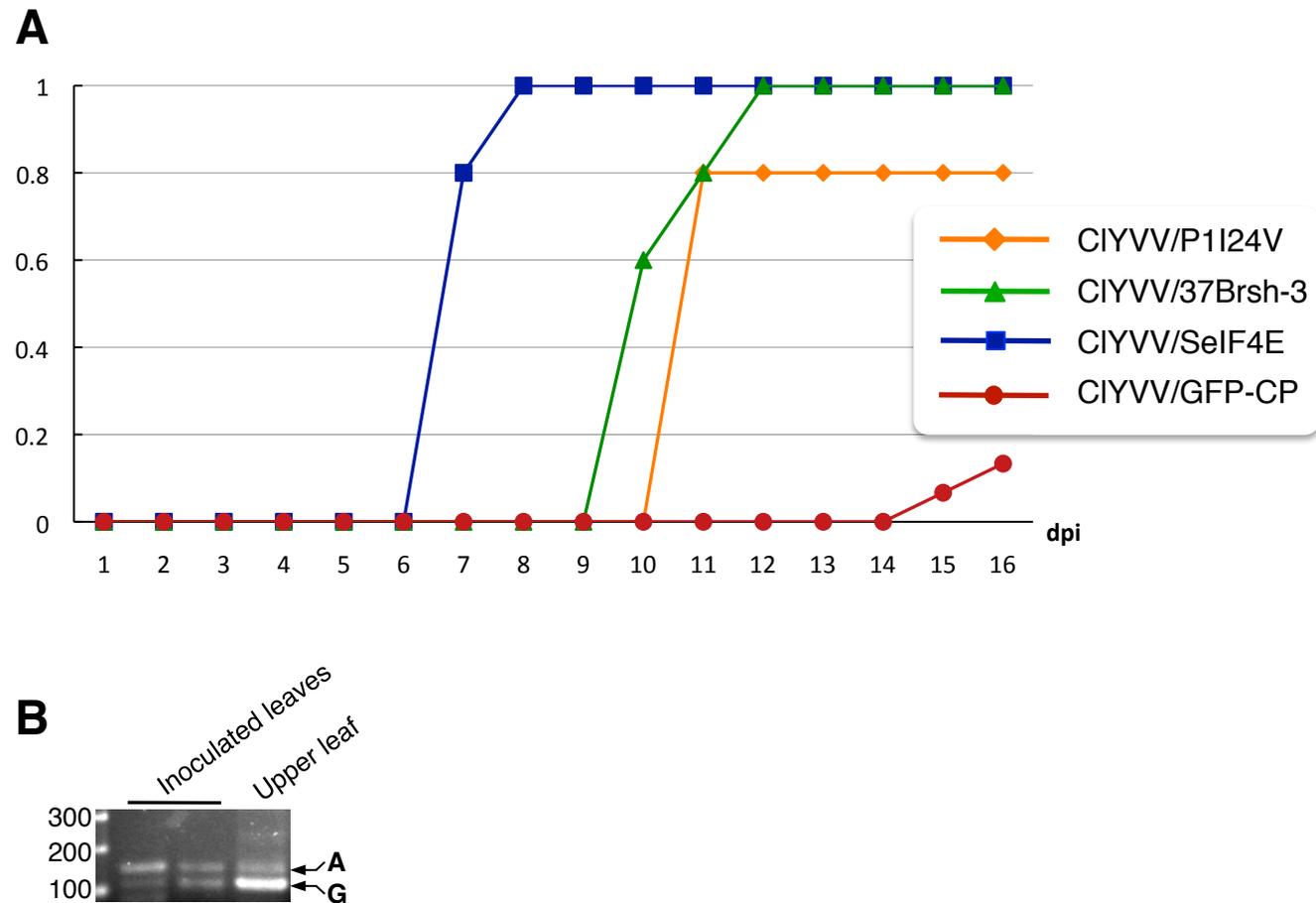




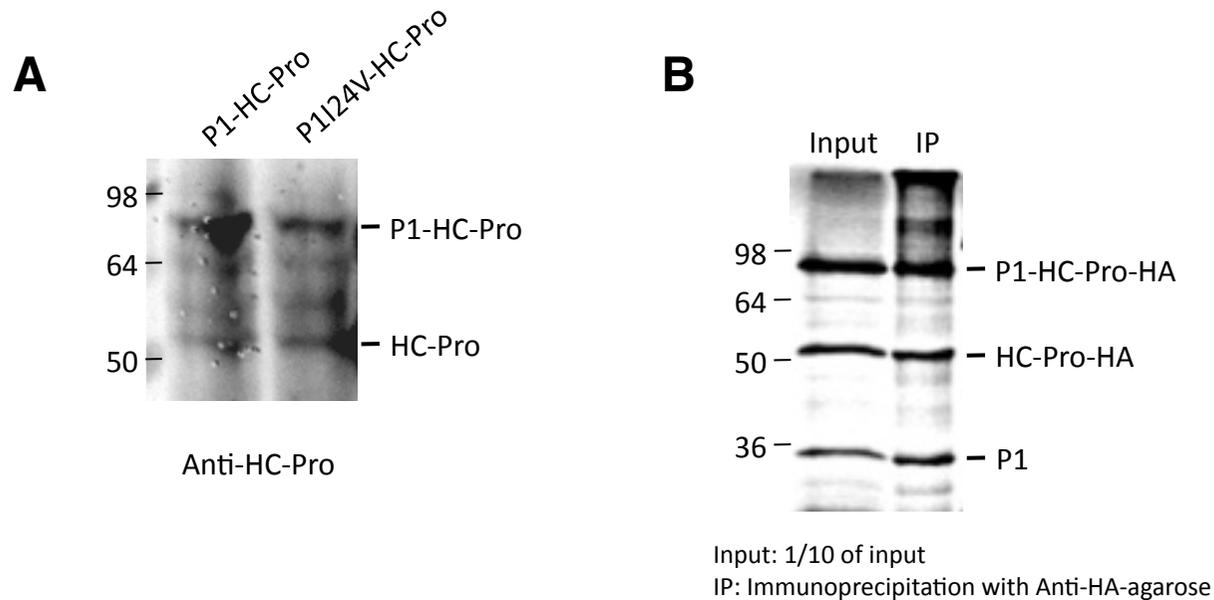
HOKKAIDO UNIVERSITY

Title	Involvement of the P1 Cistron in Overcoming eIF4E-Mediated Recessive Resistance Against Clover yellow vein virus in Pea
Author(s)	Nakahara, Kenji S.; Shimada, Ryoko; Choi, Sun-Hee et al.
Citation	Molecular Plant-Microbe Interactions, 23(11), 1460-1469 https://doi.org/10.1094/MPMI-11-09-0277
Issue Date	2010-11
Doc URL	https://hdl.handle.net/2115/44063
Type	journal article
File Information	SupFig.pdf, Supplementary Fig

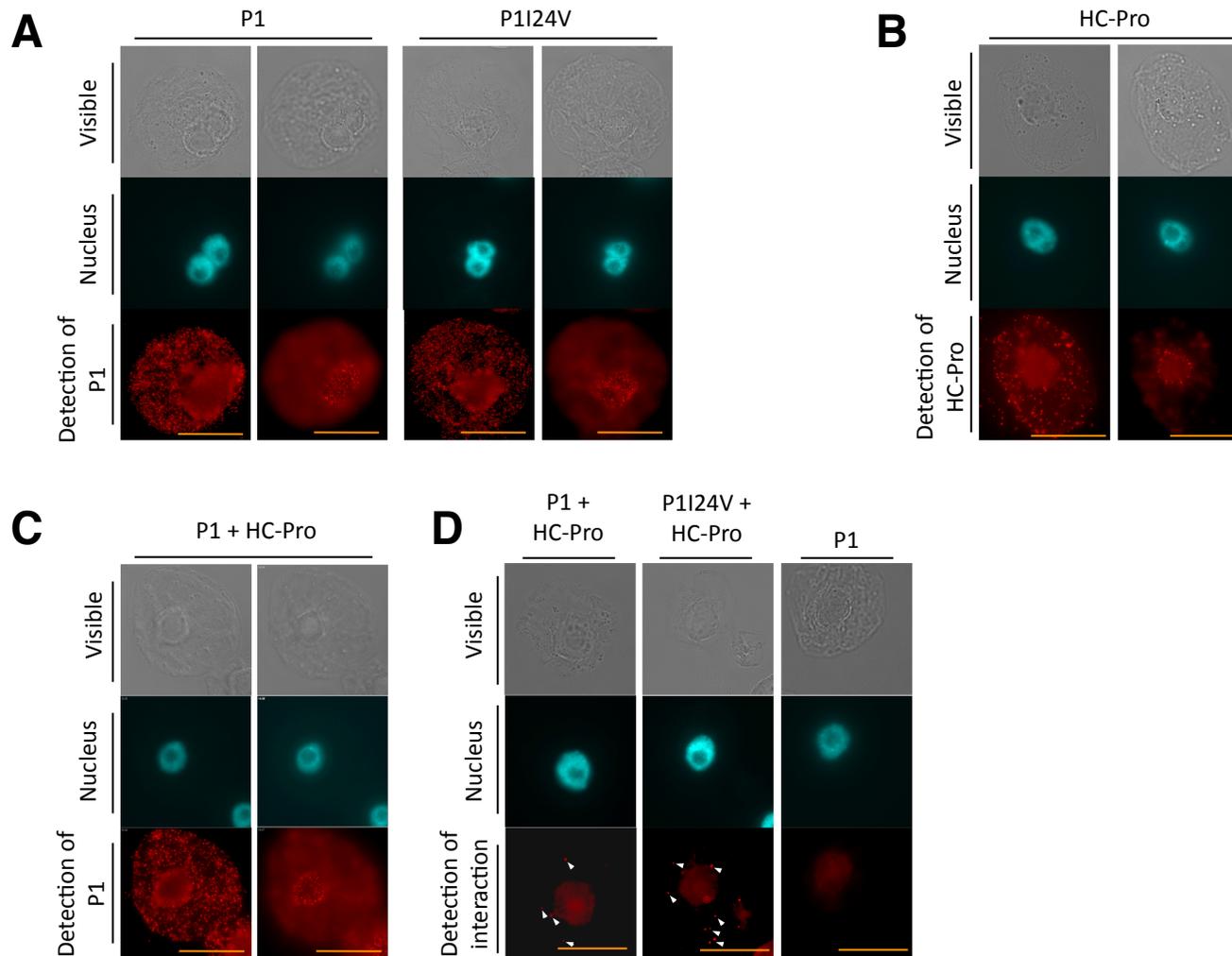




Supplementary Fig. S1. A, Comparison of systemic spread of inoculated viruses, wild-type CIYVV/GFP-CP (red circle, $n = 15$), the point mutant CIYVV/P1I24V (orange diamond, $n = 5$), the RB CIYVV/37Brsh-3 (green triangle, $n = 5$), CIYVV expressing the susceptible eIF4E, and CIYVV/SeIF4E (blue square, $n = 5$) in the resistant pea line PI 378159. Systemic infection of the inoculated viruses was monitored by observation of symptom expression and confirmed by detecting GFP fluorescence until 16 days post-inoculation (dpi). The proportion of systemically infected plants is plotted in a graph. Essentially the same result with Fig. 5B was obtained. Systemic infected plants inoculated with wild-type CIYVV/GFP-CP were unexpectedly observed. **B**, Progeny in inoculated and upper infected leaves of a plant, which was inoculated with wild-type virus, were subjected to dCAPS with *afaI* analysis. The A to G substitution mediating RB was detected in both inoculated and upper leaves, indicating that the *cyp2* resistance was broken by RB virus but not by wild-type one.



Supplementary Fig. S2. Autoproteolytic activity of P1 (A) and interaction between P1 and HC-Pro proteins shown by immunoprecipitation (B). **A**, T7 promoter followed by P1-HC-Pro-fused cDNA and 30 nucleotides of poly A with/without the A to G substitution were prepared by PCR. Using these fragments, P1 and HC-Pro fusion protein were *in vitro* translated in rabbit reticulocyte lysates and subjected to western blot analysis using anti-HC-Pro monoclonal antibody. Not only P1-HC-Pro fusion but also HC-Pro monomer were detected in both lanes, indicating that autoproteolytic activity of P1 is not affected by the substitution involved in RB. **B**, CIYVV P1-HC-Pro fusion tagged with HA (HC-Pro-HA) protein was *in vitro* translated in rabbit reticulocyte lysates containing ^{35}S -methionine. When HC-Pro-HA and its associates were immunoprecipitated by an anti-HA antibody, the P1 was precipitated, indicating the physical interaction between P1 and HC-Pro proteins.



Supplementary Fig. S3. Interactions between transiently expressed Myc tagged-P1 with/without the substitution involved in the *cyv2*-RB and HC-Pro were *in situ* detected in the proximity ligation assay (PLA) as fluorescent signals in BY2 cells (D). **A-C**, For detection of each protein, these proteins were solely detected by single recognition PLA with anti-Myc (A, P1) and anti-HC-Pro (B, HC-Pro) antibodies. Left and right panels were focused on cytoplasm and nucleus, respectively. Since PLA signals were detected in both cytoplasm and nucleus, P1 with/without the substitution were dispersed thoroughly in BY2 cells. HC-Pro was also detected in both cytoplasm and nucleus. However, in nucleus, HC-Pro tend to be border of nucleus. P1 distribution was not affected by HC-Pro expression simultaneously (C). **D**, For detection of interactions, BY2 cells expressing both proteins were subjected to PLA analysis. Weaker PLA signals (open arrowheads) were detected in both wild P1 and mutant P1 expressed cells, indicating that the substitution did not affect the P1-HC-Pro interaction. The interaction was only detected in cytoplasm though both proteins were dispersed in cells. Scale bar is 25 μ m.