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学位論文審査の要旨

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学位論文題名

The functions of the P3 cistron of *Clover yellow vein virus* in resistance breaking and cell-to-cell movement in *Pisum sativum*.

（クローバ葉脈黄化ウイルスの P3 遺伝子がエンドウにおける抵抗性打破と細胞間移行に果す役割）

本論文は英文 107 頁，図 19，表 6，8 章からなり，参考論文 6 編が付されている．

The single positive-sense RNA virus, *Clover yellow vein virus* (ClYVV), belongs to the genus *Potyvirus* and infects legume species. In pea (*Pisum sativum*), there are two recessive resistance genes, *cyv1* and *cyv2* against ClYVV, and they were mapped in linkage groups (LG) II and VI, respectively. This study focuses on characterization of *cyv1*-mediated resistance and viral proteins involved in overcoming *cyv1*-mediated resistance and cell-to-cell movement.

1. The recessive resistance gene, *cyv1*, of *Pisum sativum* against ClYVV does not encode eIF(iso)4E.

The pea lines that carry *cyv1* on LG II restricted cell-to-cell movement of ClYVV isolate no.30 (Cl-no30). Mapping of *cyv1* revealed that it was 4 cM and 5 cM from the simple sequence repeat marker AB40 and gene *PGM2*, respectively, whose loci are close to *mo* and *sbm-2*, which mediates resistance to other potyviruses. Since these resistance genes were mapped close to *eukaryotic translation initiation factor 4E* isoform [*eIF(iso)4E*], I examined the possibility that *cyv1* encoded eIF(iso)4E. Genomic sequence analyses of *eIF(iso)4E* between susceptible and resistance pea lines showed that there was no difference in the nucleotide sequence. And 2,000 nucleotides upstream of the *eIF(iso)4E* ORF were obtained and it was found only one nucleotide difference between susceptible and resistance pea lines 1,200 kb upstream from the initiation codon. Moreover, mRNA expression analyses of the *eIF(iso)4E* between susceptible and resistant pea lines showed no significant changes and the single nucleotide

difference upstream of the *eIF(iso)4E* coding region did not drastically alter the eIF(iso)4E expression. Taken together, these results suggest that *cyv1* does not encode eIF(iso)4E.

2. Quantitative and qualitative involvement of P3N-PIPO in overcoming *cyv1* resistance against ClYVV.

In contrast to Cl-no30, isolate 90-1 Br2 overcame *cyv1*-mediated resistance. The region responsible for breaking *cyv1*-mediated resistance was mapped by examining infection of *cyv1* peas with chimeric viruses constructed from parts of Cl-no30 and 90-1 Br2. The breaking of resistance was attributed to the P3 cistron, which is known to produce two proteins: P3, from the main open reading frame (ORF), and P3N-PIPO, which has the N-terminal part of P3 fused to amino acids encoded by a small ORF (called PIPO) in the +2 reading frame. I introduced point mutations that were synonymous with respect to the P3 protein but non-synonymous with respect to the P3N-PIPO protein, and vice versa, into the chimeric viruses. Infection assay of plants with these mutant viruses revealed that both P3 and P3N-PIPO were involved in overcoming *cyv1*-mediated resistance. Moreover, P3N-PIPO quantitatively affected the virulence of Cl-no30 in *cyv1* peas. Additional expression *in trans* of the P3N-PIPO derived from Cl-no30, using *White clover mosaic virus* (WClMV) as a vector, enabled Cl-no30 to infect systemically in *cyv1* peas. Susceptible pea plants infected with chimeric ClYVV possessing the P3 cistron of 90-1 Br2, and which were therefore virulent toward *cyv1* peas, accumulated more P3N-PIPO than those infected with Cl-no30, suggesting that the higher level of P3N-PIPO contributed to the breaking of resistance by 90-1 Br2.

3. P3N-PIPO is required for cell-to-cell movement of ClYVV and P3N-afs facilitates P3N-PIPO-mediated cell-to-cell movement.

Recently, a new P3 cistron product, designated as P3N-afs, was found (Hagiwara-Komoda, personal communication), suggesting that P3 cistron produces three proteins, P3, P3N-PIPO, and P3N-afs. Both P3N-PIPO and P3N-afs deficient ClYVV mutant, pCl/P3ΔPIPO, failed to move from cell to cell. Expression of the P3N-PIPO *in trans* using WClMV enable the ClYVV mutant to move to adjacent cells in susceptible pea plants. Moreover, concurrent expression of P3N-PIPO and P3N-afs enable the ClYVV mutant to form larger infection foci than those formed when only P3N-PIPO was expressed. These results suggested that P3N-PIPO of ClYVV was essential in cell-to-cell movement and P3N-afs facilitated the movement.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.