【Grant-in-Aid for Scientific Research (S)】

Broad Section F

Title of Project : Studies on molecular mechanism of effector-mediated establishment of host specificity in plant pathogens and their application

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Keyword : Plant pathogens, Effector, Host specificity, *Colletotrichum* fungi, Cucurbit

【Purpose and Background of the Research】

Both animal and plant pathogenic microorganisms generally exhibit clear host specificity (Figure 1). However, understanding on the molecular background for pathogen host specificity is still limited. More than 70% of plant diseases is caused by phytopathogenic fungi (hereafter called plant pathogens). It is speculated that series of secreted proteins, called effectors, play important roles for the establishment of host specificity in many plant pathogens. Plant pathogens secrete effectors and many effectors enter into host cells to interfere with the plant immune system, resulting in their successful infection.

In this study, to reveal molecular basis for effector-mediated establishment of host specificity, we will perform molecular and structural analyses on EPC effectors of *Colletotrichum orbiculare* that are required for host specificity of the pathogen on cucurbits (Figure 2). Also, we will try to develop new technologies to produce cultivars that exhibit durable disease resistance.

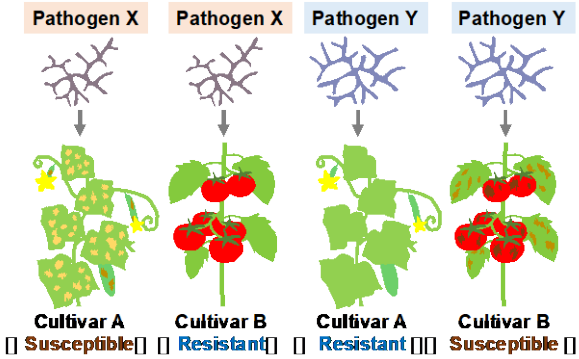

Figure 1. Host specificity of plant pathogens.

【Research Methods】

(1) Molecular and structural analyses on EPC effectors.

The ability of each EPC effector for suppression of plant immunity will be investigated. Tertiary structure of each EPC effector will be determined by NMR analysis. Based on the determined structure, we try to find out functional domains of the EPC effectors.

(2) Isolation and analyses of plant molecules targeted by the EPC effectors.

Each EPC effector containing an epitope tag will be transiently expressed in plant cell, and immunoprecipitation (IP) will be performed. Then, Co-IP fraction will be subjected to LC-MS/MS analysis to identify candidate proteins. The association between EPC effector and candidate will be then assessed. Subsequently, the virus-induced gene silencing experiment will be performed toward corresponding genes encoding the identified target proteins to reveal roles of the target proteins for plant immunity.

(3) Identification of *C. orbiculare* effectors involved in host specificity on cucurbits.

To identify *C. orbiculare* effectors involved in host specificity, comparative genomics and transcriptomics on *C. orbiculare* and multiple species inside the orbiculare clade will be performed.

(4) Generation of the modified targeted plant proteins that are resistant against the attack by the EPC effector.

Complex structure for EPC effector and its target protein will be predicted or determined. Based on the obtained information, mutational analyses will be done to generate modified targeted plant proteins that are resistant against the attack by the EPC effectors.

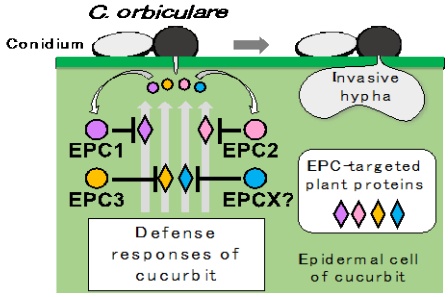

Figure 2. *C. orbiculare* EPC effectors suppress plant immunity in cucurbits.

【Expected Research Achievements and Scientific Significance】

This study reveals the molecular mechanism of effector-mediated establishment of host specificity in plant pathogens. Also, the generation of modified targeted plant proteins with the resistance against the effector attack will contribute to the development of new technologies for producing cultivars exhibiting durable disease resistance.

【Publications Relevant to the Project】

・ Conserved fungal effector suppresses PAMP-triggered immunity by targeting plant immune kinases. Irieda H, Inoue Y, Mori M, Yamada K, Oshikawa Y, Saitoh H, Uemura A, Terauchi R, Kitakura S, Kosaka A, Singkaravanit-Ogawa S, Takano Y. Proc Natl Acad Sci U S A. 116:496-505. (2019)

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