

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

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Thematic action(s) 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 jasmonate (JA) 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 JA signalling pathway by disabling the action of Poplar MYC2 transcription factors. Recent results from our research group have shown the direct control of these transcription factors upon different actors of the plant immune system, including terpene synthases (Marqués-Gálvez et al., 2022). In this project, we focus in the role of plant terpenes in the formation of ECM symbiosis.

Objectives —

The main objective of this project is to characterize the terpene emission of poplar roots to unveil their role in the molecular dialogue between roots and ECM fungi. As a specific objective, we want to address the “terpenome” differences between poplar wild type and transgenic lines with impaired formation of ECM, which will lead us to highlight specific terpenes that are related to JA signaling pathway and may play a role in the molecular cross-talk between trees and ECM fungi.

Approaches —

In this project, which will start in March 2023, we will try to measure the volatile emission profile of poplar transgenic roots overexpressing MYC2 transcription factor and compare it with wild type and empty vector control poplar. MYC2 overexpressing lines have previously shown impairment of *in planta* fungal colonization and up to 12 different terpene synthases transcriptionally upregulated. Therefore, we aim to find the specific “terpenome” profile that is related to this ECM impaired phenotype. To characterize terpene volatile emission in planta, we will capture

them using PDMS tubes located in the headspace of petri dish where the experiment will take place and further analyze them using gas chromatography coupled with mass spectrometry (GC-MS).

Key results

- The project will start in March 2023, when a postdoctoral researcher will join our team to perform the previously described experiment. Therefore, no main results have been obtained yet.
- As preliminary result, Fig. 1, shows the expression pattern of terpene synthases in MYC2 overexpression lines and their potential terpene products based on literature review.

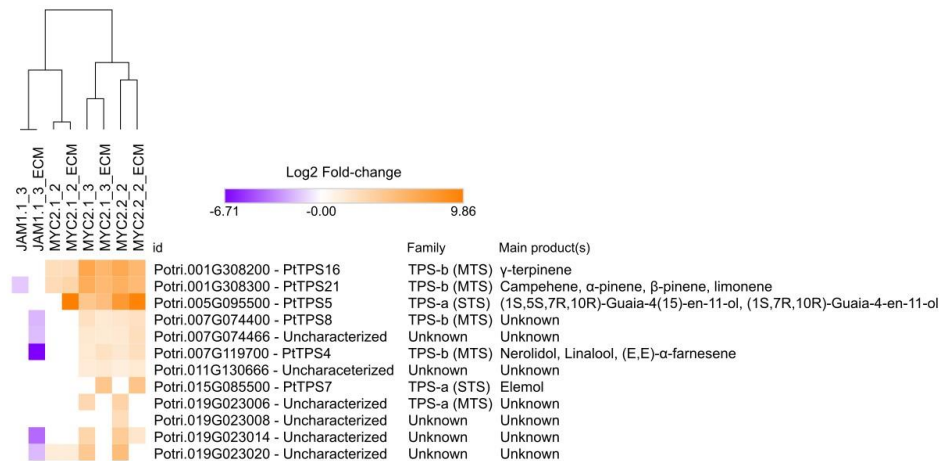


Figure 1. Terpene synthase expression profile in MYC2 overexpression lines, their family affiliation and their hypothetical products according to *in silico* analyses and literature review. Heatmaps depicts the log2 foldchange of poplar terpene synthase genes in MYC2 or JAM1 overexpression lines compared to empty vector controls. TPS = Terpene synthase; MTS = Monoterpene synthase; STS = sesquiterpene synthase.

Main conclusions including key points of discussion —

To the best of our knowledge, up to 21 TPS genes from *P. trichocarpa* have been characterized (Danner et al., 2011; Irmisch et al., 2015; Lackus et al., 2018, 2021). They have been shown to produce terpenes such as α-pinene, β-pinene, camphene, limonene or γ-terpinene.

Interestingly, we have previously demonstrated that exogenous application of some of them can lead to fungal growth arrest and ECM formation impairment (Marqués-Gálvez et al., 2020). *P. tremula x alba* genome, which is the plant model used in this experiment, contains a total of 76 (38 haplotype *tremula* and 38 haplotype *alba*) predicted TPS genes that share a high degree of similarity with *P. trichocarpa* genes. Special attention will need to be put in order to study the volatile profile of *P. tremula x alba* MYC2OE lines, which can lead us to unravel which are the specific terpene synthases that are participating in this process. Additional heterologous characterization in *E. coli*, yeast or *Nicotiana benthamiana* leaves of certain of these genes may be necessary to confirm the production of specific terpenes, since previous studies have shown that even one single amino acid difference can lead to different products (Irmisch et al., 2015; Lackus et al., 2018).

Perspectives —

The study of the “terpenome” profile associated to ECM impairment could hint specific terpene synthases implicated in ECM symbiosis and maybe more generally in beneficial plant microbe interactions. This will allow us to generate plasmids for overexpression of specific genes to study which specific terpene synthases and terpene products are implicated in ECM symbiosis. Future research will focus on how root terpene defenses mediate belowground mutualistic interactions and how they can be manipulated to engineer plants with enhanced disease resistance but stable mutualistic interactions.

Valorization —

No main results have been obtained and therefore, results have been not disseminated yet through conferences, congresses or research articles. Preliminary results have been presented at MoDiP (Molecular dialogue in plant biotic interactions) meeting 2022 and LABEX ARBRE doc postdoc day 2022. We have discussed the experimental setup and preliminary *in silico* results in group meetings and with collaborators from Jena Max Planck institute, Germany.

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, and the obtention of additional external projects comprising the recent obtention of a researcher position at University of Murcia (Spain) by Jose Eduardo Marqués-Gálvez.

References

- Danner H, Boeckler GA, Irmisch S, Yuan JS, Chen F, Gershenzon J, ..., Köllner TG (2011). Four terpene synthases produce major compounds of the gypsy moth feeding-induced volatile blend of *Populus trichocarpa*. *Phytochemistry*, 72(9), 897-908.
- Irmisch S, Jiang Y, Chen F, Gershenzon J, Köllner TG (2014). Terpene synthases and their contribution to herbivore-induced volatile emission in western balsam poplar (*Populus trichocarpa*). *BMC Plant Biology*, 14(1), 1-16.
- Lackus ND, Lackner S, Gershenzon J, Unsicker SB, Köllner TG (2018). The occurrence and formation of monoterpenes in herbivore-damaged poplar roots. *Scientific Reports*, 8(1), 1-13.
- Lackus ND, Morawetz J, Xu H, Gershenzon J, Dickschat JS, Köllner TG (2021). The Sesquiterpene Synthase PtTPS5 Produces (1 S, 5 S, 7 R, 10 R)-Guaia-4 (15)-en-11-ol and (1 S, 7 R, 10 R)-Guaia-4-en-11-ol in Oomycete-Infected Poplar Roots. *Molecules*, 26(3), 555.
- Marqués-Gálvez JE, Basso V, Kohler A, ..., Veneault-Fourrey C (2022). The establishment of *Populus x Laccaria bicolor* ectomycorrhiza requires the inactivation of MYC2 coordinated defense response with a key role for root terpene synthases. *bioRxiv*, 2022-09.