学位論文内容の要旨

博士の専攻分野名称：博士（農学）

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学位論文題目

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）

The single positive RNA virus of 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 characterizing 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 cyvl resistance against CIYVV.

In contrast to Cl-no30, isolate 90-1 Br2 overcame cyvl-mediated resistance. The region responsible for breaking cyvl-mediated resistance was mapped by examining infection of cyvl 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 cyvl-mediated resistance. Moreover, P3N-PIPO quantitatively affected the virulence of Cl-no30 in cyvl peas. Additional expression in trans of the P3N-PIPO derived from Cl-no30, using White clover mosaic virus (WCIMV) as a vector, enabled Cl-no30 to infect systemically in cyvl peas. Susceptible pea plants infected with chimeric CIYVV possessing the P3 cistron of 90-1 Br2, and which were therefore virulent toward cyvl peas, accumulated more P3N-PIPO than those infected with Cl-no30 did, suggesting that the higher level of P3N-PIPO contributed to the breaking of resistance by 90-1 Br2.


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 CIYVV mutant, pCl/P3ΔPIPO, failed to move from cell to cell. Expression of the P3N-PIPO in trans using WCIMV enable the CIYVV mutant to move to adjacent cells in susceptible pea plants. Moreover, concurrent expression of P3N-PIPO and P3N-afs enable the CIYVV mutant to form larger infection foci than those formed when only P3N-PIPO was expressed. These results suggested that P3N-PIPO of CIYVV was essential in cell-to-cell movement and P3N-afs facilitated the movement.