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Artificial induction of a plant virus protein in transgenic tobacco provides a synchronous system for analyzing the process of leaf chlorosis

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ABSTRACT

The underlying molecular mechanism of chlorosis, a typical symptom of plant viral diseases, remains poorly understood. To establish an experimental system to determine the molecular changes during chlorosis, especially in the early phase, we generated transgenic tobacco plants expressing *Cauliflower mosaic virus* Transactivator/viroplasmin (Tav) under the control of a chemically inducible promoter. Induction of Tav resulted in visible chlorosis in ten days, a statistically significant decrease in chlorophyll content in two days, decreased expression of chloroplast protein genes, and abnormal thylakoid stacks, indicating that this system reproduces the common features of chlorosis in virus-infected plants.

Introduction

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 protoporphyrin chelatase subunit I (ChlI) involved in chlorophyll biosynthesis [1,2]. 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 [3]. 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.

A possible common mechanism for chlorosis is the delayed or impaired resistance response. Delayed and reduced expression of pathogenesis-related (PR) proteins have been observed in plants infected with compatible viruses, although the dramatic induction of PR protein expression has been known as a representative molecular change during resistance response [4–6] CMV mutants with different amino acids at a particular site (129th residue) of coat protein (CP) induce various types of symptoms in the same host plants ranging from vein chlorosis, pale green mosaic, white mosaic, systemic necrosis, and vein necrosis, to local necrotic spots which resemble the resistant response [7]. Given a single host component has a role in the perception of different CMV CP mutants, the difference in symptoms could be attributed to a difference in the efficiency of CP perception, which would lead to varying responses from a resistant response to different severity of symptoms. This notion supports the idea that different severity of chlorosis responses share a common pathway and reflect the difference in pathogen perception.

Transcriptomic and proteomic studies have been conducted to understand the mechanism underlying symptom development

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[5,8–15], but there is limitation resulting from the nature of materials. Because virus infection spreads gradually cell-to-cell within an inoculated leaf, the infected leaves are a mixture of cells at different stages of virus infection. This difference in stages of virus infection makes it difficult to analyze temporal changes in the process of symptom expression and to identify a cause–effect relationship among different molecular events observed during symptom expression. To solve this problem, we established a system using transgenic tobacco plants expressing *Cauliflower mosaic virus* (CaMV) multifunctional protein, transactivator/viroplasmin (Tav) [16], under the control of a chemically inducible promoter [17]. These transgenic lines reproducibly showed virus symptom-like chlorosis, as reported for those constitutively expressing Tav [18–20], but only after induction of Tav. 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.

Materials and methods

Construction and plant transformation

The *Tav* gene was amplified using primers Sal-TavF and Tav-BlnR (Supplementary Table S1), GXL DNA polymerase (Takara Bio, Ohtsu, Japan), and pFastWt [21] as a template. The amplified fragment was

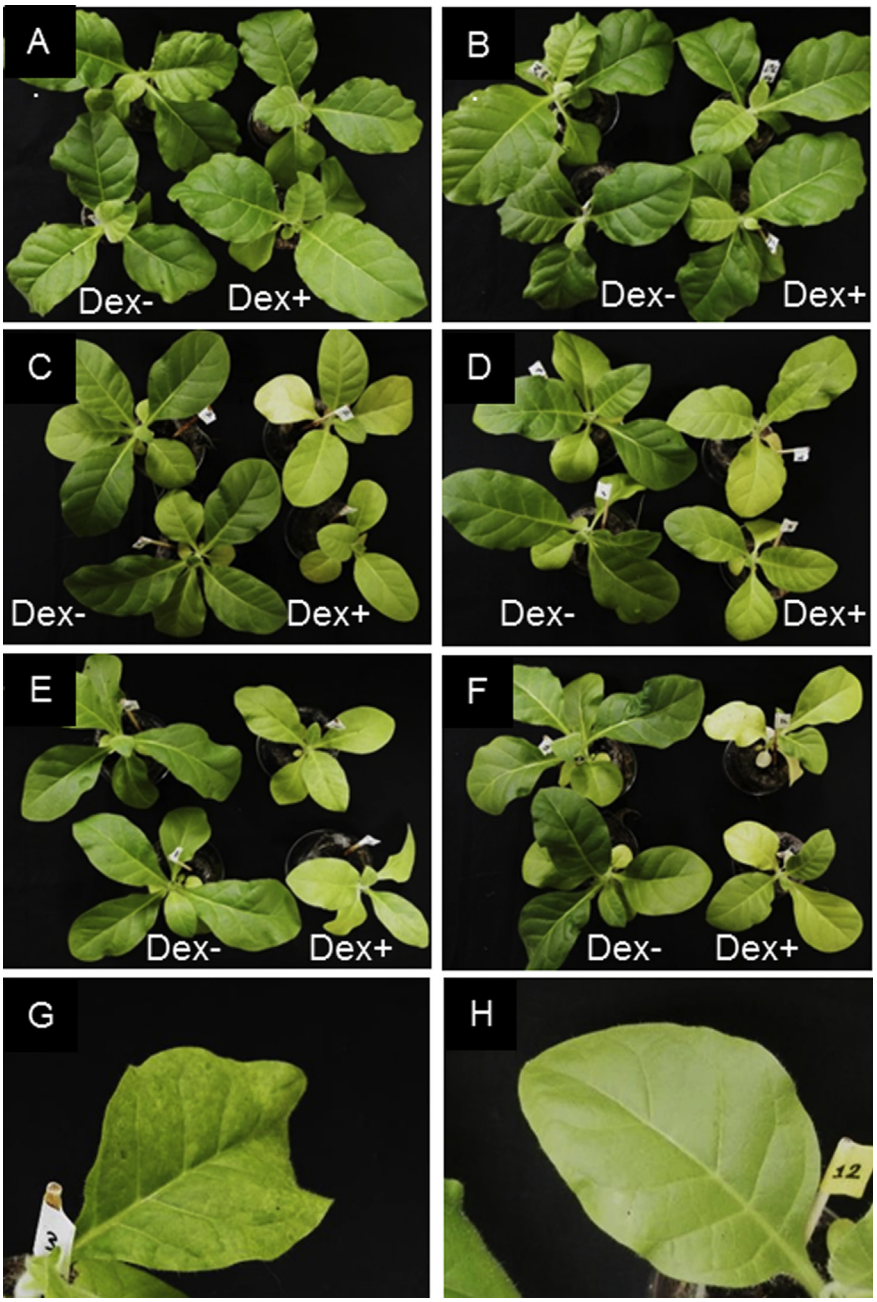

Fig. 1. Chlorosis and growth suppression in Dex-treated transgenic tobacco plants. *Nicotiana tabacum* cv. SR1, a non-transformed control (A), a control transformant with the same vector containing the L^3 tobamovirus resistance gene (B), transgenic lines 2 (C), 3 (D and G), 5 (E), and 12 (F and H) harboring the inducible *Tav* transgene at 5 weeks old received water (Dex−) or a solution containing 20 mg/L Dex (Dex+), and were photographed at 7 days post-treatment.