Molecular analysis of a necrotic cell death induced in cucumber mosaic virus-inoculated Arabidopsis thaliana

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論文内容要旨

Molecular analysis of necrotic cell death induced in cucumber mosaic virus-inoculated *Arabidopsis thaliana*

（キュウリモザイクウイルス感染シロイヌナズナに誘導される壊疽性細胞死に関する研究）

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Chapter 1 Introduction

In modern biology, the genetically programmed death of cells (PCD) is considered as a fundamental process of life. It is a process activated and actuated by cell itself and is well organized at genetic and biochemical levels [1]. In plant, cells and tissues undergo various types of cell death in several aspects of plant life [1]. Flower development is radically affected by PCD of selected cells or groups of cells [2]. At early stage of flower development, male and female flowers are indistinguishable. During flower formation, at a developmental stage that varies with plant species, either male or female parts cease growing and are eliminated via a cell death program. Senescence is a highly regulated process for maximum recovery of nutrients from the senescing tissues, in which metabolic pathways are changed through PCD [3]. Besides of plant growth and development, cell death also plays a crucial role in plant response to adverse environmental stimulations [4].

Cell death in virus-infected plants is a critical event for survival of virus because virus multiplication completely depends on host cell metabolism. The role of cell death in virus-host plant interaction is a pending issue that has been discussed for a long term [5-9]. Backing to 1923, a study on a plant invaded by a pathogen, that was the first study to introduce the concept of PCD as a process activated by plants to defend themselves against the infection of pathogens [10]. According to Barlow's opinion that if cells die at a predictable time and location, or if the death has some beneficial effect on tissue differentiation and is inherited in the next generation, this cell death will be classified as programmed cell death [11,12]. This definition excluded necrosis as cell death due to accidental or random injury such as exposure to some toxins or a lethal temperature. However, history has revealed that necrosis in many plant processes is programmed and meets many or all of Barlow's criteria [12]. Thus, programmed cell death resulting from symptom formation in incompatible interactions between viruses and plants has been described as necrotic local lesions, and that in compatible interactions was described as necrotic cell death [13,14]. The necrotic local lesions developed at primary viral infection sites on host plants that carry nucleotide-binding and leucine-rich repeat (NB-LRR) class R protein-coding virus resistance (R) genes, has been thoroughly analyzed. Furthermore, this kind of cell death has long been recognized as a hallmark of the hypersensitive response (HR) and R protein-mediated resistance to viruses [10,15-17]. Thus, the cell death, which developed at necrotic local lesions, has been named as HR cell death and well characterized [10,18-20].

Since HR cell death is critical to virus multiplication, the further spread of the virus into living cells surrounding necrotic local lesions should be prevented. However, virus still can move into the living cells surrounding necrotic local lesions [21-24]. Thus, the role of HR cell death in virus resistance remains to be explained. Meanwhile, in comparison with HR cell death, necrotic cell death seems to be poorly understood. Especially it remains unclear if necrotic cell death resulted from

non-specific damages to host cells caused by virus infection, rather than as a form of programmed cell death. To address this issue, we focused on cucumber mosaic virus (CMV), which is one of the best characterized tripartite single-strand positive-sense RNA viruses [25]. In analyzing the host response to a series of reassortant viruses between two CMV strains with different virulence in *Arabidopsis thaliana*, we discovered that cell death occurred in virus- inoculated leaves of *A. thaliana* ecotype Col-0. In this study, the feature of the cell death was analyzed by comparison with HR cell death in chapter 2, and then the viral determinant region in CMV genomes for the induction of the cell death was analyzed in chapter 3. Finally, the differences and similarities between the cell death, which was studied in this study, and other well-characterized cell death including HR cell death were discussed in chapter 4.

Chapter 2 Identification of necrotic cell death induced in CMV inoculated *Arabidopsis thaliana*

Part 1 Response of *Arabidopsis thaliana* ecotype Col-0 to a series of reassortant CMVs

A series of reassortant viruses was produced between CMV(Y) and CMV(H) (Figure 1). The reassortant CMVs containing CMV(H) RNA1: CMV(HHY), CMV(HYY) and CMV(HYH) were identified with an ability of inducing a cell death in the inoculated leaves of Col-0 at 5 days post-inoculation (dpi) (Figure 2A), whereas HR cell death (necrotic local lesions) was induced in CMV(Y)- inoculated leaves of *RCY1*-transformed Col-0 (Col::RCY1) at 3 dpi (Figure 2C). These results indicated that CMV(H) RNA1 might determine to induce the cell death in reassortant CMV-inoculated leaves through its interaction with CMV(Y) RNA2 or CMV(Y) RNA3. Further, the accumulation of the coat protein of CMV(HHY), CMV(HYY) and CMV(HYH) in virus-inoculated Col-0 leaves was detected at a similar level to the other CMVs which do not have the ability of the cell death induction (Figure 2B). Furthermore, comparison of the intensity of the norther blot analysis bands of CMV RNA1, RNA2 and RNA3 among the leaves inoculated with eight CMVs [CMV(H), CMV(Y) or one of six reassortant CMVs] suggests that there is no significant correlation between the induction of cell death and the accumulated level of CMV RNAs or the ratio of CMV RNA1, RNA2 and RNA3 (Figure 3). These results indicate that the cell death developing on the leaves inoculated with reassortant CMV carrying CMV(H) RNA1, seems to not suppress virus replication but allows it to multiply at the same level as with a susceptible interaction.

The spread of the virus was restricted to the CMV(Y)-inoculated leaves accompanying the development of HR cell death (Figure 4A). However, the reassortant CMV(HYY) spread around the whole plants and provoke systemic stunting and weak yellowing symptoms in non-inoculated upper leaves of Col-0 (Figure 4A and 4B). These results indicated that reassortant CMV carrying CMV(H) RNA1 together with CMV(Y) RNA2 and RNA3 could induce the non-HR cell death

only in inoculated Col-0 leaves, however, it did not suppress the virus multiplication or systemic spread to non-inoculated upper leaves of Col-0.

Part 2 Comparison of global gene expression pattern between two types of cell death in CMV-inoculated leaves of *A. thaliana*.

To further characterize the cell death in CMV-inoculated Col-0 leaves, global gene expression patterns were compared by RNA-Seq between CMV(HYY)-inoculated Col-0 leaves showing the non-HR cell death and CMV(Y)-inoculated Col::RCY1 leaves showing HR cell death. Differentially expressed genes (DEGs) were analyzed by DEseq2. The number of genes for which transcript expression increased >4-fold or decreased <0.25-fold in CMV(Y)-inoculated Col::RCY1 leaves showing HR cell death was much greater than that in CMV(HYY)-inoculated Col-0 leaves showing non-HR cell death (Figure 5). Furthermore, the gene ontology enrichment analysis of the DEGs also indicated a difference between non-HR cell death and HR cell death. Therefore, non-HR cell death observed in CMV(HYY)-inoculated Col-0 leaves might be a form of necrotic cell death that does not contribute to the resistance to CMV and is different from HR cell death.

Part 3 Response of *A. thaliana* ecotypes to CMV(HYY)

When 94 ecotypes of *A. thaliana* were inoculated with CMV(HYY), necrotic cell death was induced in inoculated leaves of 92 ecotypes, but not observed in ecotypes Mt-0 and Stw-0 at 14 dpi under both bright field and with trypan-blue staining (Figure 6A and 6B). The CMV coat protein was detected in CMV(HYY)-inoculated Mt-0 and Stw-0 at similar levels compared with the virus-inoculated leaves of the other ecotypes (Figure 6C). Thus, *A. thaliana* ecotypes appear to generally develop necrotic cell death in response to CMV(HYY).

Part 4 Discussion of Chapter 2

Recently, evidence is accumulating that systemic necrosis, which was thought to be symptoms in compatible interaction between virus and host plant, may be result from the induction of HR cell death with incomplete restriction of virus spread in host plants [26-31]. The lethal systemic cell death might have been caused by delayed HR cell death and escape of the virus to distant tissues [27,32]. This phenomenon indicates that virus-induced systemic necrosis could be the result of incompatible interactions between host plants carrying *R* genes and avirulent strains of virus that lead to runaway cell death. On the other hand, necrotic cell death in CMV(HYY)-inoculated Col-0 leaves did not develop systemically, even though the virus particles did systemically spread to non-inoculated upper leaves of Col-0 plants (Figure 3A). The fact further indicates that necrotic cell death developed in CMV(HYY)-inoculated Col-0 leaves, might be a symptom of a compatible interaction between *A. thaliana* Col-0 and CMV, but not a resistance response to

CMV. Furthermore, such phenomenon that different symptoms developing in inoculated and non-inoculated leaves could also be observed in *HRT* carrying *Arabidopsis* and turnip crinkle virus (TCV) interactions, tobacco cultivar Taiyan8 and CMV interaction, and potato and potato virus Y (PVY) interactions [33-36]. And in studies of potato and PVY interactions, the expression pattern of miRNA/mRNA were altered differently in the inoculated and non-inoculated upper leaves and the metabolic responses to PVY infection were less intensive in non-inoculated leaves compared to inoculated leaves [36]. Therefore, more researches were needed to analyze the disease development in Col-0 and CMV(HYY) interaction through detailly study of the symptom development in both inoculated and non-inoculated leaves with biochemical and genetical methods.

Chapter 3 Analysis of the determinant region in CMV RNA1 for induction of necrotic cell death in virus-inoculated leaves

Part 1 Analysis of viral sequence in CMV RNA1 viral sequence for induction of necrotic cell death in virus-inoculated leaves

Twenty-six non-synonymous amino acid substitutions in CMV(H) and CMV(Y) RNA1 encoding 1a protein could be observed in Figure 7. To identify the region of 1a protein encoded by CMV(H) RNA1 responsible for inducing necrotic cell death in virus-inoculated Col-0 leaves, a series of chimeric cDNAs between CMV(Y) and CMV(H) RNA1 was generated and cloned under the control of the T7 promoter (Figure 8A, 9A and 10A). Each infectious RNA1 was transcribed *in vitro* from each chimeric cDNA vector and combine with infectious RNA2 and RNA3 from CMV(Y) and used as inoculum. The determinant region of the development of necrotic cell death was first narrowed down to the region of the 1a protein that does not contain the helicase (HEL) domain (Figure 8). According to the results shown in Figure 9, the determinant for inducing necrotic cell death likely maps to the 5' region of RNA1, which corresponds to nucleotide positions 1 to 1126 in the 1a protein-coding region and includes the methyltransferase (MET) domain containing 11 amino acid substitutions (Figure 9). Then, it was found out that the development of the necrotic cell death seems to be determined by two independent regions of the 1a protein-coding region from nucleotide positions 1 to 310 or from 968 to 1126, which does not include the MET domain but each contains 3 amino acid substitutions besides the MET domain (Figure 7 and 10). Systemic cell death was not observed in the upper leaves of each reassortant CMV-inoculated plant, although the CMV coat protein was detected at similar levels in virus-inoculated leaves and non-inoculated upper leaves (Figure 12).

Part 2 Analysis of single amino acid substitutions in the CMV 1a protein for induction of necrotic cell death in virus-inoculated leaves

To determine which amino acid in each region of the 1a protein induces necrotic cell death, nucleotide substitutions resulting in single amino acid substitutions were generated in each CMV(Y) 1a protein-coding region (Figure 11A). Necrotic cell death was induced in Col-0 leaves inoculated with each of the 6 single amino acid mutated reassortant CMVs (Figure 11B).

Systemic cell death was not observed in the upper leaves of each reassortant CMV-inoculated plant, although the CMV coat protein was detected at similar levels in virus-inoculated leaves and non-inoculated upper leaves (Figure 12). These results indicated that amino acid residues 29, 49, 54, 298, 299 and 310, which are located around the MET domain in the 1a protein encoded on CMV(H) RNA1, independently determine the induction of the necrotic cell death upon Col-0 leaves with co-infection of CMV(Y) RNA2 and RNA3.

Part 3 Discussion of Chapter 3

The development of the necrotic cell death in Col-0 is necessary for co-infection of CMV(Y) RNA2 and CMV(Y) RNA3 with a CMV(Y) RNA1 encoding a 1a protein carrying single amino acid substitutions around its MET domain. This suggests that necrotic cell death must not be an artifact of a heterogenous interaction between the CMV(H) 1a protein and other proteins encoded on CMV(Y) RNA2 and RNA3. A single amino acid substitution from R to C at amino acid position 461 of the 1a protein resulting in an HR-like necrotic phenotype in virus-inoculated leaves of *Nicotiana tabacum* without affecting virus multiplication has been reported [37]. Modeling has also demonstrated this phenotype was associated with structural changes in the 1a protein caused by amino acid substitutions at position 461 [38]. The amino acid at position 461 of 1a protein of CMV was localized between the MET domain and HEL domain of 1a protein. In our experiments, single amino acid substitutions residues 29, 49, 54, 298, 299, or 310 of the 1a protein, which could independently induce the necrotic cell death in *A. thaliana* Col-0 were localized at in both the N- and C-terminal regions around the MET domain (amino acid 72 to 290). A putative hinge is located between the MET and HEL domains of the 1a protein [39]. The region of the 1a protein N-terminal to the hinge appears to self-interact to form homodimers in a yeast-two hybrid system [39], and mutation of the amino acid residues in the MET domain disrupts capping activities and virus replication [40]. However, single amino acid substitutions around the MET domain of 1a protein of CMV(Y) did not affect virus multiplication and systemic spread in the host plants in our experiment. Thus, in our experiment, changes in the degree of self-interaction or homodimer structure of the 1a protein, which could be resulted from the single amino acid substitutions around its MET domain, might be associated with the induction of necrotic cell death in *A. thaliana* Col-0 leaves.

While further study is needed to elucidate the mechanisms by which necrotic cell death is induced by single amino acid substitutions in the N- and C-terminal regions around the MET domain of the 1a protein, our results suggest that necrotic cell death can develop without preventing virus infection and is not caused by the stress of virus infection but the specific interaction between virus and host plants.

Chapter 4 Differences and similarities between necrotic cell death and other well-characterized cell death

The induction of cell death is a dominant feature during plant and virus interactions. Combining with the Figure 13, when the resistance gene of host plant recognized the avirulent gene of virus, HR cell death was induced in the virus-inoculated leaves and the virus was blocked to the HR lesion during the resistance response [41]. On the other hand, when the virus escaped from the recognition of host plant, lethal systemic necrosis was induced by virus systemic spread in the plants. Some recent studies suggesting that systemic necrosis could be induced through a delayed HR in the virus inoculated plants, is accumulating, although lethal systemic cell death is categorized in “compatible interaction” between host and virus [42]. Intriguingly, in this study, we identified necrotic cell death, which is only induced in CMV(HYY)-inoculated Col-0 leaves. CMV(HYY) systemically infected the plant and induced systemic stunting and yellowing symptoms but no systemic necrotic cell death. And the necrotic cell death could also be induced in various ecotypes of *A. thaliana* in response to the inoculation with CMV(HYY). The global gene expression of leaves showing necrotic cell death greatly differs from those showing HR cell death. Furthermore, single amino acid substitutions at residues 29，49，54，298，299 or 310 in both the N- and C-terminal regions around the MET domain of the 1a protein of CMV(HYY) could independently induce the development of necrotic cell death in Col-0 without affecting virus multiplication and systemic spread in the host plants. In conclusion, the characterization of the necrotic cell death suggests that necrotic cell death induced in CMV(HYY)-inoculated Col-0 leaves was a different type of cell death, which should be distinct to well-characterized HR cell death and systemic necrosis. The discovery of the new type of CMV 1a protein-mediated necrotic cell death could give us a novel knowledge to realize the induction of cell death in host plants to virus infection.

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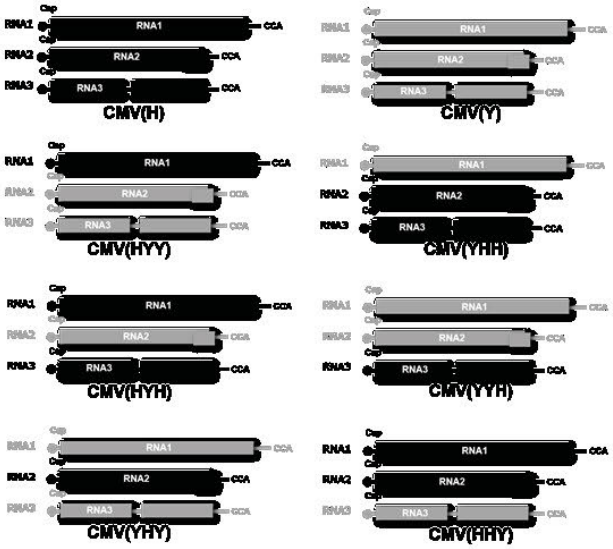

Figure 1. Schematic diagrams of the RNA genomes of the reassortant CMVs, derived from RNA1, RNA2, and RNA3 of CMV(H) and CMV(Y) (upper part of figure), used in the present study. Black indicates the CMV(H) genomes; gray, the CMV(Y) genomes.

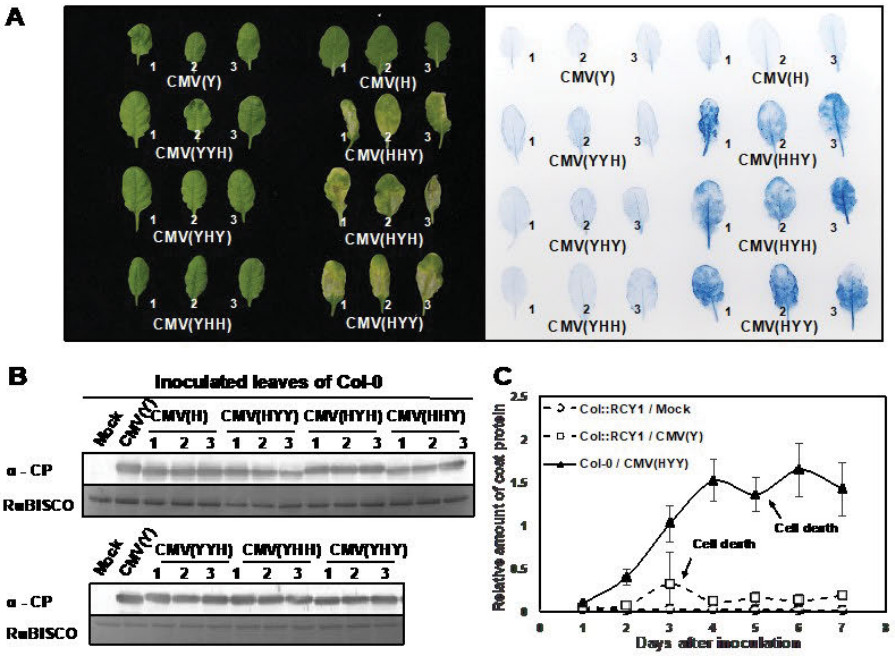

Figure 2. Response of virus-inoculated leaves of *Arabidopsis thaliana* ecotype Col-0 to CMV(H), CMV(Y) or a series of reassortant CMVs, and virus multiplication in the inoculated leaves. (A) Development of cell death in leaves with a series of reassortant CMVs, or with CMV(H) or CMV(Y) as a control. Representative virus-inoculated Col-0 of 3 independent plants (plant numbers 1, 2 and 3) under bright field (left panel) and stained with trypan blue (right panel). (B) CMV CP detected immunologically by western blotting at 7 dpi in the leaves of plants inoculated with one of a series of reassortant CMVs. CMV(Y)-inoculated Col-0 leaves and mock-inoculated Col-0 leaves were used as positive and negative control. RuBISCO protein is shown as an internal reference for protein quantity. (C) Time course of virus multiplication in Col-0 leaves inoculated with CMV(HYY) carrying CMV(H) RNA1 [Col-0/CMV(HYY)], CMV(Y)-inoculated Col::RCY1 leaves [Col::RCY1/CMV(Y)], and mock-inoculated Col::RCY1 leaves [Col::RCY1/Mock]. CMV CP quantities were measured using ELISA (mean values of relative amount of CP of three independent biological samples with standard error bars).

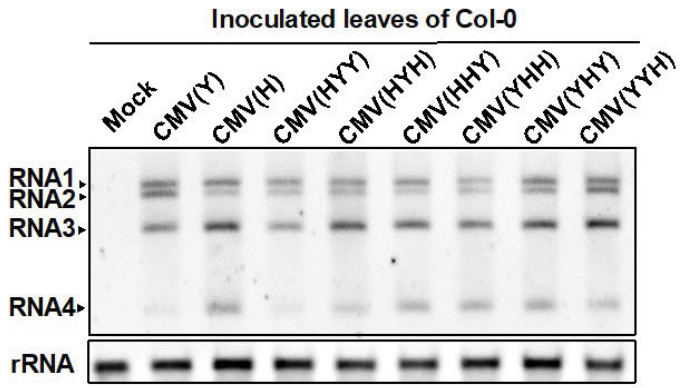

Figure 3. Detection of CMV RNA in virus-inoculated leaves of *Arabidopsis thaliana* ecotype Col-0. CMV RNA1, 2, 3 and 4 were detected by northern blot hybridization analysis of total RNA extracted from virus-inoculated leaves of Col-0 inoculated with CMV(Y), CMV(H) and a series of reassortant CMVs (as indicated) at 5 dpi. Mock-inoculated Col-0 leaves were used as a control. Total RNA was extracted from three independent samples. The position of CMV RNA is indicated at left: RNA 1, 2 and 3 represent genomic RNAs; RNA4 is subgenomic. rRNA is the loading control.

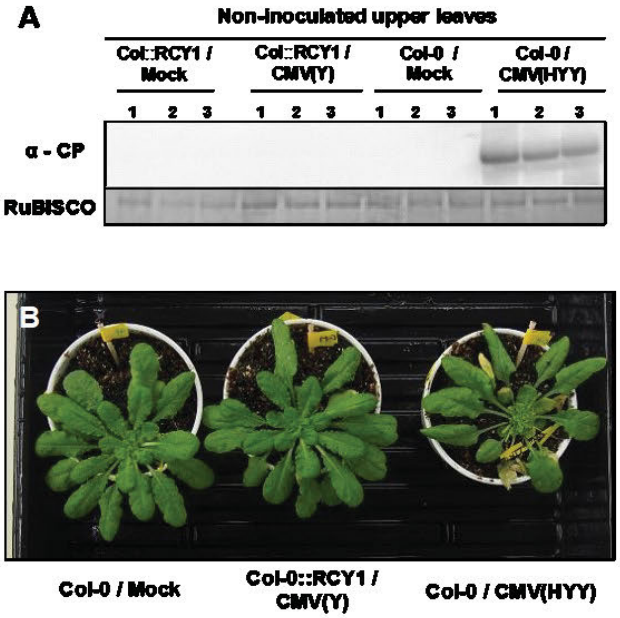

Figure 4. Detection of CMV CP in non-inoculated upper *Arabidopsis thaliana* ecotype Col-0 leaves and systemic symptom development. (A) CMV CP detected at 7 dpi, by western blot analysis, in non-inoculated upper leaves of CMV(HYY)-infected or mock-inoculated Col-0 [Col-0/CMV(HYY) and Col-0/Mock] and CMV(Y)-inoculated or mock-inoculated Col::RCY1 [Col::RCY1/CMV(Y) and Col::RCY1/Mock]. RuBISCO protein is an internal reference for protein quantity. Each experiment was conducted using three independent biological replicates (plant numbers 1, 2, and 3). (B) Symptom appearance observed at 14 dpi on CMV(HYY)-inoculated Col-0 [Col-0/CMV(HYY)], CMV(Y)-inoculated Col::RCY1 [Col::RCY1/CMV(Y)], or mock-inoculated Col-0 [Col-0/Mock] (control). Virus-inoculated leaves have been removed because they were already dead at this stage. Representative plants were photographed.

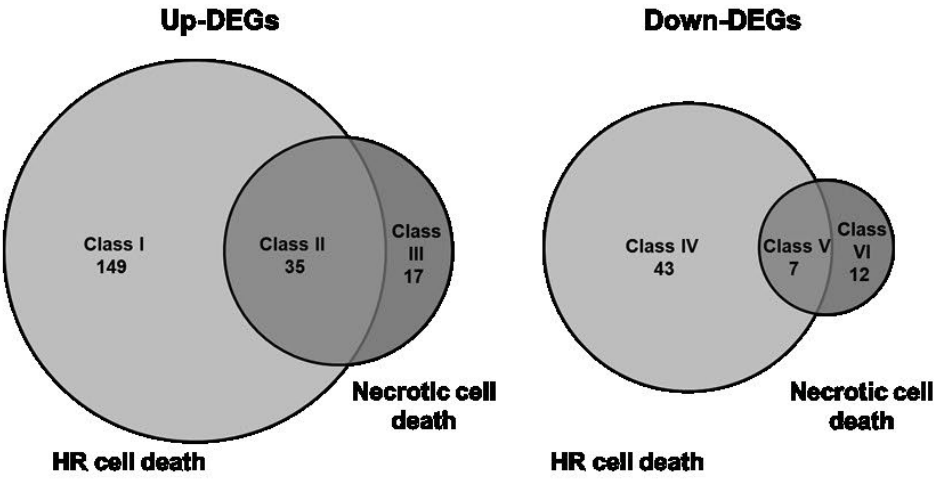

Figure 5. Venn diagram of the number of genes with increased or decreased transcript abundance in CMV(HYY)-inoculated *Arabidopsis thaliana* Col-0 leaves showing necrotic cell death and CMV(Y)-inoculated Col::RCY1 leaves showing HR cell death. The number of genes detected by RNA-Seq analysis with more than 4-fold increased expression and adjusted $p < 0.05$ in CMV(HYY)-inoculated Col-0 leaves and CMV(Y)-inoculated Col::RCY1 leaves are shown at left (Up-DEGs). Those with less than 0.25-fold decreased expression ($p < 0.05$) are shown at right (Down-DEGs). The number of genes with increased and decreased expression in leaves showing HR cell death are shown in the light gray circles; and those showing necrotic cell death in the dark gray circles.

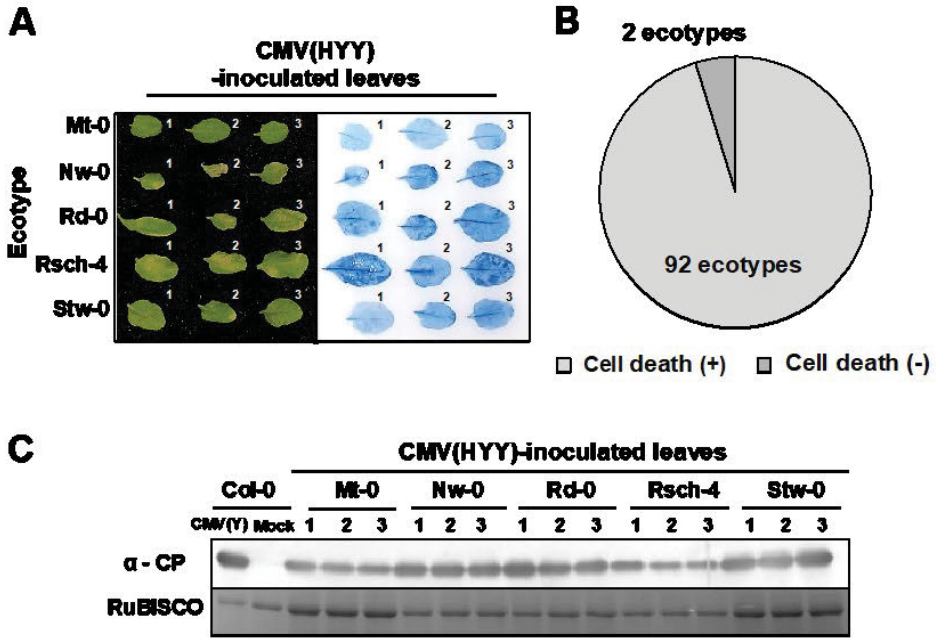

Figure 6. Survey of the response to CMV(HYY)-inoculation on the leaves of 94 ecotypes of *Arabidopsis thaliana*. (A) Representative photograph of the responses: CMV(HYY)-inoculated leaves of five ecotypes randomly selected at 14 dpi. Virus-inoculated leaves under bright field (left panel), and stained with trypan blue (right panel). (B) Pie chart summary of CMV(HYY)-inoculated leaves of the 94 ecotypes. (C) CMV CP detected immunologically by western blot analysis in virus-inoculated leaves of three independent biological replicates (numbers 1, 2, and 3) of five selected ecotypes at 7 dpi. RuBISCO protein is an internal reference for protein quantity.

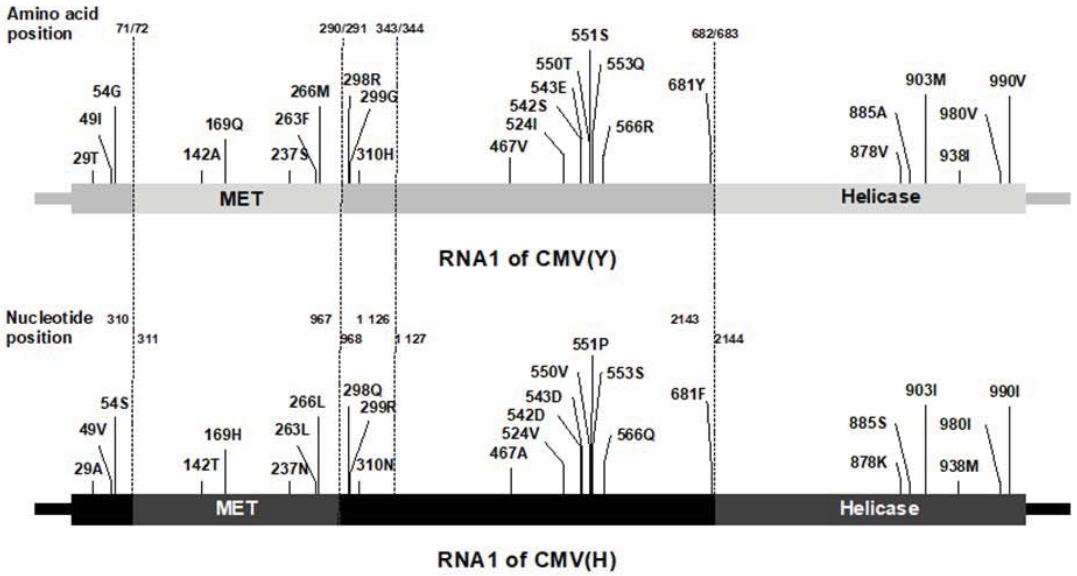

Figure 7. Schematic diagram of the CMV RNA1 encoding the 1a protein, which contains a methyltransferase (MET) and a helicase (HEL) domain. The 1a protein-coding region and corresponding 1a protein are shown as rectangles. Amino acids that differ between the 1a proteins encoded by the CMV(H) RNA1 (lower panel) and the CMV(Y) RNA1 (upper panel) and the positions of these amino acids in the 1a protein are described above each rectangle. The dotted lines connecting the two chimeric constructs indicate the amino acid and nucleotide positions of junction sites. The adjacent sequence numbers of each of these junctions are indicated above the CMV(Y) RNA1 (upper) schematic for amino acids; and above the CMV(H) RNA1 (lower) schematic for the corresponding nucleotides.

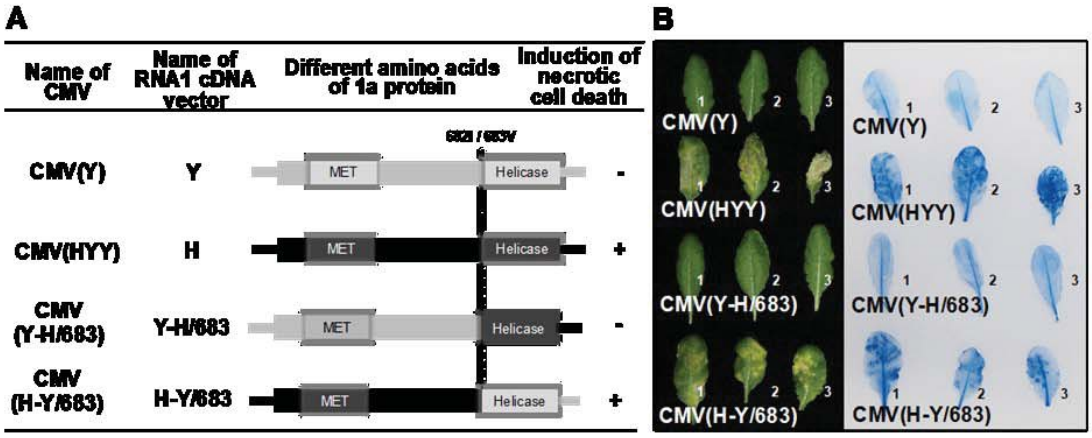

Figure 8. Induction of necrotic cell death in *Arabidopsis thaliana* ecotype Col-0 leaves inoculated with reassortant CMVs, CMV(H-Y/683) and CMV(Y-H/683), which carry chimeric 1a protein of CMV(Y) and CMV(H), and CMV(Y) and CMV(HYY). (A) Schematic diagram of the CMV RNA1 encoding the 1a protein. The 1a protein-coding region (and its corresponding 1a protein) is presented as rectangles in black for CMV(H) and in gray for CMV(Y). The dotted line indicates the junction site at amino acid position 682/683. MET, 1a protein methyltransferase domain; HEL, helicase domain. The presence (+) or absence (−) of necrotic cell death induction in virus-inoculated leaves is shown in the column on the right. (B) Responses of CMV-inoculated leaves for three independent biological replicates (plants number 1, 2, and 3). Virus-inoculated leaves to the left are under bright field and those to the right are stained with trypan blue.

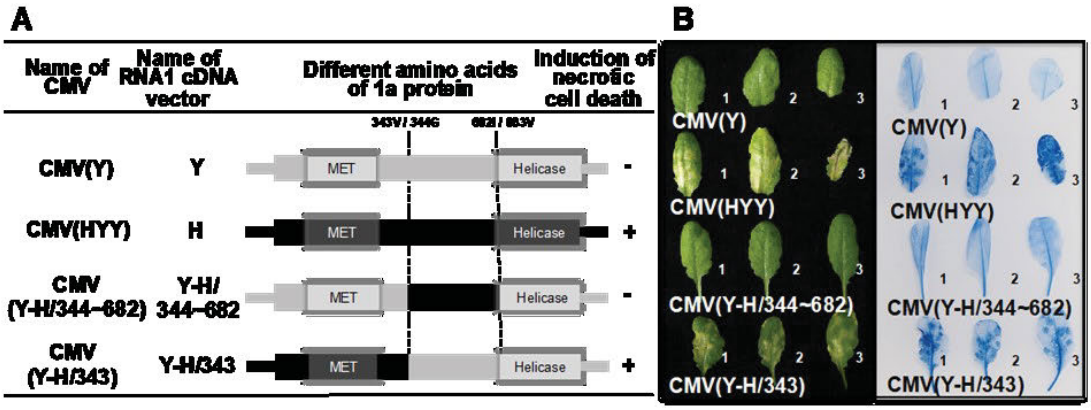

Figure 9. Induction of necrotic cell death in *Arabidopsis thaliana* ecotype Col-0 leaves inoculated with reassortant CMVs, CMV(Y-H/343) and CMV(Y-H/344~682) which carry chimeric 1a protein of CMV(Y) and CMV(H), and CMV(Y) and CMV(HYY). (A) Schematic diagram of the CMV RNA1 encoding the 1a protein (representation as in Fig.7: see legend for details). The junction sites in chimeric 1a proteins at amino acid positions 343/344 and 682/683 are indicated by dotted lines. (B) Responses of CMV-inoculated leaves (for details, see legend to Fig. 8).

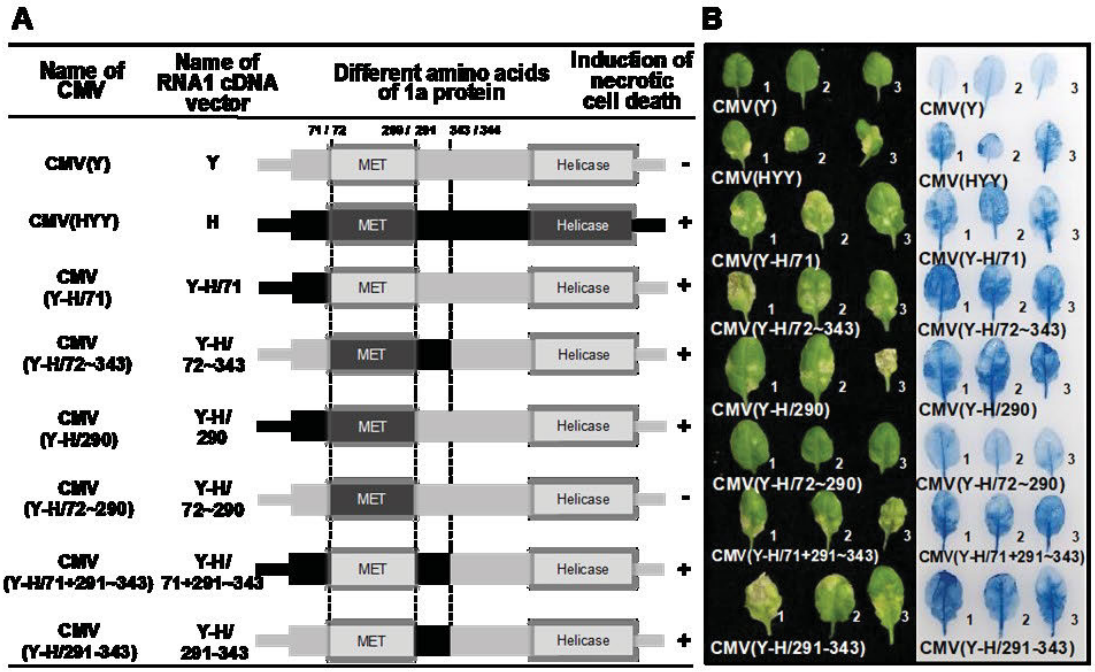

Figure 10. Induction of necrotic cell death in *Arabidopsis thaliana* ecotype Col-0 leaves inoculated with reassortant CMVs, CMV(Y-H/71), CMV(Y-H/72~343), CMV(Y-H/290), CMV(Y-H/72~290), CMV(Y-H/71+291~343) and CMV(Y-H/291~343), which carry chimeric 1a protein of CMV(Y) and CMV(H), and CMV(Y) and CMV(HYY). (A) Schematic diagram of the CMV RNA1 encoding the 1a protein (representation as in Fig.7: see legend for details). Dotted lines indicate the chimera junction sites at amino acid positions 71/72, 290/291, and 343/344. (B) Responses of CMV-inoculated leaves (for details, see legend to Fig. 8).

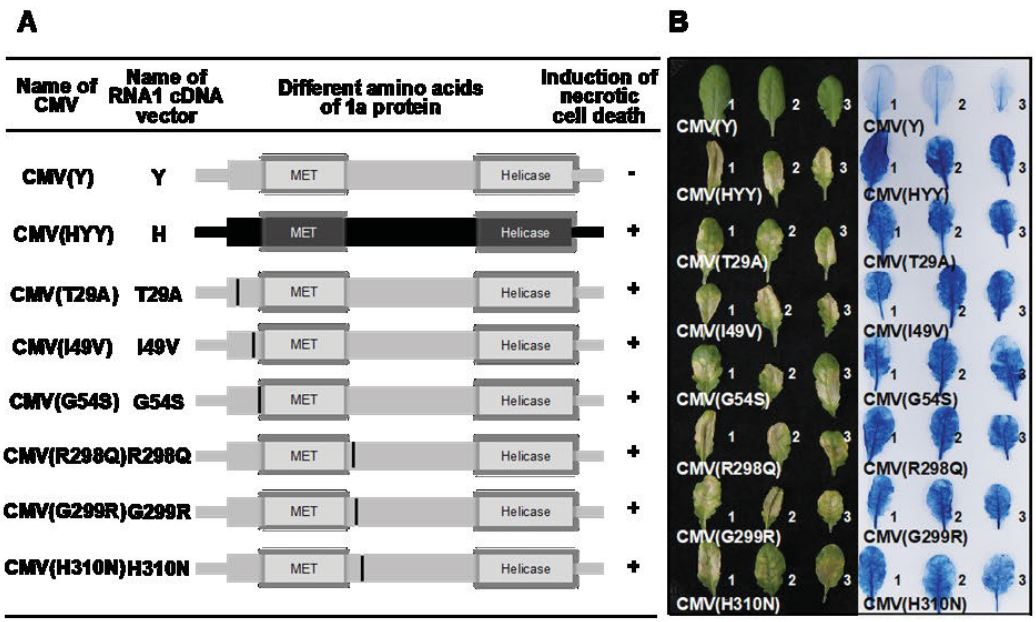

Figure 11. Induction of necrotic cell death in *Arabidopsis thaliana* ecotype Col-0 leaves inoculated with CMVs carrying single amino acid substitutions in the 1a protein of CMV(Y). (A) Schematic diaram of the CMV RNA1 encoding the 1a protein. The 1a protein-coding region and corresponding 1a protein are shown as rectangles. Deduced single amino acid substitutions and their positions in the 1a protein are shown as a black bar (representation as in Fig.7: see legend for details). (B) Responses of CMV-inoculated leaves (for details, see legend to Fig. 8).

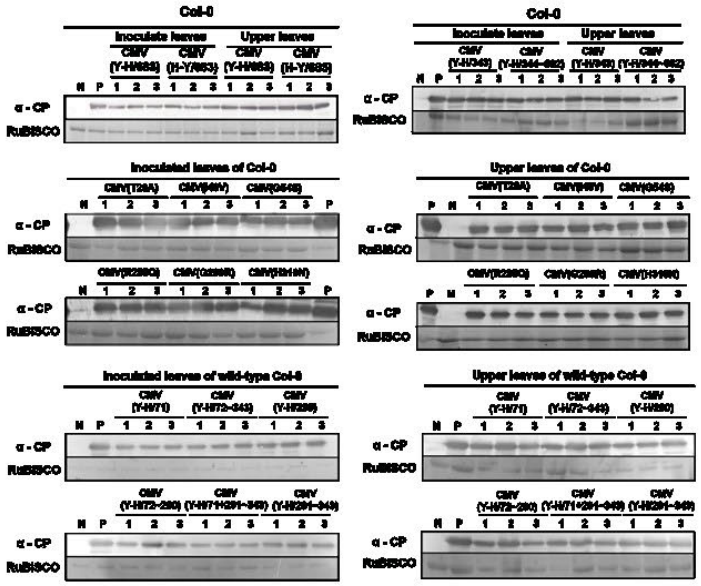

Figure 12. Detection of CMV coat protein in extracts of virus-inoculated leaves and non-inoculated upper leaves of *Arabidopsis thaliana* Col-0. Extracts from fully expanded leaves of three independent Col-0 (number 1, 2, and 3) plants inoculated with reassortant CMV were analyzed at 7 days post inoculation (dpi) using western blotting. Extracts from non-inoculated upper leaves of corresponding plants were also analyzed at 7 dpi using western blotting. RuBISCO protein is shown as an internal reference for protein quantity in each sample. CMV coat protein in CMV(Y)-inoculated leaves (P) and mock-inoculated leaves (N) was quantified as a positive and negative control, respectively.

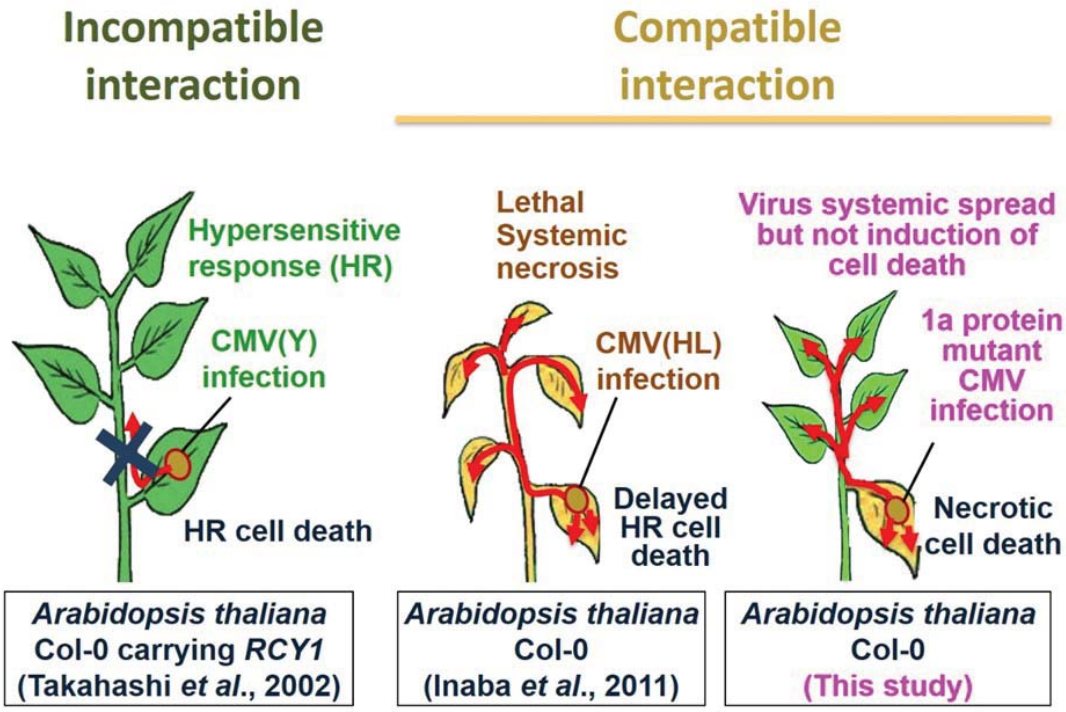

Figure 13. Cell death induced in three virus and plant interactions. Cell death induced in incompatible interactions of virus and plants was identified as HR cell death, which developed in the virus inoculated leaves and restricted the systemic spread of virus. On the other hand, cell death induced in compatible interactions of virus and plants was identified as lethal systemic necrosis. Some recent studies suggesting systemic necrosis could be induced through a delayed HR in the virus inoculated plants is accumulating. Intriguingly, in this study, a necrotic cell death was observed in 1a protein mutant CMV inoculated Col-0 leaves. The 1a protein mutant CMV could systemically infect the plant and induce systemic stunting and yellowing symptoms but not systemic necrotic cell death. The global gene expression of leaves showing necrotic cell death greatly differs from those showing HR cell death. And the necrotic cell death was also identified as a common response in various ecotypes of *A. thaliana*. Therefore, the necrotic cell death induced in the 1a protein mutant CMV inoculated Col-0 leaves was a different type of cell death from HR cell death and systemic necrosis.

論文審査の結果の要旨及び担当者

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学位論文題目	Molecular analysis of necrotic cell death induced in cucumber mosaic virus-inoculated *Arabidopsis thaliana* (キュウリモザイクウイルス感染シロイヌナズナに誘導される壊疽性細胞死に関する研究)

論 文 審 査 の 結 果 の 要 旨

ウイルスに感染した宿主植物では、ウイルスに対する応答として細胞死が誘導されることがある。宿主植物がウイルスに対して抵抗性を示す場合は、ウイルスの初期感染部位において細胞死が誘導され（過敏感細胞死)、ウイルスの死細胞組織から全身への移行が抑制されることにより、植物は発病を免れる。これまで、ウイルス抵抗性における過敏感細胞死の役割や誘導機構について、多くの研究が蓄積されてきた。一方、宿主植物がウイルスに対して罹病性を示す場合は、ウイルスの全身移行に伴い、一般的に黄化病徴が出現し、稀に全身細胞死を伴う病徴が誘導されることが知られている。近年、全身細胞死は、初期感染部位でのウイルスの移行抑制が不完全であることにより、全身移行したウイルスが全身的に過敏感細胞死を誘導した結果であり、ウイルス−宿主細胞レベルから見れば、抵抗性応答が誘導されていると解釈される報告がなされている。しかし、ウイルス感染により誘導される細胞死のすべてが過敏感細胞死によるものなのか、過敏感細胞死とは異なるメカニズムにより誘導される細胞死も存在するのかについて、十分な知見が得られているわけではない。

本研究では、分節ゲノム RNA をもつキュウリモザイクウイルス(CMV)の 2 種類の系統間で、ゲノム RNA を相互に交換した reassortant CMV シリーズを作出し、同ウイルスに対するシロイヌナズナ(*Arabidopsis thaliana*)の応答を解析する過程で、一部の reassortant CMV を接種した植物の接種葉のみに細胞死が誘導されるが、ウイルスは植物体全身に移行することを見出した。これまでに報告されているウイルス−宿主植物相互作用の中で、ウイルス接種葉のみに細胞死が誘導されながら、ウイルスは植物体全身に移行する例は報告されていない。第 1 章では、この細胞死と過敏感細胞死の比較解析を行い、細胞死誘導過程における遺伝子発現変動の網羅的解析などの結果から、reassortant CMV 接種葉のみに誘導される細胞死は、過敏感細胞死とは異なる分子機構による「壊疽性細胞死」であることを明らかにした。第 2 章では、その壊疽性細胞死の誘導を決定しているウイルス因子の解析を行い、CMV のゲノム RNA1 がコードする 1a タンパク質のメチルトランスフェラーゼ(MET)ドメインの N 末端側または C 末端側の 1 アミノ酸置換が、壊疽性細胞死誘導の有無を決定していることを見出した。これらの知見は、ウイルス−宿主植物の組み合わせによっては、過敏感細胞死とは異なる分子機構により誘導される細胞死が存在することを具体的に示すものであり、ウイルス感染宿主植物における多様な細胞死の役割を研究するための新たな実験系を提供したと言える。よって，審査員一同は，この博士論文は博士 (農学)の学位を授与するに値するものであると判断した。