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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation

Studies on rice genes involved in RNA silencing: defence against pathogens including viruses
（RNA サイレンシングに関与するイネ遺伝子の研究：ウイルスを含む病原体に対する防御）

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Dissertation Summary

RNA silencing is a universal mechanism of gene regulation in eukaryotes affecting several processes including developmental control, epigenetic modifications and antiviral defense. In these processes, small RNAs and protein factors play a role in suppressing gene transcription or increase mRNA degradation. In plants, RNA silencing is a key module of defense mechanism against viruses. The mechanism of RNA silencing involves several key protein factors that repress transcription and increase mRNA degradation in a sequence specific manner. In this process, RNA-dependent RNA polymerases and suppressor of gene silencing 3 (SGS3) plays a significant role in synthesizing RNA strands complementary to an aberrant RNA template, resulting in double-stranded RNA (dsRNA). Then dsRNAs are processed into 20–24 nucleotide (nts) small interfering RNAs (siRNAs) by Dicer-like enzymes (DCLs) that transcriptionally or post-transcriptionally target gene expression. Biotic and abiotic stresses affect the expression of *RDR*s. In *Arabidopsis* and *Nicotiana tabacum*, *RDR1* was induced by salicylic acid (SA), methyl jasmonate (MeJA), hydrogen peroxide (H_2O_2) and several viruses including *Tobaco mosaic virus*, *Potato virus Y* and *Cucumber mosaic virus* (CMV). More recently, suppressors against RNA silencing were found to be encoded by an oomycete pathogen (Qiao *et al.*, 2013). Suppression of plant immunity was observed via hijacking host RNA interference pathways by fungal small RNA suppressor (Weiberg *et al.*, 2013). Bacterial effector proteins suppress the microRNA pathway (Navarro *et al.*, 2008). Thus RDR1, RDR6 and SGS3 may play an indirect role in plant defense through silencing-mediated regulation of cellular mRNA encoding resistance factors (Katiyar-Agarwal *et al.*, 2010). In the present study, I investigated the defense role of *OsRDR1* and *OsSGS3b* against viral, bacterial and fungal pathogens by using mutant and overexpressed lines of these genes. The induction of these genes was tested in response to *Rice necrosis mosaic virus* (RNMV), CMV, *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe oryzae*. Rice mutant lines were produced by the insertion of retrotransposon Tos17. The double mutant of two genes (*OsRDR1/OsSGS3b*) was produced by crossing. The overexpressed lines of *OsRDR1* and *OsSGS3b* (OsRDR1 OX and OsSGS3b OX, respectively) were also used. These lines were inoculated with CMV, RNMV, *X. oryzae* pv. *oryzae* and *M. oryzae*, respectively. As a first step, I checked the induction of *OsRDR1* and *OsSGS3b* in response to CMV, RNMV, *X. oryzae* pv. *oryzae* and *M. oryzae*. For CMV, the expression of *OsRDR1* increased at 5 days post inoculation (dpi) and continued up to 7 dpi, while that of *OsSGS3b* increased at 3 dpi and reached a plateau at 5 to 7 dpi. For RNMV, the induction of *OsRDR1* was observed at 5 dpi and reached a plateau between 10 to 15 dpi, whereas *OsSGS3b* showed similar expression profiles to each other and their expressions were highest after 5 dpi and continued to 15 dpi. No change in the expression level was noted in the mock inoculated samples. After inoculation with *X. oryzae* pv. *oryzae*, the induction of *OsRDR1* and

OsSGS3b began early at 6 h after inoculation and reached a peak at 48 h. With a virulent *M. oryzae*, *OsRDR1* and *OsSGS3b* were induced from 2 dpi and then *OsRDR1* increased from 36 to 96 h. However, *OsSGS3* reached a plateau at 36 h, and then eventually decreased at 96 h. Although the induction patterns varied among the two genes, the expression of all two genes was induced by these pathogens.

In the next step, the accumulation level of viral RNAs and symptom severity were tested by these pathogens. In the mutant lines of *OsRDR1* and *OsSGS3b*, the higher accumulation levels of CMV or RNMV RNA were detected compared with that in the wild type (WT). The double mutant of *OsRDR1/OsSGS3b* showed the highest accumulation of viral RNA than those in the single mutant lines. As shown in *Arabidopsis*, SGS3 can bind to *OsRDR6* and then *OsRDR6* synthesizes complementary strand RNA from aberrant RNA as a template to form dsRNA . Thus, in the *OsSGS3b* mutant line, the OsRDR6 machinery could be defective in this point. *OsRDR1* OX and *OsSGS3b* OX showed an extremely low amounts of CMV RNA and RNMV RNA accumulation than in WT. Among these lines, the double mutant line showed the highest susceptibility to all pathogens tested. Single mutant lines were more susceptible than WT. Microarray analysis of the mutant lines showed down-regulation of defense related and signaling pathway related genes. These results suggest that *OsRDR1* and *OsSGS3*b have a positive defense role against viral, bacterial and fungal pathogens, implicating RNA silencing through these genes as a defense against not only viruses but also bacterial and fungal pathogens.

The rice *RDR6* gene was also investigated in response to viral, bacterial and fungal pathogens. A rice mutant line of *OsRDR6, shl2-rol* mutant*,* was inoculated with CMV, RNMV, *X. oryzae* pv. *oryzae* and *M. oryzae*, respectively. For CMV, the expression of *OsRDR6* increased at 1 day post inoculation (dpi) and continued up to 7 dpi, while for the fungal-transmitted bymovirus RNMV, the expression level increased from 5 to 15 dpi. No change in expression level was noted in the mock inoculated samples. For *X. oryzae* pv. *oryzae* and *M. oryzae*, the induction of *OsRDR6* was observed early at 6 h after inoculation and reached a peak at 72 h, then decreased up to 96 h. Compared with WT, the mutant line showed that a higher level of viral RNA accumulation and more severe symptoms by bacterial and fungal pathogens were observed. These results suggest that the *OsRDR6*-mediated RNA silencing pathway participates in defense against not only viruses, but also bacterial and fungal pathogens.

RNMV is a member of the genus *Bymovirus* in the family *Potyviridae* and transmitted by Plasmodiophorid, *Polymixa graminis*. RNMV was first reported in Japan and then in India in rice, causing mosaic symptoms characterized by yellow flecks and streaks on lower leaves. The genus *Bymovirus* in the family *Potyviridae* consists of virus particles with bipartite plus-sense RNA genomes, with 7.3–7.6 kb RNA1 and 3.5–3.7 kb RNA2. RNMV is a flexuous rod-shaped particle with its RNA encapsidated by coat protein (CP) to form two different lengths of filamentous virions: 550 and 275 nm in length and 13 nm in diameter. Both bymoviral RNAs have a viral genome-linked protein (VPg) covalently bound to the 5′ end and are polyadenylated at the 3′ end. The genus *Bymovirus* contains six known species; barley yellow mosaic virus (BaYMV) as the type species, barley mild mosaic virus (BaYMV), oat mosaic virus (OMV), RNMV, wheat spindle streak mosaic virus (WSSMV) and wheat yellow mosaic virus (WYMV). However, the complete nucleotide sequence of RNMV genomic RNAs has not yet been reported. In this study, I determined the complete nucleotide sequences of RNMV RNAs 1 and 2 and compared them with those of RNAs 1 and 2 from the other five bymoviruses, respectively. RNMV isolate Ka-1 was propagated in rice plants (*Oryza sativa* cv. Akebono) in naturally infested soil collected in Okayama, Japan. The infested soil was conserved and used as inoculum. RNMV inoculation was carried out by sowing the seeds of Akebono in RNMV-infested soil for 30 days and the young seedlings were then transferred to buckets containing regular rice soil as described previously. The seedlings were then placed in growth chambers for up to 60 days. Leaf samples were used for virus purification. The virus

was purified using cesium chloride centrifugation. The virus suspension was used for viral RNA extraction. Using the first cDNA strand as a template, cDNA fragments were PCR-amplified using the degenerated primers and cloned into the plasmid vector. To confirm the 5′ and 3′ terminal sequences and the nucleotide sequences in the regions of ambiguity the other primers were used for sequencing. The complete nucleotide sequence of RNMV RNA1 was determined to be 7,178 nucleotides long with one large open reading frame, potentially encoding a polyprotein of 258 kDa with the features P3, $7K_1$, CI, $7K_2$, NIa-VPg, NIa-Pro, NIb and CP of a typical bymovirus. The nucleotide sequence showed 56% identity to BaMMV and 46% to OMV, BaYMV and WYMV. The nucleotide sequence of RNMV RNA2 was found to be 3,579 nucleotides long, encoding 110 kDa polyprotein which is presumably processed into P1 and P2 proteins, and showed 42% and 35/26% identity to BaMMV and others, respectively. Phylogenetic analysis of each RNA showed the highest homology to BaMMV and distantly related to other four bymoviruses. Based on the sequence data, bymoviruses can be classified into two subgroups, I (BaYMV, OMV, WYMV and WSSMV) and II (RNMV and BaMMV).