2' 2829 bp (SPLCV Korea group 2; HM754635, HM754638, HM754639, HM754640, HM754634, and HM754636). Other than this difference in the single nucleotide, the IR of SPLCV Korea isolates exhibited only minimal sequence variation, in contrast to the IR region of other geminiviruses, which is known to be the most frequent site of recombination (Fauquet *et al.*, 2000).

Table 2. Percentage of nucleotide (nt) and amino acid (aa) sequence identities (%) of SPLCV Korea group 1 (Haenam 1 isolate; HM754641) and other SPLCVs

Viruses (genome size, bp)	DNA full genome	C1		C2		C3		C4		V1		V2		IR
	nt	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt
HM754638 Korea Haenam 2 (2829)	97.1	98.0	98.4	94.9	91.2	95.9	93.8	98.8	96.5	96.2	98.4	98.7	97.7	98.7
AB433788 SPLCV Japan (2828)	96.1	96.0	97.8	95.5	93.9	96.3	95.1	94.6	87.0	97.5	99.6	94.7	93.9	95.1
FJ176701 SPLCV China (2828)	95.8	95.9	98.0	93.5	88.5	94.7	92.4	95.7	89.4	97.6	99.6	84.0	84.0	77.0
AF104036 SPLCV USA (2828)	96.3	97.4	98.4	94.0	89.9	95.6	93.0	96.9	94.1	96.7	98.4	83.5	84.8	78.4
EF456744 SPLCV Spain (2829)	91.7	94.2	96.7	89.0	82.4	83.9	83.3	93.4	82.4	94.2	98.0	86.5	86.4	79.0
FJ969834 SPLCV Brazil (2829)	97.7	98.6	98.9	95.0	91.9	96.3	95.1	99.6	98.8	96.9	99.2	70.4	70.5	64.0
DQ644562 SPLCV Puerto Rico (2828)	92.3	94.7	97.3	93.3	90.5	93.1	90.3	94.2	85.9	90.6	96.5	80.0	83.3	73.0
AB433787 SPLCV Japan (2829)	94.1	95.3	95.6	81.8	74.5	85.2	84.1	95.7	89.4	92.8	99.2	96.2	94.7	64.0
FJ969836 SPLCV Brazil (2829)	97.5	98.3	98.6	94.9	91.2	96.1	94.4	99.2	97.6	96.6	99.2	70.4	70.5	79.4
DQ512731 SPLCV China (2771)	80.0	66.8	68.1	78.5	73.6	77.7	77.1	89.5	77.6	88.6	95.7	77.2	79.5	63.7
EU267799 SPLCV China (2799)	92.9	93.9	97.3	92.8	89.9	93.3	91.7	93.4	85.9	95.1	98.8	81.5	84.1	76.9
AB433786 SPLCV Japan (2844)	90.3	88.3	90.4	81.6	73.1	84.5	83.4	81.8	65.9	97.3	99.6	96.5	95.5	81.3
FN432356 SPLCV India (2823)	90.0	93.8	70.0	82.9	73.8	83.3	82.1	87.3	54.1	88.1	94.0	81.0	83.3	66.6
AF326775 SPLCV USA (2773)	81.2	80.1	81.3	79.8	73.8	75.8	75.2	72.9	44.7	90.8	97.6	82.0	84.1	59.8
FJ969832 SPLCV Brazil (2841)	89.9	85.7	90.1	93.7	92.6	94.5	91.7	80.6	60.0	96.9	98.0	68.0	69.7	50.6
FJ969833 SPLCV Brazil (2845)	91.6	89.4	92.6	91.7	89.2	93.6	90.3	82.9	64.7	97.2	99.2	69.4	70.5	52.1
FJ969835 SPLCV Brazil (2841)	97.2	98.5	98.6	92.4	88.8	93.5	91.9	99.6	98.8	96.7	99.2	70.2	70.5	64.3
AJ586885 IYVV Italy (2830)	90.2	94.2	95.9	80.9	73.8	68.0	75.2	93.8	85.9	93.6	96.0	80.5	84.8	72.9

The SPLCV Korea isolate was verified to consist of only the monopartite genome without the DNA-B genome or DNA-β satellite, and appeared to contain 6 ORFs, two in virion sense [V1 (coat protein) and V2] and four in complementary sense [C1 (Rep), C2 (TrAP), C3 (REn), and C4] (Fig. 1a). Absence of the DNA-B genome or DNA-β satellite was confirmed by PCR with specific primers capable of detecting these DNA sequences (data not shown). Both monopartite viruses with the DNA-A genome only and bipartite viruses harboring the DNA-A genome and DNA-B genome have been identified as members of the begomoviruses. Additionally, some monopartite begomoviruses harboring the DNA-β satellite, which plays an essential role in virus replication and symptom development, has also been recently reported (Saunders *et al.*, 2002; Mansoor *et al.*, 2003; Maruthi *et al.*, 2007). The SPLCV Korea isolate genome also exhibited the characteristic 'TAATATTAC' sequence motif present in the stem-loop structure that is conserved throughout all of the geminiviruses as a part of the 300 bp IR between the ORF C1 and V2.

Sequence comparison

Selecting the 'Haenam 1 isolate' (HM754641) with a genome size of 2828 bp, the complete genome of the SPLCV Korea isolates and the ORF sequences were compared with those of other known SPLCV and IYVV sequences selected from the NCBI database (SPLCV: Japan, China, USA, Spain, Brazil, Puerto Rico, and India; IYVV: Italy) in order to determine their sequence similarities at both the nucleotide and amino acid level.

The Haenam 1 isolate (HM754641), which shows 97.1% sequence identity in the full genome sequence with that of the Haenam 2 isolate (HM754638), exhibited more than 97% of identity with SPLCV Brazil isolates (FJ969834, FJ969835, and FJ969836) (Table 2). This sequence identity between SPLCV Korea isolates and SPLCV Brazil isolates was also supported by the results of phylogenetic relationship analysis, which located most of SPLCV Korea isolates with SPLCV Brazil isolate cluster (FJ969834, FJ969835, and FJ969836) when the full genome sequences were compared (Fig. 2). In contrast, SPLCV isolates of neighboring countries evidenced a slightly lower sequence identity with the Korea isolates, i.e. SPLCV Japan (AB433788: 96.1%, AB433787: 94.1%, and AB433786: 90.3%) and the China isolates (FJ176701: 95.8%, EU267799: 92.9%, and DQ512731: 80%). On the other hand, the Korea isolates evidenced sequence identity values of as high as 96.3% with SPLCV USA isolates (AF104036) and 90.2% identity with IYVV Italy isolate (AJ586885) (Table 2). As demonstrated by the 97.1% sequence identity between the Haenam 1 isolate (HM754641) and the Haenam 2 isolate (HM754638), two separate groups of SPLCV Korea isolates appear to exist; this is also reflected in the results of the phylogenetic and recombination analyses, as described later in this work.

The ORF V2 sequence evidenced the lowest levels of sequence identity (as low as 68% at the nucleotide level) even

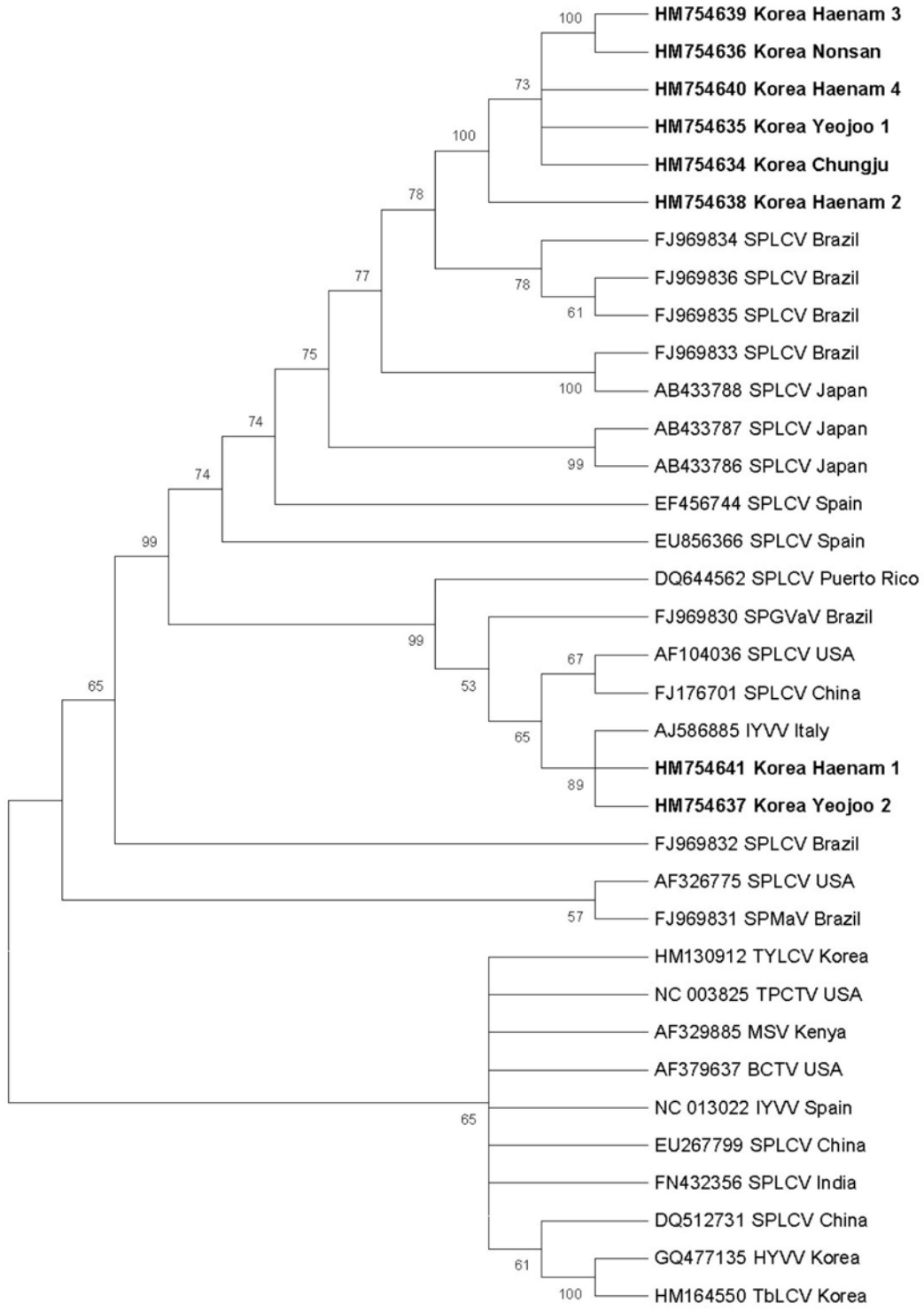

Fig. 2

Phylogenetic analysis of SPLCV Korea isolates with other SPLCVs and geminiviruses

The nucleotide sequences of DNA full genome. The tree was generated via the Bayesian method using MEGA 4.0 software and bootstrapped 1000 times. Abbreviations of virus names and accession numbers are listed in Table 1.

among the isolates with high sequence identity of full genome (Table 2). Variability in the predicted size of V2 among different groups of SPLCV isolates obtained from the NCBI database is believed to be the principal cause of the relatively low sequence identity of the V2 ORF. This heterogeneous nature of the V2 ORF may be simply due to the presence of alternative translational start sites at the 5′ proximal region of the predicted V2 ORF sequence. However, this will need to be clarified prior to further functional analysis of the V2 protein in the viral life cycle.

Phylogenetic relationship analysis

The results of full genome sequence analysis classified all of SPLCV Korea isolates into two groups, in which the Haenam 1 (HM754641) and Yeojoo 2 (HM754637) isolates were grouped together into 'Korea group 1', and the remaining Haenam 2 (HM754638), Haenam 3 (HM754639), Haenam 4 (HM754640), Yeojoo 1 (HM754635), Chungju (HM754634), and Nonsan (HM754636) isolates comprised 'Korea group 2'. SPLCV Korea group 2 in this analysis was found to form a cluster with the group of SPLCV Brazil isolates (FJ969834, FJ969835, and FJ969836), which are clustered with SPLCV Japan isolates (AB433786, AB433787, and AB433788), whereas SPLCV Korea group 1 was found to be related closely with IYVV Italy isolate (AJ586885), SPLCV China isolate (FJ176701), and SPLCV USA isolate (AF104036) (Fig. 2).

Although the exact path by which SPLCV was introduced into Korea was not elucidated in this study, the recent SPLCV outbreak may have been caused by rapidly spreading insect vector, *Bemisia tabaci,* in fields and/or as consequence of the method used for sweet potato propagation. *B. tabaci* has spread broadly throughout Korea since its initial detection in 1998 (Lee and De Barro, 2000; Lee, 2005), with a concomitant increase in the spread of other begomoviruses, including TYLCV and TbLCV (Lee *et al.*, 2010). The asexually reproductive propagation method for sweet potato may facilitate the spread of SPLCV without the need for whiteflies as an insect vector (Valverde *et al.*, 2004; Clark and Hoy, 2006).

Recombination analysis

Viral diversity is the result of mutation, recombination, reassortment and *de novo* acquisition; in particular, the viral recombination that is more prevalent in DNA viruses plays a vital role in generating virus diversity, and is therefore crucial to understanding viral evolution (Padidam *et al.*, 1999). In effort to acquire greater insights into the possible recombination events occurring in SPLCV Korea isolates, the viral sequences were compared with other begomovirus DNA genome sequences using the Recombination Detection Program Version 3.0 (RDP3) program (Martin *et al.*, 2010). RDP3 was used to identify possible recombinants and parental sequences among a number of sequences. RDP3 uses seven different automated methods - namely RDP, GENECONV, MAXIMUM CHI SQUARE, BOOTSCAN, CHIMERA, SISTER SCAN, and 3 Seq. A highest acceptable probability value of P=0.01 was employed. This result predicted that three recombination events had occurred in the viruses of SPLCV Korea group 1 (HM754637 and HM754641) and two recombination events had taken place in the viruses of SPLCV Korea group 2 (HM754634, HM754635, HM754636, HM754638, HM754639, and HM754640), showing a strong correlation with the data generated by the phylogenetic analysis described earlier.

According to these results, all of SPLCV Korea isolates contained recombination events 1 and 3 and event 2 occurred only in the Korea group 1 viruses. Recombination event 1, which occurred in the V1 region (76-175 base), appeared to have arisen with SPLCV Brazil isolate (FJ969833) as the major parent, whereas recombination event 3, which was detected in the IR sequence, appears to have occurred with IYVV Italy isolate (AJ586885) as the major parent (Fig. 3). In

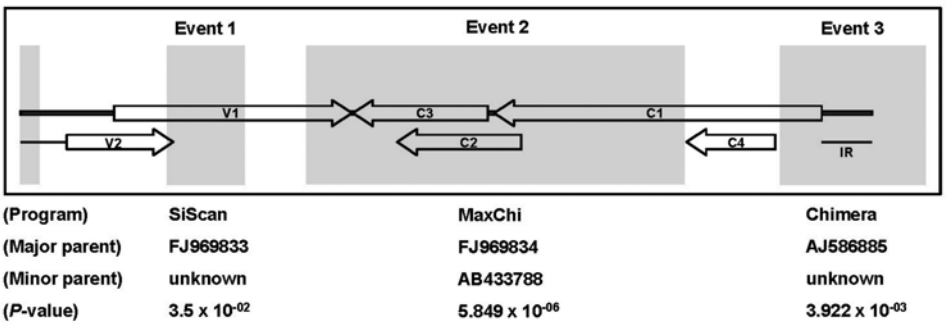

Fig. 3

Description of recombination events of SPLCV Korea isolates

The RDP3 program identifies two or three recombination events. These events were detected by SISTER SCAN, MAXIMUM CHI SQUARE, and CHIMERA. Event 1 shows recombination between the SPLCV Brazil isolate (FJ969833) as the major parent and an unknown minor parent. Event 2 shows recombination between the SPLCV Brazil isolate (FJ969834) as the major parent and the SPLCV Japan isolate (AB433788) as the minor parent. Event 3 shows recombination between the IYVV Italy isolate (AJ586885) as the major parent and an unknown minor parent.

both cases, unknown sequences appeared to serve as the minor parent. Recombination event 2, which was detected only in Korea group 1, identified SPLCV Brazil isolate (FJ969534) and SPLCV Japan isolate (AB433788) as the major and minor parents, respectively. Regions C1, C2, and C3 (683-2080 base) were identified as the recombination sites for event 2, and the sequence differences observed in these areas were identified as the principal rationale for separating SPLCV Korea isolates into two different groups (Fig. 3).

In conclusion, the results obtained from genome characterization, phylogenetic relationship and recombination analyses appear to demonstrate that the sequence variation used to separate SPLCV Korea isolates into two separate groups arose from a number of recombination events, involving both old world and new world SPLCV isolates.

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