A THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

Comparative Transcriptome Analysis of Rice stripe virus-viruliferous and Non-viruliferous *Laodelphax striatellus*

벼 줄무늬잎마름병의 매개곤충인 RSV 보독 애멸구와 비보독 애멸구의 전사체 비교 분석

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ABSTRACT

Rice stripe virus (RSV) is one of the serious plant pathogenic viruses for rice transmitted by small brown planthopper, SBPH, *Laodalphax striatellus*. So far, the studies have been mainly focused on the interaction between the host plant and the virus. In this study, for better comprehension of the interactions among Rice stripe virus, rice and small brown planthopper, the transcriptomes of the RSV-viruliferous (RVLS) and non-viruliferous *L. striatellus* (NVLS) were comparatively analyzed. For this, NVLS were collected from non-infected rice fields were fed with RSV-infected rice for 5 days. With the RNAs extracted from the RVLS and the NVLS, we conducted Illumina RNA sequencing (Hiseq 2000) and as a result two transcriptome databases were constructed

from the each sample, respectively. The transcriptome of RVLS and NVLS were compared to figure out how the gene expression of the insects was affected by Rice Stripe Virus. The RSV-dependently regulated genes analyzed from this study may have important functions in the transmission and replication of RSV.

RNA interference (RNAi) is an universal gene-knockdown mechanism in eukaryotic organisms which includes insects, and has been considered as an alternative strategy to control insect pests. Hence, we applied this technique to interfere the translation of target RNA genes to knockdown the virus gene on RVLS. Three out of seven RSV genes, RdRp, NS3, and NCP were used as target genes and each dsRNA targeting the viral genes were delivered to the insects indirectly through the rice leaves by irrigation. As a result, not only the relative expression level of target genes decreased but also those of non-target genes and the replication of RSV genome as well. In summary, leaf-mediated dsRNA feeding methods would be useful in the knockdown of target genes on piercing-sucking insects. The genes used in this experiment can be utilized for the development of pest-resistant transgenic plants based on RNAi.

Key words: Rice stripe virus, *Laodelphax striatellus*, Transcriptome, RNA interference, feeding RNAi

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1.

Comparative transcriptome analysis of Rice Stripe Virus Viruliferous and Non-Viruliferous Small Brown Planthopper, *Laodelphax striatellus*

CHAPTER 2.

RNA silencing by virus-derived small interfering RNAs

LIST OF FIGURES

CHAPTER 1.

Comparative transcriptome analysis of Rice Stripe Virus Viruliferous and Non-Viruliferous Small Brown Planthopper, *Laodelphax striatellus*

CHAPTER 2.

RNA silencing by virus-derived small interfering RNAs

LITERATURE REVIEW

1. *Laodelphax striatellus*

The small brown planthopper, SBPH, *Laodelphax striatellus* Fallen (Hemiptera:Delphacidae) is one of the most important pests that causes virus disease to rice paddy fields by transmitting the plant-pathogenic virus. It is known to transmit over 80 host plants. For this reason, controlling the rice stripe virus is almost impossible since the virus can survive at many different hosts even after all the rice get harvested. SBPH is mainly found in the Northeast Asian countries such as China, Japan, Taiwan and Korea, and is considered as a most economic pest in the rice field.

The small brown plant hopper is dimorphic, with fully winged 'macropterous' and truncate-winged 'brachypterous' forms. The macropterous forms can migrate when the insect faces a lack of food or inconvenient circumstances. Once the SBPH settles down on the rice, they produce the next generation in which almost all female insects develop as brachypters and males as macropters. Adults usually mate on the day of emergence, and the females usually lay eggs from the day following mating. Brachypterous females lay 300 to 350 eggs, while macropterous females lay fewer eggs. Eggs hatch in about six to nine days. The newly hatched nymphs are cottony white, and turn purple brown within an hour; they feed on the plant sap. They undergo five instars to become adults.

The SBPH overwinters in fourth instar nymph in the soil, root of wheat, stubble, fallen leaves, weeds and other places. The length of the life cycle is variable; only one generation per year in northern countries while more generations up to five generations

have been reported in southern countries. They can overwinter in Korea and often come flying from abroad. The eggs hatch in about seven to ten days and then live as nymphs for 18 to 20 days. After the fifth instars they become adults and can live for 20 to 25 days. SBPH normally produce five generations per year. The first generation starts from the middle of March which has overwintered. The second generation is the most important developmental stage in a pest control since it moves out from wheat field to the early rice paddy field which is very susceptible to pathogens. This generation has reported the outbreaks of SBPH. The forth instar nymph of fifth generation begins to overwinter in November.

The body size of adult females and males are approximately 3.6-3.8 mm and 3.4-3.6 mm in length, respectively. The body of adult SBPH is black to dark-brown, and the coloration varies with season. The mesonotum is black to dark-brown, as well as the pterostigma on the fore wings, and the areas between the carinae of the frons are deep black. The head is lemon yellow, and most of the compound eyes are black but often red while a stemma is dark-reddish.

As a hemipteran insect, the insect have predominant features that allow the efficient virus transmission. The distinct feature of these insects is that they have piercing-sucking mouthparts which includes a needle-like stylet bundle compounding of two maxillary and two mandibular stylets. The two maxillary stylets are interlocked and form two canals. While the wider food canal takes up plant sap into the cibarium (the sucking pump), the esophagus, and the rest of the alimentary canal, the narrower salivary canal delivers saliva with the virus into the feeding puncture in the plant tissues. There are four described mechanisms of insect transmission of plant-pathogenic viruses; nonpersistent, persistent-

circulative, persistent-propagative, and semipersistent. While in nonpersistent transmission, insects can inoculate the virus into plants for only a few minutes after the acquisition and the insect loses the virus within a few minutes and upon molting. In persistent transmission, vector insects can inoculate the acquired virus for much longer periods from days to week, transmitting the virus after molting and often for their entire lifespan. The SBPH transmits the RSV in a persistent-propagative manner which means the viruses are often transmitted to the vector's progeny through the infection of the embryos or germ cells in the female insects. (Sylvester 1980)

2. Rice Stripe Virus

Rice stripe virus (RSV) is the type member of the tenuivirus group (Falk and Tsai 1998). The virus particles are circular filaments of 290, 510, 610, 840, or 2110 nm in length and 9 nm in width. Virus particles contain five strands of ssRNA of plus and minus polarities, coat protein, and RNA polymerase. RSV is serologically related to Maize stripe virus and Rice grassy stunt virus. Infected rice plants show chlorotic stripes or mottling and necrotic streaks on their leaves, and premature wilting. Chlorotic leaves are unfolded, and later droop and wilt. Infected plants produce relatively high concentrations of virus-specific proteins, which are serologically related to the similar protein produced in RGSV-infected plants. Infected rice cells contain large masses of granular or sandy structures in the cytoplasm and nuclei and masses of needlelike and paracrystalline structures in the cytoplasm and vacuole.

RSV naturally infects rice, wheat, barley, oat, foxtail millet, and some graminaceous

weeds (Hibino 1996). These cereals, with the exception of rice and winter weeds, are not good hosts of RSV and do not serve as virus sources for the next crop season. After the rice is harvested from September to October in central and southern Japan, infective *L. striatellus* moves to grass weeds and then to wheat and barley where it transmits the virus and oviposits. Generally, the virus incidence is low in rice fields planted very early in April, high in fields planted in May, and lower again in fields planted later. Rice plants infected with the virus at the early growth stage show severe symptoms and die prematurely. Plants exposed to infective *L. striatellus* at later growth stages had less infection and showed milder symptoms and no wilting. In northern Japan (Hokkaido), RSV-infected adults of the overwintered generation appear from May to June, move to newly planted rice seedlings, and transmit the virus(Yamamura and Yokozawa 2002). RSV occurs in China, Japan, Korea, Siberia, and Taiwan. In Japan, the incidence of RSV was very high from 1960–1972, and increased again from 1977–1986(Kiritani, Plumb, and Thresh 1983). In Korea, it was high during 1964–1965 and 1973–1974 (Chong TS et al. 1975).

RSV is a single stranded RNA virus and contains four RNA segments. The genome of RSV consists of seven open reading frames (ORFs). The complementary sense of RNA1 contains a single ORF that encodes a protein of 337 kDa. Analysis of amino acid sequence of the 337 kDa protein suggested that the protein may function as an RNA-dependent RNA polymerase (RdRP).(Toriyama et al. 1994) RNAs 2, 3 and 4 are all ambisense, and each of which contains two ORFs, one ORF locates at the 5′ half of the sense viral RNA (vRNA) and the other ORF at the 5′ half of the viral complementary RNA (vcRNA). RNA2 encodes two proteins, NS2 (22.8 kDa) from the vRNA and NSvc2

(94 kDa) from the vcRNA. RNA3 encodes NS3 (23.9 kDa) from the vRNA and a nucleocapsid protein (35 kDa) from the vcRNA. RNA4 encodes a major non-structural SP protein (20.5 kDa) from the vRNA and NSvc4 (32 kDa) from the vcRNA(Kakutani et al. 1990, Toriyama et al. 1994, Takahashi et al. 1993, Zhu, Hayakawa, and Toriyama 1992) and NSvc4 had been proved as a movement protein(Xiong et al. 2008). Except the RdRp-like 337 kDa protein and NSvc4 protein, function(s) of the other RSV encoded proteins are yet to be determined.

3. RNA-Sequencing

Transcriptome is the complete set of messenger RNA (mRNA) and noncoding RNA transcripts produced by a particular cell or organism. Transcriptome analysis is a powerful strategy to connect genotype to phenotype of a cell. The phenotypic diversity has been linked to different cell activattion or repression of transcription (different sets of genes). To understand cellular transcriptome, total RNA from different organisms, tissues, or disease states for the presence and quantification of the interesting transcripts were performed by microarray technology, and sequencing-based approaches.

The first candidate gene-based studies used northern blot analysis which required the application of radioactivity and large amounts of input RNA(Alwine, Kemp, and Stark 1977). This complicated procedure and requirement for large amounts of RNA restricted northern blotting to the detection of a few known transcripts at a time from samples. With development of reverse transcription polymerase chain reaction (RT-PCR) methods, it is possible to facilitate transcription detection, increase the experimental throughput, and reduce the required quantity of input RNA(Pfaffl 2001).

Recently, the development of next generation sequencing (NGS) technologies provides a powerful alternative strategy for transcriptome analysis. The NGS technologies such as 454 GS FLX (Roche), Genome Analyzer (Illumina) and AB SOLiD (ABI) were developed to derive not only an accurate and quantitative measure of individual gene expression, but also to discover novel transcribed regions and the alternative splice events. On all three of the platforms, DNA fragments are sequenced through massively parallel RNA-sequencing approach, producing large numbers of relatively short reads of tags(Wang, Gerstein, and Snyder 2009). Read lengths range from 30-100bp for Illumina GA and ABI SOLiD to 200-500 bp for GS FLX. Although these systems were significantly different in the approached used to produce massive amounts of sequences, these flatforms were similar to rely on the work flow for the production and analysis of sequencing libraries.

In sequencing reaction, NGS technologies exploit light emitted when the correct base matches the template being sequenced at every sequencing cycle. In each cycle, the flowcell is imaged in the number of non-overlapping regions. NGS technology has an impressive application in a wide variety of fields such as solving practical challenges in medicine, engineering, agriculture and ecology(Ansorge 2009).

4. **RNA interference**

Nearly 10 years ago, Fire et al.(Fire et al. 1998) described a process in which the application of exogenous dsRNA silenced the homolog endogenous mRNA in the *Caenorhabditis elegans* and called RNA interference (RNAi). Although new for animals, the technique was already described as ‘post-transcriptional gene silencing’ in plants and

as ‘quelling’ in fungi. Moreover, those three techniques appeared to be remarkably well conserved in several eukaryotes(Fire 2007). RNAi technique soon proved to be promising in several research fields: in medicine to control cancers and viral disease amd in genomics for gene function determination and gene knockdown in eukaryotes.

In biotechnology, it shows great potential as its high specificity and might therefore serves as a new specific method to control pests in agriculture. In this branch, this new techniques are very welcome due to the continuous threat of resistance development against current insect control products and techniques. This relatively new technique of RNAi is already regularly applied in the field of entomology to study the RNA interference mechanism and the function, regulation and expression of gene cascades, mostly in *Drosophila melanogaster(*Bischoff et al. 2006, Miller, Brown, and Tomoyasu 2008), *Tribolium castaneum* (Minakuchi, Namiki, and Shinoda 2009) and *Bombyx mori(*Quan, Kanda, and Tamura 2002, Hossain et al. 2008).

However, most of these experiments have been conducted through experiments in which dsRNA is delivered by injection directly into the organism, which is difficult to apply to control insect pests in the field. For efficient insect control, the organism should be able to autonomously uptake the dsRNA, for example through feeding and digestion in the gut. The mechanism of cell-autonomous RNA interference is well known: dsRNA is cleaved by a RNase III, Dicer, into 21–25 nt-long short interfering RNA duplexes (siRNA). These siRNAs are incorporated in the RNA induced silencing complex (RISC); after abandoning the passenger strand, the RISC binds to a homolog mRNA, cutting it and thereby hindering translation.

A first approach to search for possible dsRNA uptake mechanisms in insects is to look for systems similar to those described already. Research with systemic RNAi defective mutants (sid), resulted in the description of two proteins involved in non-cell-autonomous RNAi. SID-1 is a multispan transmembrane protein essential for systemic RNAi. It functions as a multimer, transporting dsRNA into the *C. elegans* cells passively. However, it is not essential for the export of dsRNA from the cell. (Winston et al. 2007)

Another intriguing fact of this 'alternative' endocytosis based uptake mechanism is the involvement of the pattern recognition scavenger receptors, which are well known to have roles in ancestral innate immune responses(Ulvila et al. 2006). Moreover, the link between antiviral immunity and RNAi was demonstrated in *D. melanogaster* recently(Saleh et al. 2009). In this model, infected insect cells release viral dsRNA which is taken up by uninfected cells through the dsRNA uptake pathway. Subsequently, an antiviral RNAi response, limiting virus replication, is induced in those cells. This antiviral response was not present in mutants defective in genes involved in dsRNA uptake, although these mutants had a functional cell-autonomous RNAi machinery and an immune system. Furthermore, uninfected cells seem to lack a systemic RNAi pathway. Therefore, virus infection activates a virus specific intracellular immunity that prevents spreading of virus, a dsRNA uptake pathway and requiring RNAi core machinery. And, because of the observed large negative effect in the spreading deficient mutants, a spreading mechanism must be involved in this system. Thus, it was concluded that immunity in multicellular organisms requires both cell-autonomous and systemic mechanisms to create pre-existing immunity to protect uninfected cells(Saleh et al. 2009).

CHAPTER 1.

Comparative transcriptome analysis of Rice Stripe Virus Viruliferous and Non-Viruliferous Small Brown Planthopper, *Laodelphax striatellus*

Comparative transcriptome analysis of Rice Stripe Virus-Viruliferous and Non-Viruliferous Small Brown Planthopper, *Laodelphax striatellus*

1. Introduction

Rice stripe virus was found in Japan in 1931(Kuribayashi, 1931) for the first time and is the type species of the tenuivirus group which includes Maize stripe virus(Gingery, Nault, and Bradfute 1981), Rice grassy stunt virus(Hibino et al. 1985), Rice hoja blanca virus(Morales and Niessen 1983). RSV has a broad host range that includes rice, barley, wheat, rye, maize and so forth (Hibino 1996). RSV can be transmitted by *L. striatellus* in a persistent manner and can also be transmitted to the offspring by eggs transovarially at high rates(Toriyama 1986). The typical symptoms of RSV infected rice plants are chlorotic stripes or necrotic streaks on leaves with subsequent premature wilting afterwards. Rice stripe virus disease often causes significant reduction of yield in the field. Especially, *Oryza sativa* var. Japonica is susceptible to RSV(Falk & Tsai, 1998). For instance, there was a huge reduction on the field over half million hectares from 1963 to 1968 in Japan, consistently.(Toriyama, 1995) In Korea, since the RSV was first reported on 1935 around Nakdong river, the virus has been considered as a most important rice pathogen as Rice stripe virus disease has been consistently occurring in field. Rice stripe disease causes more than an estimated 600,000 ha of rice fields in Japan from 1963 to 1967 every year. Also, in Jiangsu province in China, there was a huge epidemic of this disease over 1,571,000 ha of rice fields, 80% of all rice paddy fields, which caused a reduction in seed yield of 30-40% (Wei et al. 2009).

In recent years, the next-generation high-throughput RNA- sequencing techniques have developed dramatically and improved the efficiency of discovering genes. (Ansorge 2009) Next-generation sequencing generates a huge amount of data and has been widely used not only in the study of model organisms(Filichkin et al. 2010, Graveley et al. 2011), humans(Pan et al. 2008), crops(Severin et al. 2010, Wei et al. 2011) but also in many agricultural pests(Xu et al. 2012). For instance, Graveley et al. transcriptome sequencing was conducted on 30 distinct developmental stages of *Drosophila melanogaster* and identified 111,195 functional elements. In another study, Xue et al. performed NGS to research about the different developmental stages of brown planthopper(BPH), *Nilaparvata lugens*, and obtained a large number of genes related to the sex differenciation and wing dimorphism.

So far, the researches about Rice stripe disease were only focused on the interaction between host plants and the plant-pathogenic virus. However, the interaction between the vector insects and the virus is still remained to be unknown even though the virus can replicate in a consistent manner and be transmitted by transovarially. In an effort to improve the situation, we conducted transcriptome sequencing by using Illumina Hiseq 2000 sequencing platform for the RVLS and NVLS to gain a better comprehension about the interaction between plant-pathogenic virus and vector insects. Approximately, 176,000 contigs were obtained from the transcriptome sequencing and then categorized in 23 functional groups to analyze relative expression of genes belonging to each group. With the result, we investigated the genes of *L. striatellus* affected by Rice stripe virus to figure out the interaction between the virus and the vector insect.

2. Materials and methods

2.1 *L. striatellus* rearing

The non-viruliferous small brown planthoppers were collected from the healthy rice (*Oriza sativa*) field and reared on uninfected rice in the insectary (25℃ ± 1℃, 55% ± 5% RH, 16:8 (L:D) photo period).

2.2 Inoculation of RSV to non-viruliferous *L. striatellus*

The non-viruliferous SBPH were fed on 5-6cm tall RSV-infected rice for 5 days in the insect growth chamber (28℃, 80% RH, 16:8 (L:D) photo period).

2.3 Total RNA extraction and RT-PCR

Total RNA was extracted from RVLS and NVLS using Qiazol lysis reagent (Qiagen Ltd.,Crawley, UK) according to the manufacturer's instructions. 50 of forth instars were homogenized in 1 ml of Qiazol lysis reagent and add 0.2 ml of chloroform. After waiting 3 min, centrifuge the tube with homogenized sample at 12,000 × g. Following centrifugation, the supernatant was transferred to a new tube and added 0.5 ml isopropanol per 1ml Qiazol lysis reagent. The precipitated pellet was washed with 75% ethanol and resolved in nuclease-free water. And then 1μg of total RNA used for cDNA synthesis with oligo d(T) primers using AccuPower® RocketScript™ RT PreMix (BIONEER, Korea).

To confirm the RSV infection of small brown planthoppers that fed on RSV-infected rice leaves, RT-PCR was performed. The primers used in this PCR is described in Table 1.

2.4 RNA-sequencing and EST library construction

The total RNA extracted from RVLS and NVLS were used for RNA-seq analysis of transcriptome of small brown planthopper by using Illumina Hiseq 2000 system (MACROGEN, Korea).

The raw reads of RVLS and NVLS from RNA-sequencing were filtered respectively by using NGS QC toolkit v2.3 to remove low quality sequence reads (National Institute of Plant Genome Research, India). With the filtered reads, EST library of *L. striatellus* was constructed using Trinity software (Broad Institute and the Hebrew University of Jerusalem).

2.5 Gene annotation and Gene ontology analysis

The assembled-read sequences were then used for mapping with the reference mRNA sequence of model insects using Bowtie2 software(Langmead and Salzberg 2012), with default parameters. The result of mapping by Bowtie2 were then analyzed by eXpress software(Roberts and Pachter 2013) to be quantified.

Table 1. Oligonucleotide sequences used for RT-PCR analysis

Organism	Protein name	Amplicon size (bp)	Primer sequence
L. striatellus	Cytochrome C oxidase subunit II	502	5'- GCTCATAAAGCTGATGTTG-3' 5'-GTCTTCACTTTCCCATTG-3'
RSV	NS3	443	5'-CATTCATTCATCTAGGCACCC-3' 5'-AACATAACGTGCACTAAGAGGTG-3'

The assembled-contigs were subjected to eggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups) (Jensen et al. 2008) by using BLASTX with a cut-off value of 1e-05 to identify gene function to performing gene ontology analysis.

2.6 Validation of sequencing data by Real-time PCR

To validate the RNA-sequencing data with Hiseq 2000 system, gene expression levels of each transcriptome which measured by eXpress software were compared with those of qRT-PCR. qRT-PCR was conducted by using EvaGreen qPCR Mastermix(Applied Biological Materials Inc, Canada) and CFX96^{TM} Real-Time system (BIO-RAD, USA) according to the manufacturer's instructions. The progress of PCR amplification was as following: 95℃ for 10 min, (95℃ for 15 sec, 58℃ for 15 sec, and 72℃ for 30 sec) x 40 cycles. The ADP ribosylation factor (ARF) was used for the calculation of fold change as a reference gene. The relative transcription levels were calculated by using the $2^{-\Delta Ct}$ method. (Pfaffl 2001). The primers used for validation of NGS data is described in Table 2.

3. Results

3.1 Confirmation of RSV transmission to *L. striatellus*

The total RNA from 50 4th nymphs that fed on RSV-infected rice leaves was used to conduct RT-PCR for confirmation of RSV acquisition to the *L. striatellus*.(Figure.1) RT-PCR was performed with a set of primer, CCOS-F/CCOS-R that expected to yield 502bp , correspond to the nucleotide sequence of cytochrome c oxidase II of *L. striatellus* and with a set of primer, RSV-NS3-F/ RSV-NS3-R that expected to yield 443bp, correspond to the nucleotide sequence of NS3 protein that belongs to RSV. As a result, RSV gene was detected from the total RNA of the *L. striatellus* that fed on RSV-infected rice leaves and not detected from the *L. striatellus* that fed on healthy rice leaves that weren't infected by RSV.(Fig. 1.)

3.2 RNA-sequencing data of combined transcriptome from RVLS and NVLS

As described above, total RNA from RVLS and NVLS were subjected to conduct Illumina Hiseq 2000 RNA-sequencing and obtained 175,243,488 and 146,031,348 reads, respectively. The reads from two samples were then filetered to be removed from low quality sequences and combined. The information of combined sample is described in Table 3.

Table 2. Oligonucleotide sequences used for quantitative real-time PCR

Target gene	Primer name	Oligonucleotide sequences
Serine-Type endopeptidase inhibitor	q85664-fw	TCGTCGTGGTTGTGGAAGTA
	q85664-re	AGTGTATCTGCCCACCATCC
Immunoglobulin C-2 Type	q67808-fw	CAATGGCATATTGGTTATCCG
	q67808-re	GATGGGAAACGACACAATGA
Protein involved in defense response	q76227-fw	GTGGTCAGTTTGGTGTCGTG
	q76227-re	ATGAGAGCCCTGTCAAGAGC
Protein involved in cellular iron ion homeostasis	q86923-fw	AACATGCGTCGAAGTTGTTG
	q86923-re	GCTGCAGACATAGCCTCCTC
Protein involved in innate immune response	q84874-fw	TGCTGCTGTTGGTTTACGAG
	q84874-re	AATCGGCCCAGTAGTGTTTG
Heat shock protein 1	q47305-fw	TTGGCAATGATCTCACATCG
	q47305-re	CCTGATCACTTGCACACCAT
Heat shock protein 2	q71299-fw	TGTCAAACAAGCTCTTGACGA
	q71299-re	TGTGTTGTTGTCCAGCCATC
Heat shock protein 3	q78742-fw	AGGACGAGAAGCAGAAGCAG
	q78742-re	CGGAGATCTTGTCCTTGAGC
Ribosomal protein	q66887-fw	CAGGGTGCTCTTGGTCTTGT

	q66887-re	ATCAGGAAGCATTTGGAACG
Translational initiation factor 1	q75410-fw	AAGTCCAGCAGATGTTTCGG
	q75410-re	CCACCGCAAGTAGTCCGTAT
Translational initiation factor 2	q80038-fw	GACCACGTTATCGGCAAGAT
	q80038-re	TCGAGTAACTGCCGTCCTCT
Inorganic ion transport and metabolism	q80964-fw	TATGGTGGTGATGACATGGG
	q80964-re	CCGAGAACCGCAGTACAAAT

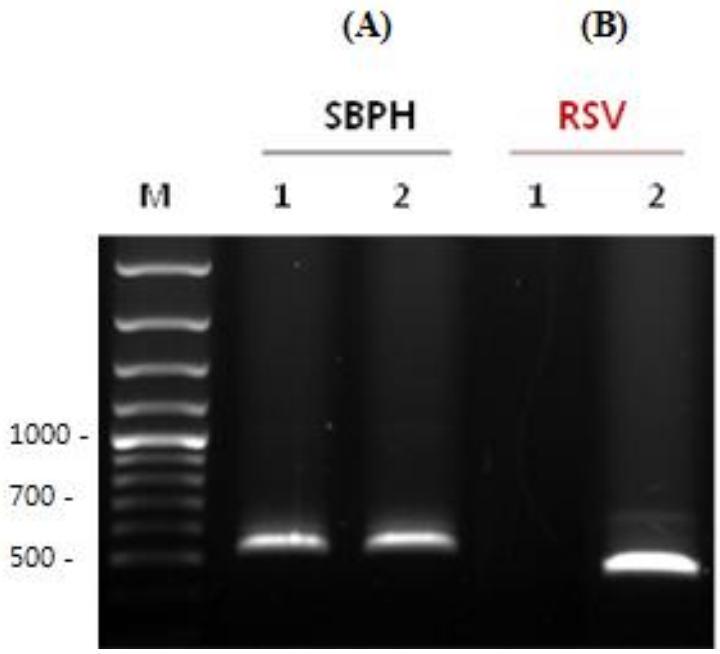

Fig. 1. RT-PCR analysis of total RNA from RSV viruliferous & non-viruliferous *L. striatellus* with specific primer sets. *L. striatellus* specific primers (A), RSV specific primers (B). Lane: M, 100bp DNA ladder; 1, Non-viruliferous *L. striatellus* ; 2, *L. striatellus* that fed on RSV-infected rice leaves for 5 days.

3.3 Gene annotation

With the filtered-combined NGS data, gene annotation analysis was performed by comparing the data with eggNOG database by using BLASTX. As a result, the filtered assembled reads were categorized in twenty three functional groups as described in Fig. 2. The categories were classified as [Q] Secondary metabolites biosynthesis, transport and catabolism, [P] Inorganic ion transport and metabolism, [I] Lipid transport and

metabolism, [H] Coenzyme transport and metabolism, [F] Nucleotide transport and metabolism, [E] Amino acid transport and metabolism, [G] Carbohydrate transport and metabolism, [C] Energy production and conversion, [O] Posttranslational modification, protein turnover, chaperones, [U] Intracellular trafficking, secretion, and vesicular transport, [W] Extracellular structures, [Z] Cytoskeleton, [N] Cell motility, [M] Cell wall/membrane/envelope biogenesis, [T] Signal transduction mechanisms, [V] Defense mechanisms, [Y] Nuclear structure, [D] Cell cycle control, cell division, chromosome partitioning, [B] Chromatin structure and dynamics, [L] Replication, recombination and repair, [K] Transcription, [A] RNA processing and modification, [J] Translation, ribosomal structure and biogenesis. From the left, [Q]-[C] are the categories related to metabolism, [O]-[D] are the categories related to cellular processes and signaling, [B]-[J] are the categories related to information storage and processing according to eggNOG's database.

Table 3. General features of combined transcriptome from RSV-viruliferous and non-viruliferous *L. striatellus*

	Combined sample
Total number of bases (bp)	32,448,758,436
Total number of reads	321,274,836
GC %	48.73 %
Average reads length (bp)	101
Total number of HQ filtered reads	277,124,968
Percentage of HQ filtered reads	86.26 %
Total number of contigs	176,194
Average contig length (bp)	856
Range of contig length (bp)	201~24,324
N25 of contigs	3,158
N50 of contigs	1,675
N75 of contigs	637

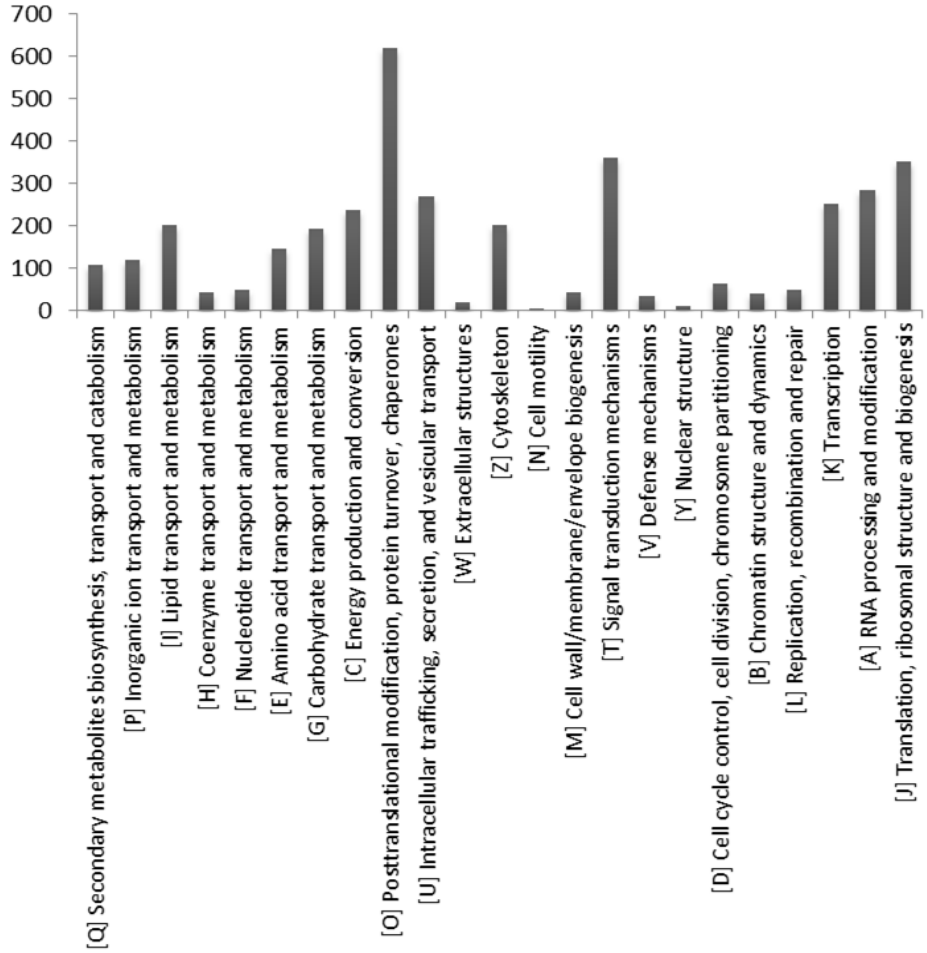

Fig. 2. Functional categories defined by eggNOG's database.

3.4 Comparative transcriptome analysis of RVLS and NVLS

Gene Ontology analysis was conducted with the functionally categorized contigs to investigate differentially expressed contigs in the non-viruliferous and RSV-viruliferous *Laodelphax striatellus*.(Table 4.) As described in Table 3, genes over two-fold differentially expressed in RVLS transcriptome are eight hundreds and eighty five that

were relatively up-regulated in RVLS compared to those of NVLS. And the genes over two-fold down-regulated in RVLS compared to those of NVLS are sixty-nine.

Table 4. **The differentially expressed genes over two folds**.

NVL; Non-viruliferous *L. striatellus*, **RVL**; RSV-viruliferous *L. striatellus*. Among the functional categorized genes, two-fold differentially over-expressed RSV-viruliferous genes were counted.

.	The simplified functional groups	NVLS > RVLS over 2 fold	RVLS > NVLS over 2 fold
METABOLISM	[Q] Secondary metabolites biosynthesis, transport and catabolism	2	50
	[P] Inorganic ion transport and metabolism	0	43
	[I] Lipid transport and metabolism	13	66
	[H] Coenzyme transport and metabolism	0	17
	[F] Nucleotide transport and metabolism	1	10
	[E] Amino acid transport and metabolism	3	54
	[G] Carbohydrate transport and metabolism	3	64
CELLULAR PROCESSES AND SIGNALING	[C] Energy production and conversion	3	72
	[O] Posttranslational modification, protein turnover, chaperones	8	142
	[U] Intracellular trafficking, secretion, and vesicular transport	1	32
	[W] Extracellular structures	0	4
	[Z] Cytoskeleton	3	79
	[N] Cell motility	0	0
	[M] Cell wall/membrane/envelope biogenesis	0	20
	[T] Signal transduction mechanisms	4	104
	[V] Defense mechanisms	1	4
	[Y] Nuclear structure	0	2
	[D] Cell cycle control, cell division, chromosome partitioning	3	5

INFORMATION STORAGE AND PROCESSING	[B] Chromatin structure and dynamics	8	8
	[L] Replication, recombination and repair	1	1
	[K] Transcription	2	45
	[A] RNA processing and modification	9	33
	[J] Translation, ribosomal structure and biogenesis	4	30
		69	885

With this two-fold differentially expressed contigs from both transcriptome, we investigate the genes uniquely differentially expressed compared to the tendency of all the two-fold differentially expressed contigs. The genes belong to three categorized in metabolism groups, [Q] Secondary metabolites biosynthesis, transport and catabolism, [P] Inorganic ion transport and metabolism, [I] Lipid transport and metabolism, and the genes belong to [Z] Cytoskeleton of cellular processes and signaling group were revealed to be expressed strangely among those of the two-fold differentially expressed genes.

Furthermore, the relative translation level of contigs encoding ribosomal proteins were analyzed to investigate the change after Rice stripe virus infection and the ribosomal proteins from the transcriptome from RVLS was somewhat up-regulated compared to those of NVLS.(Fig. 3.)

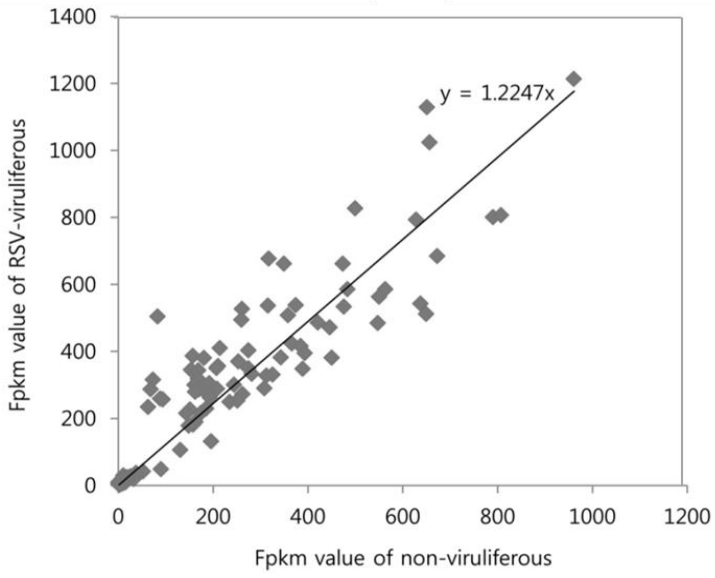

Fig. 3. Graph of the number of reads from contigs encoding ribosomal proteins.

Genes encoding ribosomal proteins were mainly up-regulated in viruliferous *L. striatellus*

3.5 Validation of sequencing data by qPCR

The real-time quantitative PCR, qPCR, was conducted to validate the transcriptome sequencing data by comparing the relative translation level of RNA-seq data and those of qPCR data. Twelve contigs were selected and comparatively analyzed based on the expression level of each gene. The calculation of the relative transcription levels of the isotigs using $2^{-\Delta Ct}$ method (Pfaffl 2001) demonstrated that the ten genes (Serine-Type endopeptidase inhibitor, Immunoglobulin C-2 Type, Protein involved in defense response, Protein involved cellular iron ion homeostasis, Protein involved in innate immune response, three Heat shock proteins, Inorganic ion transport and metabolism) and the other two genes (two translation initiation factors) were up- and down- regulated in

RVLS, respectively.(Fig. 4.) The expression levels were analyzed by FPKM value (expected fragments per kilobase of transcript per million fragments sequenced) by using eXpress software.

3.6 Expression profiling of RSV genes in RVLS

Relative expression levels of Rice stripe virus genes were investigated (Table 4.) and five out of seven RSV genes were detected from RVLS transcriptome. As a result, coat protein which belongs to RNA segment 3 was the most abundantly transcribed gene among seven RSV genes, and the putative RNA-dependent RNA polymerase protein was the lowest transcribed gene among the five genes that were detected by RNA-sequencing.

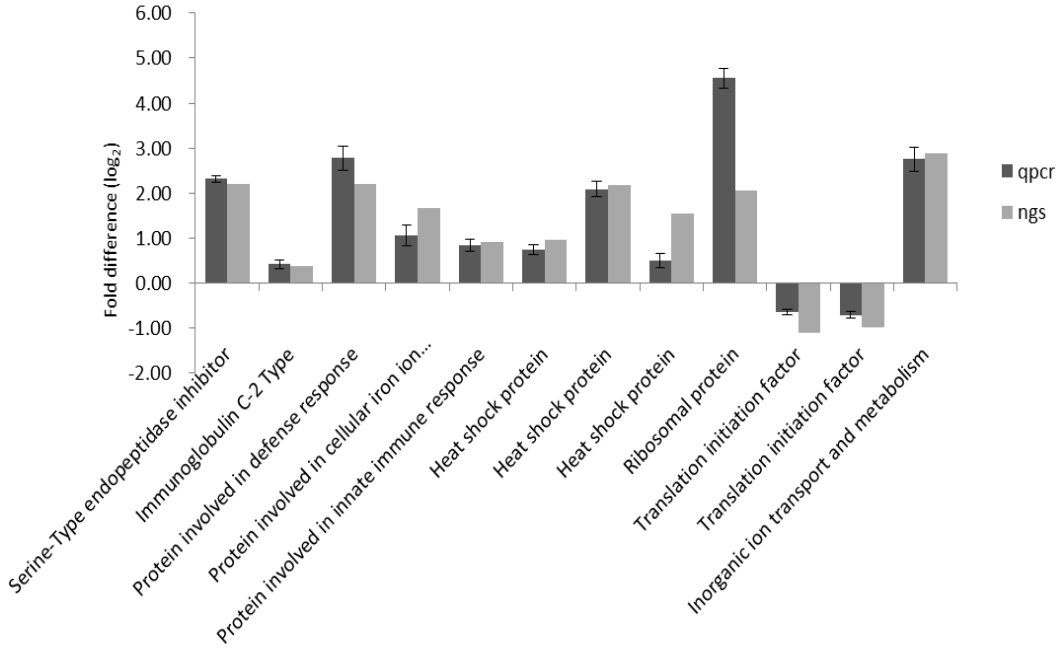

Fig. 4. Validation between NGS data and qPCR based on fold changer (Log_2) scale.

Table 5. Expression profiles of RSV genes in the transcriptome of *L. striatellus*.

	Gene	FPKM of NVL	FPKM of RVL	RVL/NVL
RdRp	RNA-dependent RNA polymerase	0	1.5	∞
NS2	Nonstructural protein	-	-	-
NSvc2	Putative membrane glycoprotein	0	2.7	∞
NS3	RNAi suppressor	-	-	-
NCP	Coat protein	0	23.2	∞
SP	Disease-specific protein	0	2.2	∞
NSvc4	Movement protein	0	1.8	∞

4. Discussion

The RNA-seq result from Illumina Hiseq 2000 system used in this experiment showed the differentially expressed genes affected by Rice Stripe Virus infection in *Laodelphax striatellus*.. As the result obtained from comparative analysis, physiology of *Laodelphax striatellus* was affected by Rice Stripe Virus. The number of genes expressed two-fold over differentially in the transcriptome of RVLS was more than ten times of those of NVLS which demonstrated RSV may affect the gene expression of the vector insect even though the virus is not considered pathogenic towards to vector insect. Among the functionally categorized genes, the genes related to 'Secondary metabolites biosynthesis', 'transport and catabolism, Inorganic ion transport and metabolism' and 'Lipid transport and metabolism' that belong to a 'Metabolism' group was expressed differently with the tendency of average expression. This suggests that the Rice stripe virus infection may affect the physiology of vector insect to activate its metabolic system because the virus needs more proteins to replicate and to transmit itself. The genes categorized in a 'Cytoskeleton' which belong to a 'Cellular processes and signaling' group was also uniquely highly expressed compared to the tendency of all genes expression level. This also suggests that RSV may affect the cellular processes and signaling system of *L. striatellus* to help its cell to cell movement.

Besides, the relative transcription levels of genes which encode ribosomal proteins were somewhat higher in the RVLS than that of NVLS. Normally, the genes encoding ribosomal proteins are not affected by stresses such as high temperature, pathogen and so forth. However, in this experiment, the comparative transcriptome analysis showed the

RNA virus affected the transcription of ribosomal protein genes of RVLS to ribosomal proteins of RVLS.

Furthermore, it is interesting to note that the genes related to immune system of RVLS that selected to validate the NGS data were highly expressed compared to those of NLVS. Although Rice stripe virus is a plant-pathogenic virus and considered to be not harmful for the vector insect, the highly expressed gene levels suggest that the virus obviously affected the physiology system of vector insect. Also, as a result from validation experiment using quantitative real-time PCR, the Illumina Hiseq 2000 RNA-sequencing system is a reliable tool and was able to reflect the characteristics of transcriptomes from RVLS and NVLS.

The result from this comparative analysis between Rice Stripe Virus-viruliferous and Non-viruliferous *L. striatellus* would be useful to find genes to be applied to control RSV and to study the interaction between vector insect and virus.

CHAPTER 2.

RNA silencing by virus-derived small interfering RNAs

RNA silencing by virus-derived small interfering RNAs

1. Introduction

Rice is the most important crop that is being consumed by over half the population across the world, however, there has been a reduction in the rice provided with many obstacles and rice stripe virus disease which caused by *Laodelphax striatellus* through transmitting rice stripe virus to the rice fields is considered as a most fatal factor for its reduction. The small brown planthopper, *Laodelphax striatellus* Fallen which scattered all around the world is one of the piercing-sucking insect pests and transmits RSV in a circulative-propagative manner(Hogenhout et al. 2008). The SBPH have distinct features that allow for the transmission of the virus efficiently. The predominant feature is that this insect has piercing-sucking mouthparts that include a needle-like stylet bundle with two mandibular and two maxillary stylets. The two maxillary stylets are interlocked and between them form two canals. Once the piercing-sucking insect starts to feed on plants, the narrower salivary canal delivers saliva which has the virus particles into the feeding puncture in plant tissue while the wider canal takes up plant sap into the sucking pump. Once the SBPH acquire RSV, that insect can inoculate the acquired virus to host plants by sucking sap and transmit the virus after molting and often for their entire

lifespan(nymphs into adults)(Hogenhout et al. 2008). The genome of Rice stripe virus is segmented to four single-stranded RNA genome of RNA1,2,3 and 4 according to the size of its molecule (Ishikawa, Omura, and Hibino 1989). The largest segment RNA1 encodes a protein which is considered as a RNA dependent RNA polymerase(Toriyama et al. 1994), RNA2 encodes glycoprotein and NS2 (Takahashi et al. 1993), RNA3 encodes NS3 and CP (Toriyama et al. 1994), and RNA4 encodes SP and NS4 (Zhu, Hayakawa, and Toriyama 1992). Except of the RNA1, negative-sense stranded, the rest of three segments are ambisense in their coding strategy (Hamamatsu et al. 1993).

RNA interference (RNAi) is an universal gene-knockdown mechanism in eukaryotic organisms includes insects(Kennerdell and Carthew 2000), and this technique has been considered as an alternative strategy to control agricultural pests(Baum et al. 2007, Price and Gatehouse 2008, Huvenne and Smagghe 2010). The first application of RNAi to control insect pests was the experiment with Coleopteran and Lepidopteran chewing insects by feeding them on dsRNA targeting the vacuolar-type H^+-ATPase, coat proteins and cytochrome P450 gene(CY-P6AE14)(Price and Gatehouse 2008, Mao et al. 2007).

Recently, feeding dsRNA via artificial saps or transgenic plant was evaluated for major Hemipteran sucking insects such as white flies(Upadhyay et al. 2011), aphids(Pitino et al. 2011) and leafhoppers(Zha et al. 2011, Chen et al. 2010, Li et al.

2011). Here, we established an RNAi system based on dsRNA delivery via leaf to knock-down the target genes belonging to RSV genome of RSV-viruliferous *L. striatellus*. We tested knockdown of target genes to figure out the possibility of applying the genes to control Rice stripe virus.

2. Materials and methods

2.1 *L. striatellus rearing*

The same strains as described in Chapter 1. Were used in this experiment and the methods were also described.

2.2 Target gene design from RSV genes

The nucleotide sequence of target genes RdRp, NS3, NCP those which belong to RSV were obtained from the RNA-sequencing. The target sequences were applied to the Invitrogen BLOCK-It RNAi Designer software to find the candidate siRNA site. Each dsRNA for the target gene was designed to include at least three putative siRNA sites.

2.3 Total RNA extraction and target gene cloning

Total RNA was extracted using Qiazol lysis reagent (Qiagen Ltd.,Crawley, UK) according to the manufacturer's instructions. The procedure is the same as described in Chapter 1.

The total RNA extracted from RVLS were then used for cDNA synthesis with QuantiTect Reverse Transcription Kit (Qiagen Ltd.,Crawley, UK). Briefly, 1μg of each RNA were incubated for 2 min at 42℃ with gDNA wipeout buffer and RNase-free water

to eliminate gDNA. The total RNA without gDNA then were added Quantiscript Reverse Transcriptase and RT buffer and incubated for 15min at 42℃ for activation of reverse transcriptase. For the last step, the entire reverse-transcription reactions were incubated 95℃ for 3min to inactivate reverse transcriptase. With the synthesized cDNA, three target genes were amplified with a set of primers added T7 promoter sequences (Table 1) through PCR reaction. The amplified amplicon were ligated into pGEM-T easy vector (PROMEGA, USA) and then transformed into TOP10 competent *E.coli* cells. After 12h incubation the plasmids were purified using QIAprep Spin Miniprep Kit (Qiagen Ltd.,Crawley, UK).

Table 1. The primers used in this experiment

Primer names	Oligonucleotide sequences	Amplicon	Remarks
T7_RSVRdRp-F	T7 promoter sequence + TGAAGGATGTAATAGAAGGTGG	RSV RdRp	Amplification of target genes for dsRNA synthesis
T7_RSVRdRp-R	T7 promoter sequence + GCATCATAATAAGCGGACAT		
T7_RSVNS3-F	T7 promoter sequence + TTCTGAGGCTTCTTCATGGC	RSV NS3	
T7-RSVNS3-R	T7 promoter sequence + TGGTAAGGATTCTAGTGCAC		
T7-RSVNCP-F	T7 promoter sequence + TAGTCATCTGCACCTTCTGC	RSV NCP	
T7-RSVNCP-R	T7 promoter sequence + GCCAAGGATATGACTATGTG		
qARF-Fw	TTGGACAGTATCAAGACCCATC	ADP ribosylation factor - Reference gene	Primers used for qRT-PCR to detect dsRNA
qARF-Re	GCAGCAATGTCATCAATAAGC		
qRdRp-RNAi-F	GGTGGGCTTTGGAAACAAGA	RSV RdRp	
qRdRp-RNAi-R	TTGTTCAGCTGTTGCTGCGT		
qNS3-RNAi-F	GCAGAGCTCTACATTGTGCCA	RSV NS3	
qNS3-RNAi-R	AACGTGCACTAAGAGGTGGTTT		
qNCP-RNAi-F	GCCAGCGCATCGAAGATAGT	RSV NCP	
qNCP-RNAi-R	CTCCTGGTCATCACATGCAA		
UBQ5-Fw	CGCCGACTACAACATCCAG	*O. sativa* Ubiquitin-5 -Reference gene	
UBQ5-Re	TCACCTTCTTGTGCTTGTGC		

2.4 dsRNA synthesis and quantification

The primers used in this experiment were given T7 promoters at the 5' ends of each strand due to the T7 RNA polymerase used to produce dsRNA synthesizes single-stranded RNA(ssRNA). The dsRNAs for the target genes were produced by Genolution Pharmaceuticals(Korea).

Each synthesized dsRNA was confirmed on a 1.8% agarose gel and quantified with Molecular Imaging Software (Eastman Kodak Company, Rochester, NY) using the Lamda DNA as a DNA mass standard.

2.5 Detection of dsRNA delivery in *O. sativa* leaves

dsRNA was delivered by leaf-mediated methods with the fabricated feeding chamber. A feeding chamber was fabricated by assembling 15ml conical centrifuge tube (Fisher Scientific, USA) with the hole on top of the tube to provide air and a 10μl micropipette tip was placed into the hole to prevent the SBPH from escaping the tube. The parafilm M membrane(American National can Company. Norwalk, CT) was placed to seal the gap between a 15ml tube and a cap consisting of a feeding chamber to make a reservoir for holding dsRNA solution. 300 μl of 50, 250 and 500 ng/μl dsRNA solution were dispensed over the reservoir and three rice seedlings were placed over the dsRNA

solution for 48 hours, respectively. Total RNA were extracted from dsRNA-treated leaves using Qiazol lysis reagent (Qiagen Ltd.,Crawley, UK) according to the manufacturer's instructions. Briefly, the root of the leaves were removed and the rest of the leaves were rinsed with nuclease free water, and then the leaves were homogenized in 1ml of Qiazol lysis reagent (Qiagen Ltd.,Crawley, UK) using Bullet Blender (Next Advance, Inc. Averill Park, NY

2.6 dsRNA delivery via dsRNA-permeated *O. sativa* leaves

The dsRNA of each target gene was delivered by a feeding chamber as described in the section 2.5. Ten of forth instars were infested over three dsRNA-permeated leaves. The dsRNA-treated leaves infested with *L. striatellus* in the feeding chamber was placed under the conditions of insect growth chamber (28℃, 80% RH, 16:8 (L:D) photo period). Total RNA from dsRNA untreated-RVLS was used as a control for the calculation of relative expression levels.

2.7 Detection of gene knockdown

dsRNA-fed small brown planthoppers were collected after 48 hours of treatment and their total RNA was extracted using Qiazol lysis reagent (Qiagen Ltd.,Crawley, UK)

according to the manufacturer's instructions. Briefly, total RNA was extracted from ten of forth instars of each sample group for cDNA synthesis. cDNA was synthesized with oligo d(T) primers using QuantiTect Reverse Transcription Kit (Qiagen Ltd.,Crawley, UK). qPCR was performed in a 20-ul reaction volume using the EvaGreen qPCR Mastermix(Applied Biological Materials Inc, Canada) and CFX96^{TM} Real-Time PCR detection system (BIO-RAD, USA) according to the manufacturer's instructions. The progress of PCR amplification was as follows : 95℃ for 10 minutes followed by 40 cycles of 95℃ for 15 seconds, 58℃ for 15 seconds, and 72℃ for 30 seconds . The ADP ribosylation factor (ARF) was used as a reference gene for the calculation of fold change. The relative transcription levels were calculated by using the $2^{-\Delta Ct}$ method. (Pfaffl 2001).

3. Results

3.1 Confirmation of dsRNA delivery to the *Oriza sativa* leaves

The dsRNA of RSV RdRp gene was tested to determine if it was delivered systematically to the rice leaves. The amount of dsRNA treated to the leaves was 300 μl of 50, 250 and 500 ng/μl dsRNA solution. After 48 hours, the amount of dsRNA were measured by qPCR and the result demonstrated that dsRNA transfer to the *Oriza sativa* leaves.(Fig. 1.)

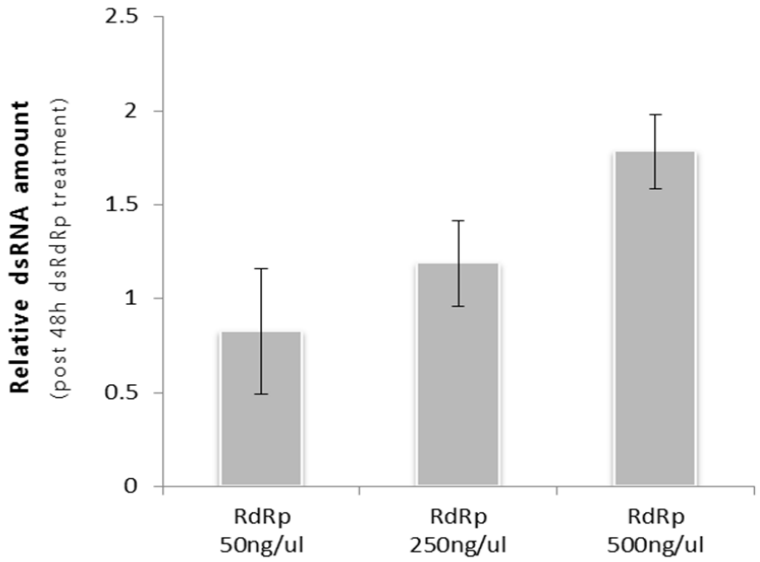

Fig. 1. Normalized amount of RdRp dsRNA against ubiquitin-5 of *O. sativa.*

RSV dsRdRp solution was treated to confirm delivery of dsRNA solution into the rice leaves. ubiquitin-5 was used as a reference gene to normalize the relative dsRNA amount.

3.2 Detection of gene knockdown by feeding dsRNA

To confirm the target gene knockdown by feeding RNAi system, qPCR was conducted using dsRNA-treated *L. striatellus.* Because the Rice Stripe Virus has its genes in one virion altogether, expression levels of non-target genes as well as those of target genes were also measured by qPCR. The result from dsNS3-treated *L. striatellus* showed that not only the transcript level of NS3 in segment 3, but also the transcript levels of non-target genes, and the replication levels of RSV genome were suppressed overall compared to that of samples from dsNS3-untreated *L. striatellus.*(Fig. 2.) The tendency of transcriptional suppression effect of dsNS3 treatment was proportionally related to the concentration of the dsRNA. Likewise, the dsRdRp and dsNCP showed transcriptional suppression effect, however, they were less concentration-dependent than that of dsNS3. (Fig. 3.4)

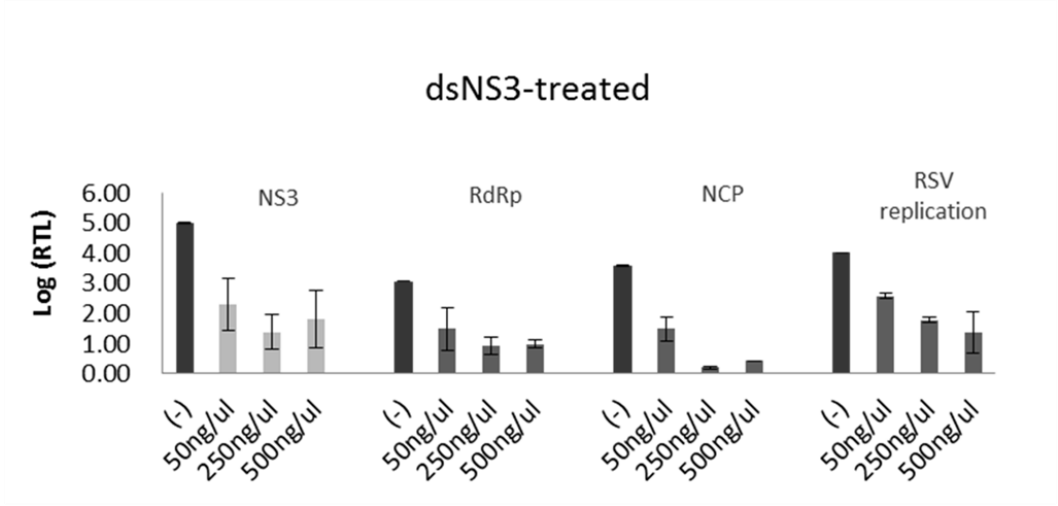

Fig. 2. Relative translation of dsNS3-treated *L. striatellus*

Relative translation levels(RTL) were measured by qRT-PCR with total RNA of RVLS fed on dsNS3 treated-rice leaves. RTL of non-target genes(RdRp, NCP) and replication level of RSV genome as well as that of NS3, target gene, were also measured.

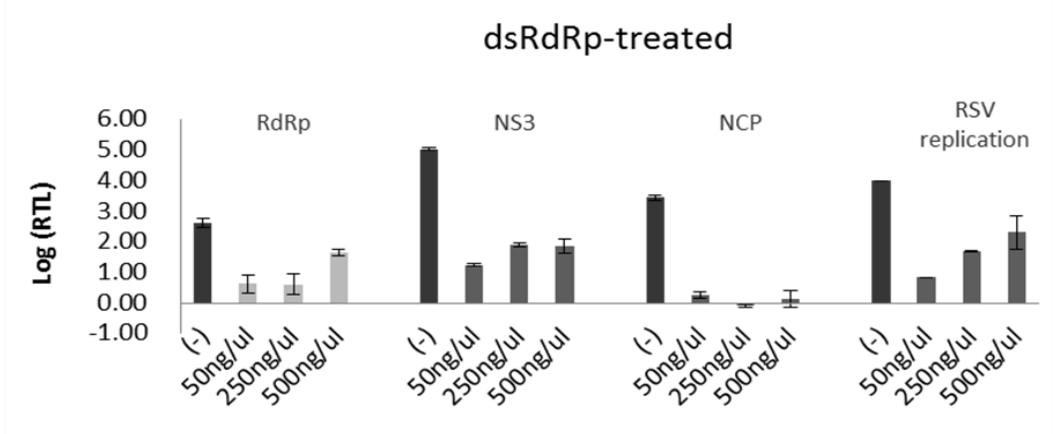

Fig. 3. Relative translation of of dsRdRp-treated *L. striatellus*

Relative translation levels(RTL) were measured by qRT-PCR with total RNA of RVLS fed on dsRdRp treated-rice leaves. RTL of non-target genes(NS3, NCP) and replication level of RSV genome as well as that of RdRp, target gene, were also measured.

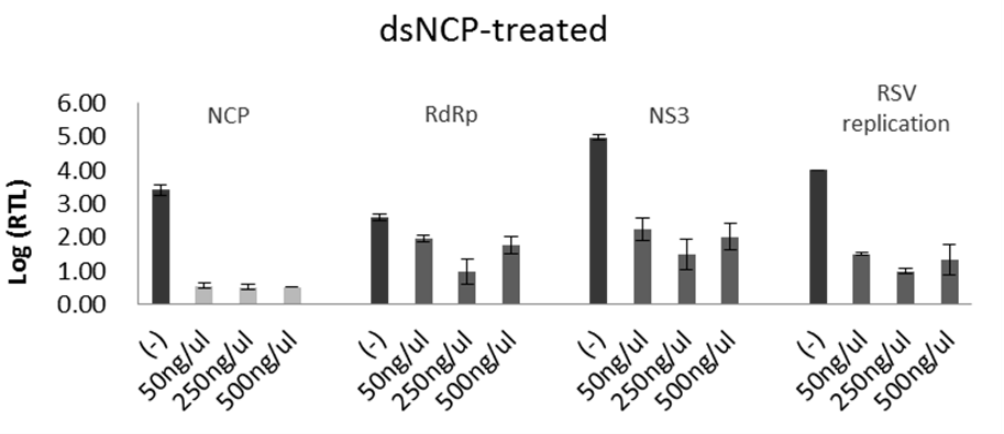

Fig. 4. Relative translation of of dsNCP-treated *L. striatellus*

Relative translation levels(RTL) were measured by qRT-PCR with total RNA of RVLS fed on dsNCP treated-rice leaves. RTL of non-target genes(RdRp, NS3) and replication level of RSV genome as well as that of RdRp, target gene, were also measured.

4. Discussion

For sucking insects, such as aphids, white flies and plant hoppers, dsRNA via artificial sap solution is well established system for feeding RNAi (Upadhyay et al. 2011). In this experiment, we established RNAi feeding system for piercing-sucking small brown planthoppers based on dsRNA ingestion via rice leaves and demonstrated that it is an efficient way to evaluate the effects of knockdown of RSV genes in RVLS. *L. striatellus* ingested target dsRNAs along with host plant sap and tissues without any feeding avoidance, which frequently observed with artificial sap feeding. Furthermore, the results from this experiment revealed that dsRNA feeding via plant actually induced gene knockdown in *L. striatellus.* All three candidate genes were significantly suppressed by the RNAi feeding system.

The RdRp is an essential gene for the replication of RNA virus and NS3, NCP belonging to the RNA segment 3 of which the transcript level was highest among seven RSV genes. A decreased amount of these three genes of RSV genome may suppress replication of RSV genome.

Although the exact tissue distribution of absorbed dsRNA in the rice leaves is not known, the presence of target dsRNA in the whole leaf was confirmed by qPCR. It is necessary to further investigate the physiological basis of dsRNA uptake. Also, further experiment to find out the most efficient and economic condition for dsRNA treatment to induce gene knockdown need to be performed.

Leaf-mediated dsRNA feeding methods applied in this experiment would be useful in the knockdown of target genes on piercing-sucking insects. Also, the target genes used in

this experiment can be utilized for the development of pest-resistant transgenic plants based on RNAi.

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KOREAN ABSTRACT

벼 줄무늬잎마름병의 매개곤충인 RSV 보독 애멸구와 비보독 애멸구의 전사체 비교 분석

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초록

Rice stripe virus (RSV)에 의해서 발생하는 벼 줄무늬잎마름병은 벼의 생산량을 감소시키는 가장 심각한 벼 바이러스 병 중의 하나이다. 매개곤충인 애멸구를 통해 벼가 RSV에 감염된 뒤, 발병하게 되면 정상적인 이삭을 출수하지 못하고 말라서 고사하게 되어 벼 수확량에 큰 손실을 초래하게 된다. 현재까지의 연구는 기주 식물인 벼와 병원체인 RSV의 상호관계에 대해서만

많이 이루어져 있어 RSV가 매개곤충인 애멸구에 미치는 영향에 대해서는 잘 알려져 있지 않다. 따라서 본 연구에서는 이 바이러스가 매개충의 생리에 미치는 영향을 조사하고 이를 새로운 RSV의 방제에 적용하고자 RSV 보독 애멸구와 비보독 애멸구의 전사체에 대하여 비교 분석을 수행하였다. Illumina Hiseq 2000 시스템을 통하여 수행한 RNA sequencing 결과 얻은 약 17만 6천여개의 contigs를 대상으로 각 contig들의 상대적인 발현량을 분석하고 총 23개의 functional group으로 세분화한 뒤, 보독 애멸구와 비보독 애멸구에서 상대적으로 2배 이상 높게 발현된 유전자들을 조사하였다. 또한 2배 이상 높게 발현된 유전자들 중에서 전체적인 양상과 상반되게 특이적으로 높게 발현된 유전자들을 P-value 10^{-3} 이하의 조건으로 분석한 결과, metabolism, cytoskeleton group에 속한 유전자들이 RSV 보독 애멸구의 transcriptome에서 특이적으로 높게 발현된 것을 확인할 수 있었다. 이는 바이러스에 감염된 뒤 기주로 하여금 바이러스의 증식이나 생존과정에 필요한 단백질들을 생산하고, 바이러스의 세포간 수송을 돕기 위한 것으로 추정된다. 본 연구에서 분석한 RSV에 의존적인 애멸구의 유전자들은 애멸구 내에서 RSV 전염과 복제에 있어 중요한 기능을 할 것으로 사료된다.

또한, RSV 보독 애멸구 내에 존재하는 RSV 유전자 중 RNA 바이러스의 복제에 필수적인 단백질로 알려진 RdRp, 가장 높은 비율로 존재하는 RNA 3 segment에 속하는 NS3와 NCP를 타겟으로 dsRNA를 합성한 뒤, 벼의 뿌리를 통해 흡수시킨 뒤 애멸구에 간접적으로 전달하는 RNA interference 실험을

수행하였다. 각각의 dsRNA를 섭취한 RSV 보독 애멸구를 대상으로 RSV의 상대적인 발현량을 qPCR을 통해 분석한 결과, 타겟 유전자의 상대적인 발현량 뿐만 아니라 비타겟 유전자와 RSV의 전체적인 증식이 복합적으로 억제된 것을 확인할 수 있었다. 효율적이고 경제적인 RNA silencing 조건을 구축하기 위하여 추가적인 연구가 필요할 것으로 생각된다. 본 연구에서 제시된 타겟 유전자들은 애멸구와 같은 흡즙성 해충 방제를 위하여 RNAi 기법을 응용한 형질전환식물체 개발에 도입한다면 새로운 RSV의 방제법으로서 방제 가능성이 높을 것으로 사료된다.

검색어 : 벼 줄무늬잎마름병, 애멸구, 전사체 분석, RNA 간섭, 섭식 RNAi 시스템

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