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Infection of Soybean Plant Roots With GFP-Expressing Phytophthora Sojae

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Introduction

Phytophthora sojae and other Phytophthora species, fungus-like eukaryotic microorganisms, are economically important pathogens in agriculture, resulting in $1 to $2 billion in damage annually[1]. P. sojae is a soil-based pathogen that infects soybean crops and is responsible for root rot. A devastating disease, root rot results in massive losses of crops due to the development of lesions which infect the root and migrate to the stem, thus stunting plant growth or killing the plant altogether[2].

A clearer understanding of the host-pathogen interaction can lead to the development of increasingly disease-resistant soybean crops or less virulent Phytophthora species. Therefore, pathogenesis study of P. sojae soybean root infection is critical and can be aided by the power of fluorescence to elucidate the molecular mechanisms of infection. Incorporation of green fluorescent protein (GFP) into P. sojae allows researchers to follow soybean root infection at all stages of the pathogen's lifecycle. Coupling the iBox Explorer[2] Microscope to the study of GFP-expressing P. sojae can greatly aid in the study of its pathogenesis. One promising research focus incorporating fluorescent molecules is the search and elucidation of effectors, virulent proteins expressed by pathogenic organisms responsible for evasion of the host immune system. For example, an effective means of following expression of effectors by a pathogen is through co-expression of a GFP gene with a protein under study. GFP co-expressed with siRNA within the host showed diminished fluorescence with infiltration by organisms that inhibited siRNA silencing by the effector protein[3].

The iBox® Explorer[2] Microscope is the ideal imager for studying fluorescently-labeled plant pathogens given the system's following key features:

- Macro to micro imaging, providing the ability to visualize a whole plant series to localized study of plant infection at the cellular level.
- A highly sensitive, cooled CCD camera for maximum light emission capture within the visible to near infrared (NIR) spectra.
- A broad spectrum excitation light source capable of generating excitation light from the UV to the NIR spectra (<400 nm to 800 nm).
- A large working distance (distance from stage to optics) for placement of roots, leaves and young plants.

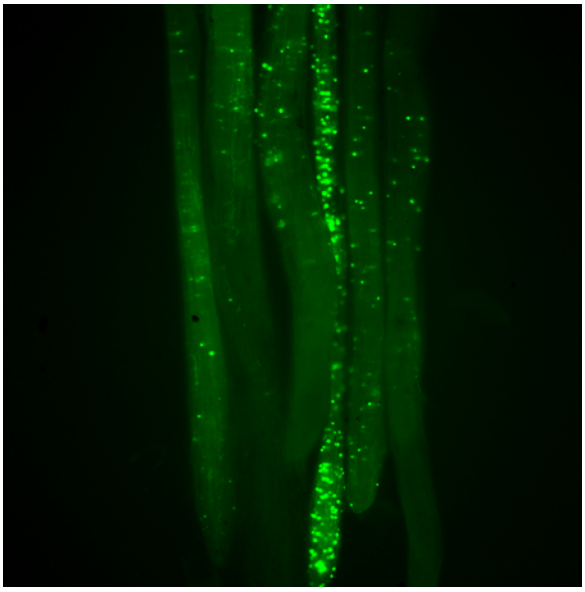

Figure 1. Phytophthora sojae infection of soybean roots at 2.5x. This image shows several soybean roots at approximately 48 hours post infection with the soil borne pathogen, Phytophthora sojae. The roots were exposed to P. sojae zoospores that express a GFP marker. Fluorescent P. sojae oospores can be seen developing inside the roots, while some zoospore cysts are attached on the surface of the roots. Field of view (FOV) corresponds to 6x6 mm^2.

Procedure

P. sojae oospores were transfected with a plasmid, pGFPH, containing a GFP construct using a ham34 promoter with a backbone conferring hygromycin resistance (Judelson Laboratory, University of California-Riverside, USA). GFP-expressing P. sojae were grown on a V8 media for several days. A mycelia plug was transferred to distilled water and starved to induce sporangia development. Zoospore release was induced by cold exposure. The zoospores were collected, quantified and inoculated onto soybean root tissue. Alternatively, mycelia plugs were directly applied to soybean roots instead of using zoospores.

Plant roots were allowed to incubate with transfected oospores or mycelia plugs prior to imaging. Infected sample roots were then placed upon the imaging stage and illuminated with a GFP excitation light source (455-495 nm) with side lighting excitation. Variable magnifications were acquired with exposure times measuring milliseconds to 10 seconds. A GFP filter (513-557 nm) was used to filter emitted light. Images were captured with a monochrome CCD camera and pseudocolored using UVP's VisionWorks®LS software.

Results

Across a wide range of magnifications, P. sojae infections can be clearly visualized on plant roots using the iBox Explorer[2]. At low magnification, infected samples can be seen harboring the transfected oospores along the length of the roots as very discreet spherical bodies (Figure 1) after 24 hours. This population of root samples shows differential infection rates as well as a diffused pattern of infection along the length of each root.

Mid to high magnification images show detailed infection patterns within the root system. Along the plant root (Figure 2), hyphae are seen extending longitudinally with zoospores present within the root itself. Further infiltration of P. sojae hyphae into the root shows a distinctive criss-cross pattern (Figure 3) as the pathogen extends into the root tissue. This root has extensive infiltration after a relatively short 8-10 hours post infection (hpi) due to the readily infectious nature of the mycelial hyphae.

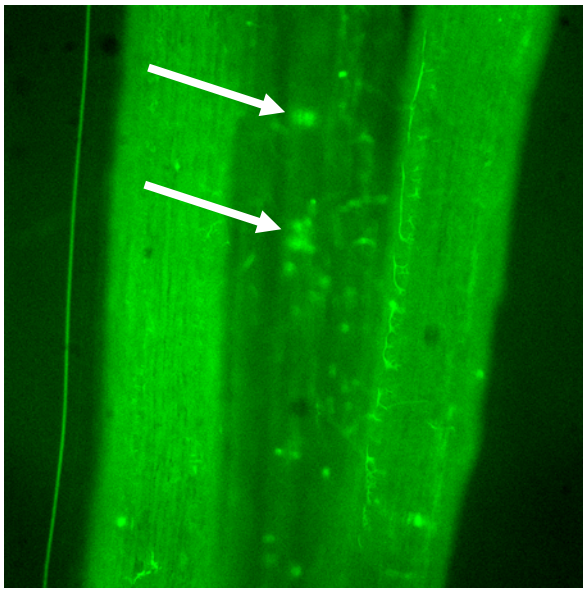

Figure 2. Soybean root infection with P. sojae zoospores at 8.8x. This image focuses on the surface of the root at 48 hpi. P. sojae oospores can be seen inside the roots (arrows) and hyphae attachment on the root surface. These roots have been transformed with a YFP fluorescent protein expressing construct by Agrobacterium transformation. Field of view (FOV) corresponds to 1.7 mm^2.

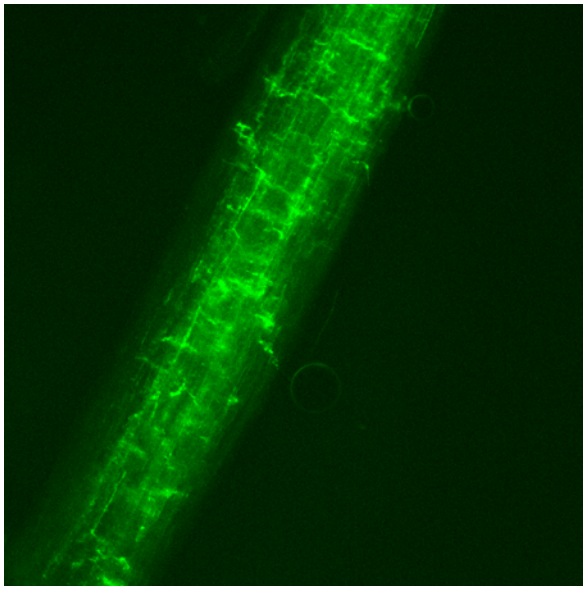

Figure 3. Phytophthora invasion of root tissue at 8.8x. This image was taken at approximately 8 hpi following infection with P. sojae mycelial plug. Note the extensive P. sojae hyphae invasion into the root tissue. Field of view (FOV) corresponds to 1.7 mm^2.

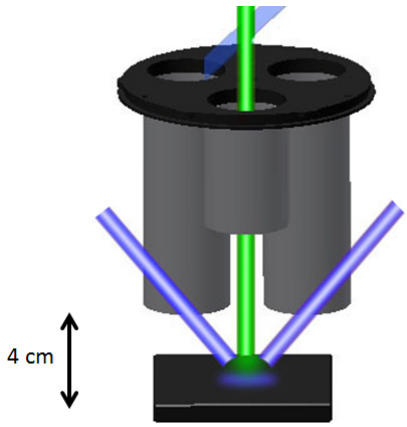

Figure 4. Working distance from stage to optics (4 cm) in the iBox Explorer[2] Imaging Microscope. Typical working distances (imaging stage to objective) for most microscopes average 0.1 to 4 mm, limiting both the field of view for imaging as well as the size of the sample that can be placed on the stage.

With a working distance of 4 cm, the iBox Explorer[2] Imaging Microscope can accommodate a wide range of samples, including roots, leaves and whole plants. In addition, the variable optics can broaden the scope of the samples imaged, ranging from low magnifications to capture whole plants to the highest magnification to visualize individual cells with a 2.24 pixel/micron resolution in a field of view of 900 microns^2.

Discussion

Through the use of fluorescent protein technology and microscopic imaging, the economically significant P. sojae can be visualized infiltrating soybean roots. Use of either zoospores or mycelial plugs results in extensive infection of the plant root, and this infiltration can be seen in vivo as extention of hyphae or formation of oospores within the root tissue.

The iBox Explorer[2] Imaging Microscope (Figure 5) offers crisp, fast and reliable image capture for many in vivo applications. In addition, the large working distance (Figure 4) provides a clearance of greater than 10 times what standard microscopes offer, designed to accommodate part of or a whole plant specimen, ideal for myriad plant imaging applications. Finally, with a large range of optical magnifications ranging from 0.17x (field of view: 9x9 cm) to 16.5x (field of view: 0.9x0.9 mm), biological phenomena can be monitored from a macroscopic to a microscopic scale.

Figure 5. iBox Explorer[2] Imaging Microscope

[1] http://magissues.farmprogress.com/OFM/OF10Oct06/ofm12.pdf
[2] http://www.extension.iastate.edu/publications/pm914.pdf
[3] Qiao, Y., Liu, L., Xiong, Q., Flores, C., Wong, J., Shi, J., Wang, X., Liu, X., Xiang, Q., Jiang, S., Zhang, F., Wang, Y., Judelson, H.S., Chen, X., Ma, W., 2013. Oomycete Pathogens Encode RNA Silencing Suppressors. *Nature Genetics*. 45: 330-333.