学位論文内容の要旨

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学位論文題名

Roles of RNA silencing-related genes in tomato tolerance to viral infection

（トマトのウイルス感染耐性におけるRNAサイレンシング関連遺伝子の役割）

In plants, RNA silencing functions as an antiviral defense mechanism triggered by virus-derived double-stranded RNAs (dsRNAs). It occurs through the action of the RNA silencing components, such as Dicer-like (DCL) nucleases, Argonaute (AGO) proteins, and RNA-dependent RNA polymerases (RDR). Plants encode multiple AGOs, DCLs, and RDRs. The functions of these components have been mainly examined in model plants, *Arabidopsis thaliana*, and *Nicotiana benthamiana*. Studying their functions and roles in antiviral defense using agriculturally important crop plants would be helpful for the control of virus-caused diseases in crop productions.

In this study, I investigated DCLs, AGOs, and RDR6 in tomato responses to viral infection. Virus inoculation tests onto transgenic tomato plants, in which the expressions of these genes were knocked down, revealed the involvement of DCL2, 4, and AGO2, 3 in tomato tolerance to potato virus X and Y (PVX and PVY). I further examined the molecular mechanism underlying the AGO-mediated tolerance to PVX infection in tomato.

1. RNA silencing-related genes contribute to tolerance against the infection of PVX and PVY in tomato

DCL2 and DCL4 (hpDCL2.4), RDR6, and AGO2 and AGO3 (hpAGO2.3)-knockdown transgenic tomato plants (*Solanum lycopersicum* cv. Moneymaker), in which dsRNAs cognate to these genes were expressed to induce RNA silencing to them, were inoculated with PVX strain UK3 and PVY strain O and N. Their reactions and the accumulation of viral coat protein (CP) and the genomic RNA were analyzed. Although reverse transcription-coupled with polymerase chain reaction confirmed systemic

infection of all PVY-inoculated plants, symptoms were observed only in the inoculated hpDCL2.4 plants. Consistently, western blot analysis revealed an increase in accumulation of PVY CP in the symptomatic upper leaves. These results indicate that DCL2 and 4 contribute to tomato tolerance, asymptomatic PVX infection by controlling the multiplication of PVY, probably through antiviral RNA silencing. More severe symptoms observed in the PVX-inoculated hpDCL2.4 plants also suggested the involvement of DCL2 and 4 in tolerance to PVX infection. On the other hand, systemic necrosis was observed only in the PVX-inoculated hpAGO2.3 plants, suggesting that AGO2, AGO3, or both are distinctly involved in tolerance to PVX infection by controlling symptom developments accompanying cell death.

2. Molecular mechanisms underlying AGO2- and 3-mediated control of systemic necrosis that PVX infection causes in tomato

A time-course investigation of CP accumulation in PVX-inoculated hpAGO2.3 plants showed a consistently higher CP accumulation level than that in wild-type tomato. Furthermore, my inoculation tests with a chimeric PVX between strains UK3 and Yatsugatake, of which infection causes severer necrosis than UK3 does, revealed that viral proteins required for viral replication and spread are involved in the systemic necrosis. I additionally found that mRNA levels of superoxide dismutase 2 (SOD2) and the copper chaperon for SOD (CCS1), which detoxicate reactive oxygen species (ROS) and are regulated by micro RNAs, decreased in the PVX-infected hpAGO2.3 plants showing necrosis. In concert with these results, DAB staining showed increased superoxide in necrotic leaves of hpAGO2.3, and ectopic expression of CCS1 from the recombinant PVX inhibited necrotic symptom expression. Taken together, my results suggest that AGO2 and AGO3 would contribute to tomato tolerance to PVX infection by regulating excessive ROS production as well as controlling viral multiplication via the RNA silencing mechanism.