（様式５）(Style5)

学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：
Name　Sumyya Waliullah

学位論文題目：
Title of Dissertation　Analysis of the pathogenic function of *Cauliflower mosaic virus* multifunctional protein Tav (Transactivator/viroplasmin) in transgenic tobacco（形質転換タバコを用いたカリフラワーモザイクウイルス Tavタンパク質の病原性機能の解析）

学位論文要約：
Dissertation Summary

One of the common features in plant virus diseases is chlorosis or yellowing, which results from the reduction in photosynthetic pigments such as chlorophyll. Because photosynthesis plays a major role in plant production, understanding the mechanism underlying chlorosis is important in avoiding crop damage caused by virus infection. Although the process of chlorosis has been extensively studied, the precise mechanism of this phenomenon remains to be clearly described. Recent studies have shown that the bright yellow symptoms in tobacco plants infected with Cucumber mosaic virus (CMV) harboring Y-satellite RNA (Y-sat) is attributed to silencing mediated by Y-sat-derived siRNA of magnesium protoporphyrinchelatase subunit I (ChlI) involved in chlorophyll biosynthesis. Another study has shown that siRNA derived from Peach latent mosaic viroid (PLMVd) directs the silencing of chloroplast heat-shock protein 90 (cHSP90), and consequently causes severe chlorosis or albinism. Although these studies showed the involvement of RNA silencing in chlorotic symptom development, this pathway is unlikely to be a common mechanism for chlorosis induced by different viruses.

Cauliflower mosaic virus (CaMV), the type member of plant pararetroviruses (Caulimoviridae), encodes a specific multifunctional protein, the transactivator/viroplasmin (Tav). A striking feature of CaMV Tav as a model to study virus pathogenesis is that it induces virus-symptom-like phenotype (SLP) in transgenic plants expressing Tav alone. However, it remains unknown how Tav induces chlorosis: whether or not something in tobacco cell perceives Tav, which signaling pathway(s) is activated during chlorosis development, and what causes the chloroplast damage. It is difficult to study the early phase events in chlorosis, which are likely to take place before chlorosis can be observed, and the temporal molecular changes during chlorosis development, because chlorosis develops along with plant growth in the transgenic plant constitutively expressing

Tav. Therefore, a novel experimental system, in which chlorosis develops synchronously in plant tissues, is needed to study precise molecular mechanisms underlying the virus-induced chlorosis.

In the first part of this study, transgenic tobacco plants expressing CaMV Transactivator/viroplasmin (Tav) under the control of chemically inducible promoter were generated to establish an experimental system which allows us to analyze the molecular changes during chlorosis. To this end, dexamethasone (Dex)-inducible promoter was employed. The expression of the Tav protein in the Dex-treated transgenic plants was examined by western blotting, revealing that the Tav protein was detectable as early as 1 day post-treatment (dpt), reached a maximum at 3 dpt, and thereafter decreased. Upon Dex treatment, all the transgenic lines showed chlorosis and growth suppression, albeit with variations in severity, at 7 days post-treatment (Fig. 1). Control transformants, which harbor the L3 tobamovirus resistance gene introduced into the same vector, as well as the non-transformed control plants (SR1) showed no visible chlorosis after Dex treatment. These results suggest that the induced expression of Tav can give rise to an SLP consisting of chlorosis and growth suppression, which were observed in transgenic tobacco constitutively expressing Tav.

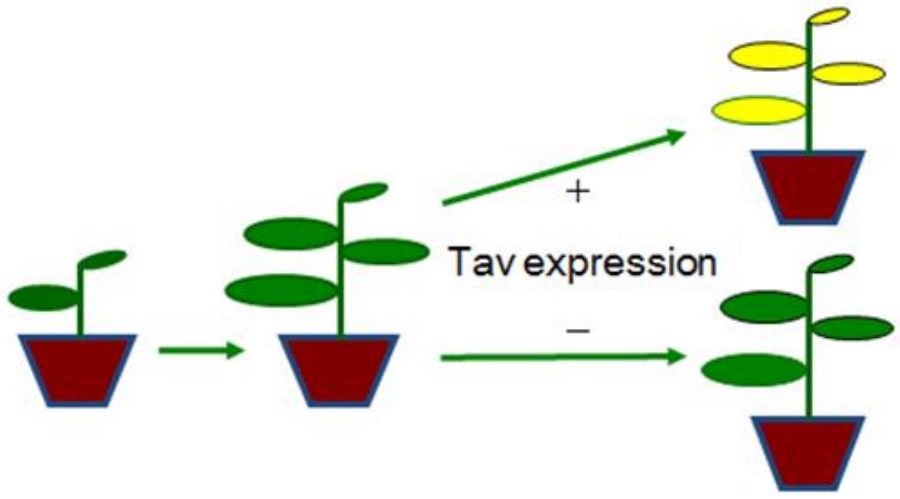

Fig. 1. Schematic representation of the experimental system. The induced expression of Tav gave rise to an SLP consisting of chlorosis and growth suppression.

The characterization of early events preceding chlorosis rather than changes found in chlorotic tissue, would lead to understanding the molecular mechanism underlying chlorosis. Because visual examination distinguished SLP as late as 7 dpt, chlorophyll content was measured to evaluate the progress of chlorosis at earlier time points. A statistically significant decrease in chlorophyll content of Dex-treated Tav transgenic plants was observed at 2 dpt and thereafter, although no

decrease in chlorophyll was observed in untreated transgenic plants, Dex-treated and untreated control plants suggest that, signaling towards chlorosis is activated within a day or two after induction of the Tav transgene.

The downregulation of chloroplast- and photosynthesis-related genes is a common feature in virus infected plants. To test if this is also the case in Tav-induced SLP, we tested the expression of a few nuclear genes encoding chloroplast proteins: a small subunit of ribulose bisphosphate carboxylase/oxygenase, light harvesting chlorophyll a/b binding protein, and ChlI. The decreased expression levels of these genes were clearly observed by 7 dpt in transgenic plants but not in untreated transgenic plants, Dex-treated and untreated control SR1 plants, supporting the idea that Tav-induced SLP shares the downregulation of chloroplast protein genes with the virus disease.

The experimental system with Tav transgenic plants presented here would enable identifying genes upregulated or downregulated early in the process of chlorosis by common transcriptome analysis. The downregulation of chloroplast protein gene expression, which is likely to be an execution phase event, was detected as early as 2 dpt. The changes in gene expression in the commitment phase may take place within a day or two and therefore, transcriptome analysis should be performed at earlier time points.

Characterization of the transgenic plants supported the idea that they would provide a good system for analyzing early events as well as temporal changes in the process of virally induced chlorosis. SLP was also observed in transgenic lines grown on a Dex-containing medium, suggesting that this system is also useful for screening tobacco mutants defective in chlorosis development and for compounds that can suppress chlorosis. In conclusion, the experimental system using transgenic tobacco with the inducible Tav transgene (hereafter referred to as iTav tobacco) would pave the way to a better understanding of the molecular mechanism underlying chlorosis.

A previous study demonstrated that pathogenesis-related (PR) protein 1a (PR1a) was upregulated in transgenic tobacco constitutively expressing Tav. In contrast, studies have shown that Tav suppresses *PR1a* gene expression. Although these results suggest the possibility that the induction of *PR1a* gene expression is a late event during chlorosis development, it was not the case: PR1a expression was upregulated shortly after the induction of Tav in iTav-tobacco. In the second part of this study, therefore, the Tav-induced chlorosis was analyzed in the light of host responses to pathogenic function of Tav.

The induction of different classes of pathogenesis related genes was analyzed. In addition to *PR1a* gene, some salicylic acid (SA)- and ethylene-responsive PR genes was up-regulated in the transgenic tobacco lines shortly after the induction of Tav expression. Change in the expression levels of jasmonic acid-responsive genes was not observed in Dex-treated iTav tobacco lines. In contrast, Tav suppressed the Agrobacterium-induced and SA-induced *PR1a* gene expression also in tobacco. The controversy in *PR1a* gene expression in iTav tobacco and transient expression system could be attributed to the low-level leaky expression of Tav transgene.

A previous study reported that some in-frame deletion mutant of Tav, namely TavD2, is defective in both VSR function and the suppression of *Tomato bushy stunt virus* (TBSV) P19-mediated cell death. To identify the domain(s) involved in Tav-induced chlorosis in iTav tobacco plants, we generated transgenic tobacco lines similar to iTav-tobacco but with in-frame deletion in different parts: TavD2 lacking amino acid residues (AA) #41 - #73; TavD6 lacking AA#167 - #200; TavD8 lacking AA #246 - #298 (Fig. 2). When the iTav-tobacco lines and its deletion mutant lines

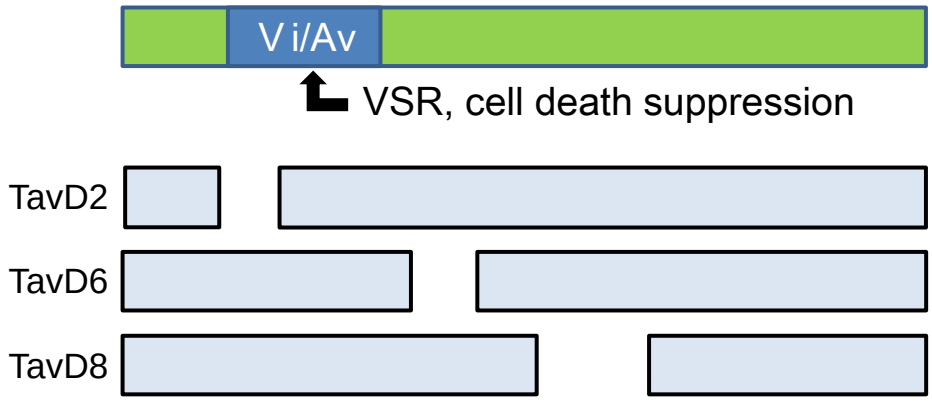

Fig. 2. Schematic representation of Tav functional domains and deletion mutants of Tav used in this study. Vi/Av, domain that virulence function in susceptible host and avirulence function in resistant host were mapped. Viral suppressor of RNA silencing (VSR) activity and the function to suppress TBSV P19-mediated cell death were also mapped to the same domain.

were treated with Dex and analyzed for Tav protein expression, protein bands of expected size were detected with varying intensity within different lines. In TavD6-expressing tobacco lines, chlorosis was induced to a similar extent to the wild-type iTav-tobacco lines as manifested by visual observation on 7 days post- treatment and the decrease in chlorophyll content. In TavD8-expressing tobacco lines, the progress of chlorosis was slower than the wild-type iTav-tobacco lines. The chlorosis in TavD8 lines was not as clear as being visually observable in day 7, but statistically significant decrease in chlorophyll content was detected at 3 and 7 dpt. By contrast, those expressing

TavD2 showed neither visible chlorosis nor statistically significant decrease in chlorophyll content. These results suggest that the region deleted in TavD2 has a role in chlorosis induction. The expression analysis of *PR1a* gene revealed that chlorosis induction positively correlates with the induction of *PR1a* gene expression. It is strongly suggested that Tav-induced chlorosis could be attributed to a host response accompanying *PR1a* gene activation to a region deleted in TavD2, which had been shown to have a role in pathogenesis in susceptible host, elicitation of hypersensitive response in resistant host, RNA-silencing suppression, and the suppression of TBSV P19-mediated cell death in tobacco.

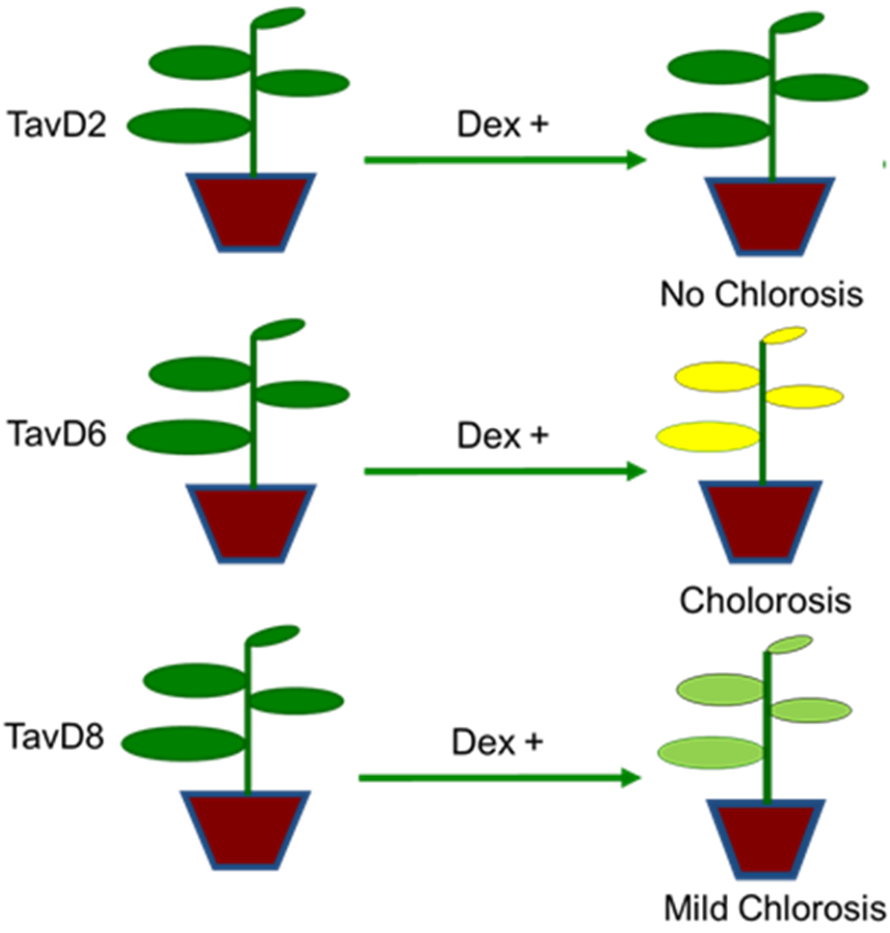

Fig. 3. Symptom-like phenotype expression after Dex-treated transgenic tobacco lines expressing Tav deletion mutants.

SA has been known to have important roles in plant defense, with special emphasis to the involvement in systemic acquired resistance (SAR), but also in local disease resistance. Because Tav-induced chlorosis could be an inefficient or a compromised form of host defense response, the

role of SA was tested in the chlorosis in iTav-tobaccos using a transient expression of SA hydrolyzing enzyme. Transient expression of *nahG* gene encoding SA hydrolyzing enzyme affected neither the Tav-induced *PR1a* expression nor Tav-induced chlorosis (Fig. 4). The results suggest that Tav-induced chlorosis results from an SA-independent host response to pathogenic function of Tav. A recent study suggested that effector-triggered immunity (ETI) involves SA-independent defense gene activation while defense gene activation in the pathogen-associated molecular patterns-triggered immunity (PTI) is SA-dependent in *Arabidopsis*. If this is also the case in tobacco, SA-independent Tav-induced PR1a expression would result from the perception of Tav by a kind of broken immune receptor that inefficiently recognize Tav and drive inefficient ETI response. Further studies examining the effect of knock-down of ETI components on Tav-induced chlorosis would help us understand the relationship between chlorosis, which has been regarded as a susceptible symptom, and the cell death, which is accepted to be a major component of plant disease resistance.

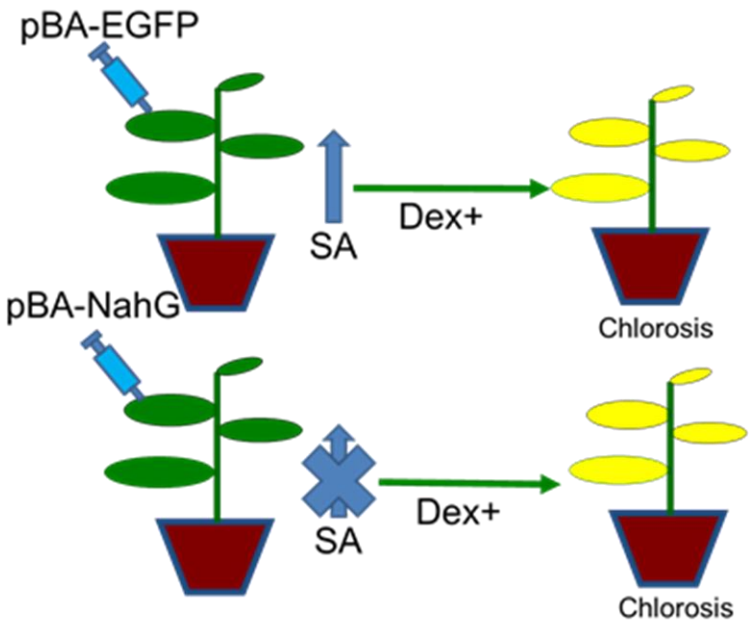

Fig. 4. Tav-induced chlorosis would result from an SA-independent host response to pathogenic function of Tav.

In this study, I have established an experimental system consist of iTav tobacco lines, in which chlorosis develops synchronously, and thus, enables us to study the mechanisms of chlorosis more in detail. Furthermore, I revealed that Tav-induced chlorosis would result from a host response accompanying *PR1a* gene activation, most likely in an SA-independent manner. The experimental system and the knowledge obtained therein could promote our understanding of plant pathogenesis.