PopSCREEN

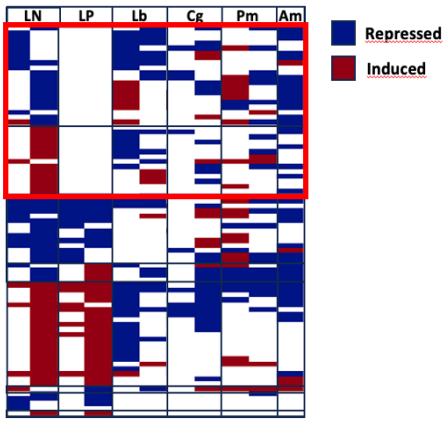

Fig. Transcriptional regulation of the gene members of the 21 SSP families of interest in response to nitrate and phosphate stresses and ectomycorrhizal associations in *Populus canescens*. Heatmaps representing the hierarchical clustering of SSP encoding genes of poplar based on their pattern of relative expression in response to nutrient stresses (nitrate starvation (LN) and phosphate (starvation) and to ectomycorrhizal associations with different ectomycorrhizal fungi (*Laccaria bicolor* (Lb), *Cenoccocum geophylum* (Cg), *Pisolithus microcarpus* (Pm) and *Amanita muscaria* (Am)). For each condition: first colum is an early time point and the second a later time point of collection.
Framed in red: the 35 genes regulated by nitrate and ectomycorrhizal

Poplar Small Secreted Peptides Candidates REgulating Ectomycorrhizal symbiosis during Nitrate stresses

Principle investigator:: Clémence BONNOT (CR), UMR Interactions Arbres/Micro-organismes (IAM) 1136

LabEx partners : Marie-Béatrice BOGEAT-TRIBOULOT (CR, UMR SILVA) sur les WP2 et WP3 du projet
Annegret KOHLER (IR, UMR IAM) sur le WP3 du projet

Collaborations : Oak Ridge National Laboratory (U.S. Department of Energy ; TN, USA) et Duke University (NC, USA)

Thematic actions concerned: WP1 (1.1 et 1.2)

Context —
Ectomycorrhizal symbioses are pivotal for carbon-sequestration and nutrient cycling in boreal and temperate forests. They enhance tree growth in providing limiting mineral nutrients and increasing photosynthesis in high-atmospheric CO_2 levels (Martin *et al.*, 2016; Steidinger *et al.*, 2019), but represent an energetic cost for the tree, which divert up to 20% of its carbon resources toward them (Farrar & Jones, 2000; Hodge *et al.*, 2009; Nehls *et al.*, 2010). To invest their resources efficiently and maintain optimal growth, trees perceive and integrate environmental nutritional cues to regulate adequately their ectomycorrhizal interactions (Plett *et al.*, 2020). Increase of the concentration of nutrients in forest soil via fertilization or pollution represses ECM associations and decreases ECM fungal populations, affecting trees growth, health, resilience and carbon-sequestration (Baum & Makeschin, 2000; Parrent *et al.*, 2006; Averill *et al.*, 2018). The signalling pathways regulating ectomycorrhizal associations in response to nutrient stresses in trees are unknown. However, in herbaceous plants it is known that Small Secreted Peptides (SSPs) transduce local and long-distance signals triggering adaptive responses to abiotic and biotic nutritive cues, including the regulation of their symbiotic associations with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (Bisseling & Scheres, 2014; de Bang *et al.*, 2017a; Roy & Müller, 2022). Also present in trees, the role of SSPs in mediating nutrient signals regulating ectomycorrhizal associations was not investigated.

Objectives —
PopSCREEN aims to discover SSP candidates involved in the regulation of ectomycorrhizal symbiosis in response to nitrate stresses (starvation or excess) in the model tree *Populus x canescens.*

Approaches —
To this aim, PopSCREEN is designed as a three steps procedure: the phylogenetic and transcriptomic *in silico* discovery of SSP candidates transcriptionally and specifically regulated by nitrate starvation or excess in ectomycorrhizal poplar; the *in vitro* assessment of their effects on ectomycorrhizal symbiosis between *P. x canescens* and the ectomycorrhizal fungus *Laccaria bicolor;* and the characterisation of these effects (specificity, transposability and dose-effect relation).

Key results —
- We analyzed the phylogeny and the expression in response to mycorrhization and to nitrate and phosphate stresses (excess and starvation) of 607 poplar genes encoding members of 21 SSP families known to regulate symbiotic associations or to be regulated by nutrient stresses in herbaceous plants.
- We identified 35 SSP encoding genes transcriptionally and specifically regulated by nitrate and ectomycorrhizal symbiosis. Among them, 20 are coding for very short peptides that can be synthesized and hence tested *in vitro* on the ectomycorrhizal symbiosis between *P. X canescens* and *L. bicolor*.
- 10 were cloned and 10 more are being cloned to verify their sequences before synthesis.
- A protocol for fungal culture on microscopy slide was set up to allow the fast and low-cost characterization of the effects of the selected peptides on fungal development.

Main conclusions including key points of discussion —
Similarly, to what is seen for herbaceous plants, all 21 of the selected SSP families harbour at least one member transcriptionally regulated by symbiosis. 81% of them harbors at least one member regulated by nutrient stresses. The expression of 6% of these genes is regulated by both ectomycorrhizal symbiosis and nitrate stresses in poplar. These specific genes are good candidates to the mediation of nitrate stresses signals regulating ectomycorrhizal symbiosis in poplar.

Perspectives —
Verifying and characterizing *in vitro* the effects of these peptides will allows us to select among them strong candidates (specific and transposable) to functionally characterize in subsequent research project aiming to dissect the molecular mechanisms of the regulation of ectomycorrhizal symbiosis in response to nitrate stresses.

Valorization —
Talk at the symposium of the INUPRAG network:
Clémence Bonnot, Alexis Chartoire, Emmanuelle Morin, Annegret Kohler, Francis Martin (2023-02-21). Do SSPs regulate ectomycorrhizal symbiosis in response to biotic and abiotic nutrient signals in trees? INUPRAG Symposium on Integrative Plant Biology 2023, Umeå, Suède. Conférence invitée. https://hal.inrae.fr/hal-04186836.

Leveraging effect of the project—
The identification of SSPs regulating ectomycorrhizal symbiosis in response to nitrate stresses, and their subsequent functional characterization, will provide insights into the regulation of ECM associations and more generally on the signalling pathways regulating tree-microbes interaction, tree nutrition and growth in a changing environment. Which will help to understand forest adaptation and evolution in the context of global change. Being the first SSPs discovered as involved in the regulation of ECM fungi associations, the functional characterization of these peptides, and of the pathway(s) to which they participate, will open a lead toward possible applications in the field of ECM fungi culture (e.g. truffle orchards) and biofertilizers accommodation for forestry and agroforestry.