学位論文審査の要旨

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学　位　論　文　題　名

Genetic studies on resistance to Rice tungro bacilliform virus and evolution of its related endogenous viruses in the genus *Oryza*

（イネのツングロ病ウイルス RTBV 抵抗性およびオリザ属における
その内在性ウイルスの進化に関わる遺伝学的研究）

This dissertation consists of six chapters in total of 126 pages, including 35 figures, 15 tables, and six supplemental data.

Rice, *Oryza sativa* L., is one of the important cereal crops that provides a staple food for a half of world's population. Rice viral diseases constitute a major obstacle to rice production. Rice is known to be infected by more than 30 viruses, but 25 viruses do not directly have economic effects in terms of rice production. Rice tungro disease (RTD) caused by Rice tungro bacilliform virus (RTBV) negatively affects rice production in South Asia and Southeast Asia. Plants have developed multilayered defense mechanisms against microbial pathogens such as the gene silencing of viral small RNA (vsRNA) or host micro RNA (miRNA). Regarding the anti-viral defense against RTBV, The author considered three factors, which were endogenous RTBV like (eRTBVL) sequence, vsRNA and rice miRNA, in this study.

In Chapter 2, the author focused on eRTBVL sequences, which have been identified in the rice genome. The distribution of eRTBVL sequences differs between the two genomes; the *O. sativa* genome contains more than 100 copies of eRTBVLs, while the *O. glaberrima* genome has only a few copies. Differences in the eRTBVL copy number in these genomes might be associated with the different reactions of these two species to RTBV. Firstly, the author confirmed innate vulnerability to RTBV of *O. glaberrima* carrying a few eRTBVL copies, then the possible function of eRTBVL-derived small RNAs were examined by the comparison between *O. sativa* and *O. glaberrima*. Small RNA sequencing analysis indicated that the expression of small RNAs from eRTBVL might be associated with the phenotypic differences between the two genotypes after RTBV infection. The author could suggest two possible ideas to explain the interaction between the susceptibility to RTBV and the presence of eRTBVL. One

is that the constitutive expression of small RNAs from eRTBVL promotes the silencing machinery mediated by vsRNA in RTBV-infected plants. The other possibility is that the small RNAs from eRTBVL respond immediately after RTBV infection to inhibit the replication of the virus at an initial stage of the infection.

In Chapter 3, the resistant (TW16) and susceptible (TN1) genotypes against RTBV were used for RTBV infection test and small RNA sequencing. To examine the RNAi-based defense machinery in rice during RTBV infection, the author characterized vsRNA and rice-derived miRNA by small RNA sequencing and compared the expression of small RNAs between TN1 and TW16. A total of 11 crucial miRNAs related to RTBV resistance were identified in this study. The expression analysis of *AGO* genes, vsRNA and rice miRNA indicated that the resistant plants against RTBV might interfere the cleavage of target RNAs by down-regulation of miRNAs and protect the rice growth from viral disease symptom. The identified miRNAs might be valuable candidates to study novel insight into the defense mechanisms of resistant genotypes against RTBV.

Chapter 4 describes evolutionary patterns of the local chromosomal regions during the *Oryza* AA-genome speciation through the comparisons of eRTBVL-D sequences among the *Oryza* AA-genome species. eRTBVL-D is known as the oldest eRTBVL family that was integrated into the *Oryza* AA-genome before the speciation. The eRTBVL-D sequences were available for the evolutionary study of the local-chromosomal regions. The author found the swerved phylogenetic relationships from the known *Oryza* speciation and chromosomal rearrangements at the eRTBVL-D loci. The author described the functional implications of eRTBVL-D loci by the evolutionary analyses for natural selection. The results of Tajima's D test indicated that natural selections might not have been acted to eRTBVL-D as the central factor. Hence, eRTBVL could exist as "genomic fossils". In this chapter, The author proposed that eRTBVL-D loci could be the useful resource as genetic neutral markers, because they have rarely selective effect. The analysis of eRTBVL-D provided novel evolutionary insight for local chromosomal regions during the AA-genome speciation.

Above all, her study gave an insight into the mechanism of the resistance against tungro disease and provided a novel idea of local evolutionary events characterized by endogenous virus segments. The committee highly evaluated Nozomi Saito's study and judged that she is worth to be conferred the Ph.D.