FUNGAL BIOLOGY

ECM fungi and all that JAZz

Ectomycorrhizal (ECM) fungi establish a mutualistic association with tree roots. Root colonization involves the formation of an invasive hyphal network, known as a Hartig net, that encases the plant epidermal cells. Many of the details of the mechanism by which such an extensive network is established in the presence of a functional plant immune response — which includes the hormone jasmonic acid — are still unknown. A new study now provides the first mechanistic insights into the negative regulation of plant jasmonic acid signalling by an ECM fungus.

The ECM species *Laccaria bicolor* establishes a symbiotic partnership with poplar species, such as *Populus trichocarpa*. *L. bicolor* secretes effectors that are known as mycorrhiza-induced small secreted proteins (MiSSPs), and it had previously been established that nuclear localization of one MiSSP, MiSSP7, was required for Hartig-net formation. Plett *et al.* carried out a detailed characterization of the interactions between MiSSP7 and plant cells, and began by using a yeast two-hybrid assay to identify potential MiSSP7 binding partners. A *P. trichocarpa* JAZ domain-containing protein that was homologous to the *Arabidopsis thaliana* JAZ protein AtJAZ6 was identified, and the authors named it PtJAZ6. The interaction between MiSSP7 and PtJAZ6 was confirmed by two additional assays: an *in vivo* DivIVa-interaction assay and an *in planta* biomolecular fluorescence complementation assay.

As JAZ domain-containing proteins are key negative regulators of the jasmonic acid signalling pathway, the authors examined the possible interactions between PtJAZ6, MiSSP7 and jasmonic acid. *Populus* spp. encode two homologues of the *A. thaliana* jasmonic acid receptor AtCOI1. Using yeast two-hybrid and DivIVa-interaction assays, the authors found that PtJAZ6 interacted with one of these homologues, PtCOI1, and that this interaction required the presence of coronatine, which is a mimic of the active form of jasmonic acid. The authors also investigated whether the presence of coronatine had a role in the interaction between PtJAZ6 and MiSSP7, and again using a yeast two-hybrid assay, they found that coronatine did not stimulate binding of MiSSP7 to PtJAZ6. Moreover, nuclear localization of MiSSP7 decreased the ability of PtJAZ6 to interact with PtCOI1 in the presence of coronatine.

Increased expression of *PtJAZ6* repressed the transcription of jasmonic acid-regulated genes, which confirmed that PtJAZ6 is a negative regulator of the jasmonic acid pathway. Could MiSSP7 inhibit the jasmonic acid-induced degradation of PtJAZ6? When *Nicotiana benthamiana* leaves that expressed a PtJAZ6–GFP fusion protein were treated with jasmonic acid, there was a loss of fluorescence, which indicates that PtJAZ6 is degraded *in planta*. This degradation was prevented in leaves that co-expressed MiSSP7. If PtJAZ6 is a negative regulator of jasmonic acid signalling and MiSSP7 prevents PtJAZ6 degradation, then MiSSP7 should repress the expression of jasmonic acid-regulated genes. Assessment of gene expression in poplar roots that overexpressed MiSSP7 confirmed that this was the case, and the repressed genes were potentially involved in cell wall modification. Taken together, these data indicate that MiSSP7 does indeed function as an inhibitor of jasmonic acid signalling in plants.

Jasmonic acid is known to suppress Hartig-net formation, so can MiSSP7 overcome this repressive effect? The addition of synthetic MiSSP7 was shown to restore *L. bicolor* Hartig-net formation in the presence of inhibitory concentrations of jasmonic acid. Finally, the authors generated transgenic poplar lines that expressed variable levels of PtJAZ6 in the roots and assessed the depth of *L. bicolor* hyphal penetration and Hartig-net formation in the presence of exogenous jasmonic acid or jasmonic acid signalling inhibitors. Increased expression of PtJAZ6 and pharmacological inhibition of jasmonic acid signalling both compensated for the lack of MiSSP7 in Hartig-net formation.

Biotrophic bacterial and fungal plant pathogens stimulate jasmonic acid signalling to promote colonization. By contrast, this work shows that the ECM fungus *L. bicolor* establishes its mutualistic interaction with plant roots by blocking this signalling pathway.

Sheilagh Molloy

> “MiSSP7 does indeed function as an inhibitor of jasmonic acid signalling in plants”

ORIGINAL RESEARCH PAPER Plett, J. M. *et al.* Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 proteins and represses jasmonic acid (JA) responsive genes. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1322671111 (2014)