APPLICATION NOTE

A1 R, AX/AX R Confocal Microscope with Water-Immersion Objective

Morphogenesis imaging of *mCherry*-expressing *Magnaporthe oryzae* transformants with DIC and confocal microscopy

Rice blast disease is the most serious rice disease. The ascomycete fungus *Magnaporthe oryzae* is known as a hemibiotrophic pathogen that causes rice blast disease. *M. oryzae* infects rice leaves, stems and panicles, and causes severe reductions in yield. To establish a new control method for this disease and to develop resistant rice varieties, it is important to clarify the details of gene-to-gene and protein-protein interactions between *M. oryzae* and rice.

In this application note, we will introduce an example of imaging with differential interference contrast (DIC) and confocal laser scanning microscopy using the CFI Apochromat Lambda S 40XC WI objective, in a paper concerning the identification of a novel pathogenic gene using the differential gene expression evaluation method during plant-pathogenic fungus interactions reported by Professor Hiromasa Saitoh at Tokyo University of Agriculture.

Keywords: DIC, confocal laser scanning microscope system, water immersion objective, *Magnaporthe oryzae*, rice plant

Overview

As a result of RNA-Seq analysis of barley cotyledons inoculated with a conidial suspension of rice blast fungus, putative secreted protein genes were identified that show expression that increased and reached its peak 12 to 24 hours post-inoculation (hpi) (at the time of appressorial penetration and invasive growth), then down-regulated at 36 or 48 hpi. Out of those, seven highly expressed genes were selected and each of the seven gene disrupt mutants of *M. oryzae* were made. A gene disrupted strain showed significantly reduced pathogenicity and that gene was named *MoSVP*.

To investigate the timing and site of *MoSVP* expression in the fungus, the blast fungus was transformed with a reporter plasmid (*MoSVPp::mCherry*) in which *mCherry* (red fluorescent protein gene) was inserted downstream of the *MoSVP* promoter, and a reporter plasmid (*Rp27p::mCherry*) in which *mCherry* was inserted downstream of the promoter of the blast fungus *ribosomal protein 27* gene as a constitutive expression control. After incubating the conidial suspension of each transformant on a cover glass or inoculating each into the inner epidermis of the rice leaf sheath (Fig. 1), infection-related morphogenesis and mCherry fluorescence were observed over time with DIC and confocal laser scanning microscopy (Fig. 2).

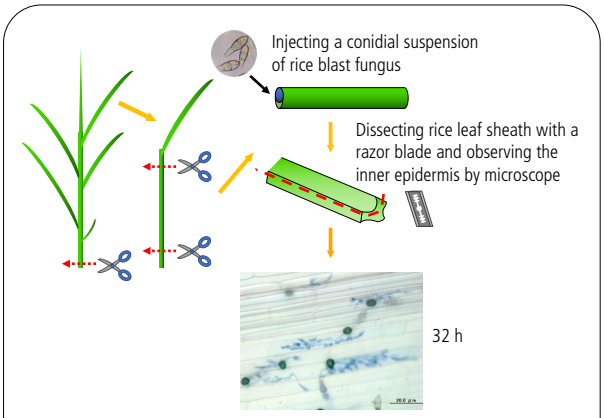
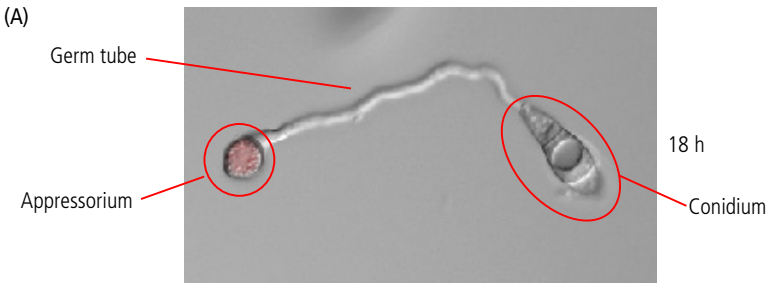


Fig. 1: Outline of rice leaf sheath inoculation and sample preparation



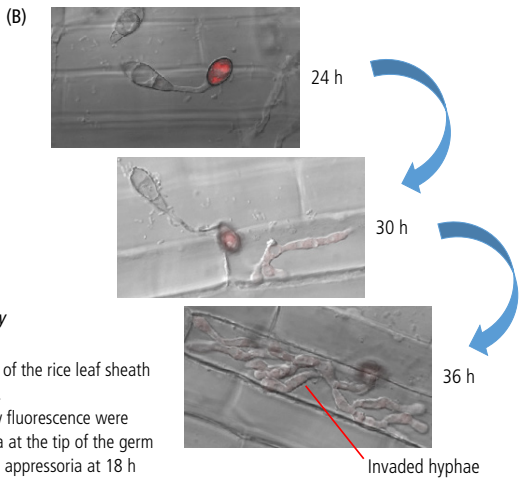


Fig. 2: Merged image of DIC and mCherry fluorescence (red) of the rice blast fungus harboring *MoSVPp::mCherry*

(A) Conidium of the blast fungus transformed with *MoSVPp::mCherry* at 18 h after spotting on a coverglass.

(B) A conidial suspension of the *MoSVPp::mCherry*-transformed blast fungus was inoculated into the inner epidermis of the rice leaf sheath and then infection-related morphogenesis was observed over time by confocal laser scanning microscope (CLSM).

After incubation of the conidial suspension-inoculated rice leaf sheath, infection-related morphogenesis and mCherry fluorescence were observed at 24, 30 and 36 hpi. After inoculation of a conidial suspension, conidia germinate and develop appressoria at the tip of the germ tubes, then penetrate into host cells and develop invasive hyphae. mCherry fluorescence was strongly detected in the appressoria at 18 h after spotting on a coverglass (A) as well as at 24 hpi on the rice leaf sheath cells (B) by DIC and fluorescence observation using the CFI Apochromat Lambda S 40XC WI objective.

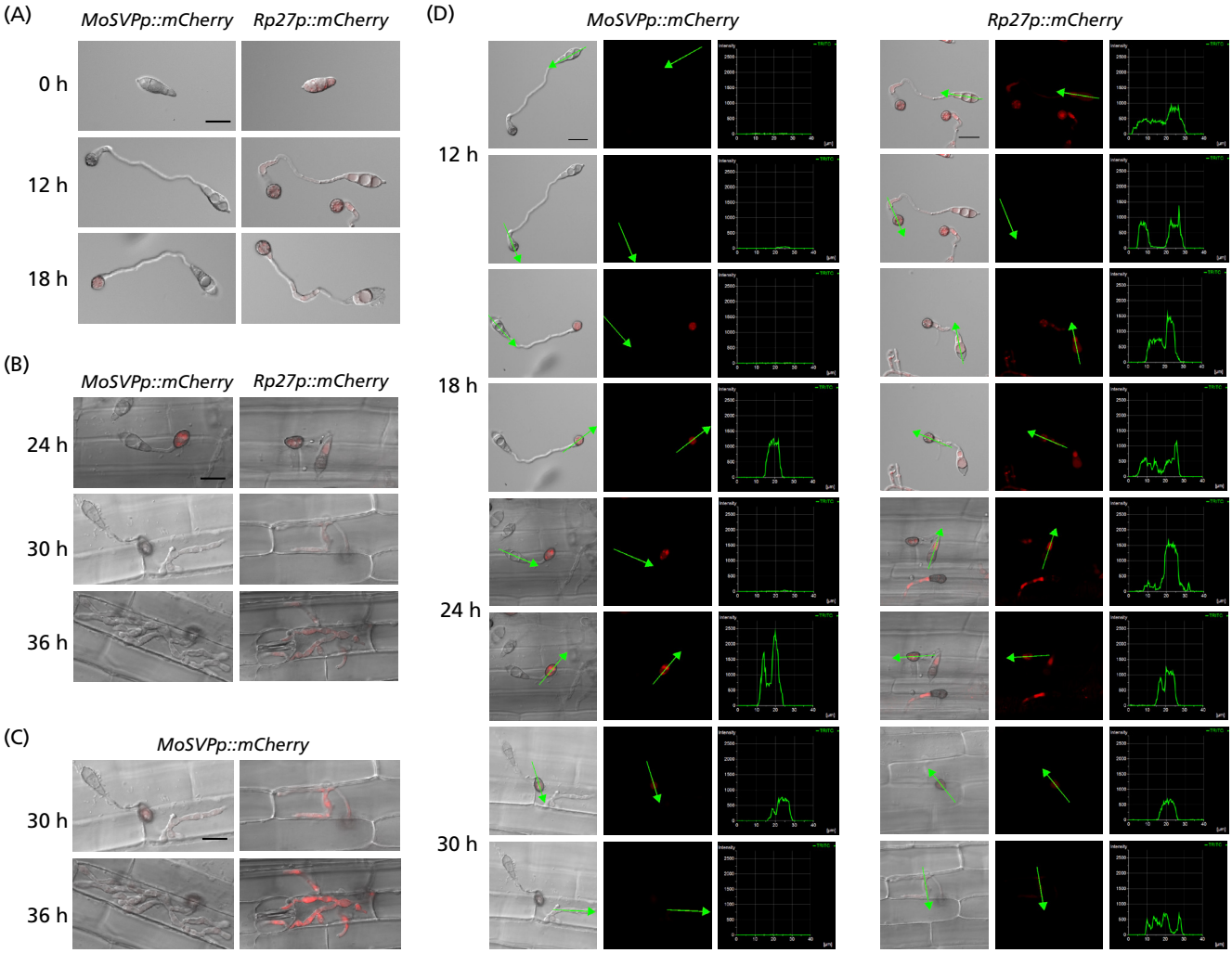


Fig. 3: Promoter assays using *mCherry* as a reporter

(A), (B), (C) and the left panels of (D) show merged DIC and mCherry fluorescence images.
(A) Conidia of the rice blast fungal strains harboring *MoSVPp::mCherry* or *Rp27p::mCherry* were spotted onto coverglasses observed at 0, 12, 18 h after incubation.
(B) Conidia of the rice blast fungal strains harboring *MoSVPp::mCherry* or *Rp27p::mCherry* were inoculated into the inner epidermis of the rice leaf sheath and observed at 24, 30 and 36 hpi.
(C) The photos at 30 and 36 hpi in (B); the pinhole was enlarged three times and mCherry fluorescence was observed.
(D) Promoter assays with fluorescence intensity distribution graphs for the images of (A), (B), (C) along the green arrows at 12, 18, 24 and 30 hpi.
In the *MoSVPp::mCherry* transformant, mCherry fluorescence was strongly detected in the appressoria at 18 or 24 hpi, and then decreased. In the *Rp27p::mCherry* transformant, mCherry fluorescence was constantly expressed at the given time points. In addition, mCherry fluorescence was strongly detected not only in the appressoria but also in conidia, in germ tubes at 12, 18, 24 hpi and in invasive hyphae at 30 hpi. Thus, it was revealed that the *MoSVP* promoter is specifically activated in the appressoria at the time when the blast fungus penetrates into the host cells.
Pinhole: 24.3 µm (A, B, D), 72.9 µm (C), Scale bar: 20 µm

Summary

An *mCherry*-expressing strain under the control of the *MoSVP* promoter was produced and its infection-related morphogenesis was observed over time with DIC and confocal laser scanning microscopy using the CFI Apochromat Lambda S 40XC WI objective. As a result, it was confirmed that the *MoSVP* is expressed in the appressorium at the early stage of rice blast fungus infection.
These results demonstrate that the combination of a high-precision objective and a confocal laser scanning microscope system can clearly visualize the timing and localization of fluorescent signals in the plant pathogenic fungus.

References

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MOTOKI SHIMIZU, YUKI NAKANO, AKIKO HIRABUCHI, KAE YOSHINO, MICHIE KOBAYASHI, KOSUKE YAMAMOTO, RYOHEI TERAUCHI AND HIROMASA SAITOH