学位論文内容の要旨

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学位論文題名

Study on cucumber mosaic virus resistance conferred by an in-frame deletion in genome-edited tomato *eIF4E1*

（トマト *eIF4E1* のゲノム編集によるインフレーム欠失変異により付与されるキュウリモザイクウイルス抵抗性に関する研究）

Plant viruses, such as the cucumber mosaic virus (CMV) pose major threats to global agriculture by infecting a wide range of economically important crops. CMV encodes a multifunctional 2b protein known to suppress host RNA silencing, interferes with plant defense pathways, and enhances viral virulence. The eukaryotic translation initiation factor 4E (eIF4E) is a known susceptibility factor exploited by many plant viruses. Mutations in eIF4E can disrupt these interactions and confer antiviral resistance. In tomato, a CRISPR/Cas9 edited 9-nucleotide in-frame deletion in the eIF4E1 gene (9DEL) was previously shown to increase resistance to CMV (Atarashi et al., 2020). However, the molecular mechanism underlying this resistance remained unclear.

In this study, I aimed to determine the viral protein that interacts with the eIF4E1-9DEL. I further studied whether this *9DEL* mutation alters the interactions and how this contributes to CMV resistance in 9DEL tomato plants.

Chapter I: Identification of interaction between the genome-edited in-frame mutant of eIF4E1 (9DEL) and CMV 2b

Given that the *eIF4E1* null mutant did not enhance CMV resistance, I hypothesized that the mutant protein eIF4E1-9DEL may interfere with specific CMV proteins. Using yeast two-hybrid assays, I found that both wild-type eIF4E1 and the eIF4E1-9DEL variant interact with CMV 2b, known as a suppressor of RNA silencing and pathogenicity factor. I also found that 9DEL did not interact with HL2b, which CMV lily isolate possesses and carries specific amino acid substitutions in the

C-terminal region, highlighting the importance of the C-terminal region of 2b for interaction. Similarly, eIF4E1 mutant variants interacted with 2b except for the one lacking C-terminal 55 amino acids, underscoring the critical role of the eIF4E1 C-terminus in 2b interaction. The interaction between eIF4E1-9DEL and 2b was further validated in planta using split-luciferase complementation assay, and subcellular co-localization tests. Inoculation assays showed that CMV-Y systemically infected both 'S8' and 9DEL tomatoes. In contrast, CMVΔ2b (lacking 2b) failed to infect both, confirming that 2b is essential for systemic infection in tomato plants and that 9DEL may interfere with the 2b function.

Chapter II: Functional significance of 2b inhibition by genome-edited eIF4E1 (9DEL) in CMV resistance

Despite binding to 2b, only eIF4E1-9DEL, not the wild-type eIF4E1, attenuated CMV virulence, suggesting a mutation-specific inhibitory effect. To evaluate the functional impact of this interaction, I examined whether eIF4E1-9DEL inhibits RNA silencing suppressor (RSS) activity of 2b. Co-expression assays in N. benthamiana revealed that eIF4E1-9DEL suppressed the enhancement of GFP fluorescence normally observed due to 2b-mediated RSS activity, whereas wild-type eIF4E1 did not affect the GFP fluorescence significantly. Consistently, western blotting showed reduced accumulation of 2b protein in the presence of eIF4E1-9DEL, indicating that 9DEL inhibits 2b function at the post-translational level. Furthermore, quantitative RT-PCR confirmed a substantial reduction (not significant) in viral accumulation in 9DEL. These results collectively indicate that eIF4E1-9DEL interferes with 2b function without affecting general host physiology, suggesting a functional gain-of-interference mutation targeting 2b. This study highlights in-frame deletions as promising tools in resistance breeding.