A 2014 Nationwide Survey of the Distribution of Soybean Mosaic Virus (SMV), Soybean Yellow Mottle Mosaic Virus (SYMMV) and Soybean Yellow Common Mosaic Virus (SYCMV) Major Viruses in South Korean Soybean Fields, and Changes from 2012 Isolate Prevalence.

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A 2014 Nationwide Survey of the Distribution of *Soybean Mosaic Virus* (SMV), *Soybean Yellow Mottle Mosaic Virus* (SYMMV) and *Soybean Yellow Common Mosaic Virus* (SYCMV) Major Viruses in South Korean Soybean Fields, and Changes from 2012 Isolate Prevalence.

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In 2014 symptomatic soybean samples were collected throughout Korea, and were tested for the most important soybean viruses found in Korea, namely *Soybean mosaic virus* (SMV), *Soybean yellow common mosaic virus* (SYCMV), and *Soybean yellow mottle mosaic virus* (SYMMV). SYMMV was most commonly detected, followed by SMV. Only a few samples were found to be infected by SYCMV; of these, three samples were positive for double infection of SYMMV and SYCMV. Phylogenetic analysis of HC–Pro of the SMV isolates collected in 2014 from the eight provinces of Korea showed that most isolates were distinct from the most common Korean isolate detected in 2012, but related to other Korean, Chinese and North American isolates. No isolates varying in HC–Pro amino acid residues implicated in efficiency of RNA silencing suppression activity were detected in 2014. Phylogenetic analysis of ORF1 of both 2012 and 2014 SYCMV isolates showed differentiation into three subgroups. However, the geographical distribution of all three viruses in 2014 was essentially the same as observed in 2012. Quantitative real time PCR data also indicated a similar pattern of dual infected viruses occurrence as existed in 2012. Results showed SMV/SYMMV double infection RNA accumulation was not changed as much as SYMMV/SYCMV double infection. However, between double infection SMV/SYMMV, SYMMV RNA accumulation level rises more than SMV, and SYCMV RNA accumulation level decline a little compare with SYMMV. In summary, the 2014 survey showed that SMV and SYMMV are still the most prevalent soybean viruses in Korea, and all three viruses were still dispersed in the same areas where they were detected in 2012, although with an apparent shift towards SMV Group I isolates compared to 2012. The reason for the shift in SMV isolates across all Korean provinces is not clear, as seed transmission through farmer–saved seed is presumed to be the main source of infection within the crop.

Key words: Double infection, *Soybean mosaic virus*, *Soybean yellow common mosaic virus*, *Soybean yellow mottle mosaic virus*, Virus survey

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) originated from East Asia, and is a natural host to more than 30 plant viruses (Demski *et al*., 1989). Eight of these soybean virus species were already found in Korea, including *Alfalfa mosaic virus* (AMV), *Cowpea mosaic virus* (CPMV), *Cucumber mosaic virus* (CMV), *Soybean dwarf virus* (SbDV), *Soybean mosaic virus* (SMV), *Soybean yellow common mosaic virus* (SYCMV), *Soybean yellow mottle mosaic virus* (SYMMV), and *Peanut stunt virus* (PSV) (Nam *et al*., 2009; Nam *et al*., 2011; Baek *et al*., 2012). Among them, SMV is one of the most prevalent pathogens of soybean (Hill, 1999). SYMMV and SYCMV are recently discovered viruses first detected in Korea (Nam *et al*., 2009; Nam *et al*., 2011). SYMMV was shown to be a member of the genus *Carmovirus* family *Tombusviridae* (Nam *et al*., 2009); SYCMV belongs to the genus *Sobemovirus* (Nam *et al*., 2011). SMV is a member of the genus *Potyvirus*, family *Potyviridae* (Cho and Chung, 1976). AMV is a member of the genus *Alfamovirus*, the family *Bromoviridae* (Bol, J. F. 2005), and CPMV of the genus *Comovirus*, family *Comoviridae* (Green *et al*., 2009). CMV belongs to the *Cucumovirus* genus in the family *Bromoviridae* (Van Regenmortel *et al*., 2000), while SbDV is a member of the genus *Luteovirus*, family *Luteoviridae* (Terauchi *et al*., 2001) and PSV is in the genus *Cucumovirus*, family *Bromoviridae* (Diaz–Ruiz *et al*., 1983).

In Korea most soybeans are grown on small farms, and most of the smallholder farmers save their own seed for replanting. This may lead to the seed acting as the largest source of primary inoculum for the new crops. However, there are also wild legumes including other Glycine species growing in Korea that may serve as reservoirs of SMV between seasons, and as sources of different isolates. Some seed is also imported from other countries, including the United States, as a source of new varieties. Surveys of virus prevalence and isolate differences over time will help to differentiate between these possibilities.

In a 2012 survey of 682 Korean soybean samples, Cho *et al* reported that most were infected by SMV (15%) or SYMMV (17%) and few are infected by SYCMV (2.4%) (Cho *et al*., 2013). One SMV isolate detected during the

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2012 survey was found to have a very weak silencing suppressor HC–Pro due to substitutions of three amino acids (Li *et al*., 2013). Others have reported that SMV caused the most severe (more than 90% in the past) damage in Korea, while more recently viruses like SYMMV, SYCMV, SbDV and PSV have been reported (Baek *et al*., 2012). Two other 2012 surveys reported detection of five kinds of viruses (SMV, PSV, SYCMV, SYMMV, and BCMV) with SMV and SYMMV found to be the most prevalent in this survey (Baek *et al*., 2012; Lim, 2013).

Following a 2012 nationwide survey Cho *et al*. (2013) suggested self–managed seed production might result in seed transmission as the primary source of inoculum in the crop. Typically, seed transmitted SMV is no longer detected in the US (Hill, 1999) as a result of screening by seed companies. HC–Pro of SMV functions as a silencing suppressor (Hill, 1999) and also in seed transmission (Blanc *et al*., 1997; Peng *et al*., 1998). The domains of HC–Pro are highly conserved in highly seed transmitted SMV (Domier *et al*., 2011), while HC–Pro conserved amino acid sequence motifs (KITC and PTK) are required for aphid transmission. (Atreya *et al*., 1990; Blanc *et al*., 1997; Peng *et al*., 1998). Virus seed transmission has different selection pressures than aphid transmission; seed transmission is mainly dependent on the seed genotype (Domier *et al*., 2011), and pathogen virulence is not a selective advantage for seed transmission (Bergstrom *et al*., 1999). In this report we performed a nationwide survey including all of the same regions as were examined in 2012. Through this work we have generated information to explain why soybean viruses occur in Korean soybean fields, and to note the importance of managing viral diseases and seed control through regular farmer education.

MATERIALS AND METHODS

Collection of virus infected plant materials. Soybean samples with virus symptoms were collected nationwide. A total of 251 soybean tissue samples were collected in 2014 from infected soybean plants from fields in all eight Korean provinces (Fig. 1). 48 samples were collected from North Gyeongsang, and 21 samples from South Gyeongsang. 36 samples were collected in North Jeolla, and 37 samples in South Jeolla. 29 samples were from Gangwon, and 30 samples from Gyeonggi, 30 samples from South Chungcheong, 20 samples from North Chungcheong (Fig. 1).

RNA extraction, RT–PCR detection, and sequencing. Total RNA was extracted from each of 251 collected plant samples using TRIzol (Invitrogen, Rockville, MD) according to the manufacturer's recommendations. Individual RNA samples were used as template to synthesize cDNA, using M–MLV reverse transcriptase according to the manufacturer's recommendations (Enzynomics, Korea). RT–PCR for detection of SMV, SYMMV, and SYCMV was performed using the primers listed in Table 1, yielding products of 890 bp, 597 bp, and 346 bp respectively (Cho *et al*., 2013). Primers for amplification of SMV HC–Pro were designed based on reference sequence NC_016033 (Li *et al*., 2013), and for SYCMV ORF1 based on NC_002634 from National Center for Biotechnology Information (NCBI) (Table 1) for PCR using high–fidelity Blend Taq polymerase (Toyobo Co., Ltd) followed manufacturer's recommendations. Amplification products were cloned in the TOPO blunt vector (Enzynomics, Korea); a minimum of five clones for each isolate were sequenced (Macrogen, Seoul, Korea). No variation was observed among clones of an isolate. One clone from each isolate was selected for further analysis.

Quantitative real–time PCR. Quantitative real–time PCR (Q–RT–PCR) was utilized to compare accumulation of double infection SMV/SYMMV and SYMMV/SYCMV RNA (primers QRT–SMV F, R; QRT–SYMMV F, R; QRT–WYCMV F, R Table 1) accumulation in infected soybean plants. Total RNA was extracted from plant tissues using an RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations, and cDNAs were synthesized using M–MLV reverse transcriptase as described above. Internal standard primers were designed for the Tubulin RNA of soybean (GenBank accession no. NM_001250372 Tubulin F and R). Q–RT–PCR was performed with SsoAdvanced SYBR Green Supermix (Bio–Rad Laboratories, CA). Each 20–μl reaction contained 10 μl SsoAdvanced SYBR Green Supermix, 5 μl 40–fold–diluted cDNA, 10 pM each gene–specific primer. Reaction conditions were 95ºC for 10 min; 40 cycles of 95ºC for 30 s, 58ºC for 20 s, 72ºC for 30 s. Transcript levels of double infection SMV/SYMMV CP, SYMMV/SYCMV CP were normalized to SYMMV, respectively. Soybean tubulin was used as an internal standard. Mean values were calculated from two biological replications (Bae *et al*., 2006). Statistical analysis of Q–RT–PCR results was carried out using Microsoft Excel to separate reactions into classes.

Phylogenetic analysis. To examine the relationships of SMV and SYCMV isolates between 2012 and 2014, phylogenetic analyses were performed on SMV HC–Pro and SYCMV ORF1 sequences (Fig. 2 and Fig. 3). SYMMV sequences from 2012 were not available. Nucleotide sequences were aligned using Clustal X2 (Thompson *et al*., 1997) and manually edited. Maximum–likelihood (ML) and maximum–parsimony (MP) analyses were implemented with MEGA v 5.05 (Tamura *et al*., 2011). Each MP analysis was carried out by a heuristic search with 1000 random stepwise additions of branches to obtain bootstrap values. The nucleotide sequence of watermelon mosaic virus (NC_006262) HC–Pro was used as outgroup to root SMV trees; SMV HC–Pro sequences from 2012 were previously examined (Li *et al*., 2013). The HC–Pro nucleotide sequence of SMV isolate A297–12 was used as an outgroup to root the tree of SYSMV ORF1.

RESULTS

SMV, SYMMV, and SYCMV were dispersed nationwide. We collected 251 soybean samples from suspected virus infected soybean based on symptom development. As indicated in Figure 1 we collected samples across the

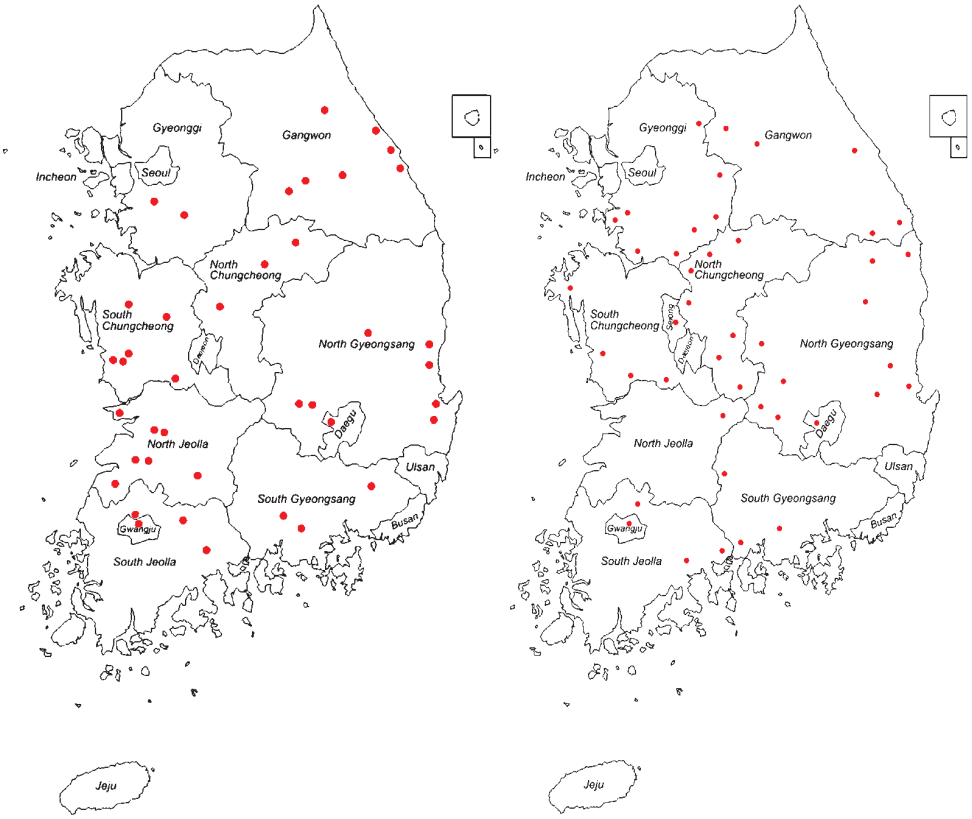

Fig. 1. Geographical distribution of collection sites of soybean samples (Left:2014, Right:2012).

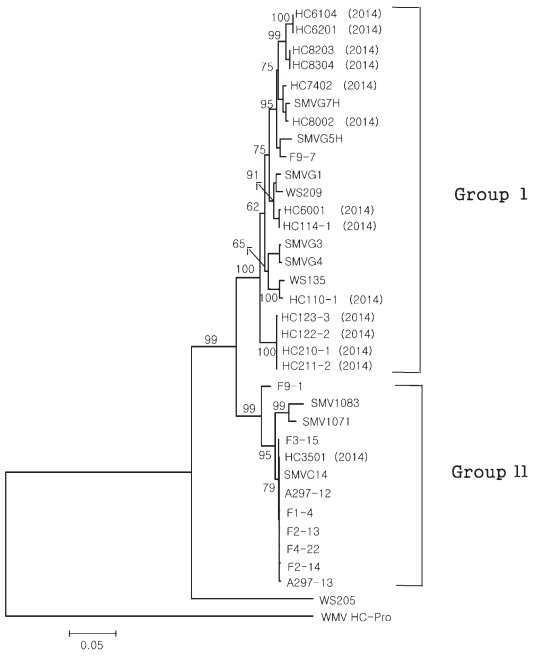

Fig. 2. The most parsimonious tree based on the HC–Pro nucleotide sequences of 39 SMV strains. Bootstrap values greater than 50% calculated from 1000 replicates are given at the nodes. The scale bar indicates the number of nucleotide substitutions. HC–Pro sequences from 2014 isolates: HC6104 isolate B61, HC6201 isolate B62, HC8203 isolate B82, HC8304 isolate B83, HC7402 isolate B74, HC8002 isolate B80, HC6001 isolate B60, HC114–1 isolate B114, HC110–1 isolate B110, HC210–1 isolate B210, HC211–2 isolate B211, HC3501 isolate B35. All other HC–Pro sequences are reference isolates or 2012 Korean isolates described in Li *et al.*, (2014).

Fig. 3. Typical symptoms of virus–infected soybeans: (A) *Soybean mosaic virus* infected plants (B) *Soybean yellow mottle mosaic virus* infected plants (C) *Soybean yellow common mosaic virus* infected plants (D) *Soybean mosaic virus*, *Soybean yellow mottle mosaic virus* double infected plants (E) *Soybean yellow mottle mosaic virus*, *Soybean yellow common mosaic virus* double infected plants.

same regions in which we performed the survey in 2012 (Cho *et al.*, 2013). RT–PCR data showed that fifty–three tissue samples were positive for SMV, six samples for SYCMV and 67 for SYMMV (Table 2 and Table 3). Among these samples B82 was positive for both SMV and SYMMV, and B183/B184/B185 were positive for both SYCMV and SYMMV (Table 4). B82 was collected in North Gyeongsang and B183/B184/B185 were from Gangwon. Compared to the survey performed in 2012, SMV/SYMMV dual infections, and SMV/SYCMV dual infection were found but not SYMMV and SYCMV (Table 4). In 2014 survey, one sample was infected by SMV and SYMMV, and three samples were infected with both SYMMV and SYCMV.

Plants dually infected by SMV+SYMMV or SYMMV+SYCMV did not show severe symptoms, however singly infected soybean produced severe symptoms regardless of which virus was present. Leaves with single infection

Table 1. Nucleic acid sequence of oligonucleotide primers used in this study

Virus	Accession No.	Sequence of Primer	PCR region
SMV_a detection	GU015011	GAACAAAGCAAGATAGCATG TGTGTAGACTATCTCAGCAT	NIb
$SYMMV_b$ detection	NC_011643	CAACCCTCAGCCACATTCAACTAT TCTAACCACCCCACCCGAAGGATT	ORF5
$SYCMV_c$ detection	NC_016033	TTGGCTGAGAGGAGTGGCTT TGCGGTCGTGTAGTCAGTG	CP
SMV HC–Pro (F, R)	GU015011	ATGTCCCAAAATCCTGAAGCTCAG TTATTCACCACCAACTCTGTAGAATTTC	HC–Pro
SYCMV ORF1(F, R)	NC_016033	ATGACCGACACAGTCGTCGTG TCACGACCAAGAGCGTGTACGCAAA	ORF1
QRT–SMV(F, R)	GU015011	AAAACACCAAACAGGGCAAG TGGTTGAGATGTTCCCATCA	CP
QRT–SYMMV (F, R)	NC_011643	TGTGTCCAATGGAGTACCGA AAGCAGGGGACTCAGTGCTA	CP
QRT–SYCMV (F, R)	NC_016033	ATGCTAACATCGCGACTCCT GTTCAAGGCTGAGGCGATAG	CP
QRT–Tubulin (F, R)	NM_001250372	TCTCTCGCATTGACCACAAG CACGAGCCTCAGAAAACTCC	Tubulin

[a] Soybean mosaic virus
[b] Soybean yellow mottle mosaic virus
[c] Soybean yellow common mosaic virus

Table 2. Incidence of viruses detected during 2012 and 2014 in Korea

Virus	2012 % incidence	2014 % incidence
SMV	100/682	52/251
SYMMV	116/682	67/251
SYCMV	17/682	6/251
SMV+SYMMV	5/682	1/251
SMV+SYCMV	1/682	–
SYMMV+SYCMV	–	3/251

Table 3. Virus infected samples collected in 2012 and 2014 in eight Provinces of Korea

Viruses infection Provinces	SMV		SYMMV		SYCMV	
	2012	2014	2014	2014	2012	2014
South Gyeongsang	+	+	+	+	+	–
Nouth Gyeongsang	+	+	+	+	+	–
South Chungcheong	–	+	+	+	+	–
North Chungcheong	+	+	+	+	+	+
South Jeolla	+	+	+	–	–	–
North Jeolla	+	+	+	+	–	+
Gyeonggi	+	+	+	+	+	+
Gangwon	+	+	+	+	+	+

Table 4. Locations of virus infection of soybean samples

Sample No.	Localization	Date	Viruses identified		
			SMV	SYCMV	SYMMV
B2, B3, B4	Dalseong–gun, Daegu	2014–06–14			●
B6	Seosan	2014–06–18			●
B18	Jeongeup	2014–06–25			●
B25	Damyang–gun	2014–06–25	●		
B31	Cheonan	2014–07–01			●
B35	Cheonan	2014–07–01	●		
B38, B40, B41, B42, B43, B44	Yesan	2014–07–04			●
B47	Cheongyang	2014–07–04			●
B51, B52, B53, B54, B55, B57	Chungju	2014–07–10			●
B60	Chungju	2014–07–13	●		
B61, B62	Jeonju	2014–07–13	●		
B63	Jeonju	2014–07–13		●	
B71, B72, B73	Jinju	2014–07–15			●
B74, B75, B76, B77, B78, B79,B80	Sancheong	2014–07–15	●		
B81, B83, B84, B85	Gimcheon	2014–07–18	●		
B82a	Gimcheon	2014–07–18	●		●
B86, B87	Gimcheon	2014–07–18	●		
B102, 103, 106	Icheon	2014–07–21			●
B108, B110, B111, B112, B114	Icheon	2014–07–21	●		
B113	Icheon	2014–07–21		●	
B118, B119	Donghae	2014–07–28			●
B122, B123, B125, B127, B128	Yeongwol	2014–07–28	●		
B124	Yeongwol	2014–07–28			●
B129, B132	Yeongwol	2014–07–28			●
B134	Jecheon	2014–07–28		●	
B135, B137	Jecheon	2014–07–28			●
B138, B139, B140, B141, B142,B143	Buyeo	2014–07–31			●
B150	Gyeongju	2014–08–07	●		
B154, B158, B159	Yeongdeok	2014–08–07			●
B161, B162, B163	Ulgin	2014–08–07			●
B166, B168	Samcheok	2014–08–07			●
B169, B172, B173, B174, B178vB179, B180	Ansan	2014–08–07			●
B171	Ansan	2014–08–07	●		
B181, B182	Yanggu	2014–08–07	●		
B183, B184, B185	Yanggu	2014–08–07		●	●
B186, B187	Yanggu	2014–08–07			●
B192	Eumseong	2014–08–07			●
B198, B200–202	Miryang	2014–08–20	●		
B209–B224	Gurye	2014–08–27	●		
B239–242, B244, B246	Jeongeup	2014–08–27			●

of each of SMV, SYMMV and SYCMV showed severe mosaic, with curling margins (Fig. 4). In order to determine the relationship between virus concentration and symptoms caused by double infection we performed Q–RT–PCR to quantify individual virus RNA copy number. Results showed SMV/SYMMV double infection RNA accumulation was not changed as much as SYMMV/SYCMV double infection. However, between double infection SMV/SYMMV, SYMMV RNA accumulation level rises constantly higher more than SMV, and SYCMV RNA accumulation level declined a little compare with SYMMV (Table 5).

Phylogenetic analysis (ML and MP methods) of HC–Pro of 14 representative new SMV isolates. Comparison of new Korean isolates with previously characterized strains from China, Korea or North America showed that the SMV HC–Pro sequences were divided into two groups (Fig. 2 and Table 6). Most U.S. strains clustered (100% bootstrap support) in group I, together with most of the Korean isolates collected in 2014. As results from ML and MP analyses were similar, only MP analyses are further described. Only one of the new isolates fell into group Ⅱ (99% bootstrap support) with one previously characterized Chinese isolate (C14) and most of the 2012 Korean SMV isolates. Phylogenetic analysis results showed that the HC–Pro sequences of most of the SMV isolates that we collected in 2014 were not similar to the isolates collected in 2012, including the SMV/SYMMV double infection isolate B 82; the majority were instead similar to American, Chinese and other reference isolates (Domier *et al*., 2003; Lim 1985; Li *et al*., 2013). Isolate B82 came from the same region as 2012 double infection SMV/SYMMV isolate A 269, from North Gyeongsang Province.

Amino acid alignment of SMV HC–Pro isolates between 2012 and 2014. We have previously identified differences of one 2012 isolate at HC–Pro amino acid residues 54, 286, 369 which we predicted were related to efficiency of HC–Pro silencing suppressor activity (Li *et al*., 2013). Alignment of HC–Pro amino acid sequences showed that all of the 2014 isolates were identical at these positions to the majority of 2012 isolates; only 2012 isolate A297–13 was different at these three positions (Li *et al*., 2013; and data not shown).

Analysis of SYMMV and SYCMV. Comparison of new isolates from 2014 with previously characterized isolates

Table 5. Real–time qPCR of soybeans double infected with between *Soybean mosaic virus* (SMV) / *Soybean yellow mottle mosaic virus* (SYMMV) and SYMMV / *Soybean yellow common mosaic virus* (SYCMV)

Location Samples	SMV/SYMMV B82	SYMMV/SYCMV		
		B183	B184	B185
Δct1[a]	2.46	0.84	2.46	5.42
Δct2[b]	–7.93	5.08	7.89	9.33
ΔΔct3[c]	10.39 (1341.8)	–4.24 (0.0529)	–5.43 (0.0232)	–3.91 (0.0665)

[a] Δct_1 (ct value of tubulin – ct value of SYMMV or SYCMV).
[b] ct_2 (ct value of tubulin – ct value of SMV or SYMMV).
[c] $\Delta\Delta ct$ shows value of $\Delta ct_1 - \Delta ct_2$.

Table 6. Country of origin and GenBank accession numbers of SMV HC–Pro nucleotide sequences used for comparisons

SMV strain/isolate	Origin	GenBank accession No.
HC3501 (2014)	Korea (South Chungchong)	KM650173
HC6001(2014)	Korea (North Chungchong)	KM650174
HC6104 (2014)	Korea (North Jeolla)	KM650175
HC6201 (2014)	Korea (North Jeolla)	KM650176
HC7402 (2014)	Korea (South Gyeongsang)	KM650177
HC8002 (2014)	Korea (South Gyeongsang)	KM650178
HC8203 (2014)	Korea (North Gyeongsang)	KM650179
HC8304 (2014)	Korea (North Gyeongsang)	KM650180
HC110–1 (2014)	Korea (Gyeonggi)	KM650181
HC114–1 (2014)	Korea (Gyeonggi)	KM650182
HC122–2 (2014)	Korea (Gangwon)	KM650183
HC123–3 (2014)	Korea (Gangwon)	KM650184
HC210–1 (2014)	Korea (South Jeolla)	KM650185
HC211–2 (2014)	Korea (South Jeolla)	KM650186
WMV	France	NC_006262

Table 7. Region of origin in Korea and GenBank accession numbers of SYCMV ORF1 nucleotide sequences used for comparisons

SYCMV strain/isolate	Region of origin	GenBank accession No.
1–1	Gyeonggi	KM881452
1–1–3	Gyeonggi	KM881453
1–1–4	Gyeonggi	KM881454
1–2–2	South Chungcheong	KM881455
1–2–3	South Chungcheong	KM881456
1–2–4	South Chungcheong	KM881457
1–3–3	North Chungcheong	KM881458
1–3–4	North Chungcheong	KM881459
1–3–5	North Chungcheong	KM881460
1–4–2	Gangwon	KM881461
1–4–3	Gangwon	KM881462
1–4–4	Gangwon	KM881463
1–5–1	South Gyeongsang	KM881464
1–5–2	South Gyeongsang	KM881465
1–5–4	South Gyeongsang	KM881466
B113 (2014)	Gyeonggi	KM881462
B134 (2014)	North Chungcheong	KM881463
B183 (2014)	Gangwon	KM881464
B184 (2014)	Gangwon	KM881465
B185 (2014)	Gangwon	KM881466

from 2012 showed that the SYCMV ORF1 sequences were divided into three groups (Fig. 3 and Table 7) correlating with geographical origin. Group I including north part of Korea (Gyeonggi, Gangwon), 2014 survey double infection SYMMV, SYCMV No. 183–185 from Gangwon province together with 2012 single SYCMV infection isolates 1–4–1, 1–4–2, 1–4–3 from northern Gangwon Province clustered (67% bootstrap support) in group I. Group Ⅱ contains single infection of SYCMV No. 113 and 134 from the middle part of Korea (North Gyeongsang, South Gyeongsang and North Chungcheong), together with 2012 isolates. Group Ⅲ included only 2012 isolates from the south part of Korea (South Gyeongsang) (Fig. 3).

DISCUSSION

We compared the distribution of the three main soybean viruses – SMV, SYMMV and SYCMV – in Korea in 2014 to their distribution in 2012. During this survey, results showed SYMMV and SMV were the most prevalent viruses in 2014, and few SYCMV isolates were detected, just as observed in 2012 (Cho *et al*., 2013). A few double infections of SMV/SYMMV, and SYMMV/SYCMV were detected, whereas in 2012 no SYMMV/SYCMV double infections were detected (Cho *et al*., 2013). Phylogenetic analysis of SMV HC–Pro showed that the most of the 2014 isolates were of a different subgroup than most 2012 isolates. However, amino acid alignment of domains associated with RNA silencing suppression efficiency (Li *et al*., 2013) showed there were no differences within any 2014 isolates; HC–Pro residues 54, 286 and 369 were important for the virus to maintain high silencing suppressor activity (Li *et al*., 2013). In order to examine the relationship between symptoms and double infection we performed Q–RT–PCR to quantify RNA copy number of each virus. Results showed SMV/SYMMV double infection RNA accumulation was not changed as much as SYMMV/SYCMV double infection. However, between double infection SMV/SYMMV, SYMMV RNA accumulation level was higher more than SMV, and SYCMV RNA accumulation level declined a little compare with SYMMV (Table 4).

In past few years, many studies have been performed to study the soybean virus incidence in Korea. Cho *et al*. reported that the most prevalent soybean viruses in Korea are SMV and SYMMV, with a few double infections of SMV/SYMMV and SMV/SYCMV also being detected (Cho *et al*., 2013). Our 2014 results were consistent with the prior report that SMV and SYMMV are the main viruses infecting soybean plants. Symptoms of single infection of SMV, SYMMV and SYCMV were severe mosaic and curling leaves, but double infection of SMV/SYMMV, SYMMV/SYCMV did not produce symptoms as severe as the single virus infections. Our 2014 results showed that single infection of SMV and SYMMV were still prevalent to the same extent as our earlier study, with similar symptom expression. However, phylogenetic analysis showed a difference in the type of SMV isolates between 2012 and 2014, and no variation in residues linked to less efficient silencing suppression activity of HC–Pro was detected. Distribution of SYCMV in 2014 was consistent with that observed in the 2012 study and there was little

evidence from the phylogenetic analysis for significant change in the distribution of types of isolates, although no group Ⅲ isolates were detected in 2014. Plants with double infections of SYMMV/SYCMV showed apparent aphid feeding damage that suggests that aphid control may be needed to minimize SYCMV transmission.

We have shown here that SMV, SYMMV and SYCMV occurred with about the same frequency in 2014 as we previously observed in 2012 (Cho *et al*., 2013), across all of the Korean provinces examined. However, we have observed a shift in the types of isolate of SMV between 2012 (predominantly group Ⅱ) and 2014 (predominantly group Ⅰ). The reason for the apparent shift in SMV isolate type across all regions of Korea is unknown, but calls into question some prior assumptions. Because many or most small–scale soybean farmers in Korea save their own seed for replanting, it has been widely assumed that SMV seed transmission was responsible for most of the initial inoculum in the crop. There are no major suppliers of soybean seed within Korea, but some farmers do periodically obtain fresh sources of seed, typically from the United States. Unfortunately, we did not obtain information about seed sources or varieties at the time of either the 2012 or 2014 sample collections, and thus cannot determine whether there has been a significant turnover in varieties between the two surveys. One other possible explanation for our observations is that a new isolate had been introduced or evolved that either replicates to higher levels (providing more inoculum for aphid transmission) or is more efficiently aphid– (or seed–) transmitted than the isolates predominating in our 2012 survey; this seems somewhat unlikely, as the group Ⅰ isolates were widely distributed both geographically in multiple Korean provinces, and also appear within multiple clades within group Ⅰ suggesting variation developing over a considerably longer period than the two years between our surveys. Yet a third possibility is transmission from wild or weedy hosts of SMV spurred by climatic conditions that encouraged atypical movement of a vector between infected wild hosts and cultivated soybean over all of the provinces surveyed; while this scenario seems unlikely, a survey for SMV infection in wild *Glycine* species and other wild legumes would address the question of the distribution and variability of SMV in non–crop species. Lee and Kim (*2013*) have already demonstrated that wild soybean (*G. soja*), *Vigna angularis, Trifolium repens*, and *Lespedeza cuneata* are naturally weed hosts of SYMMV, and there is thus a possibility that these or other wild legumes are also reservoirs of various isolates of SMV, potentially causing the observed shifts in isolates detected in cultivated soybean between 2012 and 2014. We previously reported that during the survey period of May to October, there were more SYCMV detections during the later part of the period than during the early stage (Cho *et al*., 2013). In this study, more SYCMV infections were detected after July than were identified before July. How and why this happened will need additional study, but suggests transmission of SYCMV within and between fields by an aerial vector. SMV infection has been reported to occur by both seed and aphid transmission (Browers and Goodman, 1991; Domier *et al*., 2007; Seo *et al*., 2010). SYMMV could be infected by seed transmission, and SYMMV infection of wild soybean populations was notably detected by mid–July (Lee and Kim, 2013). There is, as yet, no report about transmission of SYCMV; therefore, more study about SYCMV transmission in soybean will needed. However, it appears that wild legumes, including weedy soybean, may well serve as reservoirs of SYCMV and SMV in addition to their proven infection by SYMMV.

Whichever explanation is ultimately determined to explain the significant change in SMV isolate types in soybeans across all Korean provinces over a two year interval, it is clear that improving the seed health of soybeans in Korea will reduce the initial inoculum within the crop. Further surveys based on testing of self–saved seed obtained from farmers may be useful to track virus isolate types between seasons, and additional surveys of wild legumes would be useful to determine potential external sources of SMV, SYMMV and SCYMV infection. Such results would be useful to educate the typical small–scale farmer of the potential yield and quality improvements to be gained from using seed with a lower viral load, as is already widely available in the United States (Hill, 1999).

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