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学位論文題名

Analysis on expression controlling mechanism of AVR-Pia, an avirulence effector in
Magnaporthe oryzae

(イネいもち病菌の非病原性エフェクターAVR-Piaの発現制御メカニズムの解析)

Magnaporthe oryzae is the pathogen of rice blast disease, a devastating disease that can attack all rice-growing regions around the world. Previously, resistant cultivars of rice were used to control rice blast disease, but new pathogenic races of pathogen appear frequently, therefore the management of this disease is continuously regarded as an important issue in agriculture. Based on gene-for-gene relationship, the specific interaction between avirulence (AVR) effectors of *M. oryzae* and resistant (R) proteins of rice is required to trigger the defense response, called effector-triggered immunity (ETI), which is a major component of the disease resistance. Therefore, AVR effectors are considered to be a key to control rice blast disease and many genes for those effectors (AVR genes) were cloned and analyzed. Knowledge on the controlling mechanism of their expression is important for the construct the durable or sustainable usage of resistant rice cultivars, but little is available currently, except for that most of AVR genes are induced during infection. The aim of this study is to elucidate the expression controlling mechanism of AVR-Pia, one of AVR effectors in *M. oryzae*.

1. Construction of fluorescent reporter analysis system for AVR-Pia expression

In order to analyze AVR-Pia expression, a fluorescent reporter analysis system was constructed by fusing putative promoter of *AVR-Pia* (PPR) to eGFP as reporter gene. The construct was introduced to strain Ina168m95-1 (*avr-pia*) and the transformants were inoculated onto compatible rice leaf sheath and onion epidermis. Fluorescence visualization of inoculated rice leaf sheaths and onion epidermises showed that GFP was expressed in appressoria of transformants, suggesting that AVR-Pia expression was induced during appressorial differentiation, and onion epidermis can be used for the expression study instead of the rice leaf sheath.

2. Relationship between AVR-Pia expression and host penetration

In order to clarify the relationship between AVR-Pia expression and host penetration, observation of AVR-Pia expression in penetration-deficient mutant (*Δpls1*) was performed using the fluorescent reporter system. The results clearly demonstrated that AVR-Pia expression was independent to penetration into host cells. This study suggested that AVR-Pia expression is induced during appressorial differentiation to produce AVR-Pia effector before penetration.

3. Identification of cis elements for *AVR-Pia* expression

In order to identify regulatory cis elements for *AVR-Pia* expression, deletion series of PPR were constructed and subjected to the fluorescent reporter analysis system. Fluorescence observation and RT-PCR revealed that the level of gene expression was reduced in PDL3, while the expression of other deletion versions still was detected, suggesting that the region between PDL2-3 and PDL3 (-286 to -277 bp) is an enhancer sequence, which is required for transcription factors called activator to increase the rate of transcriptional level. In the other hand, the region between PDL3 and PDL3-1 (-277 to -261 bp) was considered as silencer, which is a protein-binding sequence for repressor to suppress gene expression.

4. Role of global transcription regulator *LaeA* in *AVR-Pia* expression

Previously, a global regulator *LaeA* was reported that it controls the expression of multiple biosynthesis gene clusters for secondary metabolites in filamentous fungi. The expression regulation of ACE1 avirulence effector, which is expected as polyketide secondary metabolites and that of AVR-Pia were similar and expected to share the same regulatory expression pattern. In order to know whether *LaeA* can regulate AVR-Pia expression or not, *LaeA*-orthologous gene (MGG08161) was identified in *M. oryzae* genome and deleted in strain Ina86-137 carrying eGFP-labeled AVR-Pia. Fluorescent reporter analysis showed that the ratio of fluorescence-expressing appressoria of Δ MGG08161 was reduced compared to the control, suggesting that MGG08161 may manipulate the expression of *AVR-Pia*.