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

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 CIYVV.

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 CIYVV 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.


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 WClMV 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.

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