学 位 論 文 要 旨

The Study of Acibenzolar-*S*-methyl-Mediated Resistance to *Potexvirus* Long-distance Movement
アシベンゾラル S-メチルに対するポテックスウイルスの長距離移行抑制機構に関する研究

生物生産科学専攻
生物制御大講座
FAWZIA NOVIANTI

Plant viruses depend on their host plant to establish their infection steps: replication, cell-to-cell, and long-distance movement. In long-distance movement, viruses translocate from the initially infected leaf to the rest of the plant through the phloem vascular tissue. Long-distance movement can be characterized by the three steps: loading into, translocation inside, and unloading from the phloem. To load into the phloem, viruses exploit the plasmodesmata, complex cytoplasmic bridges interconnecting plant cells. The permeability of plasmodesmata is highly regulated by the level of callose accumulation. Previous studies have shown that each step in long-distance movement, including loading, translocation, and unloading, can be a bottleneck during the long-distance movement of viruses.

The detailed mechanisms underlying the restriction of long-distance movement of plant viruses remain unknown; however, there is some evidence that phytohormone-mediated plant defense responses are involved. Salicylic acid (SA) is a well-studied phytohormone that induces defense responses and restricts viral cell-to-cell and long-distance movements. Acibenzolar-*S*-methyl (ASM) is a member of a group of agrochemicals called plant defense activators. It mimics the function of SA in restricting infection by plant viruses and other plant pathogens. A previous study has found that pre-treatment of *Nicotiana benthamiana* with ASM restricted infection by a potexvirus, plantago asiatica mosaic virus (PlAMV), a causal agent of devastating necrotic diseases in lilies. This treatment restricted viral replication and long-distance movement, but did not restrict cell-to-cell movement. Because the cell-to-cell movement was as efficient in ASM-treated leaves as in untreated leaves, the ASM-mediated delay in viral long-distance movement was not simply caused by the suppression of viral accumulation in the ASM-treated, inoculated leaves. Thus, it is of interest to elucidate the mechanism by which ASM delays the long-distance movement of PlAMV. In this study, *N. benthamiana* and PlAMV expressing a green fluorescent protein (PlAMV-GFP) were used as a model system to examine where and when viral movement is inhibited upon ASM treatment. I also employed fluorescence microscopy to monitor the cell-to-cell and long-distance movement of PlAMV-GFP and examine the distribution of the virus in the vasculature.

In the loading step, PlAMV-GFP entered into the vascular tissue in the inoculated leaf by around four days-post inoculations, and this step is inhibited by ASM treatment. When I observed GFP fluorescence in the vascular tissue in the inoculated leaf, PlAMV-GFP was located in the vascular tissue (i.e., xylem, adaxial/internal, and abaxial/external phloem) and the mesophyll cells in the major veins and the petiole of untreated control plants. However, in ASM-treated plants, GFP fluorescence was detected in the vascular tissues but not in the mesophyll cells. Moreover, ASM treatment drastically reduced the accumulation of PlAMV-GFP in the vascular tissues of the major veins and the petiole of the inoculated leaf.

In ASM-treated plants, translocation of PlAMV-GFP was delayed in the stem above the inoculated leaf and the basal stem compared to control plants. GFP fluorescence was located in all types of vascular tissues in the main stems of both control and ASM-treated plants. However, areas of GFP fluorescence detected in ASM-treated plants were much more limited than in the control plants. RT-qPCR-based quantification of PlAMV-RNA levels confirmed that the ASM-treated plants had lower viral accumulation in the main stem than in control plants, although there were no statistically significant differences.

A stem girdling experiment, which blocked viral movement downward into the roots through phloem tissues, demonstrated that downward movement of PlAMV-GFP from the inoculated leaf into the roots is responsible for rapid and efficient viral movement to the upper leaf. On the other hand, the movement of PlAMV-GFP to the upper leaves was restricted by ASM treatment, even though the downward movement was impaired. This result indicated that the downward movement of PlAMV-GFP in the main stem is not essential for ASM-mediated delay of systemic infection.

Viruses unload in sink organs (where the photoassimilates are imported), including the roots and the upper leaves. ASM treatment has a more restrictive effect on the unloading of PlAMV-GFP into the upper leaves than in the roots. I also found that ASM treatment allows the viral unloading and localization into the vascular tissues of uninoculated upper leaves but restricts its subsequent accumulation in mesophyll cells.

To gain insight into the function of callose in ASM-mediated inhibition of viral loading, I analyzed the levels of callose accumulation in phloem. The accumulation of callose in phloem was elevated in both control and ASM-treated plants. This result indicated that ASM-mediated restriction on loading of PlAMV is callose deposition-independent.

In conclusion, ASM treatment delays the loading of PlAMV-GFP into vascular tissues in the inoculated leaf, which leads to restricted viral translocation and unloading and reduced accumulation in sink organs. Because vascular loading is an essential and prerequisite step for viral long-distance movement of plant viruses, ASM treatment could efficiently control plant virus disease.