Synergism Between Southern rice black-streaked dwarf virus and Rice ragged stunt virus Enhances Their Insect Vector Acquisition

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ABSTRACT

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Southern rice black-streaked dwarf virus (SRBSDV), a tentative species in the genus *Fijivirus*, family *Reoviridae*, is a novel rice virus transmitted by the white-backed planthopper (*Sogatella furcifera*). Since its discovery in 2001, SRBSDV has spread rapidly throughout eastern and southeastern Asia and caused large rice losses in China and Vietnam. *Rice ragged stunt virus* (RRSV) (genus *Oryzavirus*, family *Reoviridae*) is a common rice virus vectored by the brown planthopper (*Nilaparvata lugens*). RRSV is also widely distributed in eastern and southeastern Asia

but has not previously caused serious problems in China owing to its low incidence. With SRBSDV's spread, however, RRSV has become increasingly common in China, and is frequently found in co-infection with SRBSDV. In this study, we show that SRBSDV and RRSV interact synergistically, the first example of synergism between plant viruses in the family *Reoviridae*. Rice plants co-infected with both viruses displayed enhanced stunting, earlier symptoms, and higher virus titers compared with singly infected plants. Furthermore, white-backed and brown planthoppers acquired SRBSDV and RRSV, respectively, from co-infected plants at higher rates. We propose that increased RRSV incidence in Chinese fields is partly due to synergism between SRBSDV and RRSV.

A new rice dwarf disease caused by Southern rice black-streaked dwarf virus (SRBSDV), a recently proposed novel species in the genus *Fijivirus* (family *Reoviridae*), was first discovered in Yangxi County, Guangdong Province, China, in 2001 (28,32). Over the past few years, SRBSDV has rapidly spread throughout southern China and northern Vietnam, and has become one of the most important rice pathogens in these regions (7,33). In 2009, it was estimated that up to 315,000 ha of rice (*Oryza sativa*) growing in 9 Chinese provinces and >60,000 ha in 19 provinces of northern Vietnam were infected. In 2010, SRBSDV had spread to 13 provinces in southern China, 28 in northern Vietnam, and 1 in central Vietnam, with 1,601,600 ha of rice infected. In 2011 and 2012 the disease damaged >700,000 and 500,000 ha of rice, respectively, in the two countries (33). The disease has also been found in some areas of Japan (13). Rice plants infected with SRBSDV at the early seedling stage show severe stunting, twisting of leaf tips, and leaf darkening. Infection at later stages leads to small, streaked, black or white, waxy galls on stems; aerial rootlets; branches on stem nodes; and deficient grain weight (33). SRBSDV can be efficiently transmitted by the white-backed planthopper (WBPH) (*Sogatella furcifera*) in a persistent, circulative-propagative manner but not through egg to the progeny of the insect (17,32). In Southeast Asia, WBPH is a typical long-distance migratory rice pest overwintering in warm tropical or subtropical areas. In early spring, WBPH adults migrate from overwintering sites to China, Japan, and Korea, driven by monsoons. During autumn, when the monsoon direction shifts, WBPHs return to their overwintering locations. In the disease cycle described by Zhou et al. (33), viruliferous WBPH adults carry the virus from south to north via long-distance migration in early spring, transmit the virus to early rice in the newly colonized

areas, and lay eggs on the plants. Thus, upon hatching, WBPH population sizes and infection rates on early rice are magnified. The virus then spreads to early and late summer rice, leading to severe outbreaks of the viral disease.

Rice ragged stunt virus (RRSV) (genus *Oryzavirus*, family *Reoviridae*) was first described in 1976 and is distributed in eastern and southeastern Asia (6). Following its initial discovery in China in 1978, it has been distributed sporadically, with severe occurrences in some years and localized outbreaks in others. Rice plants infected with RRSV show stunting, leaf edge serration, darkened green coloring on leaves, twisting of leaf tips, and vein swelling or galls on undersides of leaf blades and outer surfaces of leaf sheaths. RRSV is vectored by the brown planthopper (BPH) (*Nilaparvata lugens*) in a persistent, circulative-propagative manner, without transovarial passage (6). The BPH is another long-distance migratory rice pest, whose overwintering places and migration pathways are similar to those of WBPH. Rice seedlings are susceptible to RRSV; therefore, the occurrence of the disease is more serious in overwintering sites of BPH such as southern Vietnam (15). However, in contrast to WBPH, BPH immigrate to China at a later growth stage of rice when plants have developed resistance to the virus. Because of its low incidence, RRSV does not constitute a threat to rice production in China (34). With the advent of SRBSDV, however, RRSV has become increasingly common in China over the last several years. Field surveys during 2011 to 2013 indicated that the rate of co-infection with SRBSDV and RRSV was >10% in dwarf rice samples from southern China while single infections with SRBSDV and RRSV were ≈70% and <2%, respectively.

Wang et al. (27) reported that SRBSDV alters host selection preference of insect vectors to enhance its spread, and also of insects vectoring RRSV to produce co-infection with that virus. In this study, we demonstrate that SRBSDV and RRSV interact synergistically. Compared with plants singly infected with SRBSDV or RRSV, we observed that co-infected rice plants presented aggravated symptoms, increased virus titers, and ele-

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vated vector acquisition efficiencies. Consequently, the outbreak of SRBSDV threatens rice production not only directly but also through synergism with RRSV, which enhances vector acquisition and promotes the spread of RRSV.

MATERIALS AND METHODS

Test plants, viruses, and insects. The rice cultivar used in this study (‘Qiuyou-998’) was purchased from the Guangdong Academy of Agricultural Sciences, Guangzhou, China. Water planting of rice was conducted according to Yoshida et al. (31). The seed were germinated and sown in a 2-liter beaker half-filled with culture solution (pH 4.5 to 5.0) (31), where they were maintained until the three- or four-leaf seedling stage. A number of seedlings of uniform size were selected and individually transferred into culture tubes (2.5 cm in diameter by 25 cm in height). The roots of each seedling were immersed in the culture solution (pH 4.5 to 5.0). The seedlings were cultured in an illuminated growth incubator (27°C; 16 h of light and 8 h of darkness).

SRBSDV and RRSV isolates were collected from infected field rice in Guangzhou, Guangdong Province, China, and were WBPH and BPH transmitted, respectively, and maintained on Qiuyou-998 rice plants grown in insect-proof greenhouses at our laboratory.

WBPHs and BPHs were collected from field rice plants in the suburb of Guangzhou, Guangdong Province, China and reared on healthy rice plants for three generations in an illumination incubator at 25 to 27°C. After the plants on which the plant-hoppers were reared were confirmed negative for SRBSDV and RRSV by RT-PCR, as described below, WBPH nymphs were transferred to SRBSDV-infected or healthy rice plants and BPH nymphs were reared on RRSV-infected or healthy rice plants. Some of the second-generation, late-stage WBPH or BPH nymphs reared on SRBSDV- or RRSV-infected rice plants were individually subjected to reverse-transcription polymerase chain reaction (RT-PCR) using One Step RNA PCR kit (AMV) (TaKaRa, Dalian, China) and the specific primers for SRBSDV (5'-CGCGTCATCTCAAACCTACAG-3' and 5'-TTTGTGACGATCTAAAGCGC-3') and RRSV (5'-CGCCGTATCTAACGTTCCAG-3' and 5'-TGCCGCGACATAATCAAC-3') (27). Viruliferous WBPH and BPH populations showing high amplification rates of viral genetic elements (92%, 46 of 50 for SRBSDV and 80%, 40 of 50 for RRSV) were used for inoculations.

Five late-stage WBPH or BPH nymphs reared on SRBSDV- or RRSV-infected rice plants for two generations were collected and transferred to individual virus-free rice seedlings at the three- to four-leaf stage cultured separately in a tube in an illuminated growth incubator (27°C; 16 h of light and 8 h of darkness). At least 30 rice seedlings were used for each inoculation. After a 24-h exposure, all insects were removed manually. Rice plants singly infected with SRBSDV or RRSV were obtained by inoculation with viruliferous WBPHs or BPHs, respectively, and plants doubly infected with SRBSDV and RRSV were obtained by inoculation with both viruliferous WBPHs and BPHs. A small amount of second-leaf tissue was collected at 9, 15, and 20 days after the 24-h insect exposure (days postinoculation [dpi]),

wrapped with tin foil, quick-frozen in liquid nitrogen, and stored in a freezer at -70°C. All rice plants were screened by RT-PCR at 20 dpi. Virus-positive samples were defined as diseased rice plants. In all, 20 to 30 plants with single or dual infection were used for virus quantification and acquisition experiments.

Observation of symptoms. At 9, 15, and 20 dpi, plant height (from the base of the stem to the tip of the second flat leaf) of each diseased rice plant was measured, and leaf tips were examined for twisting. Rice seedlings that had been inoculated with the same number of virus-free vectors (mock inoculated) served as healthy rice controls. At least six rice plants were examined per treatment.

SRBSDV and RRSV quantification by RT quantitative PCR. Total RNA was extracted from frozen leaves using Trizol RNAiso Plus (Invitrogen, Boston), and then treated with DNase I (TaKaRa). The purity and concentration of total RNA were determined by spectrophotometry. Total RNA samples with A260/A280 ratios of 1.9 to 2.1 and A260/A230 ratios greater than 2.0 were used as templates for RT quantitative PCR (RT-qPCR).

RT-qPCR primers (Table 1) were designed using Primer Express (version 3.0; Applied Biosystems, Foster City, CA) based on the S10 sequence of an SRBSDV Guangdong isolate and the S8 sequence of an RRSV Philippine isolate. Total RNA was reverse-transcribed into cDNA using a PrimeScript RT reagent kit (TaKaRa). The RT reaction mixture consisted of 2 µl of 5× RT buffer, 0.5 µl of RT Enzyme Mix I, 1 µl of RT primers (Table 1), 6 µl of RNase-free double-distilled (dd)H₂O, and 1 µl total RNA. Thermal cycling conditions for the RT were 42°C for 15 min followed by 95°C for 2 min. qPCR was then conducted on a Cyclar Dice Real Time System TP800 (TaKaRa) using an SYBR Premix Ex Taq II kit (TaKaRa). The qPCR mixture consisted of 12.5 µl of SYBR Premix Ex Taq II, 0.6 µl each of forward and reverse primers (10 µM), 2 µl of cDNA, and sufficient RNase-free ddH₂O to yield a final volume of 25 µl. Thermal cycling conditions were as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. The U6 small nuclear RNA gene of rice was used as a control for relative quantification (14), and RNase-free ddH₂O was used as a no-template control for all detections. At least six rice plants were used from each treatment, with reactions repeated three times per sample.

Vector virus acquisition efficiencies. Newly emerged virus-free WBPH or BPH adults were released on singly or doubly infected rice plants at 9, 15, and 20 dpi. After 24 h, all insects were transferred to healthy rice plants cultured in a 2-liter beaker, as described above. Seven days after the transfer, the WBPHs and BPHs were screened individually for SRBSDV and RRSV, respectively, by RT-PCR (27) and virus acquisition rates from the original singly or doubly infected rice plants were calculated. The experiment was repeated four or five times using different infected rice plants, and 48 or 60 WBPH or BPH adults were used to determine virus acquisition for every combination.

Statistical analysis. Experimental data were collected and analyzed using the statistical software package SPSS 19.0 (IBM, Armonk, NY). Plant height data were examined by repeated measures analysis of variance. Virus titers in rice plants at the same stage were compared using a *t* test with Bonferroni correction. Pearson’s product moment correlation was used to ex-

TABLE 1. Reverse-transcription quantitative polymerase chain reaction primers used for quantification of Southern rice black-streaked dwarf virus (SRBSDV) and *Rice ragged stunt virus* (RRSV)

Purpose	Target gene (GenBank accession number)	Primer name	Sequence (5'–3')	Size of expected amplicon (bp)
SRBSDV	S10 (EU784840)	SRBSDV CP-F	tcattcattagcgcgactagtcca	64
		SRBSDV CP-R	cgctactcggcgtcgataa	
RRSV	S8 (AF486811)	RRSV CP-F	ggctgagcgtgcggtta	66
		RRSV CP-R	tcagccttgatctgttagca	
Reference	U6 (S83742)	U6-F	cgataaaattggaacgatacaga	72
		U6-R	atttgaccattctcgattgt	

amine the relationship between virus titers and symptoms of dwarfing and leaf tip twisting in rice, and the relationship between virus titers in rice and vector virus acquisition efficiency.

RESULTS

Aggravated symptoms in plants with mixed infections. At 9 dpi, rice plants co-infected with SRBSDV and RRSV were, on average, 12.3% shorter in height than healthy plants. This difference was significant ($F = 3.75$, $df = 22$, $P = 0.026$), whereas no significant difference in average height was found between singly infected and healthy rice plants. At 15 dpi, average heights of rice plants infected with SRBSDV, RRSV, or both viruses were 38.8, 24.9, and 49.2% lower, respectively, than healthy plants, with the differences between the three types of infected plants and the controls being significant ($F = 103.829$, $df = 22$, $P < 0.0001$). Rice plants co-infected with SRBSDV and RRSV were, on average, 16.7 and 32.3% shorter, respectively, than rice plants singly infected with SRBSDV and RRSV, which was a significant difference ($F = 103.829$, $df = 22$, $P < 0.0001$). At 20 dpi, rice plants infected with SRBSDV, RRSV, or both viruses were 56.8, 44.3, and 67.2% shorter, respectively, than healthy rice; the differences between the three types of infected plants and the control were significant ($F = 290.06$, $df = 22$, $P < 0.0001$). Co-infected rice plants on average were 24.5 and 41.6% shorter than rice plants singly infected with SRBSDV and RRSV, respectively, with the differences between co-infected and singly infected rice being significant ($F = 290.06$, $df = 22$, $P < 0.0001$) (Fig. 1). These results demonstrate that rice plants with mixed SRBSDV and RRSV infection presented aggravated stunted growth. At 15 dpi, 50% of co-infected rice plants showed leaf tip twisting, whereas singly infected plants were symptom-free. At 20 dpi, 66.7, 50, and 75% of rice plants infected with SRBSDV, RRSV, or both viruses, respectively, had twisted leaf tips (Table 2). Thus, rice plants co-infected with SRBSDV and RRSV exhibited symptoms earlier than rice plants singly infected with these viruses.

Increased titer of the two viruses in co-infection. Detection of SRBSDV and RRSV was achieved by RT-qPCR with cycle threshold values of target and reference genes of 23 to 38 ($R^2 = 0.993$ to 0.999) and detection efficiency of 90.6 to 103.2% with a melting temperature (T_m) of $83 \pm 0.5^\circ\text{C}$. Single peaks were observed for all dissociation curves. Therefore, we used this

method to characterize SRBSDV and RRSV titers in rice plants. At 9 dpi, no significant difference in SRBSDV titers was found between rice plants singly infected and those co-infected with RRSV ($F = 5.328$, $df = 16$, $P = 0.211$) but RRSV titers in co-infected rice were 1.94 times higher than those in singly infected rice (Fig. 2). This difference was significant ($F = 5.114$, $df = 16$, $P = 0.02$). At 15 dpi, SRBSDV and RRSV titers in co-infected rice plants were both significantly higher than in rice plants infected with either SRBSDV (2.06 times higher; $F = 5.523$, $df = 22$, $P < 0.0001$) or RRSV (1.76 times higher; $F = 4.346$, $df = 26$, $P = 0.040$). At 20 dpi, SRBSDV titers in co-infected rice were significantly higher than those in singly infected rice (1.43 times; $F = 7.538$, $df = 26$, $P < 0.0001$), whereas no significant difference in RRSV titers was found between rice plants singly infected and those co-infected with SRBSDV ($F = 0.488$, $df = 28$, $P = 0.744$).

Virus titers in infected rice plants were negatively correlated with plant height. More specifically, a negative correlation was observed between plant height and SRBSDV titers in SRBSDV-infected rice ($r = 0.583$, $df = 29$, $P < 0.0001$), RRSV titers in RRSV-infected rice ($r = 0.664$, $df = 29$, $P < 0.0001$), and SRBSDV and RRSV titers in co-infected rice ($r = 0.704$, $df = 31$, $P < 0.0001$ for SRBSDV and $r = 0.670$, $df = 31$, $P < 0.0001$ for RRSV).

Increased virus acquisition efficiency of vectors due to co-infection. At 9 dpi, $26.7 \pm 5\%$ and $34.1 \pm 6\%$ of WBPHs acquired SRBSDV from SRBSDV-infected and co-infected rice, respectively, a nonsignificant difference ($F = 1.161$, $df = 8$, $P = 0.679$). In contrast, $26.0 \pm 3\%$ and $37.5 \pm 2\%$ of BPHs acquired RRSV from RRSV-infected and co-infected rice, respectively, which was a significant difference ($F = 1.159$, $df = 8$, $P = 0.014$). At 15 dpi,

TABLE 2. Observation of twisted leaf tip symptoms in rice plants infected with Southern rice black-streaked dwarf virus (SRBSDV) and *Rice ragged stunt virus* (RRSV)

Days after infection	Infected with ^a		
	SRBSDV	RRSV	SRBSDV+RRSV
9	0 (6)	0 (6)	0 (8)
15	0 (6)	0 (6)	4 (8)
20	4 (6)	3 (6)	6 (8)

^a Numbers in each column indicate the number of plants with a twisted leaf tip and numbers in parentheses indicate the number of plants observed.

Fig. 1. Height of rice seedlings infected with Southern rice black-streaked dwarf virus (SRBSDV), *Rice ragged stunt virus* (RRSV), and SRBSDV+RRSV; and healthy rice. Five viruliferous white-backed planthopper (WBPH) or brown planthopper (BPH) nymphs at the third to fourth instar were released onto individual rice plants at the three- to four-leaf stage for 24 h. After removal of WBPHs and BPHs, plant height was checked at 9, 15, and 20 day postinfection. Mock-treated rice plants (healthy rice) were used as controls. Different letters above vertical bars (with error bars indicating standard deviation) correspond to significant differences in plant height among differently infected rice plants or controls on the indicated day postinfection (Tukey's honestly significant difference test, $P = 0.05$).

SRBSDV acquisition efficiency of WBPHs fed on co-infected rice ($73.3 \pm 5\%$) was significantly higher than in those fed on SRBSDV-infected rice ($45.3 \pm 4\%$) ($F = 7.938$, $df = 8$, $P = 0.01$). RRSV acquisition efficiency of BPHs fed on co-infected rice ($74.3 \pm 5\%$) was also significantly higher than that of BPHs fed on RRSV-infected rice ($55.0 \pm 5\%$) ($F = 2.054$, $df = 8$, $P = 0.05$). At 20 dpi, SRBSDV acquisition efficiency of WBPHs fed on co-infected rice ($92.9 \pm 5\%$) was significantly higher than in those fed on SRBSDV-infected rice ($71.1 \pm 5\%$) ($F = 1.548$, $df = 8$, $P = 0.03$). RRSV acquisition efficiency of BPHs fed on co-infected rice ($95.3 \pm 3\%$) was also significantly higher than in BPHs fed on RRSV-infected rice ($80 \pm 7\%$) ($F = 0.01$, $df = 8$, $P = 0.05$) (Fig. 3).

Virus titers in plants were positively correlated with vector virus acquisition efficiency. SRBSDV acquisition efficiency of WBPHs was positively correlated with SRBSDV titers in SRBSDV-infected rice ($r = 0.783$, $df = 14$, $P = 0.001$) and co-infected rice ($r = 0.848$, $df = 14$, $P < 0.0001$), while RRSV acquisition efficiency of BPHs was positively correlated with RRSV titers in RRSV-infected rice ($r = 0.692$, $df = 14$, $P = 0.006$) and co-infected rice ($r = 0.746$, $df = 14$, $P = 0.003$).

DISCUSSION

Compared with plants infected by SRBSDV or RRSV, co-infected rice plants characterized in this study were more stunted (Fig. 1) and had slower growth (Table 1) but no new symptoms. This situation differs from that of RRSV and *Rice grassy stunt virus* (genus *Tenuivirus*, family unassigned) co-infection, which results in yellow syndrome (3); it is instead similar to co-infection

with *Panicum mosaic virus* (genus *Panicovirus*, family *Tombusviridae*) and its satellite virus that causes aggravated dwarfing, mosaic, and necrosis (12). SRBSDV and RRSV co-infection is obviously one of the reasons why virus-infected rice plants in some fields exhibit severe dwarfism and deficient grain weight (unpublished data). SRBSDV and RRSV titers in co-infected rice were higher than titers in SRBSDV or RRSV singly infected rice, suggesting that the patterns of synergism between SRBSDV and RRSV promote their replication. Patterns of synergism in which replication is mutually stimulated are frequently observed between virus congeners, such as in synergisms between *Wheat streak mosaic virus* (WSMV) (genus *Tritimovirus*, family *Potyviridae*) and *Maize chlorotic mottle virus* (MCMV) (genus *Machlomovirus*, family *Tombusviridae*) (9), and between *Cymbidium mosaic virus* (genus *Potexvirus*, family *Alphaflexiviridae*) and *Odontoglossum ringspot virus* (genus *Tobamovirus*, family *Virgaviridae*) (8). There have also been reports of viruses from different families enhancing one another's replication, such as in the synergism between WSMV and *Triticum mosaic virus* (TriMV) (genus *Poacevirus*, family *Potyviridae*) (24). To our knowledge, however, such synergistic behavior has not been previously reported between viruses in the family *Reoviridae*. SRBSDV and RRSV are members of the family *Reoviridae*; they both exist only in phloem cells (6,32) and, thus, likely have similar mechanisms for utilizing host components to replicate. Interaction between these two viruses would consequently promote, rather than inhibit, their replication. The mechanism associated with this interaction is worthy of further study.

We found that SRBSDV and RRSV titers in rice plants were positively correlated with the symptoms of dwarfing and leaf tip

Fig. 2. A, Southern rice black-streaked dwarf virus (SRBSDV) and B, *Rice ragged stunt virus* (RRSV) titers in infected rice. Virus titers were determined as the relative ratio of SRBSDV S10 or RRSV S8 gene expression to rice U6 gene expression at 9, 15, and 20 days postinfection. Asterisks indicate a significant difference between single infection by SRBSDV or RRSV and mixed infection by both viruses on the indicated day postinfection (t test with Bonferroni correction, $P < 0.05$).

Fig. 3. Virus acquisition rates of A, white-backed planthopper and B, brown planthopper from infected rice. Planthoppers were fed 24 h on rice plants infected with Southern rice black-streaked dwarf virus (SRBSDV), *Rice ragged stunt virus* (RRSV), and SRBSDV+RRSV, and detected individually by reverse-transcription polymerase chain reaction after they were transferred to healthy rice plants for 7 days for the virus incubation. Asterisks indicate a significant difference between the two columns for each time point (t test with Bonferroni correction, $P < 0.05$). Vertical bars represent a standard error value.

twisting. Matsukura et al. (13) demonstrated that SRBSDV accumulation in plants is correlated with the appearance of symptoms. Previous research has shown that plants co-infected with WSMV and MCMV present increased virus titers, more intense chlorosis, and higher density of chlorotic spots compared with singly infected plants (20). In addition, titers of *East African cassava mosaic virus* (genus *Begomovirus*, family *Geminiviridae*) and *African cassava mosaic virus* (genus *Begomovirus*, family *Geminiviridae*) in co-infected plants were, on average, 2.2 and 5.7 times higher, respectively, than those in singly infected plants, with co-infection resulting in aggravated symptoms (5).

Rice plants co-infected with SRBSDV and RRSV exhibited symptoms earlier than singly infected plants, presumably because of synergistic interaction between both viruses promoting the transport of virus in phloem. Increasing experimental evidence and data exist showing that plant virus movement proteins (MPs) are functionally homologous, despite their lack of sequence homology. As a consequence, the MP of different species, and even different families, can complement and mediate the systematic transport and synergism of heterologous viruses (2,19, 23). The protein encoded by open reading frame 3 of *Groundnut rosette virus* (genus *Umbravirus*) represents a class of trans-acting long-distance movement factors that can facilitate trafficking of *Tobacco mosaic virus* (TMV) (genus *Tobamovirus*, family *Virgaviridae*) (19). The MPs of *Red clover mottle virus* (genus *Comovirus*, family *Secoviridae*) and TMV can complement one another and promote synergism between their viruses (25). P7-1 of SRBSDV and P6 of RRSV are recognized as MPs (10,30), and their sequence identity is 49.0%. Whether they complement one another remains to be determined.

RNA silencing serves as an antiviral defense system in response to virus challenge in plants. RNA silencing suppressor (RSS) is one of the crucial factors used by viruses to overcome host silencing and infect host plants (1,4). Studies have shown that viral synergism is often associated with the action of RSS proteins (16,18,26). The helper component proteinase of potyviruses has been shown to mediate synergism with viruses from eight other genera (16,21), while the 2b protein mediates synergism between *Cucumber mosaic virus* (genus *Cucumovirus*, family *Bromoviridae*) and *Turnip mosaic virus* (genus *Potyvirus*, family *Potyviridae*) (22). Protein P6s of SRBSDV and RRSV has been identified as the RSSs of these two viruses (11,30); their sequence homology is 46.9% but their possible role in the synergism between SRBSDV and RRSV requires further investigation.

SRBSDV-infected rice can attract RRSV-viruliferous BPHs, thus aggravating SRBSDV and RRSV co-infection (27). The green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), and the potato aphid, *Macrosiphum euphorbiae* (Hemiptera: Aphididae), both preferentially settle on plants co-infected with *Potato virus Y* (PVY) (genus *Potyvirus*, family *Potyviridae*) and *Potato leafroll virus* (PLRV) (genus *Polerovirus*, family *Luteoviridae*) rather than singly infected plants (21). Our results indicate that SRBSDV and RRSV co-infection increases virus acquisition efficiency by their respective vectors. In previous studies, WSMV and TriMV were transmitted by the wheat curl mites (*Aceria tosichella*) at higher efficiencies from co-infected plants than from singly infected plants (24). SRBSDV and RRSV mixed infection may promote higher transmission rates and accelerate epidemic of both viruses.

Increased virus acquisition by insect vectors from co-infected plants may not only be due to the increase in virus titers in plants but may also be the result of interaction between the two viruses. Studies have shown that SRBSDV titers in rice are positively correlated with WBPH virus acquisition efficiency (13). In this study, virus acquisition efficiencies of WBPHs and BPHs were positively correlated during most infection stages with SRBSDV and RRSV titers in rice plants. At 20 dpi, however, no significant difference was found between RRSV titers from singly infected

and co-infected rice, whereas RRSV acquisition efficiency of BPHs was significantly higher in co-infected plants. A similar example is that of *Nicotiana benthamiana* co-infected with *Tomato chlorosis virus* (ToCV) (genus *Crinivirus*, family *Closteroviridae*) and *Tomato infectious chlorosis virus* (genus *Crinivirus*, family *Closteroviridae*) (29), where ToCV titers decreased during co-infection compared with single infection but little change was observed in the efficiency of ToCV acquisition by the banded wing whitefly (*Trialeurodes abutilonea*). The authors of that study suggested that virus concentration in plants was not the only factor impacting vector acquisition efficiency, and that acquisition efficiency may possibly also be associated with the interaction of viral proteins in the host. In PLRV and PVY co-infected plants, no significant increase in virus titers was observed (21) but virus acquisition efficiency by green peach aphids was significantly increased. The authors of that study speculated that co-infection elevated plant amino acids and soluble carbohydrate concentrations in plants, subsequently affecting vector biology. In our study, synergistic interaction between SRBSDV and RRSV increased the virus acquisition efficiency of their respective vectors, and could promote the SRBSDV outbreaks and RRSV epidemics. To confirm this hypothesis, the significance of SRBSDV and RRSV co-infection requires further investigation.

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