Challenge

Understanding the host-pathogen interaction between soybean plant roots and Phytophthora sojae

Solution

Fluorescent microscopic imaging using the iBox Explorer²

Infection of Soybean Plant Roots With GFP-Expressing Phytophthora Sojae Using the iBox Explorer²

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. 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.

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 Explorer² Microscope to the study of GFP-expressing P. sojae can greatly aid in the study of its pathogenesis.

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Materials and Methods

Samples and reagents

- Imaging System: iBox Explorer 2 Microscope
- GFP- expressing P. sojae
- Soybean plant
- V8 agar media

Samples preparation

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.

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 pseudo colored using UVP's VisionWorks software.

Results and Discussion

Across a wide range of magnifications, P. sojae infections can be clearly visualized on plant roots using the iBox Explorer². 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 crisscross 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.

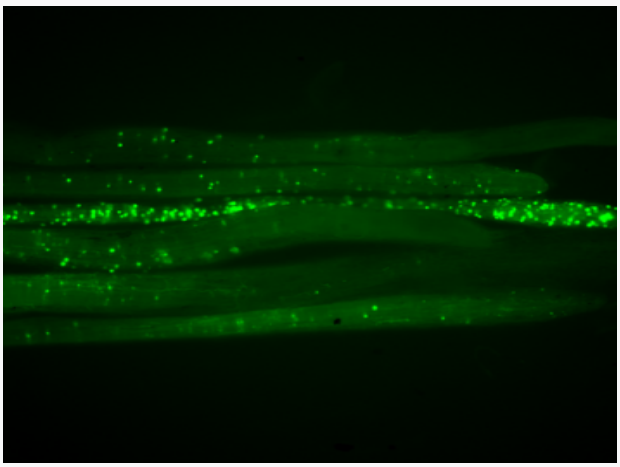

Figure 1. Phytophthora sojae infection of soybean roots at 2.5x. This image shows several soybean roots at approximately 48 hours post infection with the soil borne pathogen, Phytophthora sojae. The roots were exposed to P. sojae zoospores that express a GFP marker. Fluorescent P. sojae oospores can 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 mm²

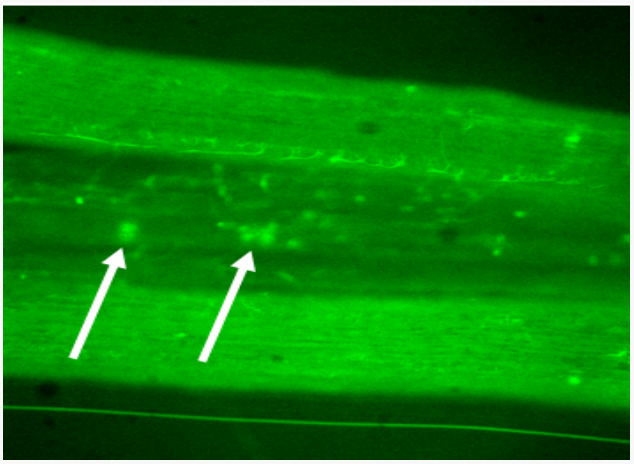

Figure 2. Soybean root infection with P. sojae zoospores at 8.8x. This image focuses on the surface of the root at 48 hpi. P. sojae oospores can be seen inside the roots (arrows) and hyphae attachment on the root surface. These roots have been transformed with a YFP fluorescent protein expressing construct by Agrobacterium transformation. Field of view (FOV) corresponds to 1.7 mm².

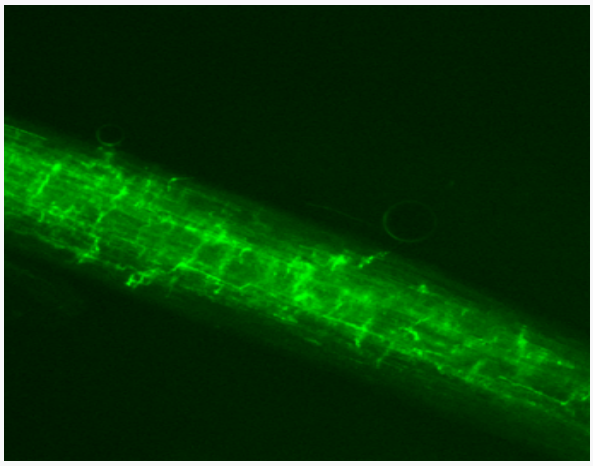

Figure 3. Phytophthora invasion of root tissue at 8.8x. This image was taken at approximately 8 hpi following infection with P. sojae mycelial plug. Note the extensive P. sojae hyphae invasion into the root tissue. Field of view (FOV) corresponds to 1.7 mm².

Conclusion

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 extension of hyphae or formation of oospores within the root tissue.

The iBox Explorer² Imaging Microscope offers crisp, fast and reliable image capture for many in vivo applications. In addition, the large working distance from stage to optics (4cm) 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. The broad-spectrum excitation light source is capable of generating excitation light from the UV to the NIR spectra (<400 nm to 800 nm). 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 and captured from a macroscopic to a microscopic scale.

References

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