学位論文内容の要旨

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学位論文題名

Study on the dynamics and inheritance of mitochondria during conidiation in *Pyricularia oryzae*

（イネいもち病菌の分生子形成時のミトコンドリアの動態と伝搬に関する研究）

Pyricularia oryzae, which causes rice blast disease in rice, is a filamentous ascomycete fungus. QoI fungicide (quinone – outside inhibitors) is one of the major fungicides to control rice blast disease. They inhibit mitochondrial respiration by binding to the Q_o site of the cytochrome (bc1) enzyme complex, blocking the electron transport chain and consequently reduce the production of ATP. In recent decades, resistance has been developed for this fungicide due to single nucleotide substitution in *cytb* gene in mitochondrial DNA (mtDNA). Therefore, it is important to understand mitochondrial dynamics and inheritance to curb the resistance development in the fungus. In this study, visualization of GFP-fused mitochondria was used to understand the movement and shape of the mitochondria during the initial stage of conidia formation. In addition, wild and the mutant allele were quantified using quantitative PCR (qPCR) to understand the process of homoplasmy during conidiation.

1. Microscopic observation of mitochondria dynamics in the initial stage of conidiation

The movement and shape of the mitochondria during initial stages of conidiation were investigated using *P. oryzae* transformant harboring GFP-tagged Citrate synthase (*CitA*) gene. Strain Ina86-137 CitA-GFP was cultured on special slide, with a thin paper soaked with oatmeal agar at 25ºC for 30-33hours. Observations were made every 20-30 minutes to investigate the movement and the shape of the mitochondria. It was observed that there is continuous flow of mitochondria from conidia to conidiophore during early conidiation. The shape of the mitochondria was initially network at zero minute of observation and later they changed into dot shape at around 100-120 minutes before the flow of the mitochondria stops. Septa formation was also observed parallel to

development of dot shaped mitochondria.

It is very important to understand about the shape and movement of mitochondria during conidiation to introduce novel strategies to control rice blast disease.

2. Establishment of qPCR for the quantification of *cytb* alleles in mtDNA

One nucleotide substitution in the cytochrome b (*cytb*) gene had been revealed to develop resistance against QoI fungicides. Absolute qPCR system was established to quantify each *cytb* allele in this study, in order to monitor the ratio of resistant and wild type allele in a strain. A linear standard curve was produced using standard DNA clone of each allele, for 10^8-10^4 copies. Using this system, *cytb* alleles were quantified in four QoI resistant field isolates and two sensitive field isolates, which were collected from Akita and Hyogo prefectures, Japan. The wild: mutant ratio of the resistant and sensitive strains were ranged from 10^2 to 10^4 :1 and 1: 10^2 to 10^4, respectively.

3. Elucidation of relationship between conidiation and homoplasmy

Occurrence of mutation in mitochondria will lead the heterogeneity of mitochondria in a cell, which is called heteroplasmy. Heteroplasmy is not stable, and often return to the condition called homoplasmy, in which one type of mitochondria dominates in a cell. Occurrence and rapid spread of QoI resistant mitochondria in the wild population can be considered as a result of homoplasmy, but no investigation on the process to be homoplasmy in *P. oryzae*. In order to study about homoplasmy and a conidiation, a artificial heteroplasmy strain having similar amount of wild type allele and mutant allele *cytb* mtDNA was produced using PEG-mediated protoplast fusion. This heteroplasmy was then used to generate single colony isolates with conidiation and without conidiation, by single conidia isolation and single protoplast isolation, respectively. The isolates with conidiation showed homoplasy when the quantity of the alleles were measured using qPCR. Those isolates had almost only the wild type allele. Isolates without conidiation retained heteroplasmy. These results suggested that conidiation is acting as an important process to maintain homoplasmy of mitochondrial DNA.

This study demonstrated that the conidiation, which is an important event for *P. oryzae* which produces the cell required for the pathogenesis, is the key event for the spread of QoI resistance as well. Further analyses using qPCR system for *cytb* alleles and fluorescent microscopic observation of mitochondria may help to mitigate building-up of QoI fungicide resistance in *P. oryzae* and will have a great impact on the developing sustainable management practices against the rice blast disease in the future.