Australian Journal of Crop Science AJCS
AJCS 5(11):1379-1387 (2011) ISSN:1835-2707

Intra-host competition and interactions between *Soybean mosaic virus* (SMV) strains in mixed-infected soybean

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Abstract

Over the past two decades, the dominant *Soybean mosaic virus* (SMV) strain in South Korea has changed from G5 to G7H. To examine the dominance of G7H, intra-host competition between G7H and G5 was evaluated in soybean plants infected with a mixture of SMV strains. The distribution patterns of the two SMV strains in soybean plants inoculated with G7H, G5 and G7H/G5 were investigated at designated time points by RT-PCR/RFLP analysis, which enables the specific differentiation of low concentrations of SMV strains and the detection of mixed infection at any given time. When leaves of 'Kwangankong' and 'Tawonkong' were infected with both strains, the upper leaves had only the G7H strain in simultaneous infections. In sequential inoculations, the leaves exhibited mosaic symptoms caused by G7H, and the G5 strain was not detected in plants pre-inoculated with the G7H strain before inoculation with the G5 strain. In the reverse treatment, both G5 and G7H were present at every vegetative stage. In addition, interactions between the virulence and dominance of G7H, G5, and G1, a less virulent strain, were investigated. Three landrace soybeans were co-inoculated with G7H/G5, G7H/G1, G5/G1, and G7H/G5/G1 sets. There was no significant difference between virulence and dominance. These results demonstrate the dominance of G7H in mixed infections and could explain the prevalence of G7H in South Korea.

Keywords: Intra-host competition; Mixed infection; RT-PCR/RFLP; Seed mottling; Soybean; Soybean mosaic virus; SMV strain G5; SMV strain G7H.
Abbreviations: ANOVA – analyses of variance, ELISA – enzyme linked immunosorbent assay, EtBr – ethidium bromide, NICS – national institute of crop science, ORF – open reading frame, PepMV – pepino mosaic virus, RB – resistance-breaking, RCBD – randomized complete block design, RT-PCR/RFLP – reverse transcriptase PCR/restriction fragment length polymorphism, SMV – soybean mosaic virus.

Introduction

There are 67 known viruses that infect soybean [*Glycine max* (L.) Merr.] at any given location, and at least 27 of these viruses pose a threat to soybean (Saghai Maroof et al., 2008). *Soybean mosaic virus* (SMV; genus *Potyvirus*, family *Potyviridae*) is one of the most destructive viral pathogens affecting soybean seed quality and yield (Kim et al., 1997; Kim et al., 1996; Ren et al., 1997). SMV has a positive-sense single-stranded RNA genome of 9588 nucleotides, and the genome contains one large open reading frame (ORF) that is translated into a polyprotein (Eggenberger et al., 1989). SMV is seed-borne and transmitted by more than 30 species of aphids in a non-persistent manner (Irwin and Schultz, 1981). SMV infected seed is recognized as the most important source of primary inoculum (Irwin and Goodman, 1981; Kim et al., 1997). Seed transmission plays a critical role in the epidemiology of SMV (Maury, 1985). Bowers and Goodman (1991) reported that resistance to SMV seed transmission is strain-specific and must be considered in breeding programs. SMV is classified on the basis of its pathogenicity to soybean varieties and the symptoms it causes in infected plants (Cho and Goodman, 1979; Takahashi et al., 1980). Cho and Goodman (1979) classified 98 SMV isolates from soybean seeds of the USDA soybean germplasm collection into seven strains (SMV-G1 through SMV-G7) on the basis of the symptoms (resistance, mosaic, or necrosis) they caused in eight soybean cultivars. In Japan, SMV was classified into five strains, A to E, also on the basis of induced symptoms in different soybean cultivars (Takahashi et al., 1980). Since these initial classifications were made, several highly virulent SMV strains, including G7A, G5H, CN, and G7H, have emerged (Jeong et al., 2008). Among these, G7H (Kim et al., 2003), found in South Korea, could induce some disease symptoms in most of the SMV-resistant soybean varieties. Interestingly, the dominant SMV strain in South Korea changed from G5 to G5H, and then from G5H to a new virulent G7H strain over a period of 20 years (Kim et al., 2003). Although G7H has become the most prevalent strain and may

cause significant yield losses in South Korea, little is known about how soybean–SMV interactions change the dominant SMV population. The ability of one virus strain to protect against infection and invasion by a second related strain plays an important role in determining the fate of mutant viral strains. When two pathogenic strains affect a single host, the strains may compete for host resources (Read and Taylor, 2001). Such intra-host competition may (Arends et al., 2005; Pepin et al., 2008) or may not (Christen et al., 1990) cause a decrease in the replication of both strains. Takahashi et al. (1980) reported that ordinary strains (A, B, and C) that cause typical mosaic symptoms are comparatively limited in host range, and that severe strains (D and E) of SMV have a wider host range, causing necrosis in varieties that have genes for resistance to the ordinary strains. Importantly, the ordinary strains are readily transmissible by seed and aphids, whereas the severe strains are not. To establish the underlying reason why SMV strain B (SMV-B) is readily transmitted via seeds and strain D (SMV-D) is not, the mode of translocation, multiplication, and inactivation of both SMV strains was investigated in a soybean cultivar (Iwai and Wakimoto, 1990). There was no significant difference in translocation between the two strains, but in every infected organ SMV-D disappeared more rapidly than SMV-B. Kosaka and Fukunishi (1994) reported that Aa15-M1 and Aa15-M2, which are attenuated isolates of SMV obtained by cold treatment, effectively imparted cross-protection against virulent strains on black soybean cv. 'Shin Tambaguro'. Although soybean cultivars resistant to SMV isolates have been developed, the resistant cultivars have become susceptible to emergent isolates (Cho, 1995; Kim et al., 2003). For example, a new virulent strain, G7H, can cause necrosis in 20 elite soybean cultivars of G5H and G7-resistant (Kim et al., 2003). Examination of intra-host competition between the current dominant strain, G7H, and the previous dominant strain, G5, would provide clues as to why G7H has become dominant. For this reason, the interactions and dominance relationships between SMV strains within individual plants were investigated. However, it is technically difficult to discriminate between G7H and G5 strains in plants infected with a mixture of SMV strains because the complete nucleotide sequences of G7H and G5 exhibit a similar genomic organization. The similarity of nucleotide and deduced amino acid sequences between G7H and G5 were 97.8% and 98.9%, respectively (Lim et al., 2003; Seo et al., 2009). In this study, reverse-transcriptase polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) analysis, as described by Kim et al. (2004), was used to investigate intra-host competition between SMV strains in mixed-infected soybean plants at four or five stages during leaf development, and then the effect of virulence and the resistant reaction on SMV strain dominance were quantified. This approach made it possible to examine the distribution patterns and interactions between SMV strains in mixed-infected plants. The results provide an insight into the competition between SMV strains and their spread in the field. Data of the interactions between SMV strains will help us understand why novel SMV strains emerge and facilitate the development of cultivars that are resistant to new SMV strains.

Results

Detection of SMV strain

The distribution patterns of two SMV strains in soybean plants inoculated with G7H, G5, and G7H/G5 sets were investigated by RT-PCR/RFLP analysis (Table 2; Fig. 1 and 2). In the first treatment, two primary leaves of a single plant were infected with both strains by the simultaneous inoculation of one strain per leaf. The leaves of Kwangankong and Tawonkong at the V2, V4, and V6 stage were doubly infected with the two strains, and the upper leaves were only infected with the G7H strain. In the second treatment, the two cultivars were inoculated with G7H and, 24 h later, with G5. The V6 and V8 leaves of all plants infected in this manner exhibited the mosaic symptoms caused by G7H, but did not contain detectable levels of the G5 strain. In contrast, different results were obtained when plants were first inoculated with G5 and then 24 h later with G7H. The pre-inoculated G5 strain was detected at every stage along with the G7H strain.

Seed mottling of Kwangankong

Infection by SMV strain G7H and G5 individually and in combination induced a high level of seed mottling (Table 3 and Fig. 3). Among 205 seeds from G7H-infected Kwangankong, 202 seeds were mottled. G7H had a seed-mottling rate of 98.5% in Kwangankong, while G5 had an incidence of seed mottling of 1.4% in the same cultivar. In Kwangankong, the incidence of seed mottling in plants infected with G7H and G5 was 98.2% and 100%, respectively. We did not investigate the incidence of seed mottling in the black soybean Tawonkong.

Effect of SMV strains on seedling emergence and seed transmission

Seed transmission of SMV strains differed in the two soybean cultivars (Table 4). In Tawonkong, seed transmission was 11.1% and 33% for single infection by G7H and G5, respectively, while those for Kwangankong were 5.6% and 0%. There was no apparent correlation between seed mottling and seed transmission. Seed transmission from the mottled seeds of the susceptible cultivar, Kwangankong, was significantly less than that from Tawonkong. In this study, no differences in seedling emergence were found among SMV strains and soybean cultivars (Table 4).

The effect of individual and combined infection with G7H and G5

The effects of SMV strains G7H and G5 individually and together on the plant growth of Tawonkong and Kwangankong are given in Table 5. G7H caused severe mosaic symptoms in Tawonkong, but produced mild mosaic symptoms in Kwangankong. In contrast, G5 produced mild mosaic symptoms in Tawonkong and severe mosaic symptoms in Kwangankong. SMV infection resulted in altered plant development, including reductions in plant height, number of pods, and weight of 100 seeds in both cultivars, but the effects were more pronounced in Tawonkong than in Kwangankong. The average reduction in plant height caused by infection was 26% and 4% for G7H and G5, respectively, in Tawonkong, whereas it was 9% and 13% in Kwangankong. The height reduction of doubly infected plants was 10% (A/B, simultaneous inoculation: Treatment 1), 11% (A/B, sequential inoculation: Treatment 2), and 6% (B/A, sequential inoculation: Treatment 3) in Tawonkong, while that for Kwangkong was 5, 7, and 14%, respectively (Table 5). The reduction in the number of pods ranged from 7 to 63% in Tawonkong and from 3 to 25% in Kwangankong, depending on the SMV strains, strain combinations, and varieties used. G7H and Treatment 2 caused the most severe reduction in the number of pods in Tawonkong, while G5 and Treatment 3 caused the most severe reduction in the number of pods in Kwangankong. Changes in the height and number of pods appeared to be correlated with differences in varietal

Table 1. Phenotypic reaction of soybean differentials to *Soybean mosaic virus* (SMV) strains G1, G5, and G7H

Differential Cultivars	Symptoms caused by SMV strains†		
	G1	G5	G7H
Rampage	-/M	-/M	-/M
Clark	-/M	-/M	-/M
Davis	-/-	-/M	-/M
York	-/-	-/M	-/M
Marshall	-/-	-/-	L/N
Ogden	-/-	-/-	L/N
Kwanggyo	-/-	L/N	L/N
Buffalo	-/-	-/-	L/N
Hwangkeumkong	-/-	L/-	L/N
Daewonkong	-/-	L/-	L/N
Duyoukong	-/M	L/-	-/M
Myeongjunamulkong	-/-	-/-	-/-

†Reaction on inoculated primary leaves/reactions on uninoculated upper leaves; – = symptomless; M = mosaic; N = necrosis; L = local lesions.

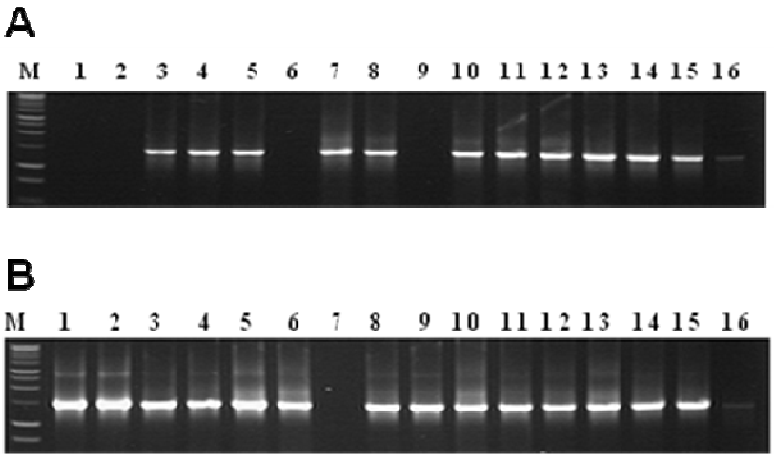

Fig 1. Detection of SMV by RT-PCR analysis of doubly infected soybean plants. (A) Kwangankong: V2 stage, lanes 1, 2, 6, 9, and 16; V4, lanes 3 and 10; V6, lanes 4, 7, 11, and 15; V8, lanes 5, 13, and 14; V10, lane 14; M, molecular size marker (1-kb ladder). (B) Tawonkong: V2, lanes 1, 3, 4, 7, 14, and 16; V4, lanes 2, 5, 8, 11, and 13; V6, lanes 9, 10, 12, and 15; V8, lanes 6.

susceptibility to SMV strains. Interactions between SMV strain and soybean cultivar were evident. The weight of 100 seeds was altered by viral infection. In Kwangankong, the seeds from plants subjected to Treatment 1 and 2 were larger than those from plants exposed to G5 and Treatment 3. The two different soybean cultivars varied in their seed yield compared to the mock-inoculated control (Fig. 4.). The variance may be due to genetic differences. In Tawonkong, the average yield loss was 63% and 12% single infections with G7H and G5, respectively. For double infections, the yield loss was 53, 65, and 28% for Treatment 1, 2, and 3, respectively. Infection with SMV also reduced seed yield in Kwangankong. Yield loss due to infection with G7H and G5 was 23% and 42%, respectively. For double infection, yield loss was 14, 17, and 36% for Treatment 1, 2, and 3, respectively. In all treatments, the range of yield reduction in Kwangankong was narrower (23 ~ 42%) than that for Tawonkong (12 ~ 63%).

Interactions between virulence and dominance

Interactions between the virulence and dominance of G7H, G5, and G1, a less virulent strain, were investigated by RT-PCR/RFLP analysis (Table 1). G7H was detected in plants infected with the G7H/G5 set, and two strains, G7H and G1, were detected in plants inoculated with the G7H/G1 and G7H/G5/G1 sets. In these experiments, the two mixed infected landrace plants used were G7H, G5, and the G1-susceptible landraces, NICS germplasm No. 793 and 640 (Fig. 5A and B). Only G7H was detected when the G1-resistant landrace NICS germplasm No. 332 was infected with G7H/G5, G7H/G1, and G7H/G5/G1 sets. For infection with the G5/G1 set, only G5 was detected in the infected plant (Fig. 5C). There was no significant difference between G7H and G1 in the two susceptible landraces. In the case of the G1-resistant landrace, G1 was not detected in plants inoculated with this strain.

Discussion

To understand the distribution patterns of G7H in mixed-infected soybean plants, SMV strains were identified in leaves sampled at four or five stages of leaf development using RT-PCR/RFLP analysis. In this study, G7H was dominant over G5 both in simultaneous and sequential inoculations with the two strains, regardless of the host cultivar tested (Table 2; Fig. 2

Table 2. Detection of SMV strains in soybean plants inoculated with G7H (7), G5 (5), G7H/G5 (D), and G5/G7H (D).

Varieties	Growth stage	G7H (A)	G5 (B)	A/B (sim.)	A/B (24 h)	B/A (24 h)	Con.
		1R,2R,3R†	1R,2R,3R	1R,2R,3R	1R,2R,3R	1R,2R,3R	all
Tawon-kong	V2	7, 7, 7	5, 5, 5	7, 7, D	N, 7, 7	D, 5, D	N‡
	V4	7, 7, 7	5, 5, 5	D, D, 7	7, 7, 7	7, D, D	N
	V6	7, 7, 7	5, 5, 5	7, 7, 7	7, 7, 7	D, D, D	N
	V8	7, 7, 7	5, 5, 5	7, 7, 7	7, 7, 7	D, D, 7	N
Kwang-ankong	V2	N, N, N	N, N, N	N, N, N	N, N, 7	7, 5, N	N
	V4	7, 7, 7	5, 5, 5	7, D, D	7, 7, 7	D, D, D	N
	V6	7, 7, 7	5, 5, 5	D, 7, 7	7, 7, 7	D, D, D	N
	V8	7, 7, 7	5, 5, 5	7, 7, 7	7, 7, 7	7, D, D	N
	V10	7, 7, 7	5, 5, 5	7, 7, 7	7, 7, 7	D, D, D	N

† R = Replication, ‡ N = No detection.

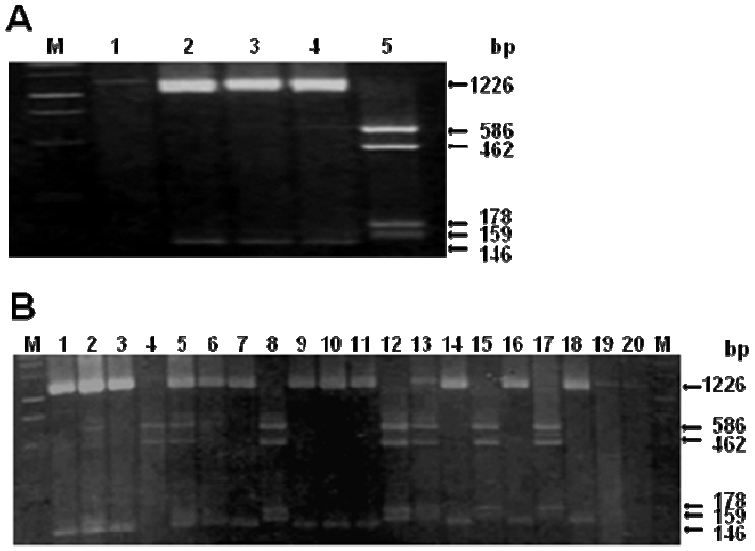

Fig 2. Electrophoresis of PCR products after *Rsa*I restriction enzyme digestion. G7H has specific band profiles at 1226 and 146 bp, and G5 has specific band profiles at 586, 462, 178, and 159 bp. (A) G7H, lanes 1-4; G5, lane 5; M, molecular size marker (1-kb ladder). (B) G7H, lanes 1, 3, 7, 9, 10, 11, 14, 16, 18, 19, and 20; G5, lanes 4, 8, 12, 15, and 17; doubly infected (G7H and G5), lanes 2, 5, 6, and 13; M, molecular size marker (1-kb ladder).

and 3). The results of all treatments showed that the translocation and multiplication abilities of G7H and G5 differed in doubly infected plants (Table 2). Moreover, there appeared to be a high correlation between the distribution patterns induced by interactions between SMV strains and seed yields of doubly infected plants. A similar asymmetrical antagonism was observed in mixed infections with *Pepino mosaic virus* (PepMV) strains, showing that the accumulation of the PS5 isolate was suppressed in the presence of the Sp13 isolate. This suggests that antagonistic mixed infections could be significantly contributing to dynamics of PepMV populations (Gomez et al., 2009). The results presented here that G5 strain is strongly suppressed in mixed infection give a clue to explain how the dominant strain has changed from G5 to G7H. Therefore, intra-host interactions between SMV strains are likely to play an important role in replacing the preexisting strain. G7H, a new strain of SMV, was identified in the plants infected with SMV (Kim et al., 2003). It induced mosaic and necrotic symptoms in the G5H and G7-resistant South Korean elite soybean cultivars. Infection with G7H was up to 36% in the northern provinces of South Korea, and necrosis by G7H has become a severe problem (Kim, 2000). Forty-four South Korean elite soybean cultivars were tested for reactions to nine SMV strains, G1, G3, G4, G5, G6, G7, G7a, G5H, and G7H, and grouped into twenty-four subgroups on the basis of the symptoms when inoculated with each of the strains. Four of the forty-four soybean cultivars, including 'Myeongjunamulkong','Ilpumgeomjeongkong','Geomjeonkong 2', and 'Pureunkong', were identified as being resistant to G7H, whereas twenty-seven and twenty-four soybean cultivars were resistant to G1 and G5, respectively (Kim, 2000). Thus, the interactions between virulence and dominance were investigated. Two strains, G7H and G1, were co-dominant in two landraces such as typical systemic mosaic symptoms varieties to SMV strains G7H, G5, and G1-susceptible. The G1 strain was not detected in a G1-resistant landrace (Fig. 5). Our results are somewhat consistent with the previous observation (Iwai and Wakimoto, 1990) that virulence and dominance are not significantly correlated between mild and severe SMV strains. However, our results showed that, when soybean

Table 3. The effect of individual and combined infection with G7H and G5 on the percentage of mottled seeds of "Kwangankong".

Percent of mottled seeds	G7H (A)	G5 (B)	A/B (sim.)	A/B (24 h)	B/A (24 h)	Con.
No. of investigated seeds	205	139	243	228	153	270
No. of mottled seeds	202	2	243	224	153	0
Percent	98.5	1.4	100	98.2	100	0.0

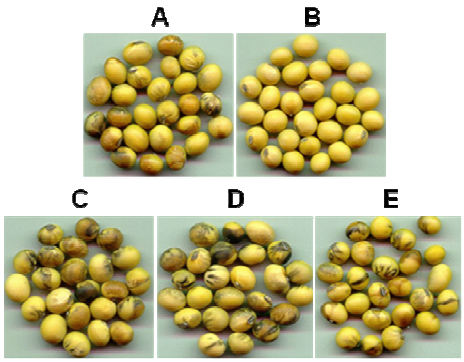

Fig 3. Mottled seeds of soybean singly and doubly infected by SMV strains, G7H and G5. (A) G7H; (B) G5; (C) G7H/G5 (sim.); (D) G7H/G5 (24 h); (E) G5/G7H (24 h).

cultivars resistant to mild SMV strains are planted, severe strains would have a higher chance of seed-transmission than would mild strains. G7H had a high level of seed-mottling in Kwangankong, while G5 had noticeably less in the same cultivar. The percent of mottled seed was not correlated with the severity of the other symptoms. G5 caused a severe reduction in seed yield, but induced minimal mottling. These results suggest that G5 has a lower affinity for infecting soybean seed tissue than does G7H. The incidence of seed mottling in plants infected with G7H/G5 (sim.), G7H/G5 (24h), and G5/G7H (24h) were similar level. These results indicate that G7H plays a key role in seed mottling of Kwangankong. SMV shows a similar genomic organization and high sequence similarities (Lim et al., 2003). Recombination between the RNA of one strain and another depends on the degree of sequence similarity between the sequences involved and the length of the viral genome (Gagarimova et al., 2008; Gallei et al., 2004; Lai, 1992). Seo et al. (2009) suggested that recombination as well as mutation is an important evolutionary process in the genetic diversification of SMV population. The occurrence of RNA recombination was evaluated in three soybean differential cultivars, Hwangkeumkong, Daewonkong, and Duyoukong (Table 1), using G7H from mixed-infected soybean cultivars Kwangankong and Tawonkong. Inoculations with G7H on the primary leaves of Hwangkeumkong and Daewonkong induced necrotic symptoms in the upper leaves, whereas inoculation of Duyoukong resulted in mosaic symptoms (data not shown). These results suggested that there were no recombination events between G7H and G5 strain. The susceptibility of resistant cultivars is attributed to the emergence of resistance-breaking (RB) virus strains (Harrison, 2002). SMV has readily evolved over time due to the selection pressure imposed by host cultivars as well as by aphid and seed transmission or changes in environmental conditions (Choi et al., 2005, Seo et al., 2010). In South Korea, 12 newly emerged Chungnam RB SMV isolates, CN1, CN2, CN3, CN7, CN9, CN10, CN12, CN13, CN15, CN18, CN31, and CN36, were collected based on their ability to infect different cultivars carrying *Rsv*1, *Rsv*3, or *Rsv*4 (Choi et al., 2005). To date, it has not been reported which of these RB isolates is prevalent in other areas of South Korea. If these RB isolates become prevalent, it will be necessary to identify the prevalent isolates and to develop a new cultivar that is resistant to these isolates. Our data suggest that an investigation of intra-host competition will be useful for determining which dominant strain among emerging RB strains can overcome *Rsv* resistance at three loci.

Materials and methods

Soybean plants and growth conditions

Two soybean cultivars, 'Tawonkong' and 'Kwangankong', were selected according to their mosaic symptom-severity in the competition experiment. Tawonkong exhibited more severe symptoms to G7H, whereas Kwangankong was highly susceptible to G5. In order to investigate the interactions between virulence and dominance, National Institute of Crop Science (NICS) germplasm landraces No. 332 (G7H and G5-susceptible, and G1-resistant), No. 640 (G7H, G5, and G1-susceptible), and No. 793 (G7H, G5, and G1-susceptible) were selected based on the presence of typical mosaic symptoms following infection with G7H, G5, and G1. The individual soybean plants were grown in 16-cm-diameter pots (four plants per pot) in the greenhouse at average daily minimum and maximum temperatures of 20 and 35 ^{o}C under natural light conditions.

SMV strains

Three SMV strains, G7H, G5, and G1, were used in this study. G1-VA (PV-571) was provided by the American Type Culture Collection (1018 University Boulevard, Manassas, Virginia 20110-2209, USA), and G7H (SC26) and G5 (SE 29) were seed-borne, and were isolated from soybean fields. Each strain was confirmed by inoculating different varieties of soybean (Table 1) and by performing RT-PCR/RFLP analysis (Kim et al., 2004). Soybean cv. 'Seokryangputkong', which is susceptible to G7H, G5, and G1, was used as the maintenance host.

Table 4. The effect of SMV strains on average seedling emergence and seed transmission from infected "Tawonkong" and "Kwangankong".

Varieties	Seedling emergence		Seed transmission	
	G7H	G5	G7H	G5
Tawonkong	94.4 a†	92.6 a	11.1 a	33.0 a
Kwangankong	97.2 a	97.6 a	5.6 a	0 b
LSD (5%)	5.2	7.6	12.95	18.78

† Mean separation within columns by least significant difference (LSD) at P ≤ 0.05.

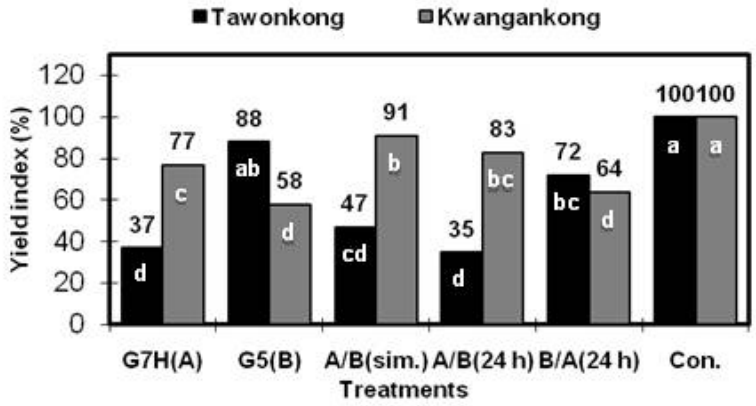

Fig 4. Changes in yield index of soybean plants in response to single and double infection with SMV-G7H and G5. The letters at the top of each column represent seed yield per plant (%). a-d: Mean separation within columns by least significant difference (LSD) at P = 0.05.

Determinant of SMVstrain concentration for inoculations

Enzyme linked immunosorbent assay (ELISA) was employed to determine the concentration of three SMV strains for inoculation. The double antibody sandwich (Sanofi) technique was used for SMV detection, as described by Sohn et al. (2004). After the reaction, optical densities at 405 nm were measured with a microplate reader.

Treatments and inoculation

All inoculations were performed by equal amount of SMV strains after ELISA test. Treatments consisting of single, simultaneous, and sequential inoculations with G7H and/or G5 strains were replicated three times. The inoculation scheme is given in Table 2. Simultaneous inoculations were performed by inoculating one unifoliolate leaf of each plant with one strain and the other unifoliolate with a second strain, and sequential inoculations were performed by inoculating one unifoliolate leaf of each plant with one strain and the other unifoliolate with a second strain 24 h later. To investigate the interactions between virulence and dominance, the strain combinations included the four combination sets, G7H/G5, G7H/G1, G5/G1, and G7H/G5/G1. For the G7H/G5/G1 set, triple strain inoculations were performed by inoculating both unifoliolate leaves of each plant with equal volumes of G7H, G5, and G1 strains. Inoculum was produced by grinding the leaves from soybean plants infected with G7H, G5, and G1 in 0.01 M sodium phosphate buffer, pH 7.0, at a ratio of 1 : 10 (wt/vol), using a chilled mortar and pestle. Fully expanded primary leaves were inoculated 20 days after planting. Sap inoculum of 100 µl was applied to the unifoliate leaf. All inoculations were made by rubbing inoculums-soaked cotton swabs on Carborundum-dusted (600 mesh) leaves and were rinsed immediately with tap water after inoculation.

Sampling and SMV strain detection

To investigate the distribution of two SMV strains in soybean plants, one of the trifoliolate leaves was sampled from the leaves of each node at vegetative stages V2, V4, V6, V8, and V10 for Kwangankong; and at stages V2, V4, V6, and V8 for Tawonkong. Growth stage V8 leaves were sampled to test the relationship between virulence and dominance in three landraces. RT-PCR/RFLP analysis was used to detect SMV strains in sampled leaves as follows. Viral RNA extraction was performed using the SV RNA Isolation System (Promega, Madison, WI, USA). A set of primers, CI5' (5'-GCATTCAACTGTGCGCTTAAAGAAT-3') and CI3' (5'-TTGAGCTGCAAAAATTTACTCACTT-3'), that amplified a 1385-bp fragment were designed at positions 4176 to 5560, which included the cylindrical inclusion region (Kim et al., 2004). RT/PCR was conducted with an Access RT-PCR System (Promega, Madison, WI, USA). Thermocycling was performed in a PTC 200 (MJ Research, Waltham, MA, USA) and programmed as follows: one cycle of DNA synthesis at 48 ^{o}C for 45 min; one cycle of AMV RT inactivation and RNA/cDNA/primer denaturation at 94 ^{o}C for 2 min; 40 cycles of template denaturation at 94 ^{o}C for 30 s, primer annealing at 60 ^{o}C for 1 min, and extension at 68 ^{o}C for 2 min; and one cycle of final extension at 68 ^{o}C for 7 min. The products were electrophoresed on a 1.2% agarose gel. After precipitation of the PCR products with ethanol and 3 M sodium acetate and collection by centrifugation, the products were resuspended in nuclease-free water. A 12-µl volume of each product was digested with 2 µl of restriction enzyme (*Rsa*I 40 U/ul). The

Table 5. The effect of individual and combined infection with G7H and G5 on soybean growth.

Varieties	Treatment†	Plant height (cm)	No. of pods per plants	Pods containing seeds (%)			Weight of 100 seeds (g)
				1	2	3	
Tawon-kong	G7H (A)	24.3 c‡	13.3 bc	78.2 a	21.8 d	0.0 c	10.3 ab
	G5 (B)	31.5 ab	22.0 a	28.8 c	65.2 b	6.0 b	10.2 b
	A/B (sim.)	26.3 bc	17.3 ab	55.6 b	42.5 c	2.0 bc	10.0 b
	A/B (24 h)	26.0 bc	8.7 c	71.4 a	26.2 d	2.4 bc	10.4 ab
	B/A (24 h)	31.0 ab	20.3 a	39.4 c	58.3 b	2.4 bc	10.1 b
	Control	32.8 a	23.7 a	6.9 d	80.4 a	12.7 a	11.0 a
	LSD (5%)	5.68	6.38	13.44	12.98	5.64	0.82
Kwang-ankong	G7H (A)	45.3 b	28.7 bc	27.3 a	67.0 a	5.7 a	13.8 ab
	G5 (B)	43.3 b	22.0 d	29.0 a	57.3 a	13.7 a	12.9 bc
	A/B (sim.)	47.3 ab	31.0 abc	18.4 a	64.3 a	16.2 a	13.9 ab
	A/B (24 h)	46.3 ab	33.3 ab	14.9 a	68.0 a	14.1 a	14.0 ab
	B/A (24 h)	43.0 b	25.7 cd	19.3 a	66.7 a	14.1 a	11.7 c
	Control	50.0 a	34.3 a	12.0 a	69.8 a	18.2 a	14.8 a
	LSD (5%)	4.62	5.47	39.65	28.65	13.53	1.78

†G7H (A): Two primary leaves were inoculated with the G7H strain. G5 (B): Two primary leaves were inoculated with the G5 strain. A/B (sim.): Two primary leaves were simultaneously inoculated with G7H and G5 strains, by means of one strain per leaf. A/B (24 h): One primary leaf was inoculated with G7H and, 24 h later, with G5 on the other side. B/A (24 h): One primary leaf was inoculated with G5 and, 24 h later, with G7H on the other side. Control: Uninoculated, healthy plants. ‡† Mean separation within columns by least significant difference (LSD) at P = 0.05.

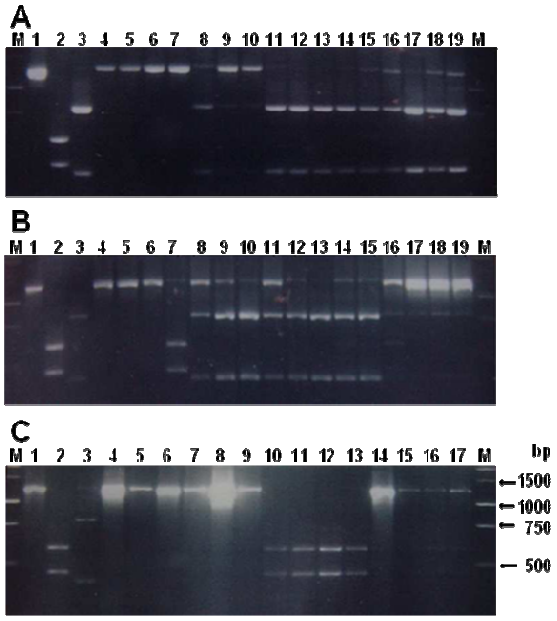

Fig 5. Electrophoresis of PCR products after *Rsa*I digestion. G7H has specific band profiles of 1226 and 146 bp; G5 of 586, 462, 178, and 159 bp; and G1 of 796, 430, and 159 bp. (A, B, and C) Lane 1, G7H; lane 2, G5; lane 3, G1; and M, molecular size marker (1-kb ladder). (A) NICS germplasm No. 793, and (B) NICS germplasm No. 640 (G7H, G5, and G1-susceptible): lanes 4, 5, 6, and 7, G7H and G5; lanes 8, 9, 10, and 11, G7H and G1; lanes 12, 13, 14, and 15, G5 and G1; lanes 16, 17, 18, and 19, G7H, G5 and G1; M, molecular size marker (1-kb ladder). (C) NICS germplasm No. 332 (G7H and G5-suscepetble, and G1-resistant): lanes 4, 5, 6, and 7, G7H and G5; lanes 8 and 9, G7H and G1; lanes 10, 11, 12, and 13, G5 and G1; lanes 14, 15, 16, and 17, G7H, G5, and G1.

digested fragments were analyzed on a 4% Nusieve 3:1 agarose gel (FMC) stained with ethidium bromide (EtBr) after digestion with *Rsa*I at 37 ^{o}C for 3 h.

Evaluation of agricultural traits

Each soybean plant was evaluated for agricultural traits (seed yield, plant height, number of pods per plants, pods containing seeds, and weight of 100 seeds). Plant height was measured at maturity as the average distance from soil surface to the apex of the main stem. When 95% of the pods on the main stem had reached their mature color, the number of pods per plant and pod containing seeds (number of one-, two-, and three-seeded pods) were recorded for each of the plants (Fehr et al., 1971). Mature seeds were harvested from infected and control plants to measure seed yield and the weight of 100 seeds. Seedling emergence and seed transmission were evaluated in the greenhouse by visually rating the percentage of the seeds that emerged, and by performing an ELISA test, respectively.

Data analyses

A randomized complete block design (RCBD) was used for agricultural traits. Each treatment was performed three times. Analyses of variance (ANOVA) for seed yield, plant height, number of pods per plants, pods containing seeds, weight of 100 seeds, seedling emergence and seed transmission were performed by SAS (SAS Institute Inc., 2003). Mean separation was done by calculating the least significant difference (LSD) at $P \leq 0.05$.

Acknowledgments

This work was supported by grants from the R&D project (PJ006681) of the National Institute of Crop Science as a Post-Doctoral Fellowship, and by a grant from the Next-Generation Biogreen 21 program (Plant Molecular Breeding Center No. PJ008060012011), Rural Development Administration, Republic of Korea.

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