Research Highlights

"Intrahyphal Hyphae and Dead Hyphae", Aberrant Hyphae Triggered by Host Immune Responses to Plant Pathogenic Fungus

Ascochyta (Mycosphaerella) blight of pea, caused by *Mycosphaerella pinodes* (Berk. et Blox.) Vestergren [syn. *Peyronellaea pinodes* (Berk. & A. Bloxam) Aveskamp, Gruyter & Verkley], is one of the most important diseases of grain legumes worldwide. Despite the economic impact and numerous studies on this disease, little is known about the cytological features during infection by *M. pinodes*, especially in resistant interactions. One reason is due to the lack of resistant cultivars of pea (*Pisum sativum* L.) as well as the available resources in the *Pisum* germplasm collection with strong resistance to this disease.

Kazuhiro Toyoda and colleagues at Okayama University examined the histology and ultrastructure of early infection events and fungal development including penetration by appressoria, vegetative growth of infection hyphae and host responses, using a recently developed model pathosystem involving *Medicago truncatula* and *M. pinodes* strain OMP-1 (Toyoda *et al.*, 2013).

On the susceptible ecotype R108-1, pycnospores germinated and grew over the surface of the epidermis, then formed an appressoria and penetrated the cuticle. Beneath the cuticle, the infection peg expanded into a hyphae that grew within the outer wall of the epidermis. Subsequently, the hyphae penetrated down within mesophyll cells and proliferated vigorously, eventually, forming asexual fruiting bodies (pycnidia) (**Fig. 1**). In contrast, successful penetration and subsequent growth of infection

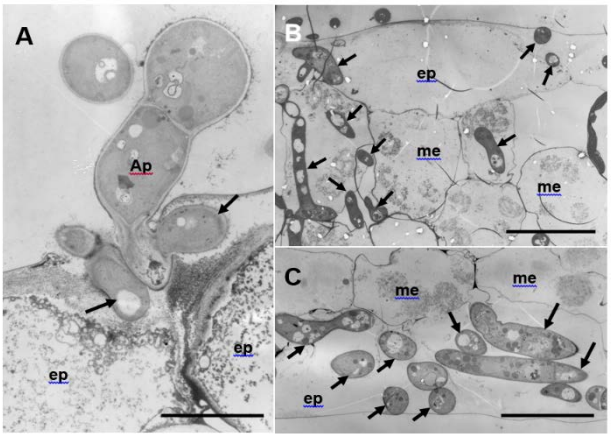

Figure 1 Transmission electron microscopy (TEM) images of susceptible R108-1 leaves at 3 days after inoculation with *M. pinodes*. (A) Infection hyphae (arrows) that look like infection vesicles formed in the cell wall of the adaxial epidermal (ep) cell. Appressorium (Ap). Bar = 5 μm. (B) Adaxial epidermis and mesophyll cells (me) invaded by hyphae. Host cell organelles were degraded. Bar = 20 μm. (C) Extensive hyphae in abaxial epidermal cells. Bar = 20 μm.

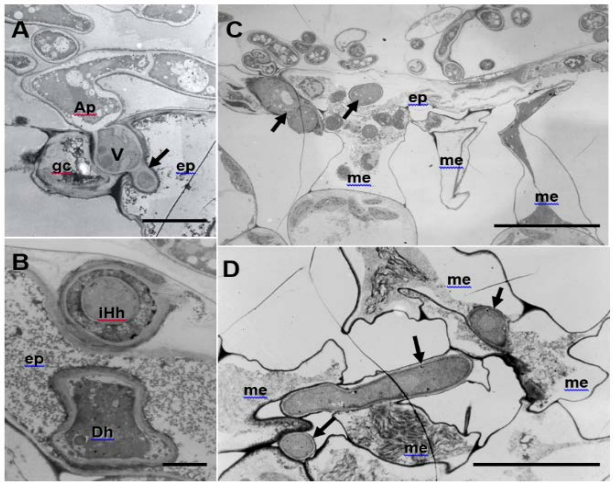

Figure 2 TEM images of resistant Caliph leaves at 3 days after inoculation with *M. pinodes*. (A) Infection vesicle (V) emerged from the tip of an appressorium (Ap) to cell walls between guard cell (gc) and epidermal cell (ep), and infection hyphae extended into epidermal cell (ep). Bar = 5 μm. (B) Aberrant hyphae in epidermal cell; intrahyphal hyphae (iHh) and dead hypha (Dh). Bar = 2 μm. (C) Epidermis invaded by hyphae and adjoining mesophyll cells had shrunk. Bar = 20 μm. (D) Mesophyll cells in contact with the hyphae had shrunk. Bar = 10 μm.

hyphae were considerably restricted in the ecotype Caliph (**Fig. 2**). Interestingly, aberrant hyphae such as intrahyphal hyphae and dead hyphae, due to a local defense elicited by the fungus, were abundant in Caliph but not in R108-1. Detected by its reaction with cerium chloride ($CeCl_3$) to generate electron-dense cerium perhydroxides in transmission electron micrographs, hydrogen peroxide (H_2O_2) accumulated in epidermal and mesophyll cells of Caliph challenged with pycnospores of *M. pinodes*. This intracellular localization was confirmed by energy-dispersive X-ray (EDX) spectroscopy (**Fig. 3**). These observations thus indicate that the oxidative burst reaction leading to the generation of reactive oxygen species is associated with a local host defense response in Caliph, since no clear H_2O_2 accumulation was detectable in susceptible R108-1.

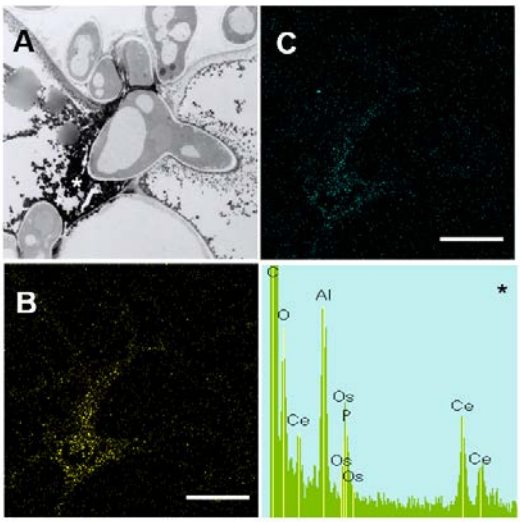

Figure 3 Elemental mapping and EDX spectrum showing the deposition of cerium perhydroxides at fungal invasion sites in Caliph epidermis. (A) TEM image. Analytical point is shown by asterisk. Ce (B) and O (C) mapping images.

The researchers conclude that the structural aberrations are likely common mechanisms of fungi to be protected from a hostile environment in a resistant host by being enclosed by another hyphae. The structural differences between susceptible and resistant interactions as well as the host responses will assist in better understanding pathogenesis of the fungus on pea, thus providing information on the breeding of the resistant cultivars of pea.

Reference

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Title of original paper
Ultrastructural and cytological studies on *Mycosphaerella pinodes* infection of the model legume *Medicago truncatula*

Journal
Frontiers in Plant Science 8, 1132 (2017)

Digital Object Identifier (DOI)
10.3389/fpls.2017.01132

Journal website
http://journal.frontiersin.org/article/10.3389/fpls.2017.01132/full

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