Molecular adaptation of *Cucumber mosaic virus* soybean strains and functional Analysis of TGS and PTGS using CMV vector system

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Systemic infection can be achieved when viruses have the ability to replicate, to move from cell to cell, and to move long distances through the vasculature of a host plant. The inability of a virus to infect a particular plant is thought result from a lack of cell to cell movement of the viruses. Such cell to cell movement is frequently mediated by virus movement proteins, which therefore might be important candidates for host specificity factors. Recently, their has been a rapid increase in knowledge concerning such movement proteins and their possible functions. In general, resistance to plant viruses has been discussed in two aspects, which are often associated with hypersensitive reaction followed by acquired resistance mediated by various defense-related genes and post-transcriptional gene silencing (PTGS) recently highlighted as plant immunity system (Ratciff et al., 1997; Racliff et al., 1999; Moore et al., 2001). In the latter defense system, some viruses even have counter-defense strategy (Brigneti et al., 1998; Kasschau et al., 1998; Voinnet et al., 1999). The other aspect is inaccessibility of virus to host factors, which are necessary for viral infection process (Schaad et al., 1996;Keller et al., 1998; Schaad et al., 2000). Since the course of viral infection requires interactions between host and viral macromolecules, lack of recognition in host cell leads to resistance to the virus. This type of viral limitation has been studied as recessive resistance. Some of such host factors have been already identified in Arabidopsis mutants and discussed as a host factor necessary for the viral replication and movement (Oshima et al., 1998; Yoshii et al., 1998; Yoshii et al., 1998; Yamanaka et al., 2000).

SSV cannot systemically infect for example cucumber that common strains of CMV can infect. We have conducted phylogenetic analysis using 3a and CP gene sequences to understand the evolutionary relationships among the collected SSVs. This is often regarded as difference in host range and explained by lack of recognition between virus and host rather than existence of a specific resistance gene. In order to characterize the nature of the the role of the 3a protein in SSV host specificity (soybean cultivar and wild soybean ecotypes) to different CMVs andsoybean cultivar resistance. We produced a series of pseudorecombinant and chimeric viruses among infectious transcripts from SSV-C, SSV-D and CMV-Y. Based on the results of their inoculations, we found that the viral systemic infection might be controlled by viral movement protein and host factors. We also discussed the molecular evolution and adaptation to non-host.

Molecular evolution and adaptation of *Cucumber mosaic virus* soybean strains in cultivated and wild soybeans

Systemic infection of soybean-adapted *Cucumber mosaic virus* (CMV) strain (namely, soybean stunt virus; SSV) and non-adapted CMV-Y requires RNA3, which encodes the 3a movement protein and coat protein. SSV was isolated from soybean (*Glycine max*) to map determinant of host specificity and cell-to-cell and long distance movement function. Systemic infections were observed on *Nicotiana benthamiana* and soybean, but cucumber (cv. Model), and tomato (cv. Rutgus) were not infected. The 3a protein and coat protein of RNA3 was indicated a closer relationship with the CMV subgroup I. Phylogenetic analysis showed that the SSVs formed a distinct cluster separated from the other CMV strains. Comparison of the rates of synonymous and nonsynonymous substitutions revealed that the SSV group had evolved faster than the subgroup IA. SSV group isa unique soybean-adapted novel strain and evolved from a common ancestor of the genus Cucumovirus. Many virus genes responsible for viral infection steps (replication, cell-to-cell and long-distance movement) have been identified in various virus-host combinations. SSV inoculation resulted in systemic infection in most of the wild soybeans used while general CMV could not. Pseudorecombinants between SSV-C and CMV-Y were constructed *in vitro* by exchanging the three genomic RNAs. Inoculation of the wild types and their pseudorecombinants to cultivated and wild soybeans suggested that the infection of the viruses in a plant comes into being through a complex interaction of the virus-host plant. Whereas, the determinant gene of SSV for in wild and cultivated soybean the systemic infections was determined to 3a gene and/or 2b gene. In the cultivar resistance to SSV, the 2b gene were necessary for the systemic movement. Furthermore, host reactions resulted from the interactions of any two, or perhaps even all three RNAs of the virus and host factors.

Induction of methylation on a transgene promoter and transcriptional repression using viral vector in plants

Sequence homology-dependent gene silencing in plants can occur either through mRNA degradation, termed post-transcriptional gene silencing (PTGS), or through repression of transcription, termed transcriptional gene silencing (TGS). Double-stranded RNAs (DsRNAs) that contain sequences identical to a promoter sequence can induce methylation on the promoter and can subsequently induce transcriptional gene silencing. The dsRNAs can be supplied to plant cells using a plant RNA virus vector. in order to analyze the process of transcriptional gene silencing in detail, we constructed a trans-methylation system using a vector derived from *Cucumber mosaic virus* (CMV) as a vector. We cloned various portions of the CaMV 35S promoter into the CMV vector, and inoculated the virus to *Nicotiana benthamiana* (line 16c) plants that express GFP under the control of the CaMV 35S promoter.

Soybean seed mottling induced by interaction between PTGS of CHS genes and viral suppressor

Plant viruses are thought to be targets of RNA silencing and have acquired silencing suppressor proteins as counterdefence machinery modulated by small interfering RNAs (siRNA). MicroRNAs (miRNA) play roles in the development of plants as well as animals. Virus-inducing symptoms may be explained by inhibition of the pathways of siRNAs and miRNAs by viral suppressors. We demonstrated that naturally occurring PTGS played a key role in expression of a distinctive phenotype in plants, and presented a simple, clear example of the elucidation of the molecular mechanism for viral symptom induction(1). Most commercial soybean varieties have yellow seeds due to loss of pigmentation in the seed coat. It has been suggested that inhibition of seed coat pigmentation in yellow soybeans may be controlled by homology-dependent silencing of chalcone synthase (CHS) genes. Our analysis of CHS mRNA and siRNAs provide clear evidence that the inhibition of seed coat pigmentation in yellow soybeans results from post-transcriptional rather than transcriptional silencing of the CHS genes. Furthermore, we showed that mottling symptoms presented on the seed coat of soybean plants infected with some viruses can be caused by suppression of CHS post-transcriptional gene silencing (PTGS) by a viral silencing suppressor protein.