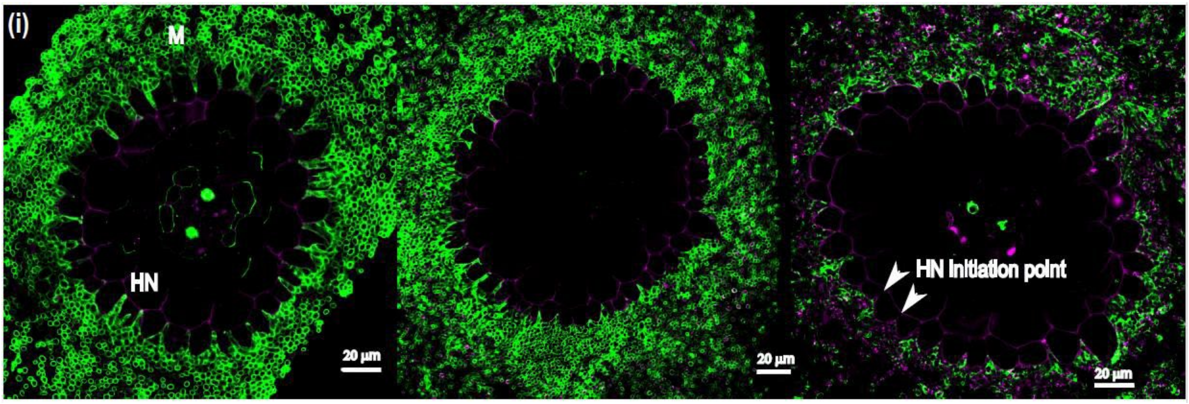
TERPECM



Monoterpenes affect ectomycorrhizal formation in a time-dependent manner. *Representative confocal microscopy images of transversal sections of poplar roots at 21 days post inoculation (dpi) with L. bicolor, treated with a monoterpene mix including γ-terpinene, (-) limonene, (-) camphene, (-) α-pinene, and (-) β-pinene. Analysis of control (left), monoterpene mix added at 14 dpi (center) and at 7 dpi (right) treatments show that these monoterpenes impact the formation of hartig net (HN) when applied early in the interaction. Propidium iodide (magenta) stains the root cell wall, while AlexaFluor WGA-488 (green) stains the fungal cell wall. Bars: 20 µm.*

Deciphering the role of plant terpenes in the modulation of ectomycorrhizal symbiosis

Principle investigator: Jose Eduardo Marqués Gálvez, UMR Interactions Arbres/Micro-organismes (IAM) *1136*

LabEx partners: Claire Veneault-Fourrey (Research director UMR IAM), Gaurav Pandharikar (Postdoctoral researcher hired for the project, UMR IAM)

Collaborations: Nathalie Lackus (Max Planck Institute for Chemical Ecology, Jena, Germany now at Lehrstuhl für Pharmazeutische Biologie, Julius-von-Sachs-Institut für Biowissenschaften, Julius-Maximilians-Universität Würzburg, Julius-von-Sachs-Platz 2, Würzburg, 97082, Deutschland)

Thematic action concerned: *WP1*

Context —

Among beneficial tree-microbe interactions the association between ectomycorrhizal (ECM) fungi and root trees is of interest because of the broad influence that these symbioses have in forest ecosystems. The role of jasmonic acid in the establishment of ectomycorrhizal symbiosis has been well established in the last decades. ECM fungi secrete fungal effectors that are capable of inactivating the jasmonic acid signalling pathway by disabling the action of Poplar MYC2 transcription factors. Here, we focus in the role of poplar MYC2s and genes under its control such as terpene synthases in the formation of ECM symbiosis.

Objectives —

In this project, we aimed to elucidate the contribution of poplar MYC2s to the establishment of ECM symbiosis and to identify which direct or indirect MYC2-controlled genes are required for ECM establishment. Since preliminary results pointed towards the importance of MYC2 control over terpene synthases, we also aimed to address the effect of exogenous terpenes over fungal growth and ECM symbiosis. Additionally, we intended to address the terpene profiles ("terpenome") of poplar wild type and transgenic lines with impaired ECM formation.

Approaches —

Transgenic poplars overexpressing or silencing the two paralogs of *MYC2* were produced, and their ability to establish ectomycorrhizal symbiosis was assessed. We coupled these analyses with transcriptomics and DNA affinity purification sequencing. We also attempted to identify root volatile terpenes using Thermal Desorption Unit (TDU)-GC-MS analysis and by Hexane extraction and GC-MS analysis.

Key results —

- Poplar MYC2s mediates *in planta* fungal growth, since its overexpression inhibits Hartig net formation and its silencing results in fungal over-colonization.
- MYC2 overexpression lines inhibiting *in planta* fungal growth are associated with a transcriptional core set of defensive genes, with a significant enrichment of terpene synthases.
- Certain monoterpene synthases, including γ-terpinene, (-) limonene, (-) camphene, (-) α-pinene, and (-) β-pinene are capable of inhibiting the fungal growth of ECM and endophyte fungi as well as *in planta* fungal growth in a time dependent manner. Despite our attempts, no root volatile terpenes were identified in *MYC2* overexpressing mutants.
- Poplar MYC2.1 and MYC2.2 directly control the expression of several other transcription factors as well as defensive genes, including some key terpene synthases such as *TPS16* or *TPS4*.

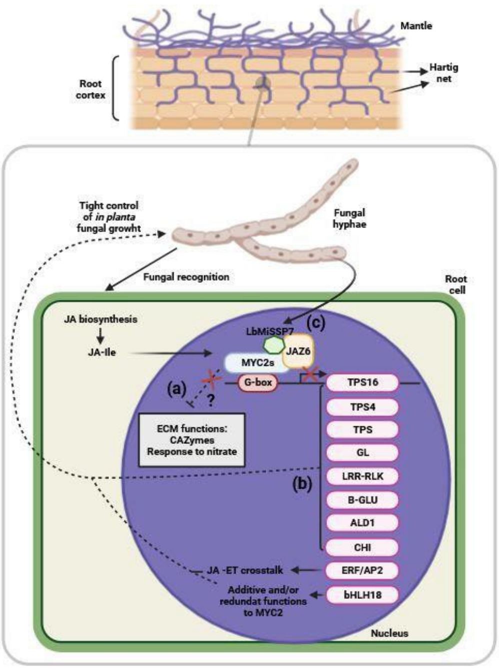


Figure 2. Proposed working model of action for Populus MYC2 transcription factors during ectomycorrhiza (ECM) development.

Main conclusions including key points of discussion —

The results of this project underscore the significant role of MYC2s as regulators of mutualistic ECM symbiosis in poplar and allows us to extend our previously proposed hypothesis based on the *L. bicolor* x *Populus* sp. Interaction (Martin et al., 2016) (Fig. 2). To successfully establish ECM symbiosis, the fungal effector LbMiSSP7 enters root cells and migrates to the nucleus. In the presence of JA-Ile, it binds to and stabilizes the JA coreceptor PtaJAZ6, preventing its degradation and counteracting PtaMYC2s activity outlined in this work (Fig. 2a, Plett et al., 2011, 2014; Daguerre et al., 2020). Using transgenic poplars, we showed that both PtaMYC2 paralogs coordinate root cell transcriptional reprogramming that results in the tight control of in planta fungal growth. On one side, these TFs directly activate the expression of other TFs and specific defence-related genes, with a prominent role for TPSs (Fig. 2b). On the other side, they induce indirect downregulation of ECM functions (i.e. cell wall metabolism and nitrate transport) by anunknown mechanism (Fig. 2c).

Perspectives —

These findings enhance our fundamental understanding of ECM symbiosis and plant-microbe interactions. They also prompt new research questions about the role of root defenses in mediating belowground mutualistic interactions and the potential for manipulating them to engineer plants with disease resistance, all while ensuring the stability of mutualistic interactions.

Valorization —
The results of this project has been published in *New Phytologist* (Marqués-Gálvez et al., 2024), as well as several oral presentations at MoDiP (Molecular dialogue in plant biotic interactions) meeting 2022 and LABEX ARBRE doc postdoc day 2022. These results have been also submitted for either a poster or oral presentation to ICOM12 (International Conference on Mycorrhiza), to be held at Manchester in August 2024.

Leveraging effect of the project —
The obtention of TERPECM project has facilitated a collaboration with a group from Max Planck Institute for Chemical Ecology in Jena (Germany), the hire of a postdoctoral researcher for 6 months (Gaurav Pandharikar), and the obtention of additional external projects including a researcher position at University of Murcia (Spain) by Jose Eduardo Marqués-Gálvez.

References —

Daguerre Y, Basso V, Hartmann-Wittulski S, Schellenberger R, Meyer L, Bailly J, Kohler A, Plett JM, Martin F, Veneault-Fourrey C. 2020. The mutualism effector MiSSP7 of Laccaria bicolor alters the interactions between the poplar JAZ6 protein and its associated proteins. Scientific Reports 10: 20362.

Martin F, Kohler A, Murat C, Veneault-Fourrey C, Hibbett DS. 2016. Unearthing the roots of ectomycorrhizal symbioses. Nature Reviews. Microbiology 14: 760–773.

Plett JM, Daguerre Y, Wittulsky S, Vayssieres A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey JL, Brun A, Veneault-Fourrey C et al. 2014. Effector MiSSP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid JA responsive genes.
Proceedings of the National Academy of Sicences, USA 111: 8299–8304.

Plett JM, Kemppainen M, Kale SD, Kohler A, Legue V, Brun A, Tyler BM, Pargo AG, Martin F. 2011. A secreted effector protein of Laccaria bicolor is required for symbiosis development. Current Biology 21: 1197–1203.