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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Comparative analysis of mechanisms underlying the chlorosis development in transgenic tobacco expressing different chlorosis triggers
(異なる退緑黄化発症因子を発現する遺伝子組換えタバコにおける退緑黄化発症機構の比較解析)

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A wide range of plant species are infected by numerous viruses and viroid which induce a variety of symptoms and the nature of the viral symptom development is specific to the host-pathogen combination. One of the most frequently occurred symptoms due to virus infection is chlorosis. The morphological and physiological alterations due to chlorosis include a reduction in chlorophyll content, and the impaired chloroplast structure and function. The compromised chloroplast function is responsible for the severe decline in plant production. Therefore, deciphering the underlying molecular events of virus-induced chlorosis would provide new acumen into crop protection.

To study the mechanisms underlying chlorosis development, two model systems were previously developed in our lab. In one of these chlorosis model system, i-hpHSP90C, chloroplast HSP90C genes was silenced in an artificially inducible manner resulting in the development of chlorosis. In another system, i-amiCHLI, artificial micro RNA is expressed in an artificially inducible manner, resulting in the dropped expression of CHLI genes encoding a subunit of a chlorophyll biosynthetic enzyme. Also in these two systems, Dex-treatment decreased the expression of chloroplast protein genes and chlorophyll content in two days and induced visible chlorosis in seven days. These findings suggested that the chlorosis in these model systems is attributed to the compromised biogenesis of chloroplast and also by the activation of plant responses to the altered chloroplast function. Therefore, I have made a comparative transcriptome analysis of these two transgenic chlorosis model systems,

by using an RNA-seq approach to elucidate the detailed mechanisms of chlorosis development.

In the case of i-hpHSP90C plants, RNA-seq data have revealed the up- and down-regulation of 2746 and 3490 genes, respectively. Gene Ontology analysis of these differentially expressed genes (DEGs) indicated the upregulation of reactive oxygen species (ROS)-responsive genes, the activation of the innate immunity and cell death pathways, and the downregulation of genes involved in photosynthesis, plastid organization, and cell cycle. Cell death was confirmed by trypan blue staining and electrolyte leakage assay and the H2O2 production by diaminobenzidine staining. The results collectively suggest that the reduced levels of HSP90C chaperone leads the plant to develop chlorosis primarily through the global downregulation of chloroplast and photosynthesis-related genes and additionally through the light-dependent production of ROS, followed by the activation of immune responses including the cell death.

In i-amiCHLI plants comparison to the inducer-treated and untreated control, non-transformants and untreated i-amiCHLI revealed that 3668 and 3307 genes were up- and down-regulated, respectively, in the inducer-treated i-amiCHLI plants. Gene Ontology enrichment analysis of these differentially expressed genes indicated the upregulation of the genes related to innate immune responses, ROS-responses, and cell death pathways and the downregulation of genes for photosynthesis, plastid organization, and primary and/or secondary metabolic pathways in the inducer-treated i-amiCHLI plants. The cell death in the chlorotic tissues with a preceding H2O2 production was observed in the inducer-treated i-amiCHLI plants, confirming the activation of the immune response. The involvement of activated innate immune response in the chlorosis development was supported by the comparative expression analysis between the two transgenic chlorosis model systems, i-amiCHLI, and i-hpHSP90C, in which nuclear genes encoding different chloroplast proteins were silenced in the same way.

Keywords: Chlorosis; Tobacco; Transcriptome; RNA-seq; CHLI; HSP90C; ROS; Cell death; Immune response.