Short communication

p2 of *Rice stripe virus* (RSV) interacts with OsSGS3 and is a silencing suppressor

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SUMMARY

A rice cDNA library was screened by a galactosidase 4 (Gal4)-based yeast two-hybrid system with *Rice stripe virus* (RSV) p2 as bait. The results revealed that RSV p2 interacted with a rice protein exhibiting a high degree of identity with *Arabidopsis thaliana* suppressor of gene silencing 3 (AtSGS3). The interaction was confirmed by bimolecular fluorescence complementation assay. SGS3 has been shown to be involved in sense transgene-induced RNA silencing and in the biogenesis of *trans*-acting small interfering RNAs (ta-siRNAs), possibly functioning as a cofactor of RNA-dependent RNA polymerase 6 (RDR6). Given the intimate relationships between virus and RNA silencing, further experiments were conducted to show that p2 was a silencing suppressor. In addition, p2 enhanced the accumulation and pathogenicity of *Potato virus X* in *Nicotiana benthamiana*. Five genes that have been demonstrated to be targets of TAS3-derived ta-siRNAs were up-regulated in RSV-infected rice. The implications of these findings are discussed.

RNA silencing is a gene regulation mechanism common to virtually all eukaryotes. In RNA silencing, Dicer or Dicer-like ribonucleases (DCLs) recognize and cleave double-stranded RNAs (dsRNAs) into small interfering RNAs (siRNAs). Then, the siRNAs are recruited by an ARGONATE (AGO) protein to form an effector complex called the RNA-induced silencing complex (RISC), which can regulate gene expression at the transcriptional, post-transcriptional and translational levels using the incorporated siRNAs as guides (Carthew and Sontheimer, 2009). Many eukaryotes, including plants, have evolved to use RNA silencing as a defensive mechanism against viral infection (Ding, 2010; Ding and Voinnet, 2007). To counter this defence, viruses encode

specific proteins called silencing suppressors (VSRs; Ding and Voinnet, 2007; Li and Ding, 2006). The most common target of VSRs seems to be virus-derived siRNA (Ding and Voinnet, 2007; Li and Ding, 2006). As exemplified by p19 of Tombuviruses, many VSRs selectively bind siRNA duplexes. By doing this, they sequester virus-specific siRNAs (vsiRNAs) and prevent the formation of functional RISCs (Lakatos *et al.*, 2006). However, VSRs can also use protein components of RNA silencing as targets. For example, diverse VSRs have been shown to interfere with the function or reduce the accumulation of antiviral AGOs through physical interactions (for a review, see Wu *et al.*, 2010). Regardless of all of these possibilities, VSRs are believed to be important in viral accumulation, spread and pathogenicity (Díaz-Pendón and Ding, 2008).

In some instances, RNA silencing can be amplified in plants by cellular enzymes called RNA-dependent RNA polymerases (RDRs; Wassenegger and Krczal, 2006). In silencing amplification, RDRs use the targets of siRNAs as templates and convert them into dsRNAs, the cleavage of which by specific DCLs gives rise to secondary siRNAs (Wassenegger and Krczal, 2006). Although the mechanism by which RDRs recognize viral RNAs remains unclear, the importance of RDRs in plant antiviral silencing has been definitively demonstrated recently by two groups using VSR-deficient Turnip mosaic virus (TuMV) and Cucumber mosaic virus (CMV), respectively (García-Ruiz *et al.*, 2010; Wang *et al.*, 2010). Not surprisingly, VSRs can strongly or even selectively block secondary siRNA production (Csorba *et al.*, 2007; Mlotshwa *et al.*, 2008; Moissiard *et al.*, 2007; Shivaprasad *et al.*, 2008). However, the molecular mechanisms underlying the inhibition of secondary siRNA production by plant viruses remain elusive.

Rice stripe virus (RSV) is the type species of the genus *Tenuivirus*, which has not been assigned to any family. It infects rice and causes large yield reductions in rice production in some countries of East Asia. The genome of RSV comprises four RNAs, named RNA1–RNA4, in decreasing order of their molecular weight (Falk

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LBL1	NSGSGIRR.GGGP.....	PGSHS....GNETMGKKKKRPGQAG	34
AtSGS3	NSSRAGPMSEKENVQGGYRPEVEQLVQGLAGTRLASSQDGGGEWEVISKNNKPCNTISGKTWVSQNSNPPRAWGGCQCG		80
OsSGS3	MASAGIRRGGGP.....	PGSGDDSGGNETVEKRVKRFACQVG	39
Consensus	m	pg q g	
LBL1	RQWAPWSSTNVTPTNARPANGSGSSSHISGTSWAQAFDHGAATRGNFRPPQSISRFLAP.....	FLANGWQWQR	105
AtSGS3	RGSNVSGRGNNVSGRGNGRGIGICANISGRGRALSRKYDNNFVAPP.....	FVSRPPLEGGNWQARGGSAHTAV	151
OsSGS3	RGQWQWNSPNAAPAPTAPRS	SGAFHFSGNTLVHRSDRRPA.RGIFRPPQNRSIEAELQAPHGVVTAFLANGWQWGR	118
Consensus	g	q	
LBL1	PRPSCSEVKKDCAPFSGSVFEVENVDGNISDCCDDCIDLSDDISDDYISDASBKSFETPKTNWFKSFEVNTLS	2	185
AtSGS3	QEFPDVEDDDVDNASEEND.....	SDALDDSDIDLA...SDDYISDVSQSHGSKFKNWFKRFFGSLDSLS	217
OsSGS3	SCPPGTSKEGGPLSGDDPETDNAEGYDTS..	DDCIDDDSDISDDYISDASBKSFETPKTNWFKSFEVNTLS	196
Consensus	e s dd dcd sddycsd s ks rk k fk ff l ls e		
LBL1	QINEPQRQWHPACNCPGCAIDWYKGLPLVSHARTKGSIRVUKHRELAPILIEEISPRCISVIPGGEQFGHWKGLCEST		265
AtSGS3	QINEPQRQWHPACNCPGCAIDWYLNHPLLRHARTKCARVUKHRELAELKELMRCISVIPGGEYQGWKGLCEDE		296
OsSGS3	QINEPQRQWHPACNCPGCAIDWYKGLPLVSHARTKGSIRVUKHRELAPILIEEISPRCISVIPGGEQFGHWKGLCEST		276
Consensus	q ne r qwhcpac ncp galdwy pl ha tkg vk hrela le l rg sv p ge wkql e		
LBL1	DR.EIUVFFMVIVMNTLEKDEDDKHWGMGNQELLDYFEDHPSKARHAYGPGSHRGMSVILFSSAVGYMAERLHKHF		344
AtSGS3	KDYEIVVFFMVIVMNTLEKDDKHWGMGNQELLDYFEDHPSKARHAYGPGSHRGMSVILFSSAVGYMAERLHKHF		376
OsSGS3	DR.EIUVFFMVIVMNTLEKDEDDKHWGMGNQELLDYFEDHPSKARHAYGPGSHRGMSVILFSSAVGYMAERLHKHF		355
Consensus	eivvppmv wnt l d dkw gmgnel yf y a arh ygp qhrgmsvl f ssa gy eaerlh		
LBL1	VNQGTHRNNSHLR.KVFFVVGGRQLYGFLAKQDMENINKECHGSRIFEMRSYNEMVVICMKMSEDNQCINYLKNN		423
AtSGS3	AEMGTHRNNSHLR.KVFFVVGGRQLYGFLAKQDMENINKECHGSRIFEMRSYNEMVVICMKMSEDNQCINYLKNN		454
OsSGS3	VRQTHRNNSHLR.KVFFVVGGRQLYGFLAKQDMENINKECHGSRIFEMRSYNEMVVICMKMSEDNQCINYLKNN		435
Consensus	cr w gg rql ycf la k d fn h gk rlk e sy emv q sedncqlny knk		
LBL1	MVKIECRSKVDESLEVVQKRLSTYENIVRSKARHAYGPGSHRGMSVILFSSAVGYMAERLHKHF		503
AtSGS3	LSKQNFHAKVDESLEIMSEKRLSTYENIVRSKARHAYGPGSHRGMSVILFSSAVGYMAERLHKHF		534
OsSGS3	MVKIECRSKVDESLEVVQKRLSTYENIVRSKARHAYGPGSHRGMSVILFSSAVGYMAERLHKHF		515
Consensus	k k e l klr t en vr k h em f i ih ke fe l g er		
LBL1	SKARREDESIGIMKDRQLRKEYVQK..	FIDCVHDEVEFEERDGLIKHEERKLRKHEYMDELEKEFEPAITGL	580
AtSGS3	AKVVGCCQNNINPSSNDCKRAEEVSSFEIEGQREMBEFVBEREMLIKCEKMEIMKKRHHEEIFDLEKEFEPAITGL		614
OsSGS3	AKARQDESIGSGSTEDRRQRKEKIQN..	FIDCVHDEVEFEERDGLIKHEERKVRKLRKHEYLAKFELEKELDPAITGL	592
Consensus	k fi q k ef er lik e k kk leke d al l		
LBL1	MEKHKIDIFEASSS		594
AtSGS3	MAKHGHNEEDD		625
OsSGS3	MEKHKIDIFKSSISPS		608
Consensus	m kh		

Fig. 1 Alignment of rice and Arabidopsis suppressor of gene silencing 3 (OsSGS3 and AtSGS3) and maize LEAFBLADELESS1 (LBL1). Regions of identity and similarity are indicated by black and blue, respectively; gaps introduced for alignment are indicated by dots. The zinc finger XS domain, true XS domain and two coiled-coil domains are denoted by dark blue, red and green horizontal bars, respectively, under their sequences. Alignment was performed using the CLUSTALW algorithm.

and Tsai, 1998; Ramírez and Haenni, 1994). RNA1 is of negative sense and encodes a putative protein with a molecular weight of 337 kDa, which was considered to be part of the RDR associated with the RSV filamentous ribonucleoprotein (RNP) (Toriyama *et al.*, 1994). RNA2–4 are ambisense, each containing two open reading frames (ORFs), one in the 5' half of viral RNA (vRNA), the proteins they encode named p2–p4) and the other in the 5' half of the viral complementary RNA (vcRNA, the proteins they encode named pc2–pc4; Kakutani *et al.*, 1990, 1991; Takahashi *et al.*, 1993; Zhu *et al.*, 1991). Owing to its reluctance to traditional virological methods, such as infectious cloning, we know little about the functions of the seven proteins encoded by RSV at present. To obtain some insight into the functions of RSV-encoded proteins, we used the yeast two-hybrid system to investigate all the potential interactions between RSV-encoded proteins and host factors. In this article, we report our identification of the interaction between RSV p2 and the rice homologue of suppressor of gene silencing 3 (SGS3), a possible

cofactor of RDRs (Dalmay *et al.*, 2000; Mourrain *et al.*, 2000). On the basis of this finding, we carried out further experiments to identify p2 as a silencing suppressor of RSV.

A rice cDNA library was screened by a galactosidase 4 (Gal4)-based yeast two-hybrid system using RSV p2 as bait. A number of positive colonies were identified among the approximately 3.6×10^6 colonies screened. Sequencing and BLAST analysis showed that the cDNA insert of one of the positive colonies corresponded to a rice protein exhibiting a high degree of identity with *Arabidopsis thaliana* SGS3 (AtSGS3) (Fig. 1). The full-length ORF of the rice protein, designated OsSGS3 (*Os12g09580*), was then cloned into the vector pGBKT7, creating pGBKT7-OsSGS3 (for experimental procedures see Appendix S1). As shown in Fig. 2A, yeast cells co-transformed with pGBKT7-OsSGS3 and pGADT7-p2 grew on SD medium lacking adenine (Ade), histidine (His), leucine (Leu) and tryptophan (Trp) (SD/-Ade/-His/-Leu/-Trp), as did those co-transformed with pGADT7-T and pGBKT7-53, which were used as positive controls. By contrast, the yeast co-transformed with

Fig. 2 (A) Interaction between full-length rice suppressor of gene silencing 3 (OsSGS3) and p2: 1, pGADT7-p2/pGBKT7-OsSGS3; 2, pGADT7-p2/pGBKT7; 3, pGBKT7-OsSGS3/pGADT7; 4, pGADT7-T/pGBKT7-53 (positive control); 5, pGADT7-T/pGBKT7-Lam (negative control); 6, pGADT7/pGBKT7. (B) Interaction between three segments of OsSGS3 and p2: 1, pGADT7-OsSGS3-3/pGBKT7-p2; 2, pGADT7-OsSGS3-2/pGBKT7-p2; 3, pGADT7-OsSGS3-2/pGBKT7; 4, pGADT7-T/pGBKT7-Lam (negative control); 5, pGADT7-T/pGBKT7-53 (positive control); 6, pGADT7/pGBKT7; 7, pGADT7/pGBKT7-p2; 8, pGADT7-OsSGS3-1/pGBKT7-p2; 9, pGADT7-OsSGS3-1/pGBKT7; 10, pGADT7-OsSGS3-3/pGBKT7.

pGBKT7/pGADT7, pGBKT7-OsSGS3/pGADT7 or pGBKT7/pGADT7-p2 failed to grow on SD/-Ade/-His/-Leu/-Trp, although they grew well on SD medium lacking His and Trp (Fig. 2A and data not shown).

A bimolecular fluorescence complementation (BiFC) assay was carried out to confirm the interaction of p2 and OsSGS3 in living plant cells (Walter *et al.*, 2004). To do this, the full-length ORF of p2 was cloned into the vector pSPYCE and that of OsSGS3 into pSPYNE. Transformed *Agrobacterium tumefaciens* EHA105 carrying each of these constructs were mixed and infiltrated into leaves of *Nicotiana benthamiana*. Strong yellow fluorescent protein (YFP) fluorescence in *N. benthamiana* leaf epidermal cells was detected as early as 2 days post-infiltration (dpi) (Fig. 3A–C). Similar results were obtained when p2 was fused with the N-terminal fragment of YFP and OsSGS3 was fused with the C-terminal fragment of YFP. By contrast, fluorescence was not detected in leaf cells co-infiltrated with pSPYNE/pSPYCE, pSPYCE-p2/pSPYNE or pSPYCE/pSPYNE-OsSGS3 (data not shown). As shown in Fig. 3, the interaction between p2 and OsSGS3 occurred in both the cytoplasm and the nucleus. In the cytoplasm, the p2–OsSGS3 complex formed distinct granules, which was consistent with the cellular localization patterns of AtSGS3 and OsSGS3 (Kumakura *et al.*, 2009 and Fig. 3H).

Three deletion mutants of OsSGS3 were constructed: OsSGS3-1 (amino acid residues 1–250), OsSGS3-2 (amino acid residues 250–405) and OsSGS3-3 (amino acid residues 405–609). Each of the three deletion mutants contained one type of the three conserved protein domains present in OsSGS3: a zinc finger XS domain, a true XS domain and two coiled-coil domains, respectively (Fig. 1). Yeast two-hybrid experiments revealed that

all three OsSGS3 fragments could interact with RSV p2 (Fig. 2B). This indicated that there were multiple p2-interacting sites on OsSGS3.

AtSGS3 is a cofactor of RDR6 and has been implicated in antiviral silencing (Dalmay *et al.*, 2000; Fukunaga and Doudna, 2009; Glick *et al.*, 2008; Mourrain *et al.*, 2000; Muangsan *et al.*, 2004). BLAST analysis showed that OsSGS3 was the rice protein that exhibited the highest homology to AtSGS3. Given the intimate relationships between virus and RNA silencing, we speculated that RSV p2 might be a silencing suppressor (Ding, 2010; Ding and Voinnet, 2007). The finding that there were multiple p2-interacting sites on OsSGS3 and the fact that RSV can infect *N. benthamiana* encouraged us to test this possibility using a popular method for VSR identification: *Agrobacterium* infiltration assay. To this end, p2 and Δ p2 were cloned into the binary vector pPZP212 and the resulting plasmids, which were named 35S-p2 and 35S- Δ p2, respectively, were introduced into *Agrobacterium*. In Δ p2, the nucleotide A from the translation start codon AUG was deleted. Each of the bacterial strains was mixed with a strain containing the green fluorescent protein (GFP) transgene (35S-GFP) at the ratio of 3:1 and then co-infiltrated into leaves of *N. benthamiana* line 16c as described previously (Hamilton *et al.*, 2002). In 21 of the 24 leaf patches infiltrated with 35S-GFP plus 35S-p2, obvious fluorescence persisted for at least 5 days, although the fluorescence intensity was much weaker in these leaf patches than in those co-infiltrated with 35S-GFP plus 35S-2b, which were used as positive controls, at 5 dpi (Fig. 4, left). By contrast, in leaf patches infiltrated with 35S-GFP plus 35S- Δ p2 or 35S-GFP alone, strong fluorescence first appeared at 2 dpi, but decreased at 3 dpi, and became

Fig. 3 Bimolecular fluorescence complementation (BiFC) assay showing the interaction between p2 and rice suppressor of gene silencing 3 (OsSGS3) and the cellular localization of OsSGS3. Leaves of *Nicotiana benthamiana* were infiltrated by *Agrobacterium tumefaciens* EHA105 harbouring pSPYCE-p2/pSPYNE-OsSGS3 (A–C), pSPYCE-p2 (D, G), pSPYNE-OsSGS3 (E, H) or OsSGS3-YFP (F, I). (A, D, E, H) Yellow fluorescent protein (YFP) fluorescence. (B, F, G, I) Bright field. (C) Overlay of (A) and (B).

Fig. 4 p2 is a silencing suppressor of *Rice stripe virus* (RSV). Left: *Nicotiana benthamiana* line 16c plants were co-infiltrated with *Agrobacterium* mixtures carrying 35S-green fluorescent protein (35S-GFP) and the individual constructs indicated in each image. GFP fluorescence was viewed under long-wavelength UV light at 5 days post-infiltration (dpi). Right: Northern blot analysis of the steady-state levels of GFP mRNA and small interfering RNA (siRNA) extracted from the different infiltrated patches shown in (A). 28S rRNA and tRNA were used as loading controls for the detection of GFP mRNA and GFP siRNA, respectively.

hardly detectable at 5 dpi (Fig. 4, left). These data indicate that p2 may be a silencing suppressor. To confirm this, Northern blotting was conducted to detect steady-state levels of GFP mRNA and GFP-specific siRNAs in infiltrated leaf patches at 5 dpi. GFP mRNA was readily detectable in leaf patches infiltrated with 35S-GFP plus 35S-p2, as well as those infiltrated with 35S-GFP plus 35S-2b. However, the accumulation levels of GFP mRNA in leaf patches infiltrated with 35S-GFP plus 35S- Δ p2 or 35S-GFP alone were very low or hardly detectable. In each treatment, the concentration of GFP-specific siRNAs was inversely correlated with that of GFP mRNA (Fig. 4, right).

Many VSRs have been shown to be able to enhance the virulence of heterologous viruses (for an example, see Xiong *et al.*, 2009). To test whether p2 had such an ability, we cloned p2 and Δ p2 into a *Potato virus X* (PVX) vector, creating PVX-p2 and PVX- Δ p2, respectively. Symptoms on newly emerged leaves of *N. benthamiana* infected with these PVX vectors were first observed at 6 dpi. At this time, no symptom differences were observed between plants inoculated with PVX, PVX-p2 or PVX- Δ p2 (data not shown). However, plants infected with PVX-p2 developed more severe symptoms at 9 dpi, which were mani-

Fig. 5 (A) p2 enhances the pathogenicity of chimeric *Potato virus X* (PVX). (B) RNA gel blot analysis of the accumulation of PVX genomic (gRNA) and subgenomic (sgRNA1 to sgRNA3) mRNAs at 9 and 22 days post-infiltration (dpi). The bottom gel shows rRNA with ethidium bromide staining as a loading control.

festated as more severe mosaics and curling of the leaves (Fig. 5A). At later times, the symptoms on the upper leaves of plants inoculated with PVX or PVX-Δp2 became very light or hardly detectable. However, the symptoms caused by PVX-p2 were sustained throughout the life of the plants (Fig. 5A). Northern blot analysis revealed that the accumulation levels of PVX RNA were much higher in plants infected with PVX-p2 than in those infected with PVX or PVX-Δp2 at 22 dpi (Fig. 5B). Thus, p2 enhanced the accumulation and pathogenicity of PVX, possibly as a result of a synergistic reaction.

It should be mentioned that, in a previous study identifying p3 of RSV as a VSR, Xiong *et al.* (2009) reported that p2 had no silencing suppressor activity. Currently, we cannot explain this disparity. However, the most likely possibility is that the silencing suppressor activity of p2 is much weaker than that of p3. Moreover, in that study, equal amounts of *Agrobacterium* suspensions expressing p2 or a GFP transgene were used in the co-infiltration assay.

In addition to antiviral defence, SGS3 is also involved in the RDR6-mediated biogenesis of *trans*-acting siRNAs (ta-siRNAs), which play an important role in plant development (Peragine *et al.*, 2004). In a primary attempt to explore the possible biological implications of the interaction between p2 and OsSGS3, we detected the expression patterns of five genes targeted by ta-siRNAs derived from *TAS3* (Liu *et al.*, 2007). All five genes encode auxin-responsive factors (ARFs). Four have been shown to be up-regulated in transgenic rice expressing *Rice yellow mottle virus* P1, possibly resulting from the reduced accumulation of *TAS3*-derived ta-siRNAs (Lacombe *et al.*, 2010). The results indicated that the overall accumulation levels of the five genes increased by 50–500% in RSV-infected rice (Fig. 6). In

Fig. 6 Quantitative reverse transcription-polymerase chain reaction (RT-PCR) of the expression of five putative target genes of rice *TAS3 trans-acting siRNA* (ta-siRNA) in *Rice stripe virus* (RSV)-infected rice. The determination of the expression levels of the assayed genes was carried out in triplicate and normalized according to the value of an 18S RNA. The expression of the wild-type was set as unity. Error bars indicate standard deviations. The y axis denotes the expression level of a particular gene in RSV-infected rice compared with that in healthy rice.

addition, the expression of OsSGS3 itself also increased. However, the expression of *SHL2* and *SHO2*, which are orthologues of Arabidopsis RDR6 and AGO7, respectively (Nagasaki *et al.*, 2007), was not altered (data not shown). The up-regulation of genes encoding ARFs might not be a general response of rice to RSV infection, because two other ARFs, *Os02g04810* and *06g48950*, showed decreased expression in RSV-infected rice.

Many viruses have been shown to encode more than one VSR (Fabozzi *et al.*, 2011; Lu *et al.*, 2004; Vanitharani *et al.*, 2004). RSV is a virus that can multiply both in its insect vectors and in its plant hosts. As mentioned above, p3 of RSV has been shown

to be a VSR (Xiong *et al.*, 2009). Most probably, p3 suppresses RNA silencing by targeting long and/or small dsRNAs (Shen *et al.*, 2010). In this study, we found that RSV p2 interacted with OsSGS3, and identified p2 as a silencing suppressor. We hypothesize that p2, which is a small protein having no RNA-binding activities, suppresses RNA silencing by inactivating the RDR–SGS3 pathway (Liang *et al.*, 2005). If this is true, it would be interesting to note that RSV has two VSRs, one targeting RNA, the most conserved element in RNA silencing across kingdoms, and the other targeting a protein involved in silencing amplification, which is specific to plants. In addition to the emerging notion that RDRs are important in antiviral RNA silencing, our finding is consistent with two recent reports regarding RSV (Qu, 2010; Shimizu *et al.*, 2011; Yan *et al.*, 2010). In rice expressing a p2-specific RNAi construct, the appearance of symptoms caused by RSV was significantly delayed, suggesting that the p2 protein might be necessary for the proliferation of RSV and might play a role at later stages of viral infection (Shimizu *et al.*, 2011). In the same report, the authors found that transgenic rice plants that harboured IR constructs specific for the gene for p3 only showed partial resistance to RSV, which was unexpected, to some extent, if p3 was the only VSR of RSV. In addition, Yan *et al.* (2010) found that the proportion of RSV-derived siRNA within the total siRNA reads from RSV-infected rice was very low. This can be partially explained by our hypothesis that RSV encodes a protein targeting silencing amplification. Interestingly, V2 of *Tomato yellow leaf curl geminivirus* (TYLCV) has been shown to interact with SGS3 of tomato (Glick *et al.*, 2008). This suggests that SGS3 may be a common target of diverse plant viruses.

The alteration of the ta-siRNA pathway has been reported in many plant–virus combinations and has been suggested to be involved in symptom development (Lacombe *et al.*, 2010; Meng *et al.*, 2008; Shivaprasad *et al.*, 2008). In this study, we found that the ta-siRNA pathway may be affected in RSV-infected rice. This provides indirect evidence suggesting that the ta-siRNA pathway is affected by RSV infection. Transgenic rice expressing RSV p2 is being produced in our laboratory to investigate whether p2 is attributable to the alteration and to investigate the role of p2 in pathogenicity.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Experimental procedures.

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