学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Study of small RNA-mediated control of gene expression and viruses in plants: Roll of a viral suppressor of RNA silencing in viral survival (Small RNA によって調節される植物体内の遺伝子発現と植物ウイルスに関する研究: ウイルス生存のために機能する RNA サイレンシングサプレッサーの役割)

RNA silencing is a conserved mechanism induced by double-stranded RNAs (dsRNAs) to regulate gene expression for control of development, genome stabilization and responses to abiotic/biotic stresses in eukaryotes. RNA silencing is operated by 21- to 24-nucleotide (nt) small RNAs (sRNAs) including microRNAs (miRNAs) and several classes of short interfering RNAs (siRNAs), and it targets RNAs based on their homologous sequences. These sRNAs are generated from dsRNA or self-complementary hairpin RNA (hpRNA) by Dicer-like (DCL) protein-mediated cleavage and captured by Argonaute (AGO) proteins forming RNA induced silencing complex (RISC) to guide them to their target RNAs for degradation. The RNA silencing is a primary antiviral response. It is strongly induced by viral infection; enormous amounts of siRNAs are synthesized from viral RNAs. To counteract the antiviral RNA silencing, viruses encode RNA silencing suppressors (RSSs), targeting key factors in the RNA silencing pathway including siRNAs, DCLs and AGOs.

So far, remarkable advancements in exploiting RNA silencing have been achieved by plant biologists for studies of reverse genetics, crop development and pathogen control. Among them, virus-induced gene silencing (VIGS) and RNA silencing-based control of plant pathogens are most widely used for studies. In this research, we include two studies on; (1) cucumber mosaic virus (CMV) VIGS for functional genomics of two spinach genes and (2) exogenous small RNA treatment to generate virus-free garlic plants. In addition, we analyzed the HC-Pro gene of onion yellow dwarf virus (OYDV), which is an RSS gene to counteract host antiviral RNA silencing.

(1) Development of a convenient virus-induced gene silencing (VIGS) system for spinach driven by efficient systemic infection of the cucumber mosaic virus vector

Although VIGS for spinach has been already developed based on two viruses, beet curly top virus (BCTV) and tobacco rattle virus (TRV), but there are several limitations especially for study of flowering related genes. Therefore, it would be better to establish more options of different VIGS systems, because various spinach cultivar may show different silencing efficiency and responses against viral infection. By combination of three genomic RNAs, CMV pseudorecombinants are easily generated, and it can modify viral pathogenicity to overcome host resistance or attenuate viral symptoms which may conceal the silencing phynotypes. In this study, we examined VIGS against the PDS and dihydroflavonol 4-reductase (DFR) genes in three spinach cultivars by using a CMV pseudorecombinant between two CMV strains (CMV-L and CMV-Y). The infected spinach plants showed clear

chlorosis on the systemic leaves of the PDS-silenced plants and decoloration of red pigment in the stem of the DFR-silenced plants. We also confirmed significantly decreased levels of PDS and DFR mRNA. Because CMV can efficiently multiply in systemic leaves and could be maintained until flowering stage, our CMV VIGS system may be a great option for functional genomics studies such as the genes related to sex expression.

(2) Helper component proteinase of onion yellow dwarf virus isolated from Japanese garlic lost a long stretch of amino acids at the N-terminal region affecting RNA silencing suppressor activity

When we investigated viral infection in garlic plants from Japanese regions, we found that a potyvirus, OYDV, was always co-infected with leek yellow stripe virus (LYSV) which is another potyvirus. Because these garlic potyviruses are transmitted by aphids, we focused on the HC-Pro, a viral determinant of vector transmission. Interestingly, our sequencing data of the HC-Pro genes from Japanese OYDV isolates showed that all of tested HC-Pros lacked ~100 amino acid of the N-terminal region including a putative crucial motif for aphid transmission (KITC). Our phylogenetic analysis based on the amino acid sequences revealed that the HC-Pros were divided into three distinct groups and they are not originated from a single ancestor, suggesting that the N-terminal deletions in HC-Pro may have been created independently between the two groups. However, at least a group of OYDV HC-Pro may be explained by a possible template-switching mechanism during viral replication. To determine whether the N-terminal deletion affects the function of OYDV HC-Pro as an RSS, we examined the RSS activity of HC-Pro in *Nicotiana benthamiana* and its natural host, onion. The tested OYDV HC-Pros showed more efficient suppression of RNA silencing in onion than *N. benthamiana*, and the three short-type HC-Pros from Japanese OYDV isolates lost their RSS activity.

Now a question remains to be answered: did the OYDV with a short-type HC-Pro lose the aphid transmissibility? Using RNA silencing-based technology, we treated garlic plants with double-stranded RNA against LYSV to obtain the garlic plants that are infected only with OYDV. Using the OYDV-infected garlic, we are planning to conduct aphid transmission experiments to test whether the OYDV isolates containing a short HC-Pro can be transmitted by aphids. Our studies on garlic viruses provide a clue of evolution of OYDV in Japanese garlic fields and the roll of an RSS for the control of viral pathogenicity.

In conclusion, our research provides two biotechnological tools based on RNA silencing in plants: (1) CMV VIGS system for functional studies of the spinach genes and (2) exogenous siRNA treatment to reduce viruses. In both systems, RSSs may play key roles in modulating viral pathogenicity and counteracting plant immunity based on RNA silencing. Additional studies about viral RSSs will be necessary for better understanding of plant-virus interaction and application of RNA silencing to practical fields.