AVERTISSEMENT

Ce document est le fruit d'un long travail approuvé par le jury de soutenance et mis à disposition de l'ensemble de la communauté universitaire élargie.

Il est soumis à la propriété intellectuelle de l'auteur. Ceci implique une obligation de citation et de référencement lors de l'utilisation de ce document.

D'autre part, toute contrefaçon, plagiat, reproduction illicite encourt une poursuite pénale.

Contact : ddoc-theses-contact@univ-lorraine.fr

LIENS

Code de la Propriété Intellectuelle. articles L 122. 4
Code de la Propriété Intellectuelle. articles L 335.2- L 335.10
http://www.cfcopies.com/V2/leg/leg_droi.php
http://www.culture.gouv.fr/culture/infos-pratiques/droits/protection.htm

Ecole doctorale SIReNA (Sciences et Ingénierie des Ressources Naturelles)

Thèse

Présentée et soutenue publiquement pour l'obtention du titre de

Docteur de l'Université de Lorraine

Mention: «Biologie et Ecologie des Forêts et des Agrosystèmes»

par Veronica BASSO

Les voies de signalisation des phytohormones dans l'établissement de la symbiose ectomycorhizienne entre racines de *Populus* et *Laccaria bicolor*: un nouveau regard sur la modulation de la perception de l'acide jasmonique au cours de la colonisation fongique

Soutenue publiquement le 04 décembre 2019

Directeur de thèse: Mr **Francis MARTIN**

Co-directeur de thèse: Mme **Claire VENEAULT-FOURREY**

Membres du jury

Rapporteurs : Mme Valérie LEGUÉ, Dr. Prof. Université Clermont Auvergne, Clermont-Ferrand

Mme Soizic ROCHANGE, Assistant Professeur Université Toulouse III Paul Sabatier

Examinateurs : Mr Yves JOLIVET, Dr. Prof. Université de Lorraine, Nancy

Mr Florian FRUGIER, Directeur de Recherche CNRS, IPS2 Paris-Saclay

Mr Francis MARTIN, Directeur de Recherche INRA Grand-Est Nancy/ Dr. Prof. Université de Pékin, **directeur de thèse**

Mme Claire VENEAULT-FOURREY, Directeur de Recherche INRA Grand-Est Nancy, **co-directeur de thèse**

UMR 1136 INRA / Université de Lorraine, Interactions Arbres – Microorganismes
INRA Centre Grand Est – Nancy – 54208 Champenoux
Faculté de Sciences et Techniques – 54500 Vandoeuvre-lès-Nancy

Doctoral School SIReNA (Sciences et Engineering of Natural Resources)

Dissertation

Publicly presented and defended to obtain the title of

Doctor of the University of Lorraine

Specialty: «Biology and Ecology of Forests and Agrosystems»

by Veronica BASSO

Phytohormone signaling in the establishment of the ectomycorrhizal symbiosis between *Populus* roots and *Laccaria bicolor*: novel insights into the modulation of jasmonate perception upon fungal colonization

Publicly defended on the 4th of December 2019

PhD supervisor: Mr **Francis MARTIN**

PhD co-supervisor: Mme **Claire VENEAULT-FOURREY**

Jury members :

Rapporteur: Mme Valérie LEGUÉ, Dr. Prof. Université Clermont Auvergne, Clermont-Ferrand

Mme Soizic ROCHANGE, Assistant Professor Université Toulouse III Paul Sabatier

Examiners: Mr Yves JOLIVET, Dr. Prof. Université de Lorraine, Nancy

Mr Florian FRUGIER, Research Director CNRS, IPS2 Paris-Saclay

Mr Francis MARTIN, Research Director INRA Grand-Est Nancy/ Dr. Prof. Beijing University, **PhD supervisor**

Mme Claire VENEAULT-FOURREY, Research Director INRA Grand-Est Nancy, **PhD co-supervisor**

UMR 1136 INRA / Université de Lorraine, Interactions Arbres – Microorganismes
INRA Centre Grand Est – Nancy – 54208 Champenoux
Faculté de Sciences et Techniques – 54500 Vandoeuvre-lès-Nancy

Acknowledgements

I wish to acknowledge Florian Frugier, Yves Jolivet, Valérie Légué, and Soizic Rochange for accepting to be part of my PhD thesis committee.
I'd like to thank Francis Martin and Claire Veneault-Fourrey for involving me in this exciting project, for their input and support to my work. A special thanks to Claire for everyday supervision, her kindness, her patience, and a lot of her time.
I want to acknowledge the funding sources that have made this work possible: the LabEx ARBRE, the Oak Ridge National Lab, and the Joint Genome Institute.

A heartfelt thank you to all the people of the IAM Ecogenomics team that have made my stay comfortable and intellectually stimulating, and allowed me to perform my research efficiently and safely. Special thanks to Sebastien, Christine, Frédéric, Agnès, Nicole, Marie-Claude and Noémie for dealing with administrative aspects of the work in and out of the laboratory. Thank you Patrice, Laure, and Frédéric for the technical support. Thank you to all the PhD students, postdoctoral fellows, and researchers that have contributed to lively discussions and exchanges of ideas: Annegret, Aurélie, Cécile, Clémence, Claude, Elena, Emmanuelle, Félix, Feng, Flora, François, Gang, Heng, Maìra, Marc, Milena, Laura, Lauralie, Liang, Lucas, Océane, Sebastien, Shingo, Siqi, Stéphane. A particular acknowledgement to Clémence, Félix, and Milena for sharing my joys and sorrows over lunch every day.

A special acknowledgement to Brice, Justine, and Jonathan, who have successfully completed their internships under my supervision. Thank you for your contribution to this work and all the best for your future.

Emanuela and Bruno, thank you for your unconditioned support. You encouraged me to follow my ambitions and dreams, even far away from you and my hometown. Your love and trust underlie everything I do.

Amit, thank you for always being there when I needed you. You showed me that the most exciting peak is always the one ahead.

To Alberta Maria and Maria Giuseppina

Table of Contents

TABLE OF CONTENTS **9**

LIST OF FIGURES **12**

LIST OF TABLES **13**

RÉSUMÉ DES TRAVAUX DE THÈSE **15**

1. INTRODUCTION 15
   1.1 Rôle des phytohormones dans le développement de la symbiose ectomycorhizienne *15*
   1.2. Objectifs de ces travaux de these *17*
2. LA PROTÉINE EFFECTRICE MISSP7 DU CHAMPIGNON ECTOMYCORHIZIEN *LACCARIA BICOLOR* IMPACTE L'INTERACTOME DE PTJAZ6, CO-RECEPTEUR DE L'AJ DANS LES RACINES DE PEUPLIER 18
3. LE FACTEUR DE TRANSCRIPTION PTAMYC2.1, ASSOCIÉ À PTJAZ6, IMPACTE LE DÉVELOPPEMENT DU RÉSEAU DE HARTIG 19
4. *LACCARIA BICOLOR* MODIFIE LA SENSIBILITÉ DES RACINES DE PEUPLIER AUX PHYTOHORMONES COMME LES JASMONATES, GIBBÉRELLINES, SALICYLATES ET ÉTHYLÈNE 21
5. CONCLUSION ET PERSPECTIVES 22

CHAPTER 1: INTRODUCTION **25**

1. THE ECTOMYCORRHIZAL SYMBIOSIS 27
   1.1. The mutualism between trees and ectomycorrhizal fungi in forest ecosystems *27*
   1.2. Morphology and molecular determinants of developing ectomycorrhizae *28*
   1.3. The genetic "symbiosis toolkit" of ectomycorrhizal lifestyle *31*
   1.4. Ectomycorrhizal symbioses and tree fitness *33*
   1.5. The modulation of tree metabolism, development and defense upon ectomycorrhizae formation depends on phytohormonal signaling *35*
2. PHYTOHORMONAL REGULATION OF PLANT DEVELOPMENT AND DEFENSE 37
   2.1. Jasmonate biosynthesis, signaling and responses *37*
   2.2. Biosynthesis and signaling of phytohormones involved in defense and growth *45*
   2.3. The molecular basis of phytohormone crosstalk during defense and growth *56*
3. DEFENSE AND DEVELOPMENT PHYTOHORMONES IN THE ESTABLISHMENT OF THE ECTOMYCORRHIZAL SYMBIOSIS 61
   3.1. Auxin, ethylene and sesquiterpenes mediate tree developmental responses to ectomycorrhizal fungi *61*
   3.2. The pivotal role of jasmonate signaling in the Populus-L. bicolor interaction *62*
   3.3. Opposing effects of ethylene and salicylic acid signaling in the development of the Hartig net *63*
4. AIMS OF THE PHD PROJECT 65
   4.1. The Populus tremula x alba - Laccaria bicolor model system *65*
   4.2. Open questions on the impact of phytohormone signaling in the establishment of the ectomycorrhizal symbiosis *65*
   4.3. Elucidating the role of JA signaling, and its interacting hormone signaling pathways, in ectomycorrhizal development *66*
5. REFERENCES 68
6. APPENDIX: JASMONATE IN BENEFICIAL MICROBE-ROOT INTERACTIONS 95
   6.1. Abstract *95*
   6.2. Introduction *96*
   6.3. JA in arbuscular mycorrhiza development *97*
   6.4. JA in root nodule symbiosis development *100*
   6.5. JA in ectomycorrhizal symbiosis development *104*
   6.6. JA in other beneficial rhizospheric/endophytic interactions *105*

6.7. JA in beneficial microbe-triggered systemic resistance *107*
6.8. Conclusion *109*
6.9. References *110*

CHAPTER 2: MISSP7 ALTERS THE INTERACTOME OF PTJAZ6 127
1. PREFACE 129
2. ARTICLE (SUBMITTED) 131
2.1. Abstract *132*
2.2. Introduction *132*
2.3. Materials and methods *135*
2.4. Results *139*
2.5. Discussion *151*
2.6. Acknowledgments *154*
2.7. References *155*
2.8. Supplemental material *161*

CHAPTER 3: THE JAZ6-ASSOCIATED TRANSCRIPTION FACTOR MYC2.1 INHIBITS HARTIG NET DEVELOPMENT 169
1. PREFACE 171
2. ARTICLE (IN PREPARATION) 172
2.1. Abstract *172*
2.2. Introduction *172*
2.3. Results *175*
2.4. Discussion *182*
2.5. Methods *187*
2.6. Acknowledgements *192*
2.7. References *192*
2.8. Supplemental material *197*

CHAPTER 4: *L. BICOLOR* ALTERS POPLAR ROOT SENSITIVITY TO JASMONATE, GIBBERELLIN, SALICYLATE, AND ETHYLENE 209
1. PREFACE 211
2. ARTICLE PUBLISHED IN PLANT, CELL & ENVIRONMENT (DECEMBER 2019) 213
2.1. Abstract *213*
2.2. Introduction *214*
2.3. Materials and methods *217*
2.4. Results *223*
2.5. Discussion *235*
2.6. Conclusion *239*
2.7. Acknowledgements *240*
2.8. Author contributions *240*
2.9. References *240*
2.10. Supplemental Material *249*
3. APPENDIX: ADDITIONAL DATA 261
3.1. Additional results *261*
3.2. Additional discussion *263*
3.3. Additional tables *265*
3.4. Additional references *266*

CHAPTER 5: DISCUSSION AND PERSPECTIVES 269
1. DISTINCT BRANCHES OF JA SIGNALING MIGHT DIFFERENTIALLY REGULATE POPLAR-*L. BICOLOR* ECM DEVELOPMENT 271
2. THE HORMONAL LANDSCAPE OF ECM DEVELOPMENT 274
3. MICROBIAL MANIPULATION OF PHYTOHORMONE SIGNALING THROUGH EFFECTORS ACROSS DIFFERENT TROPHIC STRATEGIES 278
4. FUTURE RESEARCH AND PERSPECTIVES 281

5. REFERENCES 285
RÉSUMÉ 291
ABSTRACT 292

List of Figures

CHAPTER 1: INTRODUCTION

FIGURE 1: THE MAIN STEPS OF ECM DEVELOPMENT.....25
FIGURE 2: JASMONATE BIOSYNTHESIS AND SIGNALING.....41
FIGURE 3: JAZ PROTEINS AND ASSOCIATED TRANSCRIPTION FACTORS.....43
FIGURE 4: SA BIOSYNTHESIS AND SIGNALING IN ARABIDOPSIS.....48
FIGURE 5: ET BIOSYNTHESIS AND SIGNALING IN ARABIDOPSIS.....51
FIGURE 6: AUXIN BIOSYNTHESIS AND PERCEPTION.....52
FIGURE 7: GA BIOSYNTHESIS AND SIGNALING.....55

APPENDIX: JASMONATE IN BENEFICIAL MICROBE-ROOT INTERACTIONS

FIGURE 1: THE ROLE OF JAS IN DEVELOPMENT OF AM SYMBIOSIS.....100
FIGURE 2: THE ROLE OF JAS IN RNS.....104

CHAPTER 2: MISSP7 ALTERS THE INTERACTOME OF PTJAZ6

FIGURE 1. PTJAZ6 INTERACTS WITH PTJAM1 AND PTMYC2 TRANSCRIPTION FACTORS IN YEAST AND IN PLANTA......142
FIGURE 2. PTJAZ6 IS ABLE TO FORM HOMODIMERS AND HETERODIMERS WITH PTJAZ PROTEINS THROUGH ITS TIFY DOMAIN.....145
FIGURE 3. PTJAZ6 INTERACTS DIRECTLY WITH ONE PTTOPLESS-RELATED PROTEIN AND TWO NINJA PROTEINS......147
FIGURE 4. BOTH PTJAZ6 NT AND TIFY DOMAINS ARE NECESSARY FOR PTJAZ6 INTERACTION WITH MISSP7.....149
FIGURE 5. MISSP7 IMPACTS THE STRUCTURE OF THE JASMONATE PERCEPTION COMPLEX.....151
FIGURE 6. PTJAZ6 INTERACTOME IN POPULUS ECTOMYCORRHIZAL ROOT TIPS.....154
SUPPLEMENTAL FIGURE S1. PTJAZ6 DOES NOT AUTO-ACTIVATE THE THREE REPORTER GENES USED IN THE YEAST-TWO HYBRID SYSTEM.....161
SUPPLEMENTAL FIGURE S2. FEATURES OF POPULUS MYC2 AND JAM1 PROTEINS.....162
SUPPLEMENTAL FIGURE S3. PTJAZ6 PROTEIN CONTAINS THREE TYPICAL DOMAINS OF JAZ PROTEINS.....163
SUPPLEMENTAL FIGURE S4. R209 RESIDUE OF PTJAZ6 IS REQUIRED FOR ITS CORONATINE-DEPENDENT INTERACTION WITH PTCOI1.....164
SUPPLEMENTAL FIGURE S5.....165

CHAPTER 3: THE JAZ6-ASSOCIATED TRANSCRIPTION FACTOR MYC2.1 INHIBITS HARTIG NET DEVELOPMENT

FIGURE 1. THE OVEREXPRESSION OF PTAMYC2.1 AND PTAJAM1.1 IN POPLAR ROOTS ALTERS HARTIG NET DEVELOPMENT DURING L. BICOLOR COLONIZATION.....177
FIGURE 2. L. BICOLOR COLONIZATION REPRESSES PTAMYC2.1, PTAMYC2.2, AND PTAJAM1.1 EXPRESSION IN TRANSGENIC ROOTS.....180
FIGURE 3. SILENCING OF PTAMYC2S OR PTAJAM1S DOES NOT AFFECT THE MYCORRHIZAL COLONIZATION RATE..182
SUPPLEMENTAL FIGURE 1. THE GENE MODEL OF PTAJAM1.2 DIFFERS IN ITS 3' BETWEEN TWO VERSIONS OF THE POPULUS TRICHOCARPA GENOME.....198
SUPPLEMENTAL FIGURE 2. GENERATION OF POPLAR TRANSGENIC LINES OVEREXPRESSING PTAMYC2.1, PTAMYC2.2, AND PTAJAM1.1.....200
SUPPLEMENTAL FIGURE 3. THE HARTIG NET PHENOTYPE OF MYC2.1 OE 2 AND JAM1.1 OE 3 PLANTS DOES NOT DEPEND ON ROOT DIAMETER, NUMBER OF APOPLASTIC SPACES OR FREQUENCY OF HARTIG NET INITIATION.. 201
SUPPLEMENTAL FIGURE 4. THE OVEREXPRESSION OF PTAMYC2.1 AND PTAJAM1.1 IN POPLAR ROOTS DOES NOT ALTER SHOOT PHYSIOLOGY BUT AFFECTS ROOT ARCHITECTURE.....203
SUPPLEMENTAL FIGURE 5. THE SUPPRESSION OF PTAMYC2.1 EXPRESSION IN COLONIZED GUS 6 AND MYC2.1 OE 2 TRANSGENIC ROOTS IS NOT DUE TO TECHNICAL BIAS.....205

CHAPTER 4: *L. BICOLOR* ALTERS POPLAR ROOT SENSITIVITY TO JASMONATE, GIBBERELLIN, SALICYLATE, AND ETHYLENE

Figure 1. Experimental approach.....217
Figure 2. Exogenous phytohormone treatments affect ectomycorrhizal development and physiology of poplar cuttings.....226
Figure 3. Transcriptomic analysis reveals decreased sensitivity to JA in colonized versus uncolonized poplar lateral roots.....228
Figure 4. Ectomycorrhizal development induces the activation of subsets of phytohormone-specifically differentially expressed genes.....233
Supplemental Figure 1. Replicate analysis reveals consistency of normalized poplar lateral root and L. bicolor mycelium transcriptomes.....249
Supplemental Figure 2. Weighted Gene Co-expression Network Analysis reveals four gene clusters regulated by SA-JA and ET-JA crosstalk.....250
Supplemental Figure 3. Condition-wise Tatami maps.....251
Supplemental Figure 4. ET treatment transiently affects the growth of L. bicolor colonies.....252
Supplemental Figure 5. Pictures of representative poplar cuttings.....253
Supplemental Figure 6. Groups of phytohormone-treated colonized and uncolonized poplar cuttings showing specific phenotypes.....254
Supplemental Figure 7. Expression levels of phytohormone-specifically differentially expressed genes (phytohormone-sDEGs) and crosstalk-regulated genes are constant in hormone-treated colonized and uncolonized poplar lateral roots at different time points......255

CHAPTER 5: DISCUSSION AND PERSPECTIVES
Figure 1. Working model: distinct branches of JA signaling differentially regulate ECM development......272
Figure 2. The roles of phytohormones in the development of the ECM symbiosis.....277

List of Tables

CHAPTER 2: MISSP7 ALTERS THE INTERACTOME OF PTJAZ6
Table S1. Expression level of poplar proteins involved in JA-signaling pathway in in vitro grown poplar-L. bicolor ectomycorrhiza and in poplar root tips.....167
Table S2. List of primers used in the study.....168

CHAPTER 3: THE JAZ6-ASSOCIATED TRANSCRIPTION FACTOR MYC2.1 INHIBITS HARTIG NET DEVELOPMENT
Supplemental Table 1. Primers used for cloning the genes or gene fragments employed in this study......207
Supplemental Table 2. Primers used for RT-qPCR.....207

CHAPTER 4: *L. BICOLOR* ALTERS POPLAR ROOT SENSITIVITY TO JASMONATE, GIBBERELLIN, SALICYLATE, AND ETHYLENE
Table 1. ECM accumulate salicylic acid.....224
Table 2. Notable overlaps between poplar specifically differentially expressed genes after phytohormone treatment and ectomycorrhiza-responsive genes suggest that JA and ET signaling function in ectomycorrhizal development.....235

Appendix: additional data
Additional Table 1. ECM accumulate auxins, CK ribosides, and phaseic acid.....265
Additional Table 2. Expression of genes that function in auxin and CK metabolism in early colonized poplar LRs.....266

Résumé des travaux de thèse

1. Introduction

1.1 Rôle des phytohormones dans le développement de la symbiose ectomycorhizienne

Pour pallier aux limitations nutritives des sols des forets tempérées et boréales, la plupart des espèces d'arbres forment des associations mutualistes avec les champignons présents dans le sol. Dans ces écosystèmes forestiers, environ 6000-7000 espèces d'arbres interagissent avec plus de 20 000 espèces de basidiomycètes et d'ascomycètes pour former des structures symbiotiques appelées ectomycorhizes (ECM). Ces tissus sont composés à la fois de tissus racinaires et fongiques (van der Heijden et al., 2015 ; Martin et al., 2016). Les champignons ectomycorhiziens contribuent au développement et à la santé des arbres en (i) améliorant leur nutrition minérale (N,P) et hydrique, en échange de carbone issu de la photosynthèse (Tibbett & Sanders, 2002 ; Plassard & Dell, 2010 ; Lehto & Zwiazek, 2011) ; (ii) augmentant leur résistance aux stress biotiques et abiotiques (Luo et al., 2009 ; Pena et al., 2013 ; Danielsen & Polle, 2014 ; Luo et al., 2018 ; Gonthier et al., 2019) ; (iii) participant via leur réseau de mycélium extramatriciel au réseau appelé "wood wide web", permettant l'échange de nutriments et de molécules de signalisation entre arbres voisins (Simard et al., 1997 ; Klein et al., 2016) ; (iv) participant aux cycles des éléments minéraux par la séquestration du carbone atmosphérique dans le sol et la dégradation de la matière organique (Lindahl & Tunlid, 2015 ; Nehls et al., 2016).

Les ECM sont des organes mixtes composés d'hyphes fongiques et de fines racines latérales d'arbres. L’ontogenèse d’une ECM nécessite plusieurs étapes : d’abord l’échange de signaux pré-symbiotiques, suivie de la formation d’un manteau fongique et enfin la colonisation de l’apoplaste par les hyphes pour former le réseau de Hartig, zone où les échanges de nutriments entre les partenaires symbiotiques ont supposément lieu. Ainsi avant le contact direct, les deux partenaires échangent des flavonoïdes, des phytohormones et des petites protéines sécrétées pour une reconnaissance mutuelle et pour déclencher le développement de racines latérales (Garcia et al., 2015). Ensuite, les hyphes fongiques agrégées adhèrent à la surface des racines latérales et formant un épais manteau. La formation du réseau d’Hartig s’accompagne de l'allongement radial des cellules épidermiques et corticales de la racine et d’une modification de la composition des parois des cellules fongiques et végétales permettant aux hyphes fongiques de croître dans l'apoplaste des cellules corticales (Balestrini & Kottke, 2016). Le développement d’une ECM

fonctionnelle nécessite donc un dialogue moléculaire sophistiqué et finement ajusté entre les deux partenaires de l'interaction. Ce dialogue implique un échange de molécules volatiles et solubles, ainsi que la sécrétion de protéines effectrices et d'enzymes. Ces échanges de signaux ont un double objectif : (i) reprogrammer le développement des racines de l'hôte et des cellules fongiques et (ii) détourner l'immunité des plantes hôtes.

Les premiers champignons ectomycorhiziens sont apparus il y a environ 140-180 millions d'années, associés aux racines de Pinaceae (Brundrett, 2002). Depuis lors, les champignons ectomycorhiziens ont évolué à plusieurs reprises à partir d'ancêtres saprotrophes. Malgré leur origine polyphylétique, les champignons ectomycorhiziens ont subi une perte convergente d'enzymes dégradant la paroi cellulaire des cellules végétales (PCWDE) et de peroxydases de classe II oxydant la lignine, tout en développant un arsenal de petites protéines sécrétées (SSPs) spécifiques pour chaque lignée phylogénétique (Kohler et al., 2015). L'expression au cours de la mycorhization de gènes fongiques codant des PCWDE, des transporteurs de nutriments (N, P, C) et de petites protéines sécrétées (Mycorrhiza-induced Small Secreted Proteins, MiSSPs) est nécessaire au développement de l'ECM, même si des mécanismes moléculaires spécifiques d'établissement de symbiose peuvent aussi dépendre de l'espèce et résultent probablement d'une co-évolution avec l'arbre hôte (Martin et al., 2010).

Si la formation d'une ECM nécessite une reprogrammation générale du transcriptome des cellules fongiques, celui des cellules racinaires est également fortement modifié. La modulation de l'immunité et du développement des racines des arbres se produit en partie par l'accumulation et/ou une perception modifiée des phytohormones. Par exemple, lors de la mycorhization la signalisation induite par l'auxine dans les racines de l'arbre hôte favorise le développement des racines latérales, probablement en lien avec la voie de signalisation de l'éthylène (Felten et al., 2009 ; Splivallo et al., 2009). De plus, la protéine MiSSP7 de *Laccaria bicolor* inhibe la voie de signalisation de l'acide jasmonique (AJ) dans les racines de l'hôte, en stabilisant le co-récepteur de signalisation de l'AJ du peuplier, PtJAZ6. Ainsi, la protéine effectrice MiSSP7 est nécessaire au développement du réseau de Hartig, probablement pour supprimer au moins en partie les réponses immunitaires des cellules racinaires (Plett et al., 2014a). Cependant, la reprogrammation transcriptomique des racines de peuplier déclenchée par MiSSP7 reste à identifier, ainsi que les relations pendant le développement d'ECM entre la voie de signalisation de l'AJ et les autres voies de signalisation hormonale.

1.2. Objectifs de ces travaux de these

Cette thèse a pour objectif d'élucider le rôle de la voie de signalisation de l'AJ et de celles interagissant avec l'AJ comme l'acide gibbérellique (GA), l'acide salicylique (SA) et l'éthylène (ET), dans la régulation du réseau transcriptomique associé au développement de l'interaction mutualiste entre le basidiomycète ectomycorrhizien *L. bicolor* et l'arbre angiosperme *P. tremula x alba*.

Pour ce faire, j'ai concentré mon travail sur trois aspects principaux : (i) comprendre le mécanisme moléculaire par lequel l'effecteur fongique sécrété MiSSP7 supprime les réponses du peuplier médiées par l'AJ ; (ii) évaluer si un(des) segment(s) de la signalisation de l'AJ sont sélectivement réprimés pendant le développement d'ECM ; (iii) caractériser les voies de signalisation hormonales et leurs interactions dans les racines du peuplier et clarifier leur fonction pendant la colonisation fongique.

Pour l'étude du rôle moléculaire de MiSSP7 dans le complexe de perception de l'AJ des racines de peuplier, nous avons d'abord identifié l'interactome de PtJAZ6 par des études d'interaction protéine-protéine. En effet, chez la plante modèle Arabidopsis, l'activité transcriptionnelle de différents facteurs de transcription (FT) contrôle des réponses distinctes à l'AJ (Chini et al., 2016). Ainsi, l'identification des FT interagissant avec PtJAZ6 dans les racines de peuplier apparait cruciale pour comprendre l'influence de MiSSP7 sur l'expression génique des racines hôtes. De plus, pour mieux comprendre l'activité de MiSSP7 sur la perception de l'AJ au niveau moléculaire, nous avons testé par triple-hybride si les interactions entre PtJAZ6 et chacune des autres protéines du complexe étaient modifiées ou non en présence de MiSSP7.

J'ai ensuite étudié le rôle des FT associés à PtJAZ6 pendant le développement de l'ECM, en générant des lignées transgéniques de peuplier surexprimant les FT d'intérêt, ainsi que des peupliers chimériques dont les racines expriment significativement moins (silencing) ces mêmes FT. L'analyse de l'architecture racinaire, du développement des ECM et du réseau de Hartig des ECM de ces peupliers transgéniques a fourni des indications sur l'importance de ces régulateurs de l'expression génique pour le développement de la relation mutualiste. D'autre part, j'ai évalué si la colonisation fongique supprime sélectivement un sous-ensemble des réponses dépendantes de l'AJ. L'objectif était de déterminer si une manipulation par le champignon de l'immunité des plantes et/ou d'autres réponses (développement) déclenchées par l'AJ est nécessaire pour la symbiose ECM. Ainsi, j'ai examiné, par séquençage d'ARN à haut débit, les transcriptomes de racines de peuplier colonisées ou non par *L. bicolor* et traitées par l'ajout exogène de MeJA. Dans le même contexte expérimental, j'ai également défini des gènes de réponse à l'AJ dans les racines de peuplier et inspecté la dynamique de leur expression au cours du développement de l'ECM.

Enfin, j'ai également étudié le rôle des voies de signalisation de l'acide salicylique, l'éthylène et l'acide gibbérellique et de leur interaction avec la voie de signalisation de l'AJ, dans la reprogrammation transcriptionnelle ayant cours pendant la formation de l'ECM. Dans la même série d'expériences, les concentrations de JAs, SA, GAs, cytokinines et abscisates ont été quantifiés par spectrométrie de masse, pour obtenir des informations sur la dynamique du métabolisme des phytohormones dans le développement de l'ECM.

2. La protéine effectrice MiSSP7 du champignon ectomycorhizien *Laccaria bicolor* impacte l'interactome de PtJAZ6, co-recepteur de l'AJ dans les racines de peuplier

L'établissement, le développement et le fonctionnement des racines ectomycorhiziennes nécessitent une coordination stricte du développement, de l'immunité et de la physiologie des cellules fongiques et végétales. Le contrôle de ces processus est effectué, en partie, par les phytohormones. En exploitant l'interaction mutualiste entre *L. bicolor* et *Populus* spp. comme modèle pour étudier la symbiose ECM au niveau moléculaire, il a été démontré que les hyphes du champignon *L. bicolor* sécrètent des petites protéines (MiSSPs) dans le voisinage des racines du peuplier (Martin et al., 2008 ; Martin et al., 2016). L'une d'entre elle, MiSSP7, est un effecteur symbiotique nécessaire au développement de l'ECM (Plett et al., 2011). MiSSP7 interagit physiquement avec la protéine JAZ6 de *Populus trichocarpa* (PtJAZ6) dans le noyau des cellules hôtes (Plett et al., 2014a). Cette interaction inhibe la dégradation de PtJAZ6 en présence d'AJ et, par conséquent, supprime au moins certaines réponses dépendantes de l'AJ permettant la colonisation fongique *in planta*. Cependant, l'influence de l'interaction MiSSP7-PtJAZ6 sur le réseau transcriptomique des cellules racinaires du peuplier reste encore inconnue. En particulier, il n'est pas clair si la stabilisation de PtJAZ6 a un impact sur l'activité des FT en aval. En effet, différents FT interagissant avec PtJAZ6 pourraient réguler des segments distincts de la signalisation de l'AJ, dont certains pourraient être perturbés en présence de la protéine effectrice MiSSP7.

Pour mieux comprendre l'altération de la perception et des réponses à l'AJ au cours du développement d'ECM, la composition du complexe protéique associé à PtJAZ6 dans les racines de peuplier a été déterminé. Un crible double-hybride en levure a permis d'identifier un FT de peuplier JAM1, appartenant à la famille basic helix-loop-helix (bHLH), comme partenaire d'interaction de PtJAZ6. Par la suite, les interactions entre PtJAZ6 et d'autres protéines de peuplier candidates, selon leur homologie avec Arabidopsis, ont été testées en levure et *in planta* par expression transitoire en feuilles de tabac. Enfin, j'ai évalué en système levure triple-hybride si MiSSP7 modifie les spécificités de liaison de PtJAZ6 à ses protéines associées.

Ces études d'interaction protéine-protéine ont démontré que PtJAZ6 interagit avec une protéine de la famille TOPLESS, deux protéines NINJA, plusieurs autres protéines JAZ et les homologues du peuplier des FT bHLH d'Arabidopsis MYC2 et JAM1. Les gènes codant pour PtMYC2 et PtJAM1 ont subi une duplication dans le génome du peuplier, aboutissant à l'existence de deux paralogues chacun : PtMYC2.1 (Potri.001G142200), PtMYC2.2 (Potri.003G092200), PtJAM1.1 (Potri.014G099700) et PtJAM1.2 (Potri.002G172100). Des tests en levure simple-hybride suggèrent que, comme chez Arabidopsis, les PtMYC2 agissent comme activateurs de transcription tandis que les PtJAM1 fonctionnent comme répresseurs de transcription. De plus, une étude triple-hybride en levure a montré que l'effecteur fongique MiSSP7 renforce la liaison de PtJAZ6 à PtMYC2.1 et affaiblit l'interaction PtJAZ6-PtMYC2.2. Chez Arabidopsis, les protéines JAZ agissent en qualité de répresseurs transcriptionnels par recrutement d'enzymes de remodelage de la chromatine de la famille TOPLESS soit directement soit via la protéine NINJA (Howe et al., 2018). Ainsi, notre résultat suggère qu'au contact du mycélium de *L. bicolor* l'activité transcriptionnelle de PtMYC2.1 est inhibée, tandis que PtMYC2.2 est libéré de l'inhibition par PtJAZ6. De plus, MiSSP7 affaiblit l'interaction entre PtJAZ6 et d'autres protéines PtJAZ, mais la fonction de cette inhibition de l'homo- ou hétérodimérisation PtJAZ6 reste actuellement inconnue.

Nous avons montré qu'un effecteur symbiotique sécrété par un champignon mutualiste peut favoriser l'interaction symbiotique en modifiant la dynamique des réseaux d'interaction protéine-protéine associés aux signaux de l'AJ et en maintenant la répression des gènes régulés par PtMYC2.1. Le rôle de la concurrence possible entre les PtMYC2 et les PtJAM1 pour la liaison à PtJAZ6 et aux promoteurs des gènes cibles doit être étudié dans les recherches futures.

3. Le facteur de transcription PtaMYC2.1, associé à PtJAZ6, impacte le développement du réseau de Hartig.

Les jasmonates sont une classe d'oxylipines dérivées d'acides gras impliqués dans l'allongement des racines primaires, la formation de trichomes, la sénescence des feuilles, la fertilité masculine et la défense (Wasternack & Hause, 2013 ; Howe et al., 2018). Il a été montré que l'ajout exogène d'AJ est délétère pour le développement de la symbiose ECM entre les racines de *Populus* spp. et *L. bicolor* (Plett et al., 2014a,b). De plus, nous avons découvert que le co-récepteur de l'AJ PtJAZ6, stabilisé par l'effecteur fongique MiSSP7, interagit avec les FT PtMYC2 et PtJAM1. Chez Arabidopsis, le FT de la famille bHLH MYC2 coordonne une cascade transcriptionnelle favorisant la résistance aux insectes, la tolérance au stress oxydatif et le métabolisme des flavonoïdes tout en ayant un impact négatif sur la réponse aux pathogènes nécrotrophiques et la biosynthèse des métabolites secondaires

(Dombrecht et al., 2007). D'autre part, les FT bHLH du sous-groupe IIId bHLH17/JASMONATE ASSOCIATED MYC2-LIKE1 (JAM1), bHLH13/JAM2, bHLH3/JAM3 et bHLH14 agissent comme répresseurs transcriptionnels impliqués pour atténuer les réponses dépendantes de l'AJ, et sont en compétition avec MYC2 pour leur liaison au promoteur des gènes cibles (Howe et al., 2018). Ainsi, la vaste gamme de FT qui interagissent avec les protéines JAZ, leurs différents profils d'expression et leur spectre spécifique de gènes cibles permettent une modulation précise des différentes réponses dépendantes de l'AJ aux différents signaux liés au développement ou de l'environnement (Chini et al., 2016 ; Howe et al., 2018).

Ayant montré dans le chapitre précédent que la fixation de MiSSP7 sur PtJAZ6 modifie la force des interactions entre PtJAZ6 et ses FT associés, nous avons émis l'hypothèse que les FT PtaMYC2 et PtaJAM1 de *Populus tremula x alba*, respectivement des activateurs et des répresseurs de transcription, jouent des rôles opposés dans le développement des ECM. Nous avons également émis l'hypothèse que les deux paralogues PtaMYC2.1 et PtaMYC2.2 régulent l'expression de différents sous-ensembles de gènes cibles et que l'activation ou la répression de segments distincts de la voie de signalisation de l'AJ pourrait contrôler la colonisation fongique des racines des arbres.

Pour évaluer le rôle de PtaMYC2.1, PtaMYC2.2 et PtaJAM1.1 dans le développement d'ECM, nous avons généré des lignées transgéniques de *P. tremula x alba* surexprimant ces FT, ainsi que des peupliers chimériques dont les racines n'expriment plus ces gènes par le phénomène de silencing via ARN interférent (RNAi). Les peupliers surexprimants PtaJAM1.1 ont montré un développement accru du réseau d'Hartig, tandis que le phénotype opposé a été observé pour les peupliers surexprimant PtaMYC2.1. Il manque encore des données concernant la surexpression de PtaMYC2.2 et les lignées composites RNAi pour ces FT. Ces résultats préliminaires confirment que l'activation de la signalisation de l'AJ est délétère pour la formation du réseau d'Hartig (Plett et al., 2014a), mais ne démontrent pas que les paralogues PtaMYC2.1 et PtaMYC2.2 ont un rôle différent. Étonnamment, la colonisation par *L. bicolor* des racines des lignées transgéniques témoins et surexprimant *PtaMYC2.1* réprime l'expression du gène *PtaMYC2.1*. Puisque l'expression de *PtaMYC2.1* dans les lignées surexprimant est pilotée par le promoteur CaMV 35S, et non par le promoteur natif de *PtaMYC2.1*, nous avons avancé l'hypothèse que la diminution des transcrits de *PtaMYC2.1* se produirait par un mécanisme de « silencing » basé sur l'ARN plutôt que par la répression de la transcription.

Dans l'ensemble, ces résultats suggèrent que PtaMYC2.1 et PtaJAM1.1 ont un impact opposé sur le développement du réseau d'Hartig et que de multiples mécanismes de régulation influencent l'expression génique dépendante de l'AJ lors de l'établissement d'ECM. D'autres études seront nécessaires pour évaluer si PtaMYC2.1 et PtaMYC2.2

régulent différemment la pénétration fongique dans l'apoplaste racinaire et pour identifier les gènes cibles directs et indirects de ces FT dans les racines de peuplier.

4. *Laccaria bicolor* modifie la sensibilité des racines de peuplier aux phytohormones comme les jasmonates, gibbérellines, salicylates et éthylène.

Chez de nombreuses plantes modèles, la voie de signalisation de l'AJ est au centre d'un réseau de régulation complexe impliquant l'interaction avec d'autres voies de signalisation hormonale. Par exemple, l'antagonisme entre la voie de signalisation de l'AJ et celle de SA régule l'équilibre entre la résistance des plantes aux pathogènes nécrotrophes et biotrophes (Pieterse et al., 2012). La voie de signalisation de l'ET peut amplifier les réponses médiées par l'AJ contre les pathogènes, mais contrer celles déclenchées par la blessure (Lorenzo et al., 2003). De plus, l'antagonisme entre la voie de signalisation de l'AJ et celle de GA est impliquée dans le compromis croissance-défense, empêchant les plantes de déployer des ressources en croissance végétative dans des conditions de stress environnemental (Guo et al., 2018). Au cours de l'interaction entre le peuplier et *L. bicolor*, la biosynthèse constitutive de l'ET inhibe le développement du réseau de Hartig, probablement en empêchant l'expression des gènes impliqués dans la reconnaissance des deux partenaires et dans la synthèse des parois cellulaires (Plett et al., 2014b). Dans la même étude, l'existence d'une synergie entre la voie signalisation de l'AJ et celle de l'ET a été suggérée, en raison de la signature transcriptionnelle similaire des racines de peuplier traitées par l'ajout exogène soit d'AJ, soit d'ET. Au contraire, le traitement exogène avec SA n'a pas eu d'impact sur le développement de l'ECM (Plett et al., 2014b). Cependant, de nombreuses questions sur le rôle exact de la voie de signalisation de l'AJ et son interaction avec les autres voies de signalisation hormonale pendant la symbiose ectomycorhizienne restent sans réponse. Par exemple, déterminer si l'inoculation fongique affecte les voies de signalisation hormonales à des stades particuliers du développement de l'ECM nécessite de connaître des gènes marqueurs pour les différentes hormones. Ces gènes de réponse ne sont actuellement pas connus pour les racines de peuplier. De plus, l'abondance de AJ, SA, ET et GA endogènes dans les racines de peuplier colonisées et non colonisées n'a jamais été examinée. Enfin, il n'y a pas de démonstration de l'existence d'interactions (antagonisme ou synergie) entre les voies de signalisation des phytohormones dans les racines de peuplier et du rôle de ces interactions dans le développement d'une ECM.

Ainsi, pour élucider le rôle des phytohormones impliquées dans la défense et le développement lors de l'établissement de l'ECM, j'ai d'abord examiné le paysage hormonal d'une ECM par hormonomique. De plus, j'ai identifié et défini les gènes de réponse à

différentes phytohormones et à leur antagonisme ou synergie. Les profils d'expression de ces gènes de réponse ont été ensuite examinés au cours développement de l'ECM. Enfin, une analyse transcriptomique globale par séquençage d'ARN de racines de peuplier colonisées et non colonisées, traitées ou non aux hormones, a été réalisée.

Nous avons constaté que le développement d'une ECM s'accompagne de l'accumulation d'auxines, de SA et de cytokinines (sous forme de ribosides) par rapport à des racines non mycorhizées. De plus, l'inoculation avec *L. bicolor* diminue la sensibilité des racines de peuplier à un traitement exogène par AJ, tout en augmentant leur sensibilité à SA, ET et GA. Le traitement exogène avec AJ, seul ou en combinaison avec d'autres phytohormones, entraîne une diminution du pourcentage de mycorhization et une forte inhibition du développement du manteau fongique et du réseau de Hartig. Ces inhibitions de la croissance fongique *in planta* sont probablement liées à l'activation d'un grand nombre de gènes liés à la défense dans les racines de peuplier traitées. Néanmoins, une branche de la voie de signalisation de l'AJ/ET peut être activée pendant le développement d'ECM pour assurer l'équilibre correct entre l'intégrité et l'élasticité de la paroi cellulaire pour une pénétration efficace de l'apoplaste racinaire par les hyphes fongiques. Nos résultats suggèrent qu'un déclenchement modéré de la voie de signalisation de l'AJ pourrait contribuer à un développement optimal du réseau de Hartig en modulant la composition de la paroi cellulaire de la plante et en supprimant le développement des poils racinaires, tandis qu'un déclenchement intense de cette même voie inhiberait la colonisation fongique par activation des défenses des cellules racinaires. De plus, nous avons démontré qu'une homéostasie hormonale équilibrée est essentielle au développement des structures symbiotiques. Les fonctions spécifiques des diverses voies de signalisation hormonales dans la reprogrammation transcriptomique des racines et des hyphes en interaction mériteraient plus d'attention dans les recherches futures.

5. Conclusion et perspectives

Dans le cadre de cette thèse, nous avons apporté de nouvelles connaissances sur le rôle des hormones végétales dans la reprogrammation transcriptomique associée au développement des ECM entre *Populus* spp. et le basidiomycète *L. bicolor*. Nous nous sommes particulièrement concentrés sur la phytohormone AJ. Puisque la voie de signalisation de l'AJ régule de nombreux aspects de la physiologie des plantes, notre objectif principal était de déterminer si un sous-ensemble spécifique de réponses médiées par l'AJ serait impliqué dans la régulation du développement d'une ECM. Dans une perspective plus large, nous avons également étudié les interactions possibles entre la voie de signalisation de l'AJ et d'autres voies de signalisation hormonale dans les racines de peuplier, afin de

mettre en évidence les contributions de l'équilibre entre différents signaux hormonaux au réarrangement transcriptomique des racines colonisées par les champignons.

Nous avons procédé à la réalisation de nos objectifs à travers trois axes de recherche. Tout d'abord, nous avons caractérisé le complexe de perception de l'AJ des racines de peuplier, en étudiant l'interactome de PtJAZ6 via des études d'interaction protéine-protéine. Cette approche a permis d'identifier les FT bHLH PtMYC2.1, PtMYC2.2 et PtJAM1.1 comme étant potentiellement impliqués dans la régulation du développement symbiotique. Par la suite, nous avons observé que la surexpression de PtaMYC2.1 limitait le développement du réseau d'Hartig, tandis que la surexpression de PtaJAM1.1 le favorisait. Enfin, nous avons constaté que la sensibilité des racines de peuplier à l'AJ est réduite pendant la colonisation mycorhizienne et qu'un sous-ensemble de gènes de réponse à l'AJ était régulé dans l'ECM. L'ensemble de nos résultats suggère donc que la voie de signalisation de l'AJ est une voie cruciale pour le développement du réseau de Hartig et que différents segments de la signalisation de l'AJ régulent différentiellement la colonisation fongique *in planta*. Nous émettons l'hypothèse que PtMYC2.1 contrôlerait préférentiellement l'expression des gènes liés à la défense, et que la suppression de son activité est nécessaire pour la pénétration fongique intracellulaire. D'autre part, PtMYC2.2 pourrait réguler des gènes dépendants de l'AJ impliqués dans la suppression des poils racinaires et la modification des propriétés de la paroi cellulaire végétale, des processus nécessaires au développement des ECM.

Malgré les résultats obtenus, des preuves expérimentales supplémentaires sont nécessaires pour valider notre modèle de travail. Par exemple, le séquençage d'ARN à haut débit sera utile pour déterminer les réponses transcriptionelles à la surexpression des FT PtaMYC2 et PtaJAM1 dans les racines de peuplier colonisés, et pour identifier les gènes cibles de ces FT. De plus, l'immunoprécipitation de la chromatine couplée au séquençage (ChIP-seq) en ciblant les protéines PtaMYC2 et PtaJAM1 permettrait de déterminer les cibles directes de leur régulation transcriptionnelle. D'autres axes de recherche sur l'identification des cibles d'autres effecteurs MiSSP et sur l'investigation de la communication basée sur l'ARN entre les arbres et les champignons mutualistes pourraient être poursuivis. Dans l'ensemble, les résultats de cette thèse permettent d'élucider une partie du réarrangement moléculaire complexe qui se produit lors de l'interaction entre le peuplier et *L. bicolor* et ouvrent de nouvelles pistes de recherche dans le domaine de la communication entre arbres et microorganismes.

Chapter 1: Introduction

Chapter 1: Introduction

1.The ectomycorrhizal symbiosis

1.1. The mutualism between trees and ectomycorrhizal fungi in forest ecosystems

To resist to nutrient limitations in forest soils, most tree species form mutualistic associations with soil-borne fungi (van der Heijden et al., 2015; Martin, 2016; Brundrett & Tedersoo, 2018). Indeed, the level of bioavailable phosphorus (P), nitrogen (N) and water in natural soil is often too low to sustain tree growth (Attiwill & Adams, 1993). Mycorrhizal fungi promote plant development through intimate interactions with their roots and delivery of mineral nutrients and water, in exchange for photosynthetic carbon (C; Bücking et al., 2012; Nehls et al., 2016). As such, the establishment of mycorrhizal interactions has been pivotal for the evolution of land plants (Brundrett, 2002; Bonfante & Genre, 2008; Feijen et al., 2018).

Endophytic and endomycorrhizal fungi can form beneficial relationships with both deciduous and perennial plants; however, ~6000-7000 tree species developed a unique kind of interaction with more than 20,000 species of ectomycorrhizal (ECM) basidiomycetes and ascomycetes (van der Heijden et al., 2015; Brundrett & Tedersooo, 2018). The hosts of ectomycorrhizal fungi (EMF) are mainly tree species belonging to the families of *Pinaceae*, *Fagaceae*, *Dipterocarpaceae* and *Caesalpinoidaceae* (Martin, 2016). The distribution and richness of EMF varies widely across biomes, with higher diversity in temperate than tropical ecosystems (Tedersoo et al., 2012; Steidinger et al., 2019). In fact, EMF are dominant in boreal forests where seasonal cold and dry climates inhibit the rate of decomposition of organic matter, whereas arbuscular mycorrhizal fungi (AMF) preferentially colonize trees of tropical biomes, where the rate of decomposition is constant throughout the year (Steidinger et al., 2019). Therefore, the ECM symbiosis has a broad influence on boreal forest ecosystems. In fact, EMF can (i) **enhance mineral nutrition** and possibly water acquisition of associated trees (Tibbett & Sanders, 2002; Plassard & Dell, 2010; Lehto & Zwiazek, 2011); (ii) **prime host plants for enhanced resistance** to biotic and abiotic stress (Luo et al., 2009; Pena et al., 2013; Danielsen & Polle, 2014; Luo et al., 2014; Kaling et al., 2018; Gonthier et al., 2019); (iii) **mediate ecosystem interactions** through an extraradical mycelial network informally termed the “wood-wide web”, allowing the exchange of nutrients and signaling molecules between neighboring trees (Simard et al., 1997; Klein et al., 2016); (iv) participate to **nutrient cycling** by **sequestering atmospheric carbon** in the soil and

degrading soil organic matter (SOM; Averill et al., 2014; Lindahl & Tunlid, 2015; Nehls et al., 2016); (v) modulate the composition and the functionality of root-associated microbiome by providing a **novel ecological niche: the mycorrhizosphere** (Vik et al., 2013; Xue et al., 2019); (vi) **alter soil texture and biochemistry** via oxidation and depolymerization of SOM, thereby promoting the formation of stable SOM-mineral associations (Wang et al., 2017).

In conclusion, ECM are beneficial for tree fitness, nutrient cycling and intra- and inter-kingdom communication in forest ecosystems. In the next paragraph, I will describe the morphology of ECM and introduce the tree and fungal molecular players implied in ECM development.

1.2. Morphology and molecular determinants of developing ectomycorrhizae

ECM are mixed organs composed of hyphae of EMF and fine lateral roots of trees. Most ectomycorrhizal hyphae show a distinct phenotype composed of three main features: (i) an extramatrical mycelium gathering nutrients from the soil; (ii) a mantle of aggregated hyphae ensheathing the tree's lateral roots; (iii) an apoplastic hyphal network between the rhizodermis and root cortex, the so-called Hartig net. The latter is the putative location of nutrient exchanges between the symbiotic partners (Blasius et al., 1986; Horan et al., 1988; Massicotte et al., 1987). Laser capture microdissection, coupled with microarray gene expression analysis, demonstrated that mantle and Hartig net are functionally distinct compartments in *Corylus avellana*-*Tuber melanosporum* ECM. Indeed, fungal hyphae forming the mantle expressed genes related to nutrient acquisition from the soil, synthesis of secondary metabolites and detoxification mechanisms, while hyphae from the Hartig net enhanced the expression of genes encoding carbon and nitrogen transporters (Hacquard et al., 2013). The development of ECM proceeds through subsequent steps of symbiotic signal exchange, mantle formation and fungal apoplastic colonisation (Garcia et al., 2015; Figure 1).

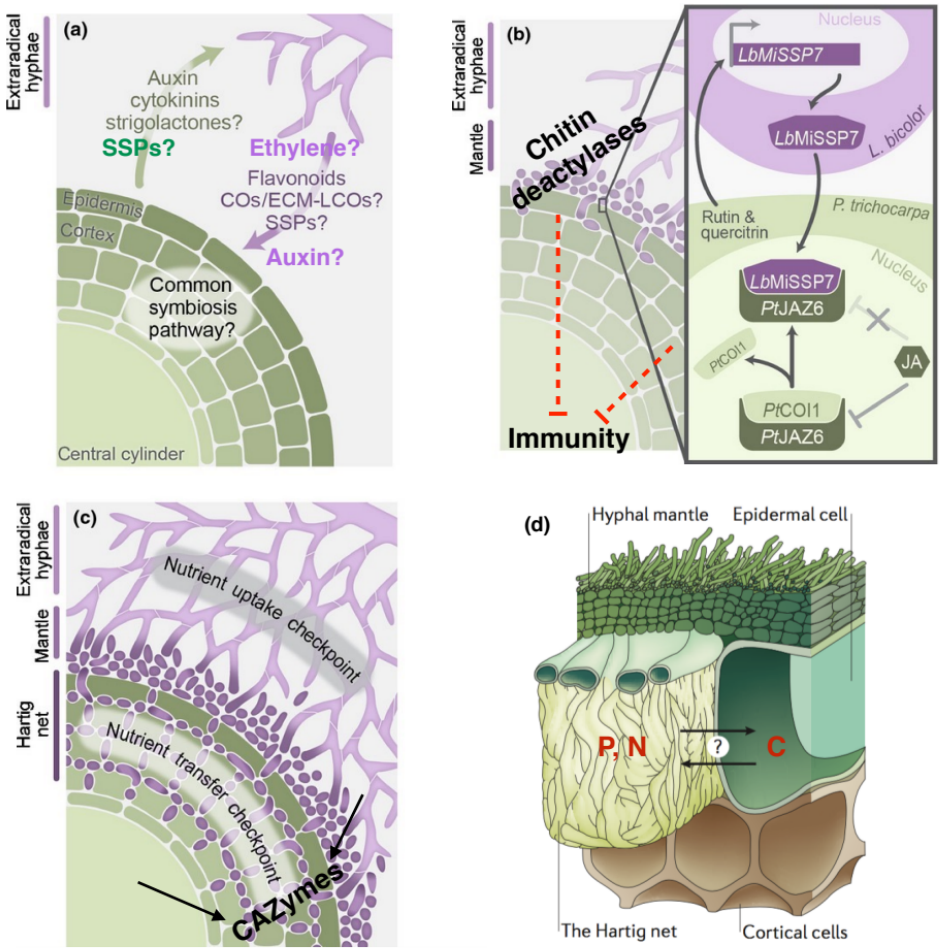


Figure 1: The main steps of ECM development.

(a) The host root and EMF mycelia exchange soluble and volatile signals, e.g. phytohormones, SSPs and chitin-derived molecules. Plant signals influence the development and metabolism of fungal hyphae, while fungal signals induce symbiotic reprogramming of host roots. (b) The physical contact between fungal hyphae and the rhizodermis culminates in the formation of the fungal mantle, ensheathing the tree's lateral root. In this phase tree immunity must be dampened to ensure full development of symbiotic interfaces. Fungal chitin deacetylases, converting chitin to the less immunogenic chitosan, may participate to this process. In addition, the small secreted protein MiSSP7 from L. bicolor, secreted upon perception of plant's flavonoids rutin and quercitin and targeting the poplar co-receptor of jasmonic acid PtJAZ6, may trigger the downregulation of plant immunity during the development of poplar-L. bicolor ECM. (c) Rhizodermal and cortical root cells elongate to increase the width of the apoplastic spaces colonized by fungal hyphae. Concerted hydrolysis of cell wall polysaccharides by plant or fungal CAZymes allows moderate cell wall loosening and subsequent formation of the Hartig net. (d) The Hartig net is the interface of symbiotic nutrient exchange. Modified from Garcia et al., 2015 and Martin et al., 2016.

Prior to direct contact, the interaction partners exchange symbiotic signals, such as flavonoids, phytohormones and small secreted proteins (Figure 1a; Garcia et al., 2015; Plett

et al., 2017). Fungal volatiles, stimulating tree auxin transport and ethylene signaling, promote lateral root initiation (Felten et al., 2009; Splivallo et al., 2009; Ditengou et al., 2015) and suppress the development of root hairs (Ditengou et al., 2000; Balestrini & Kottke, 2016). In addition, the mycelium of *L. bicolor* produces auxin, which could be taken up by host roots (Felten et al., 2009). Also, the genome of *L. bicolor* codes for 106 Mycorrhiza-induced Small Secreted Proteins (MiSSPs), specifically upregulated in symbiotic tissues (Martin et al., 2008). One of these proteins, MiSSP7, is secreted during early stages of the poplar-*L. bicolor* interaction, in response to the plant's flavonoids rutin and quercitin (Figure 1b; Plett et al., 2011). MiSSP7 penetrates the nuclei of poplar roots and hijacks plant jasmonate signaling, thereby allowing Hartig net formation (Plett et al., 2014a). On the other hand, the genes coding for 417 poplar small secreted proteins (SSPs) were shown to be induced during ECM formation. Four of these SSPs enter the nuclei of *L. bicolor* and two of them alter fungal branching (Plett et al., 2017). Therefore, pre-contact reciprocal exchange of symbiotic effectors likely triggers transcriptomic and physiologic reprogramming in both tree and fungal cells.

When in close proximity to the host root, aggregated fungal hyphae adhere to the root surface, thereby forming the mantle (Figure 1b). Root adherence may be ensured by the secretion of specialized glycoproteins (Lei et al., 1991; Tagu & Martin, 1996) and/or small hydrophobic proteins named hydrophobins (Plett et al., 2012; Doré et al., 2017) as well as other MiSSPs (Pellegrin et al., 2019). Tree lateral roots ensheathed by fungal hyphae generally become shortened and swollen, possibly because of fungal manipulation of root auxin metabolism and response (Vayssières et al., 2015). During the early contact phase, EMF need to avoid triggering plant immunity. They probably achieve so through secretion of chitin deacetylases, enzymes that convert the highly immunogenic cell wall carbohydrate chitin to chitosan, a form less recognized by plant immune receptors (Balestrini et al., 2012; Veneault-Fourrey et al., 2014; Oliveira-Garcia & Valent, 2015). Also, EMF may deter plant immunity through secretion of effector proteins (Plett et al., 2014a), similarly to pathogenic microbes (Stergiopoulos & de Wit, 2009; Kazan & Lyons, 2014).

During fungal apoplastic penetration, the morphology of root epidermal and cortical cells is modified. Cells elongate radially while decreasing their width, thereby increasing the volume of the apoplast for efficient fungal colonisation (Figure 1c,d). This process is accompanied by changes in the composition of both fungal and plant cell walls (Balestrini & Bonfante, 2014; Balestrini & Kottke, 2016; Mello & Balestrini, 2018). For instance, the cell wall of the ectomycorrhizal basidiomycete *Pisolithus tinctorius*, when colonizing *Eucalyptus grandis* roots, is enriched in symbiosis-regulated acidic polypeptides (SRAPs) and hydrophobins (Laurent et al., 1999; Martin et al., 1999; Tagu et al., 2001). In addition, fungal growth into the middle lamella likely coincides with localized pectin degradation (Sillo et al.,

2016; Mello & Balestrini, 2018). Fungal secreted phospholipases A2, expansins and cell wall degrading Carbohydrate Active enZymes (CAZymes) may play a role in this process (Balestrini et al., 2012; Hacquard et al., 2013; Veneault-Fourrey et al., 2014; Doré et al., 2017). In addition, fungal or plant CAZymes may trigger loosening of cellulose fibrils (Veneault-Fourrey et al., 2014; Sebastiana et al., 2014; Doré et al., 2017). In particular, a cellulose-binding β-1-4, endoglucanase from *L. bicolor*, GH5-CBM1, acting on cellulose, mannans and galactomannans, is implied in Hartig net development (Zhang et al., 2018).

In summary, the development of ECM requires a sophisticated and finely tuned molecular dialogue for efficient mutual recognition between trees and fungi. This likely involves an exchange of **volatile and soluble molecules**, as well as the **secretion of specialized effectors and enzymes** to ensure the correct development of symbiotic structures. These exchanges of signals serve the dual purpose of **reprogramming plant and fungal development** and **deterring plant immunity**. Since the ECM symbiosis evolved multiple times independently (Tedersoo et al., 2010; Kohler et al., 2015), the specific genes implied in the metabolic, physiologic and transcriptomic remodeling of the symbionts differ in distinct plant-fungus pairs (Martin et al., 2010). The establishment of specialized symbiotic dialogues may rely on the coevolution of EMF and their host trees (Hoeksema, 2010). In the next paragraph, I will present the hallmarks of the evolution of the ECM lifestyle in forest-associated fungi.

1.3. The genetic “symbiosis toolkit” of ectomycorrhizal lifestyle

The ancient, monophyletic group of arbuscular mycorrhizal fungi (AMF), belonging to the *Glomeromycota* phylum, evolved from a common ancestor living at least 450 million years ago (Redecker et al., 2000; Strullu-Derrien et al., 2014) and probably sustained the transition of plants from the ocean to the land (Brundrett, 2002; Feijen et al., 2018). The importance of the AM symbiosis for land plant evolution is substantiated by the fact that approximately 74% vascular plant species still form this interaction nowadays (van der Heijden et al., 2015). A common symbiosis signaling pathway is activated in all land plants upon interaction with AMF. Essentially, the perception of fungal chito-lipo-oligosaccharides, also termed Myc factors, and short chitin chains by plant leucine-repeat-rich (LRR) protein kinases generates an intracellular signaling cascade in the root. Such signal is transduced into a series of nuclear Ca^{2+} oscillations that trigger symbiotic transcriptional reprogramming (Oldroyd, 2013). AMF are obligate symbionts. Their small genome size, as well as their reduced arsenal of degrading enzymes, indicates loss of saprotrophic ability and suggests

that it is very unlikely for these species to revert to free-living lifestyle (Tisserant et al., 2012; 2013).

In contrast, ECM symbioses have arisen independently at least eight times in association to angiosperms and six-to-eight times in association to gymnosperms (Hibbett & Matheny, 2009). The first EMF appeared around 140-180 million years ago, associated to Pinaceae (Brundrett, 2002). Since then, ECM species evolved repeatedly from saprotrophic ancestors (Tedersoo et al., 2010; Kohler et al., 2015). Despite their polyphyletic origin, **EMF underwent convergent loss of plant cell wall degrading enzymes (PCWDEs) and lignin-oxidizing class II peroxidases, while expanding their arsenal of lineage-specific small secreted proteins** (SSPs, Kohler et al., 2015). The loss of cellulose and lignin degrading enzymes suggests that EMF depend on the host plant for their carbon nutrition. However, there is evidence of reversion from ECM to saprotrophic lifestyle (Hibbett et al., 2000) and several species of EMF retained the ability to degrade SOM (Lindahl & Tunlid, 2015). Altogether, these observations suggest that the ECM lifestyle has arisen repeatedly upon convergent loss of degrading enzymes and convergent gain of effector proteins, but that EMF may not depend on their host to a similar extent than AMF.

In line with the polyphyletic origin of EMF, the specific molecular mechanisms of symbiosis establishment are clade-dependent and probably the result of a co-evolution with the host tree. For example, the Agaricomycetes *L. bicolor* and *Hebeloma cylindrosporum*, as well as the Dothideomycete *Cenococcum geophilum* highly express SSPs in ECM root tips (Martin et al., 2008; Doré et al., 2015; Peter et al., 2016). In contrast, Pezizomycetes like *Tuber* spp. do not regulate SSPs during symbiosis establishment (Martin et al., 2010; Murat et al., 2018). However, all these fungi express **specific sets of PCWDEs, aquaporins and transporters**, which possibly constitute **core elements** for the development of most ECM (Martin et al., 2010). The majority of EMF are generalist, colonizing multiple tree species. However, generalist EMF can display different degrees of compatibility with distinct hosts (Tschaplinski et al., 2014; Plett et al., 2015). In addition to their core gene regulon, these fungi also display host-specific gene expression patterns, possibly to achieve the colonization of recalcitrant hosts (Plett et al., 2015; Liao et al., 2016; de Freitas Pereira et al., 2018). Likewise, the same host tree can express common and different sets of genes when colonized by distinct strains or species of EMF (Zhang et al., unpublished results; de Freitas Pereira et al., unpublished results). Within the gene regulon specific to *Populus tremula x alba* are genes belonging to the Common Symbiosis Pathway (CSP), a molecular cascade also exploited for recognition of other symbionts such as AM fungi and nitrogen-fixing rhizobia. Indeed, the CSP genes *CASTOR*, *POLLUX* and *CCaMK* contribute to fungus-induced enhanced lateral root development, as well as to efficient colonization of *P. tremula x alba* roots by *L. bicolor* (Cope et al., 2019). This study also demonstrated that *L. bicolor*,

similarly to AM fungi, produces sulfated and non-sulfated lipochitooligosaccharides (sLCOs and nsLCOs), which elicit Ca^{2+}-spiking in poplar rhizodermal cells. Moreover, it was recently found that transgenic expression of a poplar G-type lectin receptor-like kinase (LecRLK) gene in Arabidopsis roots allowed apoplastic colonization of this non-host plant by *L. bicolor* (Labbé et al., 2019). LecRLKs are transmembrane receptor proteins with roles in microbial recognition, stress response and plant development (Vaid et al., 2013). Some lysine-motif RLKs (LysM-RLKs) act as receptors of LCOs of fungal or bacterial origin for the initiation of AM and root nodule symbioses (Gough et al., 2018). It remains to be demonstrated whether LecRLKs integrate an ECM-specific version of the CSP in *P. tremula x alba* by recognizing fungal LCOs. However, many CSP genes are absent from the genome of other ECM host plants, such as *Pinus pinaster* (Garcia et al., 2015). Therefore, the CSP is likely not exploited for all ectomycorrhizal associations.

In summary, the ECM symbiosis appeared multiple times across evolutionary history. Because of their polyphyletic origin, EMF exploit different molecular mechanisms during interaction with trees and exhibit diverse ranges of host specificity. However, genome erosion for PCWDEs, and evolution of nutrient transporters and lineage-specific SSPs, appeared as hallmarks of the ECM lifestyle by convergent evolution. Investigating the molecular determinants of various EMF-tree interactions will help unravel the biochemical basis of symbiotic compatibility and will shed some light over the co-evolutionary processes of forest trees and associated fungi.

1.4. Ectomycorrhizal symbioses and tree fitness

As stated earlier, the presence of **EMF improves host tree N and P nutrition, in exchange for photosynthetic C** (Nehls et al., 2016; Nehls & Plassard, 2018). Some ECM hyphae induce weathering of mineral nutrient and/or SOM degradation, thereby producing small organic molecules that are easily taken up by belowground fungi and roots (Lindahl & Tunlid, 2015). In addition, EMF can transfer such nutrients to the plant through the Hartig net (Bücking et al., 2012). N is often the major limiting element for tree growth in boreal ecosystems (Attiwill & Adams, 1993). In forest soils N is present in mineral forms (ammonia - NH_3 and NH_4^+- and nitrates -NO_3^-) as well as organic forms (amino sugars, amino acids and proteins), about 90% of which are complexed within the SOM and unavailable for direct absorption from tree's roots (Schulten & Schnitzer, 1998). Thus, the proteolytic and oxidative activities of EMF are important for the release of N from organic sources. SOM-derived amino acids and inorganic nitrates and ammonia can be directly taken up by the tree's roots or indirectly transferred through the ectomycorrhizal network (Näsholm et al., 2009; Chalot &

Plassard, 2011). The transfer of ammonium, nitrates and amino acids from fungi to trees may occur through the aspecific transporters aquaporins (Dietz et al., 2011) or through specialized, mycorrhiza-induced transporters (Garcia et al., 2016). Regarding P nutrition, a major form of P in the soil is represented by organic P (P_o) compounds, such as phosphate esters (e.g. sugar- phosphates, inositol-phosphates) or phosphate diesters (e.g. nucleic acids and phospholipids; George et al., 2018). However, the ability of EMF to take up P_o forms has not been demonstrated experimentally. On the other hand, EMF possess inorganic P (P_i) transporters, and some high affinity H^+:P_i fungal symporters are upregulated during ECM formation (Garcia et al., 2016). Such symporters may play a dual role in P_i uptake from the soil and P_i transfer to the host (Becquer et al., 2018). In alternative, fungi may secrete polyphosphates in the symplastic space via the Vacuolar Transporter Chaperone Complex (Vtc) or through vesicle-mediated secretion (Torres-Aquino et al., 2017; Nehls & Plassard, 2018).

In the arbuscular mycorrhizal (AM) symbiosis and the root nodule symbiosis (RNS), upon which nutrient transfer also occurs, the plant systemically controls the colonization level depending on nutrient availability and/or the efficiency of nutrient exchanges (Reid et al., 2011; Kiers et al., 2011; Carbonnel & Gutjahr, 2014). However, **a nutrient-based reward/suppression mechanism might not exist for the ECM symbiosis**. Indeed, the amount of mobilized N by soil microbes towards host trees is correlated to the soil N content, but not to the amount of tree-delivered C (Näsholm et al., 2013). In contrast, reduced tree C fixation and belowground allocation, with consequent diminution of fungal biomass, increases N transfer from the soil to tree leaves (Hasselquist et al., 2016). Finally, nutrient transfer can be influenced by direct competition between EMF strains (Hortal et al., 2017). Altogether, these data suggest that the fungus, whose development is limited by the environmental supply of C and N, supports symbiotic N transfer only upon favorable C:N balance for its own growth. As a consequence, increased C sequestration in forest soils may reduce N availability for trees, posing a limit for the increase of future plant productivity upon predicted higher atmospheric CO_2 concentrations (Oren et al., 2001; Norby et al., 2010).

In addition to the amelioration of nutrient uptake, the establishment of the **ECM symbiosis provides protection against biotic and abiotic stress to the host tree**. In particular, ECM can contribute to **drought resistance** through enhanced root hydraulic conductivity (Bogeat-Triboulot et al., 2004), possibly via induced expression of tree or fungal aquaporin-coding genes (Marjanović et al., 2005; Xu et al., 2015). However, several ECM interactions do not promote drought tolerance (Parke et al., 1983; Kipfer et al., 2012; Danielsen & Polle, 2014). Therefore, the effect of ECM on water deficiency stress may depend on the genotypes of the symbionts. Indeed, drought-tolerant trees naturally select communities of EMF conferring resistance to water limitations (Gehring et al., 2017). ECM-

mediated drought resistance may rely on improved mineral nutrition (Alvarez et al., 2009; Lehto & Zwiazek, 2011) or molecular priming through the induction of abiotic stress-related genes and accumulation of the phytohormones salicylic acid (SA) and abscisic acid (ABA; Luo et al., 2009).

Moreover, **ECM enhance tree resistance to belowground and aboveground pathogens and pests** (Sylvia & Sinclair, 1983; Kaling et al., 2018) **as well as toxic concentrations of heavy metals** (Luo et al., 2014) **and osmotic stress** (Luo et al., 2011; Li J. et al., 2012). Such systemic responses likely rely on ECM-dependent promotion of phytohormone signaling, enzymatic activities and biosynthesis of secondary metabolites. For example, enhanced resistance of poplar leaves to herbivores might depend on ECM-induced upregulation of several jasmonate-responsive genes as well as on the accumulation of protease inhibitors, chitinases and insect-repellent aldoximes in the aerial part of colonized trees (Kaling et al., 2018).

Despite its centrality in forest ecology and plant fitness, the ECM symbiosis has been poorly investigated with respect to other types of beneficial plant-microbe interactions. Indeed, only in the last two decades considerable effort has been invested to unravel the genomic bases of the ectomycorrhizal lifestyle and the molecular mechanisms underpinning the establishment of this interaction (van der Heijden et al., 2015; Garcia et al., 2015; Martin et al., 2016).

1.5. The modulation of tree metabolism, development and defense upon ectomycorrhizae formation depends on phytohormonal signaling

The formation of ECM includes a general reprogramming of fungal and tree metabolism, development and transcriptome. For example, the compatible interaction between *Populus trichocarpa* and *L. bicolor* results in the accumulation of specific organic acids and fatty acid metabolites, as well as in decreased accumulation of defense compounds in poplar roots (Tschaplinski et al., 2014). In addition, colonized roots initiate specific developmental programs, leading to altered root system architecture, ontogeny of symbiotic surfaces and establishment of nutrient exchanges (Garcia et al., 2015; Balestrini & Kottke, 2016). Also, defense responses must be dampened in order to accept mutualistic colonization (Plett et al., 2014a). In the model species Arabidopsis root morphogenetic processes and initiation of plant defense are mainly driven by alterations in phytohormone content, distribution and/or signaling. In particular, lateral root initiation is obtained through the interplay between auxin basipetal transport, ethylene (ET) biosynthesis and cytokinin (CK) signaling (Aloni et al., 2006). Phytohormone signaling also influences cell wall

composition and elasticity. Indeed, auxin, gibberellin (GA), abscisic acid (ABA), ET and brassinosteroid (BR) signaling induce the expression and/or the activation of hemicellulose remodeling enzymes and expansins, promoting cell elongation. Conversely, JA signaling is induced in cellulose-deficient mutants and is implied in lignin deposition (Sánchez-Rodríguez et al., 2010; Nafisi et al., 2015). On the other hand, Arabidopsis defense to belowground and aboveground pathogens and pests is modulated through a finely tuned crosstalk between JA, salicylate (SA) and ET signaling (Pieterse et al., 2009; 2012). In Section 2, I will describe the biosynthetic and signaling pathways of selected phytohormones involved in defense and root development of Arabidopsis. In addition, I will provide current information of the role of such signaling pathways in regulating physiology and development of poplar species (Paragraphs 2.1 and 2.2). I will also illustrate the molecular basis of the interaction between JA, SA, ET, GA and auxin signaling in the regulation of Arabidopsis defense and growth (Paragraph 2.3). Given the role of phytohormone signaling in remodeling of root architecture, modification of cell wall properties and regulation of defense in the model plant Arabidopsis, it is likely that tree hormones are involved in ECM development. Current knowledge about the impact of phytohormone signaling on ECM interactions is provided in Section 3 of this Chapter.

2. Phytohormonal regulation of plant development and defense

2.1. Jasmonate biosynthesis, signaling and responses

2.1.1. Jasmonate signaling in plant defense and development

Jasmonates (JAs) are a class of **fatty acid-derived oxylipins** implied in primary root elongation, male fertility and defense (Wasternack & Hause, 2013; Howe et al., 2018). JAs have a well-acknowledged role in stimulating **plant immunity** upon attack from **herbivorous insects and necrotrophic pathogens** (Howe & Jander, 2008; Antico et al., 2012; Yan & Xie, 2015). In the model plants Arabidopsis and tomato, JAs confer resistance to leaf chewing insects, such as caterpillars and beetles, as well as to a wide panel of piercing-sucking insects, such as leafhoppers, spider mites and aphids. In addition, JAs mediate defense against necrotrophic fungal pathogens such as *Alternaria brassicicola*, *Botrytis cinerea*, *Plectosphaerella cucumerina* and *Fusarium oxysporum* (Campos et al., 2014; Yan & Xie, 2015), as well as against some (hemi)biotrophic pathogens, e.g. the fungus *Xanthomonas oryzae* in rice and the bacterium *Pseudomonas syringae* in Arabidopsis (De Vleesschauwer et al., 2013; de Torres Zabala et al., 2016). Plant mechanical wounding results in a quick accumulation of JA via the activation of mitogen-activated protein kinase (MAPK) cascades (Glauser et al., 2009; Koo et al., 2009; Chauvin et al., 2013). Depending on the main transcription factor (TF) driving the response, in Arabidopsis and tomato JA signaling can proceed along the **MYC2 branch**, eliciting responses to wounding and insects, or along the **ERF branch**, resulting in responses against necrotrophic pathogens (Lorenzo et al., 2003; 2004; Dombrecht et al., 2007; Pré et al., 2008; Du et al., 2017). Interestingly, in Arabidopsis the TF MYC2 represses the ERF branch of JA signaling, resulting in enhanced defenses against *Pieris rapae* (Verhage et al., 2011). However, tomato resistance to *Botrytis cinerea* requires simultaneous activation of both the MYC2 and the ERF branch of JA signaling (Du et al., 2017). MYC2 negatively regulates the biosynthesis of tryptophan (Trp)-derived indole glucosinolates (IGs), while enhancing the biosynthesis of flavonoids. In addition, MYC2 positively regulates responses to wounding, insects and oxidative stress (Dombrecht et al., 2007; Kazan & Manners, 2013). On the other hand, the AP2/ERF-family transcription factors ERF1 and ORA59 trigger the expression of defense-related genes, such as PLANT DEFENSIN1.2 (PDF1.2; Penninckx et al., 1998).

In addition to their role in plant immunity, JAs are also implied in several developmental processes, such as the inhibition of primary root growth, trichome formation, leaf senescence and male fertility (reviewed in Wasternack & Hause, 2013). JA signaling can

also affect the progression of the cell cycle (Pauwels et al., 2007; Noir et al., 2013), resulting into growth arrest upon pathogen attack in the model plants Arabidopsis and *Nicotiana attenuata* (Huang et al., 2017; Machado et al., 2013). However, it was recently shown that JA signaling also acts through a RETINOBLASTOMA-RELATED (RBR)- SCARECROW (SCR)-SHORT ROOT (SHR) protein network to promote stem cell activation and regeneration upon wounding (Zhou et al., 2019). Finally, through a complex network of downstream TFs, JAs regulate the biosynthesis of several secondary metabolites (Wasternack & Strnad, 2019).

In leaves of *Populus* spp. herbivory and wounding trigger the activation of the JA biosynthetic pathway, as well as the expression of many genes implied in defense (Major & Constabel, 2006; Frost et al., 2008; Hu et al., 2009). Also, wounding and exogenous methyl-jasmonate (MeJA) on poplar leaves induce the expression of the putative antiherbivore enzyme polyphenol oxidase, with consequent protection from forest tent caterpillars (Constabel et al., 2000). In addition, JA treatment of leaves triggers carbon allocation towards roots and young sink leaves (Arnold & Schultz, 2001; Babst et al., 2005; Appel et al., 2012), possibly to prevent herbivores from consuming the plant's carbohydrates and/or to deploy energy into the biosynthesis of defensive compounds. These findings suggest that, similarly to Arabidopsis, JA signaling controls plant defenses against insects in poplar leaves. However, it is still unknown whether JA signaling is important for poplar immunity against root pathogens or necrotrophs, or for poplar development.

Interestingly, **JA signaling also plays a role in the establishment of root-microbe mutualistic symbioses** (Gutjahr & Paszkowski, 2009; Basso & Veneault-Fourrey, 2019). In particular, it has been shown that JA accumulates in arbusculated cells during AM symbiosis development, possibly enhancing their carbon sink status (Hause et al., 2002; Stumpe et al., 2005). Moreover, JA signaling contributes to the systemic autoregulation of nodulation (Kinkema & Gresshoff, 2008) and to the induction of microbe-induced systemic resistance (ISR) against biotic and abiotic stress (Pieterse et al., 2014). The role of JA signaling in the ECM symbiosis will be discussed in paragraph 3.2.

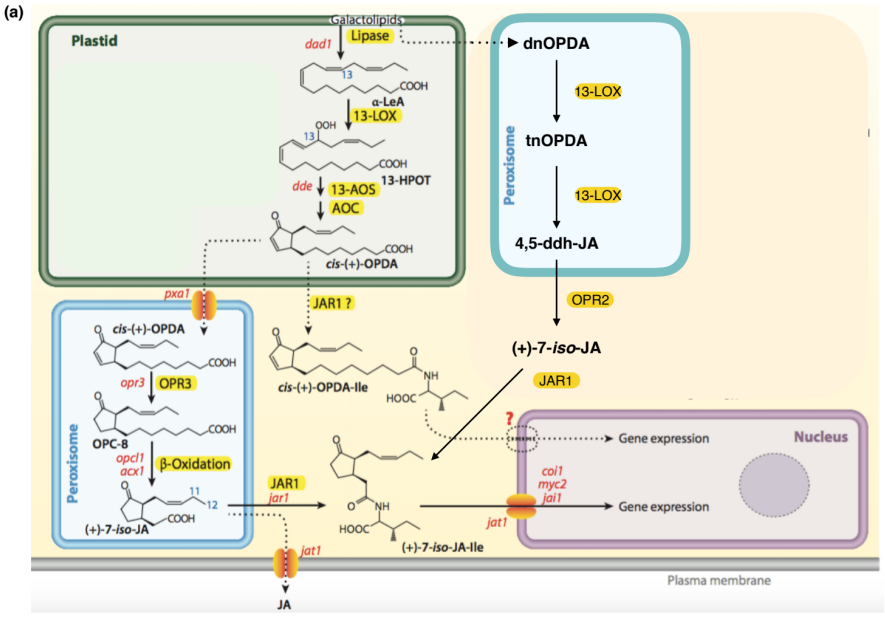
JA signaling is a central hub of an extensive crosstalk network with other hormone signaling pathways, such as GA, SA, and ET signaling, regulating plant immunity and growth (Pieterse et al., 2012; Guo et al., 2018a). The molecular mechanisms at the base of such crosstalks will be discussed in paragraphs 2.3.1 and 2.3.2.

2.1.2. Jasmonate metabolism and perception

The bioactive JAs in dicotyledons are the amino acid conjugated forms (+)-7-iso-JA-Ile, (+)-7-iso-JA-Leu, (+)-7-iso-JA-Val (+)-7-iso-JA-Met, (+)-7-iso-JA-Ala (Fonseca et al., 2009; Yan et al., 2016). However, plants can respond to exogenous JA and MeJA by converting them into bioactive JA-Ile (Stitz et al., 2011).

In Arabidopsis the biosynthesis of JA occurs through the 13-LOX branch of the lipoxygenase (LOX) pathway, starting from esterified or free fatty acids of the chloroplast membrane (Figure 2a; Wasternack & Feussner, 2017). To liberate JA precursors, these fatty acids need to be processed by either specific or aspecific acyl-lipid hydrolases and thiolases. The main free fatty acid precursor of JA is α-linolenic acid (18:3) (α-LeA). α-LeA is oxidized into (13S)-hydroperoxy octadecatrienoic acid (13-HPOT) by the action of 13-lipoxygenase (13-LOX) enzymes. Subsequently, 13-HPOT is rearranged into *cis*-(+)-12-oxo-phytodienoic acid (*cis*-(+)-OPDA) by action of the atypical cytochrome P450 allene oxide synthase (13-AOS) and the enzyme allene oxide cyclase (AOC). OPDA is exported from the chloroplast and imported into the peroxisome. Here OPDA is converted into 3-oxo-2-(2-pentenyl)-cyclopentane-1-octanoic acid (OPC-8) by the action of the enzyme 12-oxophytodienoic acid reductase3 (OPR3). Subsequent cycles of β-oxidation shorten the carboxyl side chain of JA, giving rise to (+)-7-iso-JA, which is then exported in the cytosol. Here, the jasmonoyl amino acid conjugate synthase JASMONATE RESPONSE LOCUS1 (JAR1) conjugates JA to isoleucine, producing the main bioactive form of jasmonate (Staswick & Tiryaki, 2004). JA-Ile is transported into the nucleus by the ABC transporter JAT1 (Li et al., 2017). The observation that the Arabidopsis *opr3* mutant could still accumulate JA in response to necrotrophic pathogens like *Botrytis cinerea* (Chehab et al., 2011) prompted the discovery of a second, OPR3-independent pathway for biosynthesis of bioactive JA, starting from the oxidation of the fatty acid hexadecatrienoic acid (16:3) into dinor-OPDA (dnOPDA) and the conversion of the latter into 4,5-didehydro-JA (4,5-ddh-JA), subsequently reduced into JA in the cytosol (Figure 2a; Chini et al., 2018). On the other hand, OPDA is by itself an active compound and stimulates a set of responses distinct from JA (Wasternack & Strnad, 2016).

Bioactive JA-Ile is bound by a co-receptor complex formed by a **JAZ signaling repressor** and the **CORONATINE INSENSITIVE1 (COI1) F-box protein** belonging to the Skip/Cullin/F-box SCF^{COI1} ubiquitin E3 ligase complex (Figure 2b; Xie et al., 1998; Xu et al., 2002; Chini et al., 2007; Thines et al., 2007; Yan et al., 2007). **JA-Ile perception triggers ubiquitin-tagging of JAZ proteins and their degradation through the 26S proteasome** (Chini et al., 2007; Thines et al., 2007). Upon JAZ degradation, downstream TFs, belonging prevalently to basic helix-loop-helix (bHLH) and MYB families, are released from repression (Chini et al., 2016; Howe et al., 2018). Within this wide array of TFs, the mechanism of transcriptional activation mediated by MYC2 and APETALA2/ETHYLENE RESPONSE FACTORS (AP2/ERFs) has been unraveled. MYC2, AP2/ERF59 and ERF1 interact with the MED25 subunit of the mediator complex, recruiting the RNA polymerase II as well as the co-activator histone acetyltransferase1 (HAC1) to initiate JA-dependent gene expression (Figure 2b; Çevik et al., 2012; Chen et al., 2012; An et al., 2017).


(a)
Plastid
Galactolipids
Lipase
dad1
α-LeA
13-LOX
13-HPOT
dde
13-AOS
AOC
cis-(+)-OPDA
dnOPDA
13-LOX
tnOPDA
13-LOX
4,5-ddh-JA
Peroxisome
OPR2
(+)-7-iso-JA
JAR1
pxa1
JAR1 ?
cis-(+)-OPDA-Ile
oprs
OPR3
OPC-8
opcl1
acx1
β-Oxidation
(+)-7-iso-JA
JAR1
jar1
(+)-7-iso-JA-Ile
jat1
jat1
JA
?
Gene expression
Nucleus
coi1
myc2
jai1
Gene expression
Plasma membrane

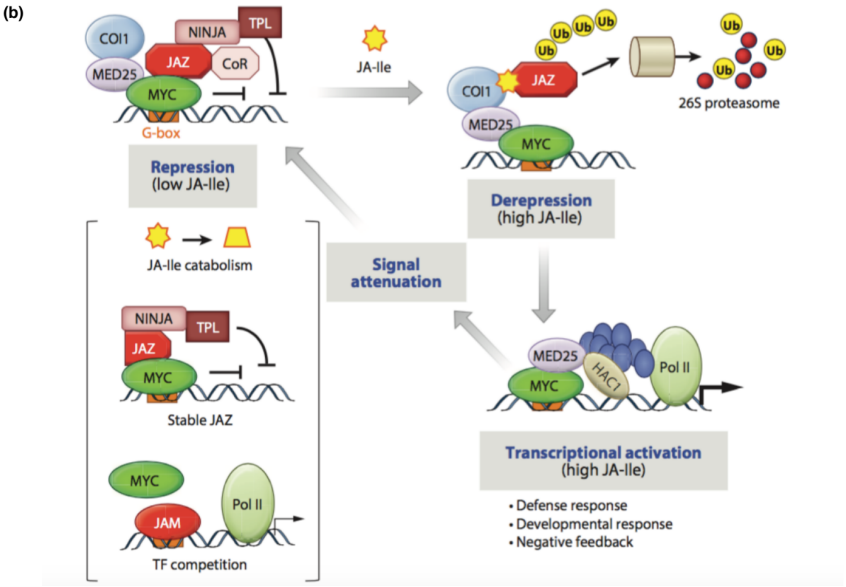

(b)
COI1
NINJA
TPL
JAZ
CoR
MED25
MYC
G-box
Repression
(low JA-Ile)
JA-Ile
COI1
JAZ
MED25
MYC
Ub
26S proteasome
Derepression
(high JA-Ile)
JA-Ile catabolism
Signal
attenuation
NINJA
TPL
JAZ
MYC
Stable JAZ
MYC
JAM
Pol II
TF competition
MED25
MYC
HAC1
Pol II
Transcriptional activation
(high JA-Ile)
• Defense response
• Developmental response
• Negative feedback

Figure 2: Jasmonate biosynthesis and signaling.

(a) The main steps of JA biosynthesis in Arabidopsis. The OPR3-dependent pathway begins with several cycles of β-oxidation of plastid membrane-derived galactolipids, followed by 13-HPOT oxidation by the AOS and AOC enzymes. The resulting cis-(+)-OPDA is exported to the peroxisome where it undergoes reduction by OPR3 and further β-oxidationto 7-(+)-iso-JA. This molecule is exported to the cytosol, where the enzyme JAR1 conjugates it with isoleucine. The resulting JA-Ile is the bioactive JA. The OPR3-independent pathway proceeds along the oxidation of galactolipids to dnOPDA, tnOPDA and 4,5-ddh-JA in the peroxisome. The latter is exported to the cytosol, where OPR2converts it to 7-(+)-iso-JA. (b) JA signaling. At low JA-Ile concentrations, the co-repressors of the JAZ family recruit, either directly or indirectly, the general repressors TPL to repress MYC2-dependent gene expression. In presence of high JA-Ile, the perception of this molecule by the co-receptor complex formed by a JAZ protein and the F-box COI1, part of a SCF E3 ubiquitin ligase, triggers proteasomal degradation of the JAZ protein. As a consequence, the activity of MYC2 is derepressed. MYC2 interacts through the bridge protein MED25 with the RNA polymerase II and the coactivator HAC1, thereby inducing JA-dependent gene expression. Among JA-induced genes are degradation-resistant JAZ proteins and JAM transcriptional repressors, which hamper MYC2 activity to ensure signal attenuation. Modified from Wasternack & Feussner, 2017 and Howe et al., 2018.

2.1.3. Structure and function of JAZ repressors

JAZ proteins are key repressors of JA signaling belonging to the larger family of TIFY proteins (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007) and they have partially redundant roles in controlling responses to JA (Pauwels & Goossens, 2011; Chini et al., 2016). The Arabidopsis genome contains 12 canonical *JAZ* genes, bearing conserved domains, as well as the divergent *JAZ13*, classified into five phylogenetic groups (Chico et al., 2008; Thireault et al., 2015). **Canonical JAZ proteins show three structural domains** mediating protein-protein interactions: a conserved **C-terminal JA-associated (Jas) domain**, a highly conserved **ZIM domain** and a variable **N-terminal (NT) domain** (Figure 3a). The Jas motif mediates the hormone-dependent interaction of JAZ with COI1. A 20-amino acid string within this domain is necessary for JAZ degradation upon JA perception and has thus been named JAZ degron. Two positively charged amino acids on the JAZ1 degron, R^{205} and R^{206}, are crucial for its binding to COI1 (Melotto et al., 2008). On the other hand, the Jas domain is also necessary for the interaction between JAZ and several TFs that mediate different subsets of JA responses (Pauwels & Goossens, 2011; Chini et al., 2016; Howe et al., 2018) and may be implied in the nuclear localization of JAZ proteins (Thines et al., 2007; Grunewald et al., 2009). **The ZIM domain contains a highly conserved TIFY motif** (TIF(F/Y)XG) that mediates homo- and heterodimerization of JAZ proteins (Chung & Howe, 2009), as well as their interaction with **Novel Interactor of JAZ (NINJA)**, an adaptor protein recruiting the general repressors **TOPLESS (TPL) and TOPLESS-RELATED (TPR)**

through a conserved Ethylene-responsive element binding factor-associated Amphiphilic Repression (EAR) domain (Pauwels et al., 2010). The weakly conserved NT domain is required for interaction with signal transduction factors from other hormonal signaling pathways (e.g. DELLA proteins repressing GA signalling; Hou et al., 2010). Five Arabidopsis JAZ proteins (JAZ5, JAZ6, JAZ7, JAZ8 and JAZ13) contain an EAR motif (Kagale et al., 2010; Thireault et al., 2015) and can directly interact with TPL and TPR (Causier et al., 2012; Shyu et al., 2012; Thireault et al., 2015; Ke et al., 2015; Thatcher et al., 2016). Remarkably, JAZ8 and splice variants of JAZ2, JAZ3 and JAZ10 bear a mutated or truncated version of the canonical degron and are therefore resistant to ubiquitin-mediated degradation (Shyu et al., 2012; Chung & Howe, 2009; Chung et al., 2010). The corresponding transcripts are accumulated upon active JA signaling and mediate de-sensitization to this hormone (Chung et al., 2010; Moreno et al., 2013). The fact that these proteins could still bind the downstream TF MYC2 led to the discovery of a cryptic MYC2-interaction domain (CMID) in the NT region of the JAZ10.4 protein (Moreno et al., 2013), binding MYC2 with higher affinity than the Jas domain and effectively hampering its transcriptional activity (Zhang F. et al., 2017).

JAZ proteins can repress downstream gene expression through multiple mechanisms: (i) direct inhibition of MYC TFs activity through clamping of their transcriptional activation domain and prevention of their interaction with MED25 (Zhang F. et al., 2015; 2017); (ii) recruitment of the general repressors TPL and TPR, either directly or through the adaptor protein NINJA (Pauwels et al., 2010); (iii) direct interaction with the RPD3-type histone deacetylase 6 (HDA6) (Wu et al., 2008; Zhu et al., 2011). TPL and TPR also mediate the interaction with histone deacetylases (HDACs), which induce chromatin modifications leading to gene silencing (Krogan et al., 2012; Wang et al., 2013).

2.1.4. JAZ-interacting transcription factors and signaling components

The wide range of JAZ-interacting TFs, their differential regulation and their specific spectrum of target genes allow precise modulation of different JA-dependent responses to developmental or environmental cues (Figure 3b; Pauwels & Goossens, 2011; Chini et al., 2016; Howe et al., 2018).

The first TF found to be interacting with JAZ proteins is MYC2, also known as JA-INSENSITIVE1 (JIN1; Lorenzo et al., 2004). This TF is a master regulator of JA-mediated responses (Kazan & Manners, 2013). In particular, **MYC2 coordinates a transcriptional cascade involving a number of other TFs, like AP2/ERFs, MYBs, zinc fingers and WRKYs** (Dombrecht et al., 2007). Through these downstream TFs, MYC2 promotes JA-dependent insect resistance, oxidative stress tolerance and flavonoid metabolism while negatively impacting JA-dependent response to necrotrophic pathogens and biosynthesis of secondary metabolites (e.g. IG metabolism; Dombrecht et al., 2007). The structure of MYC2

is typical of bHLH proteins, harboring a conserved bHLH domain in its carboxyl terminus (Figure 3c). The HLH domain consists of two amphipathic α-helices joined by a loop region and is required to form homo- and heterodimers, functional in transcription (Fernandez-Calvo et al., 2011). Finally, a transcriptional activation domain (TAD) and a neighboring JAZ-interacting domain (JID) in the amino terminus are essential for the regulation of the transcriptional activity of MYC2: indeed the JID and the TAD domains form a continuous groove that is bound by the Jas helix or the cryptic MYC-interacting domain (CMID) of JAZ repressor proteins (Zhang F. et al., 2015; 2017). Structural and biochemical analyses revealed that JAZ proteins compete with the transcriptional cofactor MED25 for binding to the TAD domain of MYC2 (Çevik et al., 2012; Chen et al., 2012; An et al., 2017).

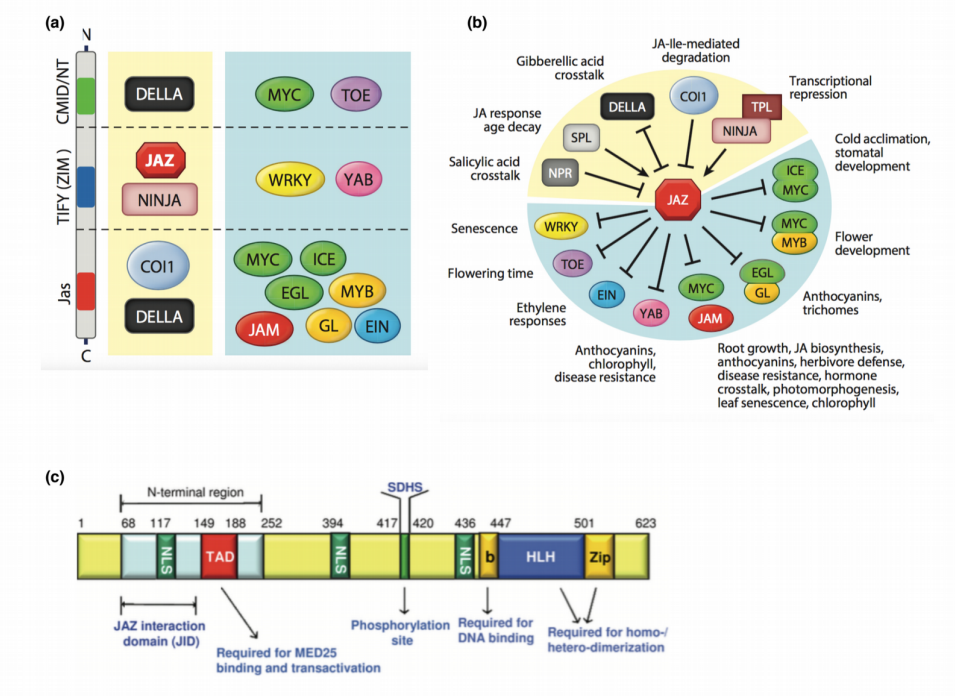


Figure 3: JAZ proteins and associated transcription factors.

(a) Schematic model of the structure of canonical Arabidopsis JAZ proteins. Functional domains are highlighted in colors. Beside the domains the signaling proteins (orange box) and the TFs (blue box) interacting with each domain are schematically represented. (b) Overview of the signaling proteins (orange section) and the TFs (blue section) interacting with JAZ proteins and their function. (c) Schematic model of the Arabidopsis TF MYC2. Functional domains are highlighted in colors. Modified from Howe et al., 2018 and Kazan & Manners, 2013.

The observation that JA-insensitive phenotypes associated to *myc2* mutants were weaker than those of *coi1-1* (Lorenzo et al., 2004) prompted the discovery of MYC3, MYC4 and MYC5 TFs (Cheng et al., 2011; Fernandez-Calvo et al., 2011; Niu et al., 2011). MYC2-5 are functionally redundant with respect to leaf senescence, resistance to herbivores and necrotrophic pathogens and contribute to the production of IGs and anthocyanins as well as

to the regulation of stamen development and pollen viability (Howe et al., 2018). All these TFs belong to the same subgroup IIIe of bHLH proteins (Heim et al., 2003) and bind to the G-box or G-related elements with very similar DNA-binding specificity (Fernandez-Calvo et al., 2011; Figueroa & Browse, 2015; Godoy et al., 2011). Despite their functional redundancy, MYC TFs retain some specificity, mostly through tissue- or stage-specific accumulation. For example, *MYC2* and *MYC3* are expressed in epidermis, cortex and endodermis of Arabidopsis roots, while the expression of *MYC4* is restricted to the columella and lateral root cap (Gasperini et al., 2015). Consistently, MYC2 and MYC3 play a more significant role than MYC4 in JA-dependent root growth inhibition (Fernandez-Calvo et al., 2011; Gasperini et al., 2015). Another source of specificity is due to the ability of MYC proteins to dimerize with other TFs. For instance, specific MYC-MYB complexes are implied in JA-derived biosynthesis of glucosinolates (Schweizer et al., 2013) and the regulation of stamen development (Figure 3b; Qi et al., 2015).

Phylogenetic analyses and interactome studies of Arabidopsis JAZ proteins revealed the bHLH subgroup IIId TFs **bHLH17/JASMONATE ASSOCIATED MYC2-LIKE1(JAM1), bHLH13/JAM2, bHLH3/JAM3 and bHLH14**. In agreement with the lack of activation domains in these TFs, genetic and biochemical studies demonstrated that they are **negative regulators of JA responses** (Song et al., 2013; Nakata et al., 2013; Sasaki-Sekimoto et al., 2013; Fonseca et al., 2014). They interact with JAZ proteins and antagonize transcriptional activators, such as MYC2 and the WD-repeat/bHLH/MYB complex, by competitively binding their target promoter sequences (Song et al., 2013; Nakata et al., 2013). The corresponding genes are regulated by JA in a COI1- and MYC2-dependent manner (Sasaki-Sekimoto et al., 2013; Fonseca et al., 2014; Liu et al., 2019). Therefore, JAM proteins are likely part of a feedback loop mechanism required for the deactivation of JA signaling (Figure 2b). The ability of JAM proteins to bind JAZ repressors through a conserved JID domain suggests that their activity is controlled by the abundance of regulatory JAZ proteins. Indeed, it has been shown that JAM-mediated transcriptional repression is counteracted by JAZ in a dose-dependent manner (Song et al., 2013).

In addition to MYC and JAM transcriptional regulators, JAZ proteins also interact with clade IIIf bHLH TFs **TRANSPARENT TESTA8 (TT8), GLABRA3 (GL3) and ENHANCER OF GL3 (EGL3)**. Moreover, several JAZ proteins interact directly with the **R2R3 MYB proteins** PRODUCTION OF ANTHOCYANIN PIGMENT1 (PAP1)/MYB75 and GL1. These proteins are part of a MYB-bHLH-WD40 (MBW) complex regulating anthocyanin accumulation and trichome formation (Qi et al., 2011). Interactions between JAZ repressors and other TFs mediate JA-dependent stamen maturation, senescence and regulation of flowering time (Song et al., 2011; Jiang et al., 2014, Zhai et al., 2015).

Moreover, JAZ proteins directly interact with **ET-responsive TFs** and the **GA signaling repressors DELLAs**, thereby mediating molecular crosstalk between different hormone signaling pathways. In Arabidopsis at least JAZ1, JAZ3, and JAZ9 can bind ETHYLENE INSENSITIVE3 (EIN3) and EIN3-LIKE1 (EIL1). Since ET is needed for EIN3/EIL1 stabilization and JA for their release from repression, this mechanism may be at the basis of the ET-JA synergy (Zhu et al., 2011). Also, the DELLA proteins GIBBERELLIC ACID INSENSITIVE (GAI), REPRESSOR OF GA (RGA) and RGA-LIKE1 (RGL1) interact with JAZ proteins and compete with MYC2 for JAZ binding, providing the molecular basis of GA-JA antagonism during plant immune responses (Navarro et al., 2008; Hou et al., 2010). On the other hand, both JAZ and DELLA proteins interact with members of the MBW complex and work in synergy in the regulation of trichome development (Qi et al., 2014). Also, the interaction between DELLA and MYC2 was proposed to explain JA-GA signaling synergy in the expression of two sesquiterpene biosynthetic genes (Hong et al., 2012).

In summary, the multifaceted role of JA signaling in plant development and defense relies on the multitude of JAZ-interacting transcriptional regulators and their specific targets (Chini et al., 2016). bHLH TFs are master regulators of JA-mediated responses to wounding, pathogens and root development. On the other hand, bHLH/MYB complexes execute JA-dependent anthocyanin biosynthesis and trichome emergence. Finally, the interaction of JAZ repressors with EIN3/EIL1 and DELLA proteins provide the molecular basis of the JA-ET and the JA-GA crosstalks.

2.2. Biosynthesis and signaling of phytohormones involved in defense and growth

2.2.1. Salicylic acid biosynthesis and signaling

SA is a major phytohormone regulating disease resistance in plants (Vlot et al., 2009; Klessig et al., 2018). Indeed, SA is important for the activation of both pathogen-associated or microbe-associated molecular pattern (PAMP/MAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI), respectively the first, aspecific and the second, specialized layer of plant immunity (Chisholm et al., 2006; Jones & Dangl, 2006; Vlot et al., 2009). Plant perception of specialized effector proteins from fungal and bacterial pathogens induces the accumulation of reactive oxygen species (ROS), which trigger SA biosynthesis and consequent expression of *pathogenesis-related* (*PR*) genes. SA signaling during ETI can trigger the hypersensitive response (HR), in which necrotic lesions appear at the site of pathogen entry (Vlot et al., 2009; Pieterse et al., 2012). Plants under pathogen attack often

display a long-lasting high basal expression level of *PR* genes, leading to quicker and more efficient immune response to subsequent infections by a wide array of pathogens. This process is called systemic acquired resistance (SAR) and SA signaling is one of its central components (Shah & Zeier, 2013; Klessig et al., 2018).

SA biosynthesis in Arabidopsis is triggered upon pathogen attack through regulation by the interacting lipase-like proteins ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and PHYTOALEXIN DEFICIENT 4 (PAD4; Aarts et al., 1998; Wiermer et al., 2005) and downstream transcription factors calmodulin binding protein 60g (CBP60g) and its related protein SYSTEMIC ACQUIRED RESISTANCE DEFICIENT1 (SARD1; Zhang et al., 2010; Wang et al., 2011). SA biosynthesis occurs through one of two distinct pathways dependent on the primary precursor chorismate (Figure 4; Wildermuth, 2006). Chorismate-derived L-phenylalanine can be converted into SA through the activity of phenylalanine ammonia lyases (PAL) and benzoic acid-2-hydroxylases (BA2H). Alternatively, SA can be synthetized by isochorismate synthases (ICS) and isochorismate pyruvate lyases (IPL) through the intermediate isochorismate. Recently, a third SA biosynthetic pathway was discovered, mediated by avrPphB Susceptible 3 (PBS3)-dependent conjugation of isochorismate to glutamate and subsequent spontaneous decay of Isochorismate-9-glutamate (ISC-9-Glu) without the requirement of enzymatic catalysis (Rekhter et al., 2019). Therefore, IPL is dispensable for pathogen-induced SA biosynthesis, while Arabidopsis *ics1* and *ics2* loss-of-function mutants are severely impaired in pathogen resistance (Wildermuth et al., 2001; Garcion et al., 2008). SA can be deactivated upon conversion to SA *O*-β-glucoside (SAG) by the activity of SA glucosyltransferases (SAGT; Lee & Raskin, 1999); SAG can be also stored in the vacuole and be converted back to SA (Dean & Mills, 2004; Hennig et al., 1993). SA can also be methylated into MeSA, a biologically inactive form (Koo et al., 2007; Song et al., 2008). However, systemic transport of MeSA and its reversion to SA may be implied in SAR under certain environmental conditions (Park et al., 2007; Attaran et al., 2009).

SA receptors remained elusive for many years. Indeed, biochemical screens in tobacco led to the identification of several SA-binding proteins, consisting mainly of catalase, ascorbate peroxidase, glutathione-S-transferases and other enzymes implied in redox reactions (Durner & Klessig, 1995; Tian et al., 2012). However, genetic evidence for the role of these enzymes as *bona fide* SA receptors is lacking. In alternative, it has been proposed that SA signaling may be transduced through modifications of the cell redox state (Caarls et al., 2015). In fact, the transcriptional cofactor NONEXPRESSOR OF *PR1* (NPR1), fundamental for SA-dependent defense (Pieterse & Van Loon, 2004; Wu et al., 2012), responds to SA-induced biphasic fluctuations in cell redox with switches between monomeric and oligomeric state (Figure 4; Mou et al., 2003; Tada et al., 2008). Oligomerization is induced by the activity of S-nitrosoglutathione (GSNO), while the SA-induced NPR1

monomerization is catalyzed by thioredoxins (TRXs, Tada et al., 2008). NPR1 monomers enter the nucleus and interact with TGA TFs to initiate the expression of SA-dependent genes (Figure 4; Kinkema et al., 2000; Zhang et al., 2003; Després et al., 2003). Also, the activity of NPR1 requires direct binding to SA (Wu et al., 2012; Ding et al., 2018). Additionally, NPR1-related NPR3 and NPR4 have been identified as SA receptors (Fu et al., 2012). NPR3/4 are negative regulators of SA responses and have been proposed to either function as E3 ubiquitin ligases regulating cytosolic NPR1 levels (Fu et al., 2012) or as NPR1-independent co-repressors of TGAs (Ding et al., 2018). Other negative regulators of NPR1 include the NIM1-INTERACTING1 (NIMIN1) and its related proteins NIMIN2 and 3 and the SUPPRESSOR of *npr1* INDUCIBLE 1 (SNI1; Li et al., 1999; Pape et al., 2010; Weigel et al., 2005). Interestingly, proteasome-mediated turnover of NPR1 is necessary for both prevention of SAR in absence of signal and its execution upon SA perception (Spoel et al., 2009). Therefore, the mechanism through which NPR receptors carry out SA responses is not yet completely understood. Downstream of NPR proteins, TGA TFs, in complex with the proteins SUPPRESSOR OF sni1 2 (SSN2), RAS ASSOCIATED WITH DIABETES51D (RAD51D) and BREAST CANCER2A (BRCA2A), regulate the expression of *PR* genes, as well as of several WRKY TFs (Figure 4; Johnson et al., 2003; Wang et al., 2005). PR proteins are small (5-75 kDa) secreted or vacuole-targeted proteins with antimicrobial activity (Sels et al., 2008) and their secretory system is also controlled by NPR1 (Wang et al., 2005). On the other hand, distinct WRKY TFs may be involved in the amplification or in the termination of SAR (Wang et al., 2006; van Verk et al., 2011).

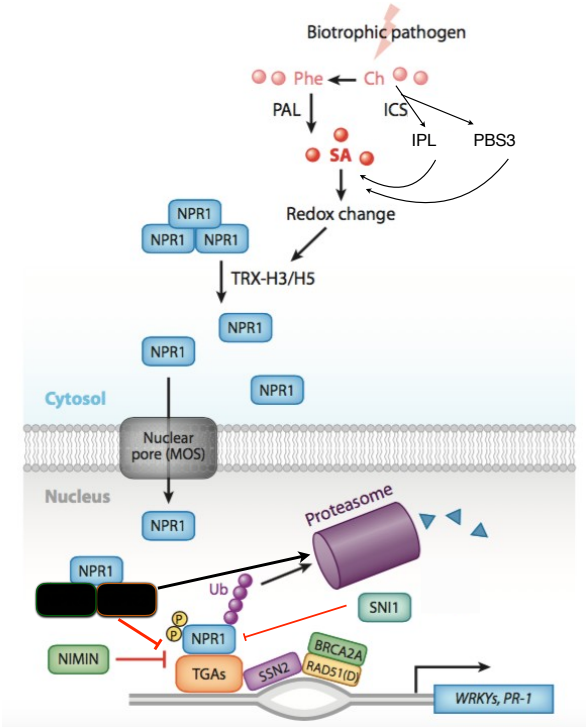


Figure 4: SA biosynthesis and signaling in Arabidopsis.

Upon pathogen perception, SA biosynthesis is initiated via the phenylalanine ammonia lyase (PAL) or the isochorismate synthase (ICS) pathway. The latter can proceed through the isochorismate pyruvate lyase (IPL) or the avrPphB Susceptible 3 (PBS3) branch. SA alters the redox status of the cytosol prompting thioredoxins (TRXs) to release monomers of NONEXPRESSOR OF PR1 (NPR1). In the monomeric form NPR1 can enter the plant cell nucleus, where it interacts with TGA TFs. Both nuclear entry and proteasomal degradation of NPR1 are required for the activation of SA-dependent gene expression. Such degradation may depend on the activity of NPR3 and NPR4, which could also serve as independent repressors of TGAs. The activity of NPR1 is further repressed by other regulator proteins such as NIM1-INTERACTING1 (NIMIN1) and SUPPRESSOR of npr1 INDUCIBLE 1 (SNI1). Downstream of NPR1, the cofactors SUPPRESSOR OF sni1 2 (SSN2), RAS ASSOCIATED WITH DIABETES51D (RAD51D), and BREAST CANCER2A (BRCA2A) associate with TGA TFs to promote the expression of pathogenesis-related (PR) genes and WRKY TFs. Modified from Pieterse et al., 2012.

Not much is known about SA biosynthesis and signaling in poplar roots or shoots. However, poplars and other Salicaceae are characterized by high basal levels of salicin-based phenolic glycosides in leaves and bark (Palo, 1984). These compounds may derive from SA and function as herbivore toxins, but their mechanisms of biosynthesis and action are not yet elucidated (Constabel & Major, 2005; Yuan et al., 2009). Other major compounds implied in poplar defense against herbivores are flavonoids and proanthocyanidins (PA or

condensed tannins). Recently, the biosynthesis of PA and their precursors flavan-3-ols (catechin, epicatechin and gallocatechin) has been shown to be triggered by increased endogenous SA levels upon infection of poplar leaves by the biotrophic pathogen *Melampsora larici-populina* (Ullah et al., 2019). The expression of the poplar orthologs of Arabidopsis *NPR1*, *MYB*, *WRKY* and *PR* genes was also enhanced by pathogen colonization, indicating active SA signaling through the MYB-bHLH-WD40 complex (Ullah et al., 2019). SA biosynthesis is also implied in poplar response against the fungal agent of the apple bot rot, *Botryosphaeria dothidea* (Li et al., 2018). In fact, inoculation of poplar bark with *B. dothidea* induced SA and MeSA accumulation in both infected and uninfected sites. In addition, the expression patterns of SA methylases and de-methylases suggest that SA produced at the infection site is converted into MeSA for systemic transport; MeSA is then de-methylated to SA in uninfected sites, allowing systemic activation of plant defenses (Li et al., 2018). Finally, genetic manipulation of SA biosynthesis and degradation demonstrated that this phytohormone is involved in responses to oxidative stress in poplar, but, unlike in Arabidopsis, such response is not accompanied by growth inhibition (Xue et al., 2013). Altogether, these studies suggest that SA signaling is a major pathway mediating poplar defense against oxidative stress, aboveground herbivores and pathogens.

2.2.2. Ethylene biosynthesis and signaling

ET is a gaseous hormones implied, among other processes, in plant development, fruit ripening, senescence and defense against biotic and abiotic stressors (Broekaert et al., 2006; Iqbal et al., 2013; Dubois et al., 2018). For instance, MAPK cascades, in response to oxidative burst, induce ET biosynthesis upon nutritional stress (Lynch & Brown, 1997; Liu & Zhang, 2004). In particular, ET signaling mediates low P-related promotion of Arabidopsis root hair development (Zhang et al., 2003), through the induction of the key transcription factor ROOT HAIR DEFECTIVE 6-LIKE4 (RSL4; Song et al., 2016; Feng et al., 2017) and alterations in auxin transport or metabolism (Rahman et al., 2002; Růžička et al., 2007). On the other hand, ET is a major modulator of plant immunity, and especially promotes plant resistance to necrotrophic pathogens (Broekaert et al., 2006). The role of ET in disease resistance depends on an extensive crosstalk network involving mainly JA and SA signaling (Glazebrook et al., 2005; Pieterse et al., 2009; 2012).

ET biosynthesis occurs through conversion of the precursor methionine into S-adenosyl-methionine and subsequently into 1-Aminocyclopropane-1-carboxylic acid (ACC), the direct precursor of ET. Two classes of enzymes, ACC synthases (ACS) and ACC oxidases (ACO), catalyze the rate limiting steps of ACC production and ACC conversion into ET, respectively (Wang et al., 2002). The activity of these enzymes is regulated both transcriptionally and post-translationally by MAPKs in response to pathogens (Figure 5; Liu &

Zhang, 2004; Li G. et al., 2012). The pool of ACC is also modulated by reversible conjugation (van de Poel & van der Straeten, 2014), as well as by LYSINE HISTIDINE TRANSPORTER1 (LHT1)-mediated xylematic transport (Shin et al., 2014).

ET is perceived by receptors localized in the endoplasmic reticulum (ER) and Golgi membrane: ERS1 (ETHYLENE RESPONSE SENSOR1), ERS2, ETR1 (ETHYLENE RESISTANT1), ETR2 and EIN4 (ETHYLENE INSENSITIVE4). In the absence of ET, these proteins bind and activate the CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) serine/threonine kinase, which inhibits downstream signaling through phosphorylation of the ER transmembrane protein ETHYLENE INSENSITIVE2 (EIN2; Merchante et al., 2013; Lacey & Binder, 2014). The presence of ET inactivates the receptors and CTR1. As a consequence, EIN2 is dephosphorylated and cleaved, releasing a C-terminal fragment that either moves to P-bodies or to the nucleus (Li et al., 2015; Merchante et al., 2015). The C-terminal fragment of EIN2 affects the translation of the EIN3-BINDING F-BOX proteins EBF1 and EBF2. EBF1/2 target the primary ET-responsive TFs EIN3 and EIL1 for proteasomal degradation in the absence of ET (Guo & Ecker, 2003; Potuschak et al., 2003). ET-induced degradation of EBF1/2 triggers EIN3/EIL1 activity and the consequent expression of numerous secondary TFs, the ETHYLENE RESPONSE FACTORS (ERFs; Figure 5; Nakano et al., 2006).

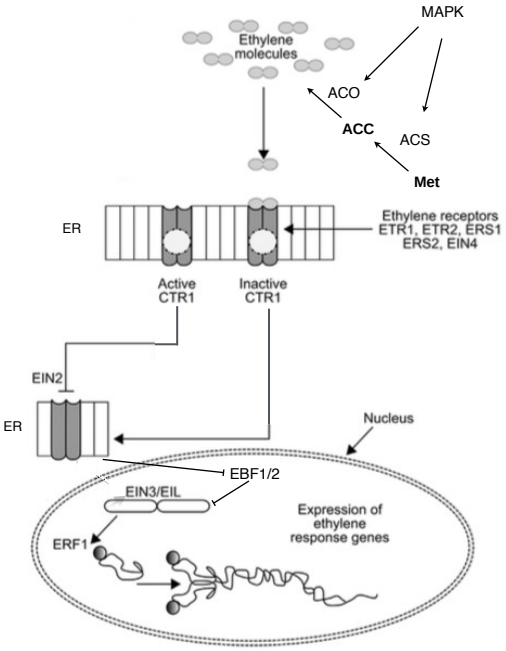


Figure 5: ET biosynthesis and signaling in Arabidopsis.

MAPK signaling cascades induce the expression of 1-Aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO), coding for two rate-limiting enzymes of ethylene biosynthetic pathway. The ET receptors ETHYLENE RESPONSE SENSOR1 (ERS1), ERS2, ETHYLENE RESISTANT1 (ETR1), ETR2 and ETHYLENE INSENSITIVE4 (EIN4) are localized in the endoplasmic reticulum (ER) and Golgi membrane. Upon ET perception, such receptors are prevented to activate the CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) serine/threonine receptor kinase. CTR1 inactivation results in the dephosphorylation of the ER transmembrane protein EIN2, cleaving its C-terminal fragment. The fragment of EIN2 enters the nucleus and affects the translation of the EIN3-BINDING F-BOX proteins EBF1 and EBF2. This results in the stabilization of the TFs EIN3 and EIL1, which trigger the transcription of the TFs ETHYLENE RESPONSIVE FACTORS (ERFs). ERFs mediate the expression of ET-responsive genes. Modified from Iqbal et al., 2013.

Current knowledge on the functions of ET signaling in poplar is limited to its role in wood formation. Indeed, both ACC content and the expression of the poplar *PttACO1* and two *PttACS* genes, orthologous to the Arabidopsis *ACC oxidase* and *ACC synthase*, respectively, are enhanced in developing secondary xylem, especially during wood formation (Andersson-Gunneråas et al., 2003; Seyfferth et al., 2018). Moreover, *PttEIN3D* and 11 *ERF* genes were identified as hubs in transcriptional networks related to xylem development, suggesting that different modules of ET signaling perform different functions during secondary growth (Seyfferth et al., 2018). The use of transgenic ET-insensitive and ET-overproducing hybrid aspens demonstrated that the main functions of ET signaling during wood development are to stimulate cell divisions in the cambial meristem (Love et al., 2009) and to induce the formation of the gelatinous layers (G-layers) typical of tension wood of aspen trees (Felten et al., 2018). In addition, JA/ET signaling is likely implied in poplar resistance to salt stress. Indeed, hybrid poplars transformed with a tomato *ERF* gene, encoding a TF mediating the crosstalk between JA and ET signaling, were more resistant in soil with excessive salt concentration (Li et al., 2008).

2.2.3. Auxin biosynthesis and signaling

Auxin is a major phytohormone implied in most, if not all, developmental and physiological processes of plants (Benjamin & Scheres, 2008). Auxin gradients are particularly important for the maintenance of quiescent cells in shoot and root meristem and the differentiation of cell types (Wang & Jiao, 2018). For instance, auxin gradients are implied in lateral root patterning and emergence (De Smet et al., 2007; Laskowski et al., 2008). Such differential accumulation of auxin is achieved through finely tuned regulation of PIN efflux transporters and AUX/LAX influx transporters (Kramer, 2004; Swarup & Peret, 2012).

The bioactive auxin form, indole-3-acetic acid (IAA), is synthesized mainly via four Trp-dependent pathways: the indole-3-pyruvic acid (IPyA), the indole-3-acetamide (IAM), the tryptamine (TRA) and the indole-3-acetaldoxime (IAOx) pathway (Figure 6; Ljung, 2013). IAA catabolism can occur via conjugation to amino acids or sugars through the action of GH3 auxin-amido-synthetases (Staswick et al., 2005) or via degradation to oxIAA through the activity of dioxygenases (Zhang et al., 2016; Porco et al., 2016; Mellor et al., 2016).

IAA is perceived by a co-receptor complex formed by the F-box protein TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX PROTEIN (TIR1/AFB) and a transcriptional inhibitor of auxin signaling of the Auxin/INDOLE ACETIC ACID (Aux/IAA) family. Upon IAA binding, The SCF^{TIR1} E3 ubiquitin ligase complex labels Aux/IAA proteins for proteasome 26S-mediated degradation, thereby releasing the repression over AUXIN RESPONSE FACTORS (ARFs) and allowing the expression of IAA-responsive genes (Figure 6; Dharmasiri et al., 2005; Kepinski & Leyser, 2005). In Arabidopsis, combinatorial interactions between six TIR1/AFBs, 29 Aux/IAAs and 23 ARFs contribute to the complexity of auxin responses (Calderón Villalobos et al., 2012).

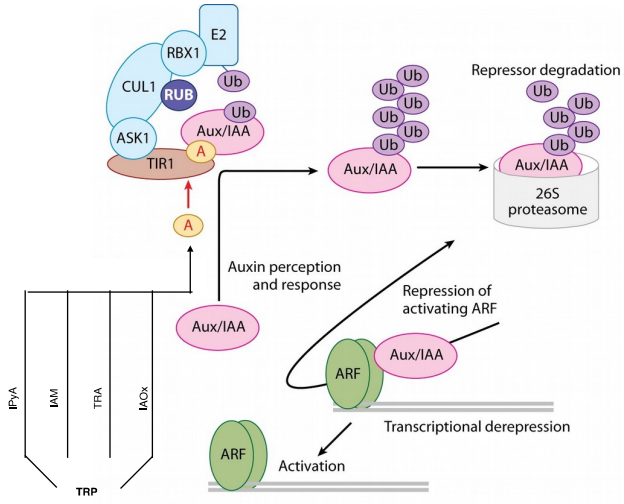


Figure 6: auxin biosynthesis and perception.

The biosynthesis of auxin starts from the precursor tryptophan (TRP) along one of four pathways: the indole-3-pyruvic acid (IPyA), the indole-3-acetamide (IAM), the tryptamine (TRA) or the indole-3-acetoaldoxime (IAOx) pathway. In the nucleus auxin is perceived by a co-receptor complex formed by one of the Auxin/INDOLE ACETIC ACID (Aux/IAA) repressor proteins and the F-box protein TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX PROTEIN (TIR1/AFB). The latter is part of a Cullin-Skip-F-box E3 ubiquitin ligase complex, which targets Aux/IAA proteins for proteasomal degradation. Consequently, Aux/IAA-mediated repression over AUXIN RESPONSE FACTORS (ARFs) is released and auxin-dependent transcription can take place. Modified from Mockaitis & Estelle, 2008.

In poplar roots the repression of auxin signaling is implied in the decrease of vessel lumina and in the synthesis of lignin and phenolic compounds during abiotic stress. Indeed, the concentration of free IAA decreases in the xylem of different poplar species under salt stress (Junghans et al., 2006), while the IAA-conjugating enzyme GH3 displays enhanced expression in the xylem of cadmium-stressed poplar roots (Elobeid et al., 2012). In Arabidopsis, auxin signaling enhances the production of reactive oxygen species (ROS) upon stress perception while auxin conjugation contributes to attenuate this stress signal (Peer et al., 2013). It is not yet clear whether a similar mechanism is conserved in woody plants. Interestingly, even though IAA levels do not vary during the formation of tension wood in poplar (Hellgren et al., 2004), exogenous IAA application enhances xylem width (Björklund et al., 2007), suggesting a role of auxin signaling in the proliferation of the cambial meristem. Finally, auxin signaling through an AP2/ERF TF may play a role in the development of adventitious and lateral roots of poplar cuttings (Trupiano et al., 2013).

2.2.4. Gibberellin biosynthesis and signaling

The plant hormone GA controls many aspects of plant development, from pollen maturation and seed germination to stem elongation, leaf expansion and trichome development (Yamaguchi, 2008; Davière & Achard, 2013; Hedden & Sponsel, 2015). More than 130 chemical species of GAs have been discovered in plants, but evidence of biological activity has been obtained only for GA_1, GA_3, GA_4 and GA_7 (Yamaguchi, 2008). In addition, GA_5 and GA_6 are effective during floral development of the grass *Lolium* spp. (King et al., 2003; 2008). In the past decade, GA signaling has been shown to constitute a regulatory hub for many other signaling pathways, mediating developmental responses to external stimuli (Campos et al., 2016).

GA biosynthesis begins from the diterpenoid precursor geranylgeranyl diphosphate (GGDP; Figure 7; Yamaguchi et al., 2008; Hedden & Thomas, 2012). Three classes of enzymes are required for the conversion of GGDP into bioactive GAs: terpene synthases (TPSs), cytochrome P450 monooxygenases (P450s) and 2-oxoglutarate-dependent dioxygenases (2ODDs). Two plastidial TPSs, *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS) mediate the conversion of GGDP into *ent*-kaurene. This tetracyclic intermediate is then converted into GA_{12} by action of two P450s: *ent*-kaurene oxidase (KO) and *ent*-kaurenoic acid oxidase (KAO). During these reactions the location of GA biosynthesis switches from the plastidial membrane to the endoplasmic reticulum. GA_{12}, the precursor of all bioactive GAs, can be the substrate of different types of cytoplasmic 2ODDs, according to the C residue they oxidize. Sequential oxidation of G_{12} on C-20 by GA 20-oxidase (GA20ox) leads to the eventual loss of the C-20. Therefore, GA20ox catalyzes the biosynthesis of C-19 GAs starting from C-20 GAs. Finally, the addition of a 3β-hydroxyl

group by the action of GA 3-oxidase (GA3ox) converts inactive precursors into bioactive GAs. The biosynthetic pathway of GAs is divided into two main branches, depending on whether the general precursor GA_{12} is oxidized on C-13 by GA 13-oxidase (GA13ox) or not before undergoing a series of C-20 oxidation reactions. In the first case, the early 13-hydroxylation (13-OH) branch of GA biosynthesis culminates with the production of bioactive GA_1, GA_3, GA_5 and GA_6 through the precursor GA_{20}. Alternatively, GA biosynthesis can proceed along the non-13-hydroxylation (13-H) branch, leading to the conversion of the precursor GA_9 into bioactive GA_4 and GA_7 (Figure 7). The inactivation of GAs occurs through C2-hydroxylation by GA 2-oxidases (GA2oxs), but GA_3, GA_5, GA_6 and GA_7 cannot be substrates of such enzymatic reaction. Alternatively, 16α,17-epoxidation and methylation of GAs have been proposed as inactivation mechanisms (Yamaguchi et al., 2008; Hedden & Thomas, 2012).

Many of the components of the GA signaling pathway have been identified through genetic screens in rice and Arabidopsis (Figure 7; Sun & Gubler, 2004; Davière & Achard, 2013). Bioactive GAs bind the nuclear receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1), inducing a conformational change of its N-terminal extension domain (N-ext). Such spatial rearrangement allows GID1 to interact with DELLA proteins, GRAS family repressors of GA signaling. The formation of the GA-GID1-DELLA complex enables the interaction between DELLA and the $SCF^{SLY1/GID2}$ complex through the F-box protein SLEEPY1 (SLY1, in Arabidopsis)/ GIBBERELLIN INSENSITIVE DWARF2 (GID2, in rice). The SCF complex is a E3 ubiquitin ligase catalyzing the polyubiquitination of the DELLA protein and its subsequent degradation through the proteasome 26S. The degradation of DELLA releases the repression over several GA-responsive TFs, leading to the activation of GA-dependent gene expression. In particular, the activation of PHYTOCHROME INTERACTING FACTOR (PIF) transcriptional activators mediates many of the GA-dependent growth responses (Figure 7; Sun & Gubler, 2004; Davière & Achard, 2013).

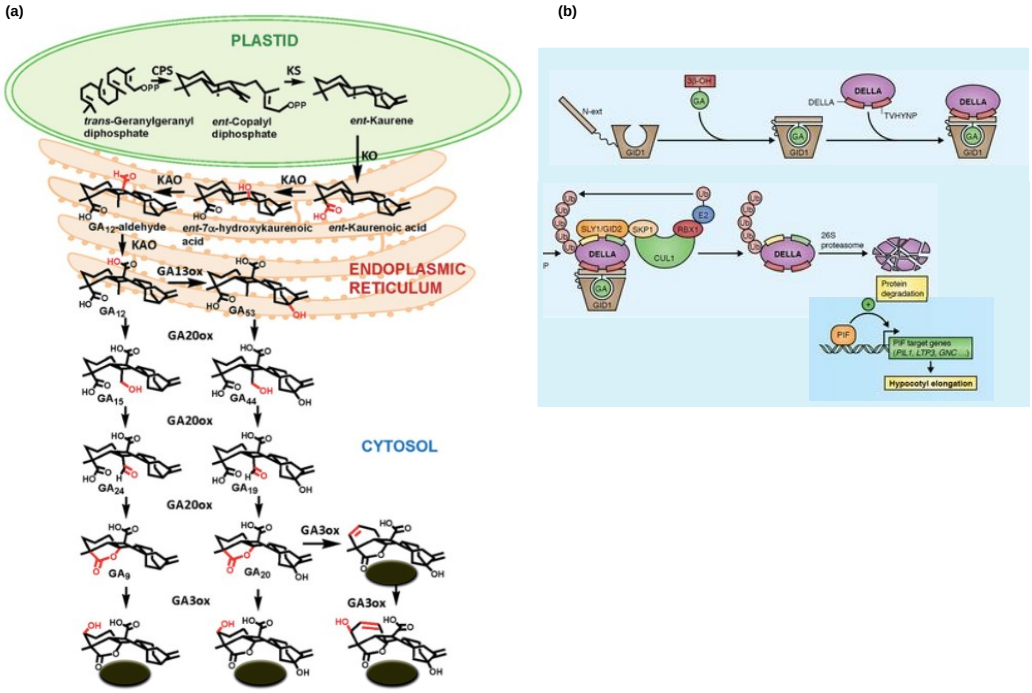


Figure 7: GA biosynthesis and signaling.

(a) GA biosynthesis proceeds from intermediates of the carotenoid biosynthetic pathway. In the plastid the enzymes ent-copalyl diphosphate synthase (CPS) and ent-kaurene synthase (KS) induce the synthesis of ent-kaurene. This molecule is translocated to the endoplasmic reticulum, where it undergoes a series of oxidation reactions by the ent-kaurene oxidase (KO) and the ent-kaurenoic acid oxidase (KAO). The resulting molecule is GA_{12}, the precursor of all bioactive GAs. In the cytosol GA_{12} is oxidized in its C-13, C-20 and C-3 by GA 13-, 20- or 3-oxidases (GA13ox, GA20ox, GA3ox), originating the various GA species, among which the bioactive GA_1, GA_3, GA_4 and GA_5 (highlighted in yellow). (b) In the nucleus GA is perceived by the receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1), inducing a conformational change of its N-terminal extension domain (N-ext). GID1 then interacts with DELLA transcriptional repressors, enabling its interaction with the F-box protein SLEEPY1(SLY1)/GID2. This triggers proteasomal degradation of DELLA proteins and release of repression for GA-dependent TFs, such as the PHYTOCHROME INTEGRATING FACTOR (PIF). Modified from Hedden & Thomas, 2012 and Davière & Achard, 2013.

In poplar GA signaling regulates shoot and root growth in response to environmental stimuli, similarly to Arabidopsis (Colebrook et al., 2014). Indeed, genetic manipulation of tissue-specific GA2ox catabolic enzymes differentially altered poplar shoot and root development. In particular, the overexpression of shoot-specific *GA2ox* genes produced a dwarfing phenotype, while silencing of root-specific *GA2ox* genes reduced root biomass (Busov et al., 2003; Gou et al., 2011). Similarly, transgenic poplars bearing degradation-

resistant versions of the DELLA proteins gai and rgl1 were dwarfed and showed enhanced root growth, possibly mediated by increased respiration (Busov et al., 2006). Constitutive suppression of GA signaling also resulted in diminished feeding of the lignin biosynthesis pathway and in preferential resource allocation towards secondary storage compounds and defense metabolites in poplar shoots (Busov et al., 2006). Physiological and genetic evidence showed that the suppression of GA signaling mediates poplar responses to drought stress and short-day-induced bud dormancy (Zawaski & Busov, 2014), while active GA signaling may promote shoot growth through increased IAA basipetal transport (Björklund et al., 2007). Altogether, these results suggest that in poplar the repression of GA signaling drives shoot growth inhibition, increased root system development and activation of chemical defenses in response to abiotic stress. Whether this effect is due to a negative crosstalk between GA and JA signaling, similarly to Arabidopsis (Guo et al., 2018a), is yet to be demonstrated.

2.3. The molecular basis of phytohormone crosstalk during defense and growth

2.3.1. The crosstalk between jasmonate, salicylate and ethylene signaling in the defense against biotrophic and necrotrophic pathogens

Activation of specific plant defenses in Arabidopsis shoots relies on the prevailing activity of determined hormone signaling pathways, with SA signaling inducing immunity mainly against biotrophs and JA/ET signaling preferentially promoting resistance against necrotrophic pathogens and herbivores (Glazebrook, 2005; Pieterse et al., 2009; 2012). However, additional layers of defense are guaranteed by concerted induction of both signaling pathways (van Wees et al., 2000; Mur et al., 2006). Remarkably, adapted pathogenic microbes learned how to take control of the JA/SA negative crosstalk in order to promote their own infection. Indeed, the virulent strain of *P. syringae* pv. *tomato* DC3000 (*Pst*DC3000) produces the phytotoxin coronatine (COR), a molecular mimic of JA-Ile, in order to suppress SA-dependent production of PR proteins (Kloek et al., 2001; Brooks et al., 2005). However, the output of the crosstalk between JA and SA signaling in defense relies on the respective concentration of the two hormones (Mur et al., 2006), as well as on the timing and the sequence of signaling initiation (Koorneef et al., 2008; Leon-Reyes et al., 2010a). In any case, the relationship between SA and JA signaling is mostly antagonistic in several dicotyledonous plant species (Thaler et al., 2012), while SA-JA antagonism seems less conserved in monocots (Tamaoki et al., 2013).

The molecular mechanism of JA/SA antagonism in Arabidopsis has been partially unraveled and occurs through the interaction of several components of JA and SA signaling along the signaling cascade (Figure 8a). For instance, the MAP KINASE4 (MPK4) is a negative regulator of SA signaling and a positive regulator of JA signaling (Petersen et al., 2000). It has been shown that the phosphorylation of MPK4 SUBSTRATE1 (MKS1) and the suppression of EDS1 and PAD4 are involved in MPK4-mediated suppression of SA signaling (Andreasson et al., 2005; Brodersen et al., 2006). However, the mechanism of MPK4-mediated promotion of JA signaling has not yet been elucidated. The redox status of the cell is also a major regulator of defense responses. In fact, both SA and JA affect the levels of the redox buffer glutathione (Spoel & Loacke, 2011) and SA-mediated suppression of JA signaling depends on glutathione biosynthesis (Koornneef et al., 2008). Since glutathione-dependent reduction of NPR1 is necessary for its nuclear import (Tada et al., 2008) and several glutaredoxins (GRXs) interact with TGA transcription factors (Ndamukong et al., 2007; Zander et al., 2012), cell redox status may indirectly influence JA signaling through promotion of the SA pathway. Interestingly, nuclear localization of NPR1 is necessary for SA-dependent gene expression, but its suppressive activity over JA signaling is mainly cytosolic (Spoel et al., 2003). However, its molecular mechanism is not yet known. In addition, the activation of nuclear SA pathway participates to this process. Indeed, SA-responsive TGA TFs are necessary for the JA/SA crosstalk (Zander et al., 2012; Leon-Reyes et al., 2010a). Interestingly, the expression of the TF ORA59, the master regulator of the ERF branch of JA signaling, is promoted upon ACC treatment through functional class II TGAs, while SA supply inhibits ORA59 transcript and protein accumulation (van der Does et al., 2013; Zander et al., 2014). The same TGAs are also required for JA- and OPDA-dependent gene expression (Zander et al., 2010; Stotz et al., 2013). Therefore, some TGAs may serve as hubs for the promotion of SA-sensitive, JA/ET-dependent transcription. WRKY50 and WRKY51 are also necessary for SA-mediated inhibition of JA signaling, possibly through downregulation of JA biosynthesis (Gao et al., 2011), while other WRKY factors may act redundantly in this process (Li et al., 2004; Mao et al., 2007; Ren et al., 2008).

The interaction between JA and ET signaling can also influence the output of plant immunity (Figure 8b). Indeed, through concerted activation of ERF TFs, simultaneous production of JA and ET can reinforce resistance against necrotrophs; on the other hand, in Arabidopsis ET signaling antagonizes the MYC branch of JA signaling, implied in response to wounding (Lorenzo et al., 2004; Pré et al., 2008). However, ABA signaling acts in synergy with JA signaling for the promotion of MYC-dependent defenses (Abe et al. 2003; Anderson et al. 2004). In addition, ET signaling can also influence the outcome of the JA/SA crosstalk: indeed, ET applied exogenously or produced by pathogens renders NPR1 redundant for SA-mediated suppression of JA responses (Leon-Reyes et al., 2009). However, simultaneous

activation of JA and ET signaling impedes subsequent SA-mediated suppression of the JA pathway in Arabidopsis (Leon-Reyes et al., 2010b). Conversely, the attenuation of JA signaling can restrict also ET-related responses through JAZ-mediated repression of EIN3/EIL1 TFs (Zhu et al., 2011). Since EIN3/EIL1 repress the expression of the SA biosynthetic gene *ICS1* (Chen et al., 2009), this may constitute a possible mechanism for JA/ET-driven suppression of SA signaling.

In conclusion, plant modulation of immunity through phytohormone signaling is determined by a complex network of interacting proteins, with specific outputs depending on hormone levels, timing and combinations. In general, SA and JA signaling act antagonistically, inhibiting each other, while ET signaling contributes to the activation of the ERF branch of JA signaling. The existence of crosstalk between JA, SA and ET signaling in poplar has not yet been investigated.

2.3.2. Jasmonate, auxin and gibberellin signaling control defense-to-development trade-offs

As sessile organisms, plants must rely on drastic immune and stress responses to optimize fitness in challenging conditions. On the other hand, the biosynthesis and transport of defensive chemicals can represent a considerable energetic cost for the plant. Therefore, the balance between the activities of different phytohormone signaling pathways is exploited for punctual activation of defense and stress responses. In particular, JA signaling constitutes a hub for defense-to-development energetic trade-offs (Figure 8c; Guo et al., 2018a).

JA signaling can promote plant defense at the expense of root and shoot growth. In fact, JA signaling represses root elongation through several mechanisms, some of which linked to auxin signaling and transport. On one hand, JA treatment suppresses the expression and membrane accumulation of the auxin efflux carrier PIN2 (Sun et al., 2011). Additionally, MYC2 represses the expression of auxin-responsive AP2-domain PLETHORA (PLT) transcription factors, essential for the maintenance of the quiescent centre (Aida et al., 2004; Gasperini et al., 2015; Chen et al., 2011). In leaves, MYCs repress the expression of photosynthesis-related genes (Attaran et al., 2014) and possibly constraint the completion of the cell cycle (Pauwels et al., 2007; Noir et al., 2013). Finally, a decuple Arabidopsis JAZ deficient mutant (*jazQ*), displaying constitutive responses to JA, showed robust resistance against herbivores and fungal pathogens, but slow vegetative growth and poor reproductive performance (Guo et al., 2018b). Interestingly, genetic manipulation allowed uncoupling the activation of defense mechanisms from growth repression upon pathogen challenge (Campos et al., 2016), indicating that the coordination of immunity and development is under genetic control, rather than determined by energetic constraints.

The main mechanism for preferential allocation of resources towards defense or development is represented by the crosstalk between JA and GA signaling (Figure 8c; Guo et al., 2018a). Arabidopsis DELLA and JAZ proteins interact, sequestering each other in the cytoplasm. The degradation of DELLA proteins releases JAZs, thus inhibiting downstream JA signaling, and vice versa (Hou et al., 2010; Yang et al., 2012). Therefore, the JAZ-DELLA interaction effectively mediates JA-GA signaling antagonism, as evidenced by several genetic studies (Navarro et al., 2008; Hou et al., 2010; Yang et al., 2012). The release of DELLA proteins, inhibiting GA-mediated growth responses, upon activation of JA signaling may thus explain the correlation between defense induction and growth suppression (Guo et al., 2018a). On the other hand, the JAZ-DELLA interaction supports promotion of JA signaling in Arabidopsis via a positive feedback loop. Indeed, upon JA perception, MYC2 induces the expression of the gene coding for the DELLA protein RGA-like3 (RGL3; Wild et al., 2012). This protein sequesters JAZ repressors in the cytosol, further promoting JA-dependent responses (Navarro et al., 2008; Wild et al., 2012). Adding further compexity, the JA-GA signaling network is integrated with the shade avoidance response through phytochrome B (phyB)-mediated perception of changes in the ratio of red to far red (R:FR) light. Low R:FR ratios indicative of leaf shading reduce phyB activity to relieve repression on PIFs, thereby promoting rapid growth through the concerted action of hormones such as auxin and brassinosteroid, as part of the shade avoidance syndrome (Figure 8c; Casal, 2013; Ballaré, 2014; Oh et al., 2012). In addition, inactivation of phyB is associated with depletion of DELLA proteins, increased JAZ stability, accelerated turnover of MYC TFs, and suppression of JA-triggered immune responses (Moreno et al., 2009; Leone et al., 2014). This effect of light signaling may be indirect, since PIFs enhance auxin accumulation (Franklin et al., 2011; Li L. et al., 2012) and auxin signaling induces the expression of JAZ1 (Grunewald et al., 2009). Remarkably, the concomitant activation of the Arabidopsis MYC2 and the PIF transcriptional modules through genetic engineering results in the rescue of developmental responses without diminished resistance against insects (Campos et al., 2016). These data suggest that the hardwired molecular network of JA, GA and phyB signaling can integrate developmental cues, stress stimuli and light perception in order to optimize plant fitness (Guo et al., 2018a).

In conclusion, JA signaling emerged as a central regulatory hub in the energetic trade-off between growth and immunity. Rather than by actual resource constraints, the balance between development and defense relies on hardwired molecular crosstalk between JA, GA and phyB signaling. Such complex signaling network integrates developmental and environmental cues in order to regulate the activity of the MYC2 and PIF transcriptional modules for optimal plant fitness. It remains to be demonstrated whether a similar regulation of the growth-defense trade-off is conserved in woody plants.

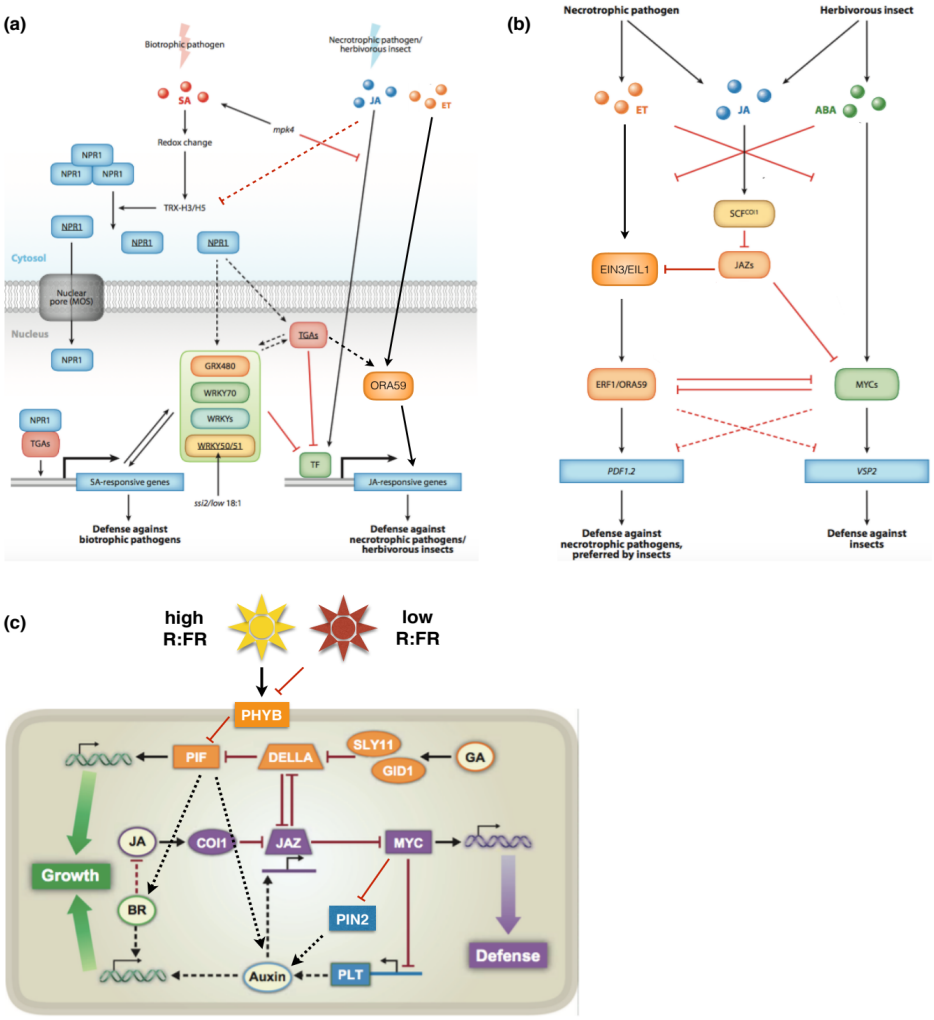


Figure 8: Molecular mechanisms underlying the crosstalk between JA, SA, ET, auxin and GA signaling in defense and development.

(a) The antagonism between JA and SA signaling in the activation of plant defenses is based on (I) MPK4-dependent suppression of SA signaling, (ii) differential regulation of the cell redox status by JA and SA, and (iii) the repression of some SA-responsive TGA and WRKY TFs on JA-dependent transcription. (b) JA and ET signaling act in synergy in the defense against necrotrophic pathogens, but they antagonistically regulate responses against insects. Synergy depends on the control by both ET and JA signaling over the activity of EIN3/EIL1, while antagonism is based on the reciprocal negative regulation of the MYC branch and the ERF branch of JA signaling. (c) The crosstalk between JA, GA and phyB signaling, involving also auxin and BR signaling, regulates the trade-off between plant growth and defense. Such crosstalk largely depends on the interaction between JAZ and DELLA proteins as well as on the activity of light signaling. Modified from Pieterse et al., 2012 and Huot et al., 2014.

3. Defense and development phytohormones in the establishment of the ectomycorrhizal symbiosis

3.1. Auxin, ethylene and sesquiterpenes mediate tree developmental responses to ectomycorrhizal fungi

The phytohormones auxin and ET are implied in root architecture remodeling upon perception of EMF by the host tree. Several species of EMF have been reported to produce IAA from Trp (Frankenberger & Poth, 1987; Niemi et al., 2002; Splivallo et al., 2009; Vayssières et al., 2015). Fungal auxin is beneficial for root colonization, since an IAA-overproducing strain of *Hebeloma cylindrosporum* increases lateral root density and develops deeper Hartig net when colonizing *Pinus pinaster* (Gay et al., 1994; Gea et al., 1994; Tranvan et al., 2000). Indirect contact with fungal mycelium enhances auxin signaling in columella and epidermal cells, suggesting perception of fungal auxin (Splivallo et al., 2009). However, fungal volatiles and/or soluble signals may also alter tree auxin homeostasis, transport and signaling. Indeed, differential expression of GH3 conjugating enzymes, of auxin influx carriers (AUX/LAX) and auxin efflux carriers (PIN), as well as of auxin-responsive Aux/IAA proteins has been reported in ECM (Reddy et al., 2006; Felten et al., 2009; Heller et al., 2012). Induced auxin signaling in host roots promotes lateral root development (Felten et al., 2009), possibly in crosstalk with ET. Indeed, a mixture of exogenous ET and IAA was necessary to reproduce the full range of root morphogenetic responses to *Tuber* spp. soluble molecules (Splivallo et al., 2009). However, it is not yet clear whether ET production occurs in the fungal mycelium or in the host root in response to fungal signals.

On the other hand, other fungal volatile organic compounds (VOCs) may be involved in root morphogenetic processes. Among these molecules, the sesquiterpene (-)-tujopsene was identified as a biologically active agent in *L. bicolor*-mediated lateral root proliferation (Ditengou et al., 2015). Sesquiterpenes are a class of terpenoid compounds produced by plants, fungi and insects; they play roles in both plant protection and signaling (Cheng et al., 2007).

Fungal manipulation of root auxin biosynthesis and signaling has also been demonstrated in mature ECM between poplar and *L. bicolor* (Vayssières et al., 2015). Indeed, progressive shortening and swelling of colonized lateral roots coincided with the diminution of auxin-responsive *DR5:GUS* activity in the lateral root meristem, while the content of bioactive auxin, its precursors and degradation products increased. Moreover, a series of auxin-responsive genes was activated upon *L. bicolor* colonization, while others

were repressed. These results indicate that fungal signals stimulate poplar auxin metabolism and trigger the activation of specific auxin responses, possibly involved in the arrest of lateral root growth (Vayssières et al., 2015).

In conclusion, fungus-driven modifications of tree hormone metabolism, transport and responses affect root architecture in both the pre-contact phase and the maturation phase of the ECM symbiosis. Altered auxin gradients and responses seem to play a major role in this process (Splivallo et al., 2009; Felten et al., 2009; Vayssières et al., 2015). In addition, the perception of mycelial volatile sesquiterpenes and the activation of tree ET signaling may contribute to EMF-induced remodeling of root architecture (Splivallo et al., 2009; Ditengou et al., 2015). To date, it remains to be assessed whether other phytohormone signaling pathways may be implied in root morphogenetic responses to EMF.

3.2. The pivotal role of jasmonate signaling in the *Populus-L. bicolor* interaction

During the past decade the fundamental role of JA signaling in controlling the establishment of the ECM symbiosis between *Populus* spp. and *Laccaria bicolor* has been unraveled (Plett et al., 2011; 2014a,b). The genome of *L. bicolor* revealed an expansion in the repertoire of < 300 amino acid-long cysteine-rich proteins carrying a secretory signal peptide (Martin et al., 2008). The genes coding for some of these small secreted proteins are upregulated upon contact with roots of the host tree *Populus trichocarpa*: thus, the correspondent products have been named Mycorrhiza-induced Small Secreted Proteins (MiSSPs; Martin et al., 2008).

The most highly upregulated *MiSSP* gene in colonizing *L. bicolor* hyphae is *MiSSP7* (MiSSP of 7 kDa). *L. bicolor* hyphae secrete MiSSP7 upon perception of diffusible signals from the host root. The small protein is then imported into root cells via phosphatidylinositol 3-phosphate-mediated endocytosis and targets the nuclei, where it alters the transcriptome of the plant cell (Plett et al., 2011). The fact that *L. bicolor* strains with reduced expression of *MiSSP7* via RNAi-mediated gene silencing failed to form the Hartig net indicates that this protein is essential for the completion of ECM development (Plett et al., 2011). Pathogenic bacteria and fungi utilize similar small secreted proteins as virulence factors: these proteins are named effectors. Some of them act in the cell wall, for instance by masking chitin residues that trigger plant basal immunity, while other hamper immunity by targeting signaling components inside the host cell (Stergiopoulos & de Wit, 2009; Shames & Finlay, 2012). Effector proteins often alter the perception of phytohormones implied in plant defense (Kazan & Lyons, 2014). Altogether, the role of virulence effectors is to hijack plant immunity

and promote pathogen infection. Similarly, MiSSP7 has been proposed to be a mutualistic effector, altering plant phythohormone-related responses in order to promote the establishment of symbiotic structures (Plett et al., 2011).

A yeast-two-hybrid screen for poplar targets of MiSSP7 identified the co-receptor of JA PtJAZ6 (Potri.003G068900) as a MiSSP7-interacting protein (Plett et al., 2014a). As mentioned in paragraph 2.1.2, JAZ proteins are repressors of JA-induced gene expression, targeted for proteasome-mediated degradation when interacting with the F-box protein COI1 in a JA-dependent fashion. MiSSP7 inhibits the interaction between PtJAZ6 and PtCOI1, thus enhancing the stability of PtJAZ6, in a dose-dependent manner (Plett et al., 2014a). Therefore, this small secreted protein likely impairs JA signaling. Indeed, *MiSSP7*-overexpressing transgenic poplar lines showed reduced expression of a subset of JA marker genes, mainly coding for predicted cell wall active enzymes (Plett et al., 2014a). The importance of suppressed JA signaling for Hartig net development is highlighted by the observation that *PtJAZ6*-overexpressing poplar transgenic lines display deeper Hartig nets, while *PtJAZ6*-silencing transgenic lines show an opposite phenotype (Plett et al., 2014a). Also, exogenous MeJA treatment of colonized poplar cuttings impairs Hartig net development and induces the expression of genes with a putative role in defense and cell wall remodeling (Plett et al., 2014b). Therefore, it has been proposed that MiSSP7 hijacks JA-mediated plant immunity and/or regulates cell wall composition to ensure apoplastic growth of fungal hyphae (Plett et al., 2014a,b).

3.3. Opposing effects of ethylene and salicylic acid signaling in the development of the Hartig net

The discovery that fungal manipulation of JA signaling is necessary for Hartig net development prompted the investigation of the function of SA and ET signaling during the establishment of ECM. Indeed, as described in paragraph 2.3.1, JA, SA and ET signaling in Arabidopsis regulate an extensive crosstalk network activating selected branches of plant immunity. It was found that exogenous SA treatment of *L. bicolor-P. tremula x alba* ECM does not impact fungal colonization or Hartig net depth. On the other hand, ET treatment, in the form of the ET precursor ACC, as well as transgenic overexpression of the ET biosynthetic enzyme PttACO1, greatly reduced Hartig net depth (Plett et al., 2014b). These plants displayed enhanced expression of pathogen-related genes and repression of genes implied in plant-microbe recognition and cell wall biosynthesis (Plett et al., 2014b). Therefore, it was proposed that JA/ET signaling may reduce fungal colonization through induction of plant defense and inhibition of cell wall expansion. It remains to be demonstrated whether *L.*

bicolor produces additional MiSSPs targeting ET signaling, similarly to the AMF *Rhizophagus irregularis* (Kloppholz et al., 2011).

4. Aims of the PhD project

The overarching goal of this PhD project is to elucidate the function(s) of JA signaling and of its manipulation by fungal signals in the regulation of the transcriptomic network associated to the development of the ECM symbiosis between *Populus tremula x alba* and *Laccaria bicolor*. Novel findings will complement current knowledge of the molecular mechanisms driving the establishment of this fundamental interaction between trees and fungi.

4.1. The *Populus tremula x alba* - *Laccaria bicolor* model system

The model system consisting of *Populus* spp. and the ECM basidiomycete *L. bicolor* has proven useful to investigate the genomic, physiologic and transcriptomic basis of ECM development (Martin et al., 2016). These model organisms present a series of advantages: (i) *P. trichocarpa* and *L. bicolor* were the first ECM partners whose genomes were fully sequenced (Tuskan et al., 2006; Martin et al., 2008) and nowadays the genomes of other poplar species, such as *P. tremula x alba*, *P. alba* var. *pyramidalis, P. tremula* and *P. tremuloides* are also available (Xue et al., 2015; Malder et al., 2016; Lin et al., 2018; Ma et al., 2019); (ii) a specific database assembles publicly available genomic and transcriptomic data for several poplar species (PopGenIE; Sundell et al., 2015); (iii) both *L. bicolor* and several *Populus* spp. can be genetically manipulated through *Agrobacterium*-mediated transformation (Parsons et al., 1986; Confalonieri et al., 2000; Kemppainen et al., 2005; Cseke et al., 2007; et al., 2010); (iv) the symbiosis can be reproduced *in vitro* or in greenhouse (Felten et al., 2009; Veneault-Fourrey et al., 2014). For all the reasons cited above, we selected this model system to pursue detailed studies on the role of plant hormones in the establishment of the ECM symbiosis.

4.2. Open questions on the impact of phytohormone signaling in the establishment of the ectomycorrhizal symbiosis

Current knowledge on the role of phytohormones like auxin, ET and JA in the establishment of the ECM symbiosis between poplar and *L. bicolor* is significant but not exhaustive. In this respect, many research questions remain open.

First of all, knowledge on the dynamics of phytohormone metabolism in developing ECM is limited to auxins (Felten et al., 2009; Vayssières et al., 2015). Monitoring hormone

biosynthesis, degradation and signaling in different stages of ECM establishment may disclose new pathways/mechanisms differentially regulated by fungal signals.

Regarding the fundamental importance of the MiSSP7-PtJAZ6 interaction, the molecular mechanism through which the fungal effector stabilizes PtJAZ6 is not yet clear. Indeed, MiSSP7 could inhibit the PtJAZ6-PtCOI1 interaction by competitive binding to the JAZ degron. Alternatively, MiSSP7 may disrupt or enhance interactions of PtJAZ6 with other components of the JA perception complex via competition, bridging or induction of conformational changes. Such differences in the composition of the PtJAZ6 interactome may prevent proteasomal degradation of this co-receptor.

Moreover, the impact of MiSSP7 on the modulation of JA signaling during ECM development has not been fully elucidated. Indeed, even if the transcriptome of MeJA-treated ECM has been investigated, the extent to which MiSSP7 hijacks JA signaling in unknown. JA responses in Arabidopsis require the activity of specific TFs, driving the expression of distinct branches of JA signaling (Howe et al., 2018). It remains to be demonstrated whether similar JA signaling subsets exist in poplar roots and, if so, which of them are differentially regulated by MiSSP7 activity.

Finally, the molecular crosstalk between JA, GA, SA and ET signaling during ECM development has not yet been comprehensively explored. In fact, it is still unclear whether interactions between JA, GA, SA and ET signaling exist in poplar roots, and what their role might be during fungal colonization.

4.3. Elucidating the role of JA signaling, and its interacting hormone signaling pathways, in ectomycorrhizal development

This doctoral project aimed at elucidating the role of JA signaling, as well as of JA-interacting GA, SA and ET signaling, in the mutualistic interaction between the ECM basidiomycete *L. bicolor* and the angiosperm tree *P. tremula x alba*. For this purpose, we focused on three main aspects: (i) understanding the molecular mechanism through which the secreted fungal effector MiSSP7 suppresses poplar reponses to JA; (ii) assessing whether specific branches of JA signaling are selectively repressed during ECM development; (iii) verifying the presence of phytohormone crosstalk in poplar roots and elucidating its function during fungal colonization.

For the study of the molecular role of MiSSP7 within the poplar JA perception complex, we first set out to define the structure of such complex. A yeast-two-hybrid screen allowed identifying one poplar TF interacting with PtJAZ6. Subsequently, based on homology with Arabidopsis, we selected other candidate interaction partners of PtJAZ6. Yeast-two-

hybrid and co-immunoprecipitation showed that JA perception in poplar roots is ensured by an ensemble of PtJAZ proteins, bHLH-like TFs and a TOPLESS co-repressor. Further, to comprehend the activity of MiSSP7 on root JA perception at the molecular level, we tested via yeast-three-hybrid whether the interactions between PtJAZ6 and any of the other proteins of the poplar JA perception complex were altered in presence of MiSSP7 (Chapter 2).

Secondly, we sought whether different branches of JA signaling specifically affect ECM colonization. On one side, we altered the expression level of the bHLH TFs PtMYC2 and PtJAM1, both interacting with PtJAZ6, in transgenic poplar roots. We then determined the phenotype of these transgenic plants in terms of root architecture and ECM morphology (Chapter 3). On the other hand, we wished to assess whether fungal colonization selectively suppressed a subset of JA-dependent responses. Thus, we examined, through high-throughput RNA sequencing, the transcriptomes of *L. bicolor*-colonized and uncolonized poplar roots under exogenous MeJA treatment. This analysis allowed to highlight symbiosis-dependent differences in JA sensitivity. In the same experimental setting, we also defined JA-responsive genes in poplar roots and inspected the dynamics of their expression during ECM development (Chapter 4).

Finally, we investigated the role of SA, ET and GA, and of their crosstalk with JA signaling, in the transcriptional reprogramming occurring during ECM formation (Chapter 4). In the same series of experiments, we also quantified through mass spectrometry the concentration of JAs, SA, GAs and some additional phytohormone species, like cytokinins and abscisates, to get hints on the dynamics of phytohormone metabolism in developing ECM (Chapter 4).

5. References

Aarts, N., Metz, M., Holub, E., Staskawicz, B.J., Daniels, M.J., and Parker, J.E. (1998). Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene-mediated signaling pathway in Arabidospsis. Proc. Natl. Acad. Sci. U. S. A. **95**: 10306–10311.

Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2003). Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) Function as Transcriptional Activators in Abscisic Acid Signaling. Plant Cell **15**: 63–78.

Aida, M., Beis, D., Heidstra, R., Willemsen, V., Blilou, I., Galinha, C., Nussaume, L., Noh, Y.S., Amasino, R., and Scheres, B. (2004). The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. Cell **119**: 109–120.

Aloni, R., Aloni, E., Langhans, M., and Ullrich, C.I. (2006). Role of cytokinin and auxin in shaping root architecture: Regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann. Bot. **97**: 883–893.

Alvarez, M., Huygens, D., Olivares, E., Saavedra, I., Alberdi, M., and Valenzuela, E. (2009). Ectomycorrhizal fungi enhance nitrogen and phosphorus nutrition of nothofagus dombeyi under drought conditions by regulating assimilative enzyme activities. Physiol. Plant. **136**: 426–436.

An, C., Li, L., Zhai, Q., You, Y., Deng, L., Wu, F., Chen, R., Jiang, H., Wang, H., Chen, Q., and Li, C. (2017). Mediator subunit MED25 links the jasmonate receptor to transcriptionally active chromatin. Proc. Natl. Acad. Sci. **114**: E8930–E8939.

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R., and Kazan, K. (2004). Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell **16**: 3460–3479.

Andersson-Gunnerås, S., Hellgren, J.M., Björklund, S., Regan, S., Moritz, T., and Sundberg, B. (2003). Asymmetric expression of a poplar ACC oxidase controls ethylene production during gravitational induction of tension wood. Plant J. **34**: 339–349.

Andreasson, E. et al. (2005). The MAP kinase substrate MKS1 is a regulator of plant defense responses. EMBO J. **24**: 2579–2589.

Antico, C.J., Colon, C., Banks, T., and Ramonell, K.M. (2012). Insights into the role of jasmonic acid-mediated defenses against necrotrophic and biotrophic fungal pathogens. Front. Biol. (Beijing). **7**: 48–56.

Appel, H.M., Arnold, T.M., and Schultz, J.C. (2012). Effects of jasmonic acid, branching and girdling on carbon and nitrogen transport in poplar. New Phytol. **195**: 419–426.

Arnold, T.M. and Schultz, J.C. (2002). Induced sink strength as a prerequisite for induced tannin biosynthesis in developing leaves of Populus. Oecologia **130**: 585–593.

Attaran, E., Major, I.T., Cruz, J.A., Rosa, B.A., Koo, A.J.K., Chen, J., Kramer, D.M., He, S.Y., and Howe, G.A. (2014). Temporal dynamics of growth and photosynthesis suppression in response to jasmonate signaling. Plant Physiol. **165**: 1302–1314.

Attaran, E., Zeier, T.E., Griebel, T., and Zeier, J. (2009). Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in Arabidopsis. Plant Cell **21**: 954–971.

Attiwill, P. M., & Adams, M. A. (1993). Nutrient cycling in forests. New Phytol., **124**: 561–582.

Averill, C., Turner, B.L., and Finzi, A.C. (2014). Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature.

Babst, B.A., Ferrieri, R. a, Gray, D.W., Lerdau, M., Schlyer, D.J., Schueller, M., Thorpe, M.R., and Orians, C.M. (2005). Jasmonic acid induces rapid changes in carbon transport and partitioning in Populus. New Phytol. **167**: 63–72.

Balestrini, R. and Bonfante, P. (2014). Cell wall remodeling in mycorrhizal symbiosis: a way towards biotrophism. Front. Plant Sci. **5**: 1–10.

Balestrini, R. and Kottke, I. (2016). Structure and development of ectomycorrhizal roots. In Molecular Mycorrhizal Symbiosis.

Balestrini, R., Sillo, F., Kohler, A., Schneider, G., Faccio, A., Tisserant, E., Martin, F., and Bonfante, P. (2012). Genome-wide analysis of cell wall-related genes in Tuber melanosporum. Curr. Genet.

Ballaré, C.L. (2014). Light Regulation of Plant Defense. Annu. Rev. Plant Biol. **65**: 335–363.

Bank, S. (2009). The experience of the passive house . Concept , cost and users behaviour . **55**: 979–988.

Basso, V. and Veneault-Fourrey, C. The role of jasmonates in beneficial microbe-root interactions. In Jasmonate in Plant Biology.

Becquer, A. et al. (2018). The Hebeloma cylindrosporum HcPT2 Pi transporter plays a key role in ectomycorrhizal symbiosis. New Phytol. **220**: 1185–1199.

Benjamins, R. and Scheres, B. (2008). Auxin: The Looping Star in Plant Development. Annu. Rev. Plant Biol. **59**: 443–465.

Björklund, S., Antti, H., Uddestrand, I., Moritz, T., and Sundberg, B. (2007). Cross-talk between gibberellin and auxin in development of Populus wood: Gibberellin stimulates polar auxin transport and has a common transcriptome with auxin. Plant J. **52**: 499–511.

Blasius, D., Feil, W., Kottke, I., and Oberwinkler, F. (1986). Hartig net structure and formation in fully ensheathed ectomycorrhizas. Nord. J. Bot. **6**: 837–842.

Bogeat-Triboulot, M.B., Bartoli, F., Garbaye, J., Marmeisse, R., and Tagu, D. (2004). Fungal ectomycorrhizal community and drought affect root hydraulic properties and soil adherence to roots of Pinus pinaster seedlings. Plant Soil **267**: 213–223.

Bonfante, P. and Genre, A. (2008). Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. Trends Plant Sci.

Brodersen, P., Petersen, M., Nielsen, H.B., Zhu, S., Newman, M.A., Shokat, K.M., Rietz, S., Parker, J., and Mundy, J. (2006). Arabidopsis MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. Plant J. **47**: 532–546.

Broekaert, W.F., Delauré, S.L., De Bolle, M.F.C., and Cammue, B.P.A. (2006). The Role of Ethylene in Host-Pathogen Interactions. Annu. Rev. Phytopathol. **44**: 393–416.

Brooks, D.M., Bender, C.L., and Kunkel, B.N. (2005). The Pseudomonas syringae phytotoxin coronatine promotes virulence by overcoming salicylic acid-dependent defences in Arabidopsis thaliana. Mol. Plant Pathol. **6**: 629–639.

Brundrett, M.C. (2002). Coevolution of roots and mycorrhizas of land plants. New Phytol.

Brundrett, M.C. and Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol.

Bücking, H., Liepold, E., and Ambilwade, P. (2012). The Role of the Mycorrhizal Symbiosis in Nutrient Uptake of Plants and the Regulatory Mechanisms Underlying These Transport Processes. Plant Sci.

Busov, V., Meilan, R., Pearce, D.W., Rood, S.B., Ma, C., Tschaplinski, T.J., and Strauss, S.H. (2006). Transgenic modification of gai or rgl1 causes dwarfing and alters gibberellins, root growth, and metabolite profiles in Populus. Planta **224**: 288–299.

Busov, V.B., Meilan, R., Pearce, D.W., Rood, S.B., and Strauss, S.H. (2003). Activation Tagging of a Dominant Gibberellin Catabolism Gene (GA 2-oxidase) from Poplar That Regulates Tree Stature. Plant Physiol. **132**: 1283–1291.

Caarls, L., Pieterse, C.M.J., and Van Wees, S.C.M. (2015). How salicylic acid takes transcriptional control over jasmonic acid signaling. Front. Plant Sci. **6**: 170.

Calderón Villalobos, L.I.A. et al. (2006). The PB1 Domain in Auxin Response Factor and Aux/IAA Proteins: A Versatile Protein Interaction Module in the Auxin Response. Nat. Chem. Biol. **8**: 477–485.

Campos, M.L., Kang, J.-H., and Howe, G.A. (2014). Jasmonate-triggered plant immunity. J. Chem. Ecol. **40**: 657–675.

Campos, M.L., Yoshida, Y., Major, I.T., De Oliveira Ferreira, D., Weraduwage, S.M., Froehlich, J.E., Johnson, B.F., Kramer, D.M., Jander, G., Sharkey, T.D., and Howe, G.A. (2016). Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. Nat. Commun. **7**: 12570.

Carbonnel, S. and Gutjahr, C. (2014). Control of arbuscular mycorrhiza development by nutrient signals. Front. Plant Sci. **5**: 462.

Casal, J.J. (2013). Photoreceptor Signaling Networks in Plant Responses to Shade. Annu. Rev. Plant Biol. **64**: 403–427.

Causier, B., Ashworth, M., Guo, W., and Davies, B. (2012). The TOPLESS Interactome: A Framework for Gene Repression in Arabidopsis. Plant Physiol. **158**: 423–438.

Çevik, V., Kidd, B.N., Zhang, P., Hill, C., Kiddle, S., Denby, K.J., Holub, E.B., Cahill, D.M., Manners, J.M., Schenk, P.M., Beynon, J., and Kazan, K. (2012). MEDIATOR25 Acts as an Integrative Hub for the Regulation of Jasmonate-Responsive Gene Expression in Arabidopsis. Plant Physiol. **160**: 541–555.

Chalot, M. and Plassard, C. (2011). Ectomycorrhiza and nitrogen provision to the host tree. In Ecological Aspects of Nitrogen Metabolism in Plants, J.C. Polacco and C.D. Todd, eds (John Wiley & Sons, Inc.), pp. 69–94.

Chauvin, A., Caldelari, D., Wolfender, J.L., and Farmer, E.E. (2013). Four 13-lipoxygenases contribute to rapid jasmonate synthesis in wounded Arabidopsis thaliana leaves: A role for lipoxygenase 6 in responses to long-distance wound signals. New Phytol. **197**: 566–575.

Chehab, E.W., Kim, S., Savchenko, T., Kliebenstein, D., Dehesh, K., and Braam, J. (2011). Intronic T-DNA Insertion Renders Arabidopsis opr3 a Conditional Jasmonic Acid-Producing Mutant. Plant Physiol. **156**: 770–778.

Chen, H., Xue, L., Chintamanani, S., Germain, H., Lin, H., Cui, H., Cai, R., Zuo, J., Tang, X., Li, X., Guo, H., and Zhou, J.M. (2009). ETHYLENE INSENSITIVE3 and ETHYLENE INSENSITIVE3-LIKE1 repress SALICYLIC ACID INDUCTION DEFICIENT2 expression to negatively regulate plant innate immunity in Arabidopsis. Plant Cell **21**: 2527–2540.

Chen, Q. et al. (2011). The basic helix-loop-helix transcription factor MYC2 directly represses PLETHORA expression during jasmonate-mediated modulation of the root stem cell niche in Arabidopsis. Plant Cell **23**: 3335–3352.

Chen, R., Jiang, H., Li, L., Zhai, Q., Qi, L., Zhou, W., Liu, X., Li, H., Zheng, W., Sun, J., and Li, C. (2012). The Arabidopsis Mediator Subunit MED25 Differentially Regulates Jasmonate and Abscisic Acid Signaling through Interacting with the MYC2 and ABI5 Transcription Factors. Plant Cell **24**: 2898–2916.

Cheng, Z., Sun, L., Qi, T., Zhang, B., Peng, W., Liu, Y., and Xie, D. (2011). The bHLH transcription factor MYC3 interacts with the jasmonate ZIM-domain proteins to mediate jasmonate response in arabidopsis. Mol. Plant **4**: 279–288.

Cheng, A. X., Lou, Y. G., Mao, Y. B., Lu, S., Wang, L. J., & Chen, X. Y. (2007). Plant terpenoids: Biosynthesis and ecological functions. J. Int. Plant Biol., **49**(2): 179–186.

Chico, J.M., Chini, A., Fonseca, S., and Solano, R. (2008). JAZ repressors set the rhythm in jasmonate signaling. Curr. Opin. Plant Biol. **11**: 486–494.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J.M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L., and Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. Nature **448**: 666–671.

Chini, A., Gimenez-Ibanez, S., Goossens, A., and Solano, R. (2016). Redundancy and specificity in jasmonate signalling. Curr. Opin. Plant Biol. **33**: 147–156.

Chini, A., Monte, I., Zamarreño, A.M., Hamberg, M., Lassueur, S., Reymond, P., Weiss, S., Stintzi, A., Schaller, A., Porzel, A., García-Mina, J.M., and Solano, R. (2018). An OPR3-independent pathway uses 4,5-didehydrojasmonate for jasmonate synthesis. Nat. Chem. Biol. **14**: 171–178.

Chisholm, S.T., Coaker, G., Day, B., and Staskawicz, B.J. (2006). Host-microbe interactions: Shaping the evolution of the plant immune response. Cell **124**: 803–814.

Chung, H.S., Cooke, T.F., Depew, C.L., Patel, L.C., Ogawa, N., Kobayashi, Y., and Howe, G.A. (2011). Alternative splicing expands the repertoire of dominant JAZ repressors of jasmonate signaling. Plant J. **63**: 613–622.

Chung, H.S. and Howe, G.A. (2009). A Critical Role for the TIFY Motif in Repression of Jasmonate Signaling by a Stabilized Splice Variant of the JASMONATE ZIM-Domain Protein JAZ10 in Arabidopsis. Plant Cell **21**: 131–145.

Colebrook, E.H., Thomas, S.G., Phillips, A.L., and Hedden, P. (2014). The role of gibberellin signalling in plant responses to abiotic stress. J. Exp. Biol. **217**: 67–75.

Confalonieri, M., Belenghi, B., Balestrazzi, A., Negri, S., Facciotto, G., Schenone, G., and Delledonne, M. (2000). Transformation of elite white poplar (Populus alba L.) cv. "Villafranca" and evaluation of herbicide resistance. Plant Cell Rep. **19**: 978–982.

Constabel, C.P. and Major, I.T. (2005). Molecular biology and biochemistry of induced insect defense in Populus (Elsevier Masson SAS).

Constabel, C.P., Yip, L., Patton, J.J., and Christopher, M.E. (2000). Polyphenol Oxidase from Hybrid Poplar. Cloning and Expression in Response to Wounding and Herbivory. Plant Physiol. **124**: 285–296.

Cope, K.R. et al. (2019). The ectomycorrhizal fungus Laccaria bicolor produces lipochitooligosaccharides and uses the common symbiosis pathway to colonize Populus. Plant Cell **In Press**.

Cseke, L.J., Cseke, S.B., and Podila, G.K. (2007). High efficiency poplar transformation. Plant Cell Rep. **26**: 1529–1538.

Danielsen, L. and Polle, A. (2014). Poplar nutrition under drought as affected by ectomycorrhizal colonization. Environ. Exp. Bot.

Davière, J.M. and Achard, P. (2013). Gibberellin signaling in plants. Development **140**: 1147–1151.

Dean, J. V. and Mills, J.D. (2004). Uptake of salicylic acid 2-O-β-D-glucose into soybean tonoplast vesicles by an ATP-binding cassette transporter-type mechanism. Physiol. Plant. **120**: 603–612.

Després, C., Chubak, C., Rochon, A., Clark, R., Bethune, T., Desveaux, D., and Fobert, P.R. (2003). The Arabidopsis NPR1 Disease Resistance Protein Is a Novel Cofactor That Confers Redox Regulation of DNA Binding Activity to the Basic Domain/Leucine Zipper Transcription Factor TGA1. Plant Cell **15**: 2181–2191.

Dharmasiri, N., Dharmasiri, S., and Estelle, M. (2005). The F-box protein TIR1 is an auxin receptor. Nature **435**: 441–445.

Dietz, S., von Bülow, J., Beitz, E., and Nehls, U. (2011). The aquaporin gene family of the ectomycorrhizal fungus Laccaria bicolor: Lessons for symbiotic functions. New Phytol. **190**: 927–940.

Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, Y., Li, X., and Zhang, Y. (2018). Opposite Roles of Salicylic Acid Receptors NPR1 and NPR3/NPR4 in Transcriptional Regulation of Plant Immunity. Cell **173**: 1454–1467.

Ditengou, F.A., Béguiristain, T., and Lapeyrie, F. (2000). Root hair elongation is inhibited by hypaphorine, the indole alkaloid from the ectomycorrhizal fungus Pisolithus tinctorius, and restored by indole-3-acetic acid. Planta **211**: 722–728.

Ditengou, F.A., Müller, A., Rosenkranz, M., Felten, J., Lasok, H., Van Doorn, M.M., Legué, V., Palme, K., Schnitzler, J.-P., and Polle, A. (2015). Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. Nat. Commun. **6**: 6279: 1–9.

Van der Does, D., Leon-Reyes, A., Koornneef, A., Van Verk, M.C., Rodenburg, N., Pauwels, L., Goossens, A., Körbes, A.P., Memelink, J., Ritsema, T., Van Wees, S.C.M., and Pietersea, C.M.J. (2013). Salicylic acid suppresses jasmonic acid signaling downstream of SCFCOI1-JAZ by targeting GCC promoter motifs via transcription factor ORA59. Plant Cell **25**: 744–761.

Dombrecht, B., Xue, G.P., Sprague, S.J., Kirkegaard, J.A., Ross, J.J., Reid, J.B., Fitt, G.P., Sewelam, N., Schenk, P.M., Manners, J.M., and Kazan, K. (2007). MYC2 Differentially Modulates Diverse Jasmonate-Dependent Functions in Arabidopsis. Plant Cell Online **19**: 2225–2245.

Doré, J. et al. (2015). Comparative genomics, proteomics and transcriptomics give new insight into the exoproteome of the basidiomycete Hebeloma cylindrosporum and its involvement in ectomycorrhizal symbiosis. New Phytol. **208**: 1169–1187.

Doré, J., Kohler, A., Dubost, A., Hundley, H., Singan, V., Peng, Y., Kuo, A., Grigoriev, I. V., Martin, F., Marmeisse, R., and Gay, G. (2017). The ectomycorrhizal basidiomycete Hebeloma cylindrosporum undergoes early waves of transcriptional reprogramming prior to symbiotic structures differentiation. Environ. Microbiol.

Du, M. et al. (2017). MYC2 Orchestrates a Hierarchical Transcriptional Cascade That Regulates Jasmonate-Mediated Plant Immunity in Tomato. Plant Cell **29**: 1883–1906.

Dubois, M., Van den Broeck, L., and Inzé, D. (2018). The Pivotal Role of Ethylene in Plant Growth. Trends Plant Sci. **23**: 311–323.

Durner, J. and Klessig, D.F. (1995). Inhibition of ascorbate peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense responses. Proc. Natl. Acad. Sci. U. S. A. **92**: 11312–11316.

Elobeid, M., Göbel, C., Feussner, I., and Polle, A. (2012). Cadmium interferes with auxin physiology and lignification in poplar. J. Exp. Bot. **63**: 1413–1421.

Feijen, F.A.A., Vos, R.A., Nuytinck, J., and Merckx, V.S.F.T. (2018). Evolutionary dynamics of mycorrhizal symbiosis in land plant diversification. Sci. Rep.

Felten, J., Kohler, A., Morin, E., Bhalerao, R.P., Palme, K., Martin, F., Ditengou, F.A., and Legue, V. (2009). The Ectomycorrhizal Fungus Laccaria bicolor Stimulates Lateral Root Formation in Poplar and Arabidopsis through Auxin Transport and Signaling. Plant Physiol. **151**: 1991–2005.

Felten, J., Vahala, J., Love, J., Gorzsás, A., Rüggeberg, M., Delhomme, N., Leśniewska, J., Kangasjärvi, J., Hvidsten, T.R., Mellerowicz, E.J., and Sundberg, B. (2018). Ethylene signaling induces gelatinous layers with typical features of tension wood in hybrid aspen. New Phytol. **218**: 999–1014.

Feng, Y., Xu, P., Li, B., Li, P., Wen, X., An, F., Gong, Y., Xin, Y., Zhu, Z., Wang, Y., and Guo, H. (2017). Ethylene promotes root hair growth through coordinated EIN3/EIL1 and RHD6/RSL1 activity in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. **114**: 13834–13839.

Fernández-Calvo, P. et al. (2011). The Arabidopsis bHLH Transcription Factors MYC3 and MYC4 Are Targets of JAZ Repressors and Act Additively with MYC2 in the Activation of Jasmonate Responses. Plant Cell **23**: 701–715.

Figueroa, P. and Browse, J. (2015). Male sterility in Arabidopsis induced by overexpression of a MYC5-SRDX chimeric repressor. Plant J. **81**: 849–860.

Fonseca, S., Chico, J.M., and Solano, R. (2009). The jasmonate pathway: the ligand, the receptor and the core signalling module. Curr. Opin. Plant Biol. **12**: 539–547.

Fonseca, S., Fernández-Calvo, P., Fernández, G.M., Díez-Díaz, M., Gimenez-Ibanez, S., López-Vidriero, I., Godoy, M., Fernández-Barbero, G., Van Leene, J., De Jaeger, G., Franco-Zorrilla, J.M., and Solano, R. (2014). bHLH003, bHLH013 and bHLH017 are new targets of JAZ repressors negatively regulating JA responses. PLoS One **9**: e86182.

Frankenberger, W.T. and Poth, M. (1987). Biosynthesis of Indole-3-Acetic Acid by the Pine Ectomycorrhizal Fungus Pisolithus tinctorius. Appl. Environ. Microbiol. **53**: 2908–29013.

Franklin, K.A., Lee, S.H., Patel, D., Kumar, S.V., Spartz, A.K., Gu, C., Ye, S., Yu, P., Breen, G., Cohen, J.D., Wigge, P.A., and Gray, W.M. (2011). Phytochrome-Interacting Factor 4 (PIF4) regulates auxin biosynthesis at high temperature. Proc. Natl. Acad. Sci. U. S. A. **108**: 20231–20235.

Frost, C.J., Mescher, M.C., Dervinis, C., Davis, J.M., Carlson, J.E., and De Moraes, C.M. (2008). Priming defense genes and metabolites in hybrid poplar by the green leaf volatile cis-3-hexenyl acetate. New Phytol. **180**: 722–734.

Fu, Z.Q., Yan, S., Saleh, A., Wang, W., Ruble, J., Oka, N., Mohan, R., Spoel, S.H., Tada, Y., Zheng, N., and Dong, X. (2012). NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. Nature **486**: 228–232.

Gao, Q.M., Venugopal, S., Navarre, D., and Kachroo, A. (2011). Low oleic acid-derived repression of jasmonic acid-inducible defense responses requires the WRKY50 and WRKY51 proteins. Plant Physiol. **155**: 464–476.

Garcia, K., Delaux, P.-M., Cope, K.R., and Ané, J.-M. (2015). Molecular signals required for the establishment and maintenance of ectomycorrhizal symbioses. New Phytol. **208**: 79–87.

Garcia, K., Doidy, J., Zimmermann, S.D., Wipf, D., and Courty, P.E. (2016). Take a Trip Through the Plant and Fungal Transportome of Mycorrhiza. Trends Plant Sci. **21**: 937–950.

Garcion, C., Lohmann, A., Lamodière, E., Catinot, J., Buchala, A., Doermann, P., and Métraux, J.P. (2008). Characterization and biological function of the Isochorismate Synthase2 gene of Arabidopsis. Plant Physiol. **147**: 1279–1287.

Gasperini, D., Chételat, A., Acosta, I.F., Goossens, J., Pauwels, L., Goossens, A., Dreos, R., Alfonso, E., and Farmer, E.E. (2015). Multilayered Organization of Jasmonate Signalling in the Regulation of Root Growth. PLoS Genet. **11**: e1005300.

Gay, G., Normand, L., Marmeisse, R., Sotta, B., and Debaud, J.C. (1994). Auxin overproducer mutants of Hebeloma cylindrosporum Romagnesi have increased mycorrhizal activity. New Phytol. **128**: 645–657.

Gea, L., Normand, L., Vian, B., and Gay, G. (1994). Structural aspects of ectomycorrhiza of Pinus pinaster (Ait .) Sol . formed by an IAA-overproducer mutant of Hebeloma cylindrosporum Romagnesi. New Phytol. **128**: 659–670.

Gehring, C.A., Sthultz, C.M., Flores-Rentería, L., Whipple, A. V., and Whitham, T.G. (2017). Tree genetics defines fungal partner communities that may confer drought tolerance. Proc. Natl. Acad. Sci. **114**: 11169–11174.

George, T.S. et al. (2018). Organic phosphorus in the terrestrial environment: a perspective on the state of the art and future priorities. Plant Soil **427**: 191–208.

Gil-Martínez, M., López-García, Á., Domínguez, M.T., Navarro-Fernández, C.M., Kjøller, R., Tibbett, M., and Marañón, T. (2018). Ectomycorrhizal Fungal Communities and Their Functional Traits Mediate Plant–Soil Interactions in Trace Element Contaminated Soils. Front. Plant Sci.

Glauser, G., Dubugnon, L., Mousavi, S.A.R., Rudaz, S., Wolfender, J.L., and Farmer, E.E. (2009). Velocity estimates for signal propagation leading to systemic jasmonic acid accumulation in wounded Arabidopsis. J. Biol. Chem. **284**: 34506–34513.

Glazebrook, J. (2005). Contrasting Mechanisms of Defense Against Biotrophic and Nectrotrophic Pathogens. Annu. Rev. Phytopathol. **43**: 205–227.

Godoy, M., Franco-Zorrilla, J.M., Pérez-Pérez, J., Oliveros, J.C., Lorenzo, Ó., and Solano, R. (2011). Improved protein-binding microarrays for the identification of DNA-binding specificities of transcription factors. Plant J. **66**: 700–711.

Gonthier, P., Giordano, L., Zampieri, E., Lione, G., Vizzini, A., Colpaert, J. V., and Balestrini, R. (2019). An ectomycorrhizal symbiosis differently affects host susceptibility to two congeneric fungal pathogens. Fungal Ecol.

Gou, J., Strauss, S.H., Tsai, C.J., Fang, K., Chen, Y., Jiang, X., and Busov, V.B. (2010). Gibberellins regulate lateral root formation in Populus through interactions with auxin and other hormones. Plant Cell **22**: 623–639.

Gough, C., Cottret, L., Lefebvre, B., and Bono, J.-J. (2018). Evolutionary History of Plant LysM Receptor Proteins Related to Root Endosymbiosis. Front. Plant Sci. **9**: 923.

Grunewald, W., Vanholme, B., Pauwels, L., Plovie, E., Inzé, D., Gheysen, G., and Goossens, A. (2009). Expression of the Arabidopsis jasmonate signalling repressor JAZ1/TIFY10A is stimulated by auxin. EMBO Rep. **10**: 923–928.

Guo, H. and Ecker, J.R. (2003). Plant Responses to Ethylene Gas Are Mediated by SCF(EBF1/EBF2)-dependent proteolysis of EIN3 Transcription Factor. Cell **115**: 667–677.

Guo, Q., Major, I.T., and Howe, G.A. (2018a). Resolution of growth-defense conflict: mechanistic insights from jasmonate signaling. Curr. Opin. Plant Biol. **44**: 72–81.

Guo, Q., Yoshida, Y., Major, I.T., Wang, K., Sugimoto, K., Kapali, G., Havko, N.E., Benning, C., and Howe, G.A. (2018b). JAZ repressors of metabolic defense promote growth and reproductive fitness in Arabidopsis . Proc. Natl. Acad. Sci. **115**: E10768–E10777.

Gutjahr, C. and Paszkowski, U. (2009). Weights in the Balance: Jasmonic Acid and Salicylic Acid Signaling in Root-Biotroph Interactions. Mol. Plant-Microbe Interact. **22**: 763–772.

Hacquard, S., Tisserant, E., Brun, A., Legué, V., Martin, F., and Kohler, A. (2013). Laser microdissection and microarray analysis of Tuber melanosporum ectomycorrhizas reveal functional heterogeneity between mantle and Hartig net compartments. Environ. Microbiol. **15**: 1853–1869.

Hasselquist, N.J., Metcalfe, D.B., Inselbacher, E., Stangl, Z., Oren, R., Näsholm, T., and Högberg, P. (2015). Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a boreal forest. Ecology **Accepted A**.

Hause, B., Maier, W., Miersch, O., Kramell, R., and Strack, D. (2002). Induction of Jasmonate Biosynthesis in Arbuscular Mycorrhizal Barley Roots. Plant Physiol. **130**: 1213–1220.

He, X., Critchley, C., Ng, H., and Bledsoe, C. (2005). Nodulated N_2-fixing Casuarina cunninghamiana is the sink for net N transfer from non- N_2-fixing Eucalyptus maculata via an ectomycorrhizal fungus Pisolithus sp. using $^{15}NH_4^+$ or $^{15}NO_3$. New Phytol. **167**: 897–912.

Hedden, P. and Sponsel, V. (2015). A Century of Gibberellin Research. J. Plant Growth Regul. **34**: 740–760.

Hedden, P. and Thomas, S.G. (2012). Gibberellin biosynthesis and its regulation. Biochem. J. **444**: 11–25.

van der Heijden, M.G.A., Martin, F.M., Selosse, M.A., and Sanders, I.R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. New Phytol.

Heim, M.A., Jakoby, M., Werber, M., Martin, C., Weisshaar, B., and Bailey, P.C. (2003). The basic helix-loop-helix transcription factor family in plants: A genome-wide study of protein structure and functional diversity. Mol. Biol. Evol. **20**: 735–747.

Heller, G., Lundén, K., Finlay, R.D., Asiegbu, F.O., and Elfstrand, M. (2012). Expression analysis of Clavata1-like and Nodulin21-like genes from Pinus sylvestris during ectomycorrhiza formation. Mycorrhiza **22**: 271–277.

Hellgren, J.M., Olofsson, K., and Sundberg, B. (2004). Patterns of auxin distribution during gravitational induction of reaction wood in poplar and pine. Plant Physiol. **135**: 212–220.

Hennig, J., Malamy, J., Grynkiewicz, G., Indulski, J., and Klessig, D.F. (1993). j.1365-313X.1993.04040593.x.pdf. Plant J. **4**: 593–600.

Hibbett, D.S., Gilbert, L.B., and Donoghue, M.J. (2000). Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature **407**: 506–508.

Hibbett, D.S. and Matheny, P.B. (2009). The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. BMC Biol. **7**: 13.

Hoeksema, J.D. (2010). Ongoing coevolution in mycorrhizal interactions. New Phytol.

Hong, G.J., Xue, X.Y., Mao, Y.B., Wang, L.J., and Chen, X.Y. (2012). Arabidopsis MYC2 interacts with DELLA proteins in regulating sesquiterpene synthase gene expression. Plant Cell **24**: 2635–2648.

Horan, D.P., Chilvers, G.A., and Lapeyrie, F.F. (1988). Time sequence of the infection process in eucalypt ectomycorrhizas. New Phytol. **109**: 451–458.

Hortal, S., Plett, K.L., Plett, J.M., Cresswell, T., Johansen, M., Pendall, E., and Anderson, I.C. (2017). Role of plant-fungal nutrient trading and host control in determining the competitive success of ectomycorrhizal fungi. ISME J. **11**: 2666–2676.

Hou, X., Lee, L.Y.C., Xia, K., Yan, Y., and Yu, H. (2010). DELLAs Modulate Jasmonate Signaling via Competitive Binding to JAZs. Dev. Cell **19**: 884–894.

Howe, G.A. and Jander, G. (2007). Plant Immunity to Insect Herbivores. Annu. Rev. Plant Biol. **59**: 41–66.

Howe, G.A., Major, I.T., and Koo, A.J. (2018). Modularity in Jasmonate Signaling for Multistress Resilience. Annu. Rev. Plant Biol. **69**: 387–415.

Hu, Z. hui, Zhang, W., Shen, Y. bai, Fu, H. jun, Su, X. hua, and Zhang, Z. yi (2009). Activities of lipoxygenase and phenylalanine ammonia lyase in poplar leaves induced by insect herbivory and volatiles. J. For. Res. **20**: 372–376.

Huang, H., Liu, B., Liu, L., and Song, S. (2017). Jasmonate action in plant growth and development. J. Exp. Bot. **68**: 1349–1359.

Huot, B., Yao, J., Montgomery, B.L., and He, S.Y. (2014). Growth-defense tradeoffs in plants: A balancing act to optimize fitness. Mol. Plant **7**: 1267–1287.

Iqbal, N., Trivellini, A., Masood, A., Ferrante, A., and Khan, N.A. (2013). Current understanding on ethylene signaling in plants: The influence of nutrient availability. Plant Physiol. Biochem. **73**: 128–138.

Jennifer L. Parke, R. G. Linderman, and C. H. Black (1983). The Role of Ectomycorrhizas in Drought Tolerance of Douglas-Fir Seedlings. New Phytol. **95**: 83–95.

Jiang, Y., Liang, G., Yang, S., and Yu, D. (2014). Arabidopsis WRKY57 functions as a node of convergence for jasmonic acid- and auxin-mediated signaling in jasmonic acid-induced leaf senescence. Plant Cell **26**: 230–245.

Johnson, C., Boden, E., and Arias, J. (2003). Salicylic Acid and NPR1 Induce the Recruitment of trans-activating TGA factors to a defense gene promoter in Arabidopsis. Plant Cell **15**: 1846–1858.

Jones, J.D.G. and Dangl, J.L. (2006). The plant immune system. Nature **444**: 323–329.

Junghans, U., Polle, A., Düchting, P., Weiler, E., Kuhlman, B., Gruber, F., and Teichmann, T. (2006). Adaptation to high salinity in poplar involves changes in xylem anatomy and auxin physiology. Plant, Cell Environ. **29**: 1519–1531.

Kagale, S., Links, M.G., and Rozwadowski, K. (2010). Genome-Wide Analysis of Ethylene-Responsive Element Binding Factor-Associated Amphiphilic Repression Motif-Containing Transcriptional Regulators in Arabidopsis. Plant Physiol. **152**: 1109–1134.

Kaling, M., Schmidt, A., Moritz, F., Rosenkranz, M., Witting, M., Kasper, K., Janz, D., Schmitt-Kopplin, P., Schnitzler, J.-P., and Polle, A. (2018). Mycorrhiza-Triggered Transcriptomic and Metabolomic Networks Impinge on Herbivore Fitness. Plant Physiol.

Kazan, K. and Lyons, R. (2014). Intervention of Phytohormone Pathways by Pathogen Effectors. Plant Cell.

Kazan, K. and Manners, J.M. (2013). MYC2: The master in action. Mol. Plant **6**: 686–703.

Ke, J., Ma, H., Gu, X., Thelen, A., Brunzelle, J.S., Li, J., Xu, H.E., and Melcher, K. (2015). Structural basis for recognition of diverse transcriptional repressors by the TOPLESS family of corepressors. Sci. Adv. **1**: e1500107.

Kemppainen, M., Circosta, A., Tagu, D., Martin, F., and Pardo, A.G. (2005). Agrobacterium-mediated transformation of the ectomycorrhizal symbiont Laccaria bicolor S238N. Mycorrhiza **16**: 19–22.

Kepinski, S. and Leyser, O. (2005). The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature **435**: 446–451.

Kiers, E.T. et al. (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science **333**: 880–882.

King, R.W., Evans, L.T., Mander, L.N., Moritz, T., Pharis, R.P., and Twitchin, B. (2003). Synthesis of gibberellin GA6 and its role in flowering of Lolium temulentum. Phytochemistry **62**: 77–82.

King, R.W., Mander, L.N., Asp, T., MacMillan, C.P., Blundell, C.A., and Evans, L.T. (2008). Selective deactivation of gibberellins below the shoot apex is critical to flowering but not to stem elongation of Lolium. Mol. Plant **1**: 295–307.

Kinkema, M., Fan, W., and Dong, X. (2000). Nuclear localization of NPR1 is required for activation of PR gene expression. Plant Cell **12**: 2339–2350.

Kinkema, M. and Gresshoff, P.M. (2008). Investigation of Downstream Signals of the Soybean Autoregulation of Nodulation Receptor Kinase GmNARK. Mol. Plant-Microbe Interact. **21**: 1337–1348.

Kipfer, T., Wohlgemuth, T., van der Heijden, M.G.A., Ghazoul, J., and Egli, S. (2012). Growth response of drought-stressed pinus sylvestris seedlings to single- and multi-species inoculation with ectomycorrhizal fungi. PLoS One **7**: e35275.

Kirkpatrick, D.S., Bustos, D.J., Dogan, T., Chan, J., Phu, L., Young, A., Friedman, L.S., Belvin, M., Song, Q., Bakalarski, C.E., and Hoeflich, K.P. (2014). Erratum: Phosphoproteomic characterization of DNA damage response in melanoma cells following MEK/PI3K dual inhibition (Proceedings of the National Academy of Sciences of the United States of America (2013) 110:48 (19426-19431) DOI: 10.1073/pnas.130947311. Proc. Natl. Acad. Sci. U. S. A. **111**: 562.

Klein, T., Siegwolf, R.T.W., and Körner, C. (2016). Belowground carbon trade among tall trees in a temperate forest. Science (80-.).

Klessig, D.F., Choi, H.W., and Dempsey, D.A. (2018). Systemic acquired resistance and salicylic acid: Past, present, and future. Mol. Plant-Microbe Interact. **31**: 871–888.

Kloek, A.P., Verbsky, M.L., Sharma, S.B., Schoelz, J.E., Vogel, J., Klessig, D.F., and Kunkel, B.N. (2001). Resistance to Pseudomonas syringae conferred by an Arabidopsis thaliana coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms. Plant J. **26**: 509–522.

Kloppholz, S., Kuhn, H., and Requena, N. (2011). A secreted fungal effector of glomus intraradices promotes symbiotic biotrophy. Curr. Biol. **21**: 1204–1209.

Kohler, A. et al. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat. Genet. **47**: 410.

Koo, A.J.K., Gao, X., Daniel Jones, A., and Howe, G.A. (2009). A rapid wound signal activates the systemic synthesis of bioactive jasmonates in Arabidopsis. Plant J. **59**: 974–986.

Koo, Y.J. et al. (2007). Overexpression of salicylic acid carboxyl methyltransferase reduces salicylic acid-mediated pathogen resistance in Arabidopsis thaliana. Plant Mol. Biol. **64**: 1–15.

Koornneef, A., Leon-Reyes, A., Ritsema, T., Verhage, A., Den Otter, F.C., Van Loon, L.C., and Pieterse, C.M.J. (2008). Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. Plant Physiol. **147**: 1358–1368.

Kramer, E.M. (2004). PIN and AUX/LAX proteins: Their role in auxin accumulation. Trends Plant Sci. **9**: 578–582.

Krogan, N.T., Hogan, K., and Long, J.A. (2012). APETALA2 negatively regulates multiple floral organ identity genes in Arabidopsis by recruiting the co-repressor TOPLESS and the histone deacetylase HDA19. Development **139**: 4180–4190.

Labbé, J. et al. (2019). Mediation of plant–mycorrhizal interaction by a lectin receptor-like kinase. Nat. Plants **5**: 676–680.

Lacey, R.F. and Binder, B.M. (2014). How plants sense ethylene gas - The ethylene receptors. J. Inorg. Biochem. **133**: 58–62.

Laskowski, M., Grieneisen, V.A., Hofhuis, H., Ten Hove, C.A., Hogeweg, P., Marée, A.F.M., and Scheres, B. (2008). Root system architecture from coupling cell shape to auxin transport. PLoS Biol. **6**: e307.

Laurent, P., Voiblet, C., Tagu, D., de Carvalho, D., Nehls, U., De Bellis, R., Balestrini, R., Bauw, G., Bonfante, P., and Martin, F. (1999). A Novel Class of Ectomycorrhiza-Regulated Cell Wall Polypeptides in Pisolithus tinctorius. Mol. Plant Microbe Interact. **12**: 862–871.

Lee, H. Il and Raskin, I. (1999). Purification, cloning, and expression of a pathogen inducible UDP-glucose:salicylic acid glucosyltransferase from tobacco. J. Biol. Chem. **274**: 36637–36642.

Lehto, T. and Zwiazek, J.J. (2011). Ectomycorrhizas and water relations of trees: A review. Mycorrhiza.

Lei, J., Wong, K.K.Y., and Piché, Y. (1991). Extracellular Concanavalin A-binding sites during early interactions between Pinus banksiana and two closely related genotypes of the ectomycorrhizal basidiomycete Laccaria bicolor. Mycol. Res. **95**: 357–363.

Leon-Reyes, A., Van der Does, D., De Lange, E.S., Delker, C., Wasternack, C., Van Wees, S.C.M., Ritsema, T., and Pieterse, C.M.J. (2010a). Salicylate-mediated suppression of jasmonate-responsive gene expression in arabidopsis is targeted downstream of the jasmonate biosynthesis pathway. Planta **232**: 1423–1432.

Leon-Reyes, A., Du, Y., Koornneef, A., Proietti, S., Körbes, A.P., Memelink, J., Pieterse, C.M.J., and Ritsema, T. (2010b). Ethylene signaling renders the jasmonate response of arabidopsis insensitive to future suppression by salicylic acid. Mol. Plant-Microbe Interact. **23**: 187–197.

Leon-Reyes, A., Spoel, S.H., De Lange, E.S., Abe, H., Kobayashi, M., Tsuda, S., Millenaar, F.F., Welschen, R.A.M., Ritsema, T., and Pieterse, C.M.J. (2009). Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. Plant Physiol. **149**: 1797–1809.

Leone, M., Keller, M.M., Cerrudo, I., and Ballaré, C.L. (2014). To grow or defend? Low red: Far-red ratios reduce jasmonate sensitivity in Arabidopsis seedlings by promoting DELLA degradation and increasing JAZ10 stability. New Phytol. **204**: 355–367.

Li, G., Meng, X., Wang, R., Mao, G., Han, L., Liu, Y., and Zhang, S. (2012). Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in arabidopsis. PLoS Genet. **8**: e1002767.

Li, J., Bao, S., Zhang, Y., Ma, X., Mishra-Knyrim, M., Sun, J., Sa, G., Shen, X., Polle, A., and Chen, S. (2012). Paxillus involutus Strains MAJ and NAU Mediate K + /Na + Homeostasis in Ectomycorrhizal Populus × canescens under Sodium Chloride Stress . Plant Physiol.

Li, J., Brader, G., and Palva, E.T. (2004). The WRKY70 Transcription Factor: A Node of Convergence for Jasmonate-Mediated and Salicylate-Mediated Signals in Plant Defense. Plant Cell **16**: 319–331.

Li, L. et al. (2012). Linking photoreceptor excitation to changes in plant architecture. Genes Dev. **26**: 785–790.

Li, Q., Zheng, J., Li, S., Huang, G., Skilling, S.J., Wang, L., Li, L., Li, M., Yuan, L., and Liu, P. (2017). Transporter-Mediated Nuclear Entry of Jasmonoyl-Isoleucine Is Essential for Jasmonate Signaling. Mol. Plant **10**: 695–708.

Li, W., Ma, M., Feng, Y., Li, H., Wang, Y., Ma, Y., Li, M., An, F., and Guo, H. (2015). EIN2-Directed Translational Regulation of Ethylene Signaling in Arabidopsis. Cell **163**: 670–683.

Li, X., Zhang, Y., Clarke, J.D., Li, Y., and Dong, X. (1999). Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of npr1-1. Cell **98**: 329–339.

Li, Y., Su, X., Zhang, B., Huang, Q., Zhang, X., and Huang, R. (2009). Expression of jasmonic ethylene responsive factor gene in transgenic poplar tree leads to increased salt tolerance. Tree Physiol. **29**: 273–279.

Li, Y. xia, Zhang, W., Dong, H. xia, Liu, Z. yu, Ma, J., and Zhang, X. yao (2018). Salicylic acid in Populus tomentosa is a remote signalling molecule induced by Botryosphaeria dothidea infection. Sci. Rep. **8**: 14059.

Liao, H.L., Chen, Y., and Vilgalys, R. (2016). Metatranscriptomic Study of Common and Host-Specific Patterns of Gene Expression between Pines and Their Symbiotic Ectomycorrhizal Fungi in the Genus Suillus. PLoS Genet. **12**: e1006348.

Lin, Y.C. et al. (2018). Functional and evolutionary genomic inferences in Populus through genome and population sequencing of American and European aspen. Proc. Natl. Acad. Sci. U. S. A. **115**: E10970–E10978.

Lindahl, B.D. and Tunlid, A. (2015). Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprotrophs. New Phytol.

Liu, Y., Du, M., Deng, L., Shen, J., Fang, M., Chen, Q., Lu, Y., Wang, Q., Li, C., and Zhai, Q. (2019). MYC2 regulates the termination of jasmonate signaling via an autoregulatory negative feedback loop. Plant Cell **31**: 106–127.

Liu, Y. and Zhang, S. (2004). Phosphorylation of 1-Aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in Arabidopsis. Plant Cell **16**: 3386–3399.

Ljung, K. (2013). Auxin metabolism and homeostasis during plant development. Development **140**: 943–950.

Lorenzo , O, Chico, J., Sánchez-Serran, J., and Solano, R. (2004). JASMONATE-INSENSITIVE1 Encodes a MYC Transcription Factor Essential to Discriminate between Different Jasmonate-Regulated Defense Responses in Arabidopsis Author (s): Oscar Lorenzo , Jose M . Chico , Jose J . Sánchez-Serrano and Roberto Solano Published. Plant Cell **16**: 1938–1950.

Lorenzo, O., Piqueras, R., Sánchez-Serrano, J.J., and Solano, R. (2003). ETHYLENE RESPONSE FACTOR1 Integrates Signals from Ethylene and Jasmonate Pathways in Plant Defense. Plant Cell **15**: 165–178.

Love, J., Björklund, S., Vahala, J., Hertzberg, M., Kangasjärvi, J., and Sundberg, B. (2009). Ethylene is an endogenous stimulator of cell division in the cambial meristem of Populus. Proc. Natl. Acad. Sci. U. S. A. **106**: 5984–5989.

Luo, Z.-B., Janz, D., Jiang, X., Göbel, C., Wildhagen, H., Tan, Y., Rennenberg, H., Feussner, I., and Polle, A. (2009). Upgrading Root Physiology for Stress Tolerance by Ectomycorrhizas: Insights from Metabolite and Transcriptional Profiling into Reprogramming for Stress Anticipation. Plant Physiol.

Luo, Z. Bin, Li, K., Gai, Y., Göbel, C., Wildhagen, H., Jiang, X., Feußner, I., Rennenberg, H., and Polle, A. (2011). The ectomycorrhizal fungus (Paxillus involutus) modulates leaf physiology of poplar towards improved salt tolerance. Environ. Exp. Bot.

Luo, Z. Bin, Wu, C., Zhang, C., Li, H., Lipka, U., and Polle, A. (2014). The role of ectomycorrhizas in heavy metal stress tolerance of host plants. Environ. Exp. Bot.

Lynch, J. and Brown, K.M. (1997). Ethylene and plant responses to nutritional stress. Physiol. Plant. **100**: 613–619.

Ma, J., Wan, D., Duan, B., Bai, X., Bai, Q., Chen, N., and Ma, T. (2019). Genome sequence and genetic transformation of a widely distributed and cultivated poplar. Plant Biotechnol. J. **17**: 451–460.

Machado, R.A.R., Ferrieri, A.P., Robert, C.A.M., Glauser, G., Kallenbach, M., Baldwin, I.T., and Erb, M. (2013). Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. New Phytol. **200**: 1234–1246.

Mader, M., Le Paslier, M.C., Bounon, R., Bérard, A., Rampant, P.F., Fladung, M., Leplé, J.C., and Kersten, B. (2016). Whole-genome draft assembly of Populus tremula x P. Alba clone INRA 717-1B4. Silvae Genet. **65**: 74–79.

Major, I.T. and Constabel, C.P. (2006). Molecular analysis of poplar defense against herbivory: Comparison of wound- and insect elicitor-induced gene expression. New Phytol. **172**: 617–635.

Major, I.T., Yoshida, Y., Campos, M.L., Kapali, G., Xin, X.F., Sugimoto, K., de Oliveira Ferreira, D., He, S.Y., and Howe, G.A. (2017). Regulation of growth–defense balance by the JASMONATE ZIM-DOMAIN (JAZ)-MYC transcriptional module. New Phytol. **215**: 1533–1547.

Mao, P., Duan, M., Wei, C., and Li, Y. (2007). WRKY62 transcription factor acts downstream of cytosolic NPR1 and negatively regulates jasmonate-responsive gene expression. Plant Cell Physiol. **48**: 833–842.

Marjanović, Ž., Uehlein, N., Kaldenhoff, R., Zwiazek, J.J., Weiß, M., Hampp, R., and Nehls, U. (2005). Aquaporins in poplar: What a difference a symbiont makes! Planta **222**: 258–268.

Martin, F. (2016). Molecular Mycorrhizal Symbiosis.

Martin, F. et al. (2010). Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature **464**: 1033–1038.

Martin, F. et al. (2008). The genome of Laccaria bicolor provides insights into mycorrhizal symbiosis. Nature **452**: 88–92.

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., and Hibbett, D.S. (2016). Unearthing the roots of ectomycorrhizal symbioses. Nat. Rev. Microbiol. **14**: 760–773.

Martin, F., Laurent, P., De Carvalho, D., Voiblet, C., Balestrini, R., Bonfante, P., and Tagu, D. (1999). Cell wall proteins of the ectomycorrhizal basidiomycete Pisolithus tinctorius: Identification, function, and expression in symbiosis. Fungal Genet. Biol.

Massicotte, H.B., Peterson, R.L., Ackerley, C.A., and Ashford, A.E. (1987). Ontogeny of Eucalyptus pilularis – Pisolithus tinctorius ectomycorrhizae. II. Transmission electron microscopy. Can. J. Bot. **65**: 1940–1947.

Mello, A. and Balestrini, R. (2018). Recent Insights on Biological and Ecological Aspects of Ectomycorrhizal Fungi and Their Interactions. Front. Microbiol. **9**: 216: 1–13.

Mellor, N. et al. (2016). Dynamic regulation of auxin oxidase and conjugating enzymes AtDAO1 and GH3 modulates auxin homeostasis. Proc. Natl. Acad. Sci. U. S. A. **113**: 11022–11027.

Melotto, M., Mecey, C., Niu, Y., Chung, H.S., Katsir, L., Yao, J., Zeng, W., Thines, B., Staswick, P., Browse, J., and Howe, G. (2008). Critical role of two positively charged amino acids in the Jas motif of Arabidopsis JAZ proteins. Plant J. **55**: 979–988.

Merchante, C., Alonso, J.M., and Stepanova, A.N. (2013). Ethylene signaling: Simple ligand, complex regulation. Curr. Opin. Plant Biol. **16**: 554–560.

Merchante, C., Brumos, J., Yun, J., Hu, Q., Spencer, K.R., Enríquez, P., Binder, B.M., Heber, S., Stepanova, A.N., and Alonso, J.M. (2015). Gene-Specific Translation Regulation Mediated by the Hormone-Signaling Molecule EIN2. Cell **163**: 684–697.

Mockaitis, K. and Estelle, M. (2008). Auxin Receptors and Plant Development: A New Signaling Paradigm. Annu. Rev. Cell Dev. Biol. **24**: 55–80.

Moreno, J.E., Shyu, C., Campos, M.L., Patel, L.C., Chung, H.S., Yao, J., He, S.Y., and Howe, G.A. (2013). Negative Feedback Control of Jasmonate Signaling by an Alternative Splice Variant of JAZ10. Plant Physiol. **162**: 1006–1017.

Moreno, J.E., Tao, Y., Chory, J., and Ballaré, C.L. (2009). Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. Proc. Natl. Acad. Sci. U. S. A. **106**: 4935–4940.

Mou, Z., Fan, W., and Dong, X. (2003). Inducers of plant systemic acquired resistance Regulate NPR1 function through redox changes. Cell **113**: 935–944.

Mur, L.A.J., Kenton, P., Atzorn, R., Miersch, O., and Wasternack, C. (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. **140**: 249–262.

Murat, C. et al. (2018). Pezizomycetes genomes reveal the molecular basis of ectomycorrhizal truffle lifestyle. Nat. Ecol. Evol. **2**: 1956–1965.

Nafisi, M., Fimognari, L., and Sakuragi, Y. (2015). Interplays between the cell wall and phytohormones in interaction between plants and necrotrophic pathogens. Phytochemistry **112**: 63–71.

Nakano, T., Suzuki, K., Ohtsuki, N., Tsujimoto, Y., Fujimura, T., and Shinshi, H. (2006). Identification of genes of the plant-specific transcription-factor families cooperatively regulated by ethylene and jasmonate in Arabidopsis thaliana. J. Plant Res. **119**: 407–413.

Nakata, M., Mitsuda, N., Herde, M., Koo, A.J.K., Moreno, J.E., Suzuki, K., Howe, G.A., and Ohme-Takagi, M. (2013). A bHLH-type transcription factor, ABA-INDUCIBLE BHLH-TYPE TRANSCRIPTION FACTOR/JA-ASSOCIATED MYC2-LIKE1, acts as a repressor to negatively regulate jasmonate signaling in Arabidopsis. Plant Cell **25**: 1641–1656.

Näsholm, T., Högberg, P., Franklin, O., Metcalfe, D., Keel, S.G., Campbell, C., Hurry, V., Linder, S., and Högberg, M.N. (2013). Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? New Phytol. **198**: 214–221.

Näsholm, T., Kielland, K., and Ganeteg, U. (2009). Tansley review: Uptake of organic nitrogen by plants. New Phytol. **182**: 31–48.

Navarro, L., Bari, R., Achard, P., Lisón, P., Nemri, A., Harberd, N.P., and Jones, J.D.G. (2008). DELLAs Control Plant Immune Responses by Modulating the Balance of Jasmonic Acid and Salicylic Acid Signaling. Curr. Biol. **18**: 650–655.

Ndamukong, I., Abdallat, A. Al, Thurow, C., Fode, B., Zander, M., Weigel, R., and Gatz, C. (2007). SA-inducible Arabidopsis glutaredoxin interacts with TGA factors and suppresses JA-responsive PDF1.2 transcription. Plant J. **50**: 128–139.

Nehls, U., Das, A., and Neb, D. (2016). Carbohydrate metabolism in ectomycorrhizal symbiosis. In Molecular Mycorrhizal Symbiosis.

Nehls, U. and Plassard, C. (2018). Nitrogen and phosphate metabolism in ectomycorrhizas. New Phytol.

Niemi, K., Vuorinen, T., Ernstsen, A., and Häggman, H. (2002). Ectomycorrhizal fungi and exogenous auxins influence root and mycorrhiza formation of Scots pine hypocotyl cuttings in vitro. Tree Physiol. **22**: 1231–1239.

Niu, Y., Figueroa, P., and Browse, J. (2011). Characterization of JAZ-interacting bHLH transcription factors that regulate jasmonate responses in Arabidopsis. J. Exp. Bot. **62**: 2143–2154.

Noir, S., Bömer, M., Takahashi, N., Ishida, T., Tsui, T.-L., Balbi, V., Shanahan, H., Sugimoto, K., and Devoto, A. (2013). Jasmonate Controls Leaf Growth by Repressing Cell Proliferation and the Onset of Endoreduplication while Maintaining a Potential Stand-By Mode. Plant Physiol. **161**: 1930–1951.

Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E., and McMurtrie, R.E. (2010). CO2 enhancement of forest productivity constrained by limited nitrogen availability. Proc. Natl. Acad. Sci. **107**: 19368–19373.

Oh, E., Zhu, J.Y., and Wang, Z.Y. (2012). Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat. Cell Biol. **14**: 802–809.

Oldroyd, G.E.D. (2013). Speak, friend, and enter: Signalling systems that promote beneficial symbiotic associations in plants. Nat. Rev. Microbiol. **11**: 252–263.

Oliveira-Garcia, E. and Valent, B. (2015). How eukaryotic filamentous pathogens evade plant recognition. Curr. Opin. Microbiol.

Oren, R., Ewers, B.E., Schäfer, K.V.R., McCarthy, H., Katul, G.G., Ellsworth, D.S., Hendrey, G., Johnsen, K.H., Maier, C., Phillips, N., and McNulty, S.G. (2001). Soil fertility limits carbon sequestration by forest ecosystems in a CO2-enriched atmosphere. Nature **411**: 469–472.

Palo, T.R. (1984). Distribution of birch (Betula SPP.), willow (Salix SPP.) and poplar (Populus SPP.) secondary metabolites and their potential role as chemical defense against herbivores. J. Chem. Ecol. **10**: 499–520.

Pape, S., Thurow, C., and Gatz, C. (2010). Exchanging the as-1-like element of the PR-1 promoter by the as-1 element of the CaMV 35S promoter abolishes salicylic acid responsiveness and regulation by NPR1 and SNI1. Plant Signal. Behav. **5**: 1669–1671.

Park, S.W., Kaimoyo, E., Kumar, D., Mosher, S., and Klessig, D.F. (2007). Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science (80-.). **318**: 113–116.

Parsons, T.J., Sinkar, V.P., Stettler, R.F., Nester, E.W., and Gordon, M.P. (1986). Transformation of Poplar by Agrobacterium tumefaciens. Bio/Technology **4**: 533–536.

Pauwels, L. et al. (2010). NINJA connects the co-repressor TOPLESS to jasmonate signalling. Nature **464**: 788–791.

Pauwels, L. and Goossens, A. (2011). The JAZ Proteins: A Crucial Interface in the Jasmonate Signaling Cascade. Plant Cell **23**: 3089–3100.

Pauwels, L., Morreel, K., Witte, E. De, Lammertyn, F., Montagu, M. Van, Boerjan, W., Inze, D., and Goossens, A. (2008). Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured Arabidopsis cells. Proc. Natl. Acad. Sci. U. S. A. **105**: 1380–1385.

Peer, W.A., Cheng, Y., and Murphy, A.S. (2013). Evidence of oxidative attenuation of auxin signalling. J. Exp. Bot. **64**: 2629–2639.

Pellegrin, C., Daguerre, Y., Ruytnix, J., Guinet, F., Kemppainen, M., Frei dit Frey, N., Puech-Pagès, V., Hecker, A., Pardo, A.J., Martin, F., and Veneault-Fourrey, C. (2019). Laccaria bicolor MiSSP8 is a small-secreted protein decisive for the establishment of the ectomycorrhizal symbiosis. Env. Microbiol. doi:10.1111/1462-2920.14727.

Pena, R., Simon, J., Rennenberg, H., and Polle, A. (2013). Ectomycorrhiza affect architecture and nitrogen partitioning of beech (Fagus sylvatica L.) seedlings under shade and drought. Environ. Exp. Bot.

Penninckx, I.A.M.A., Thomma, B.P.H.J., Buchala, A., Metraux, J.-P., and Broekaert, W.F. (1998). Concomitant Activation of Jasmonate and Ethylene Response Pathways Is Required for Induction of a Plant Defensin Gene in Arabidopsis. Plant Cell **10**: 2103–2113.

Pereira, M. de F. et al. (2018). Secretome analysis from the ectomycorrhizal ascomycete Cenococcum geophilum. Front. Microbiol. **9**: 141.

Peter, M. et al. (2016). Ectomycorrhizal ecology is imprinted in the genome of the dominant symbiotic fungus Cenococcum geophilum. Nat. Commun. **7**: 12662.

Petersen, M. et al. (2000). Arabidopsis MAP kinase 4 negatively regulates systemic acquired resistance. Cell **103**: 1111–1120.

Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C.M. (2012). Hormonal Modulation of Plant Immunity. Annu. Rev. Cell Dev. Biol. **28**: 489–521.

Pieterse, C.M.J., Leon-Reyes, A., Van Der Ent, S., and Van Wees, S.C.M. (2009). Networking by small-molecule hormones in plant immunity. Nat. Chem. Biol. **5**: 308–316.

Pieterse, C.M.J. and Van Loon, L.C. (2004). NPR1: The spider in the web of induced resistance signaling pathways. Curr. Opin. Plant Biol. **7**: 456–464.

Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M., and Bakker, P.A.H.M. (2014). Induced Systemic Resistance by Beneficial Microbes. Annu. Rev. Phytopathol. **52**: 347–375.

Plassard, C. and Dell, B. (2010). Phosphorus nutrition of mycorrhizal trees. Tree Physiol.

Plett, J.M. et al. (2017). Populus trichocarpa encodes small, effector-like secreted proteins that are highly induced during mutualistic symbiosis. Sci. Rep.

Plett, J.M., Daguerre, Y., Wittulsky, S., Vayssieres, A., Deveau, A., Melton, S.J., Kohler, A., Morrell-Falvey, J.L., Brun, A., Veneault-Fourrey, C., and Martin, F. (2014a). Effector MiSSP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes. Proc. Natl. Acad. Sci. **111**: 8299–8304.

Plett, J.M., Gibon, J., Kohler, A., Duffy, K., Hoegger, P.J., Velagapudi, R., Han, J., Kües, U., Grigoriev, I. V., and Martin, F. (2012). Phylogenetic, genomic organization and expression analysis of hydrophobin genes in the ectomycorrhizal basidiomycete Laccaria bicolor. Fungal Genet. Biol.

Plett, J.M., Kemppainen, M., Kale, S.D., Kohler, A., Legué, V., Brun, A., Tyler, B.M., Pardo, A.G., and Martin, F. (2011). A secreted effector protein of Laccaria bicolor is required for symbiosis development. Curr. Biol. **21**: 1197–1203.

Plett, J.M., Khachane, A., Ouassou, M., Kohler, A., and Martin, F. (2014b). Ethylene and jasmonic acid act as negative modulators during mutualistic symbiosis between Laccaria bicolor and Populus roots. New Phytol. **202**: 270–286.

Plett, J.M., Tisserant, E., Brun, A., Morin, E., Grigoriev, I. V, Kuo, A., Martin, F., and Kohler, A. (2015). The Mutualist Laccaria bicolor Expresses a Core Gene Regulon During the Colonization of Diverse Host Plants and a Variable Regulon to Counteract Host-Specific Defenses. Mol. Plant Microbe Interact. **28**: 261–273.

de Poel, B. Van and Van Der Straeten, D. (2014). 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: More than just the precursor of ethylene! Front. Plant Sci. **5**: 640.

Porco, S. et al. (2016). Dioxygenase-encoding AtDAO1 gene controls IAA oxidation and homeostasis in arabidopsis. Proc. Natl. Acad. Sci. U. S. A. **113**: 11016–11021.

Potuschak, T., Lechner, E., Parmentier, Y., Yanagisawa, S., Grava, S., Koncz, C., and Genschik, P. (2003). EIN3-Dependent Regulation of Plant Ethylene Hormone Signaling by Two Arabidopsis F Box Proteins: EBF1 and EBF2. Cell **115**: 679–689.

Pré, M., Atallah, M., Champion, A., De Vos, M., Pieterse, C.M.J., and Memelink, J. (2008). The AP2/ERF Domain Transcription Factor ORA59 Integrates Jasmonic Acid and Ethylene Signals in Plant Defense. Plant Physiol. **147**: 1347–1357.

Qi, T., Huang, H., Wu, D., Yan, J., Qi, Y., Song, S., and Xie, D. (2014). Arabidopsis DELLA and JAZ proteins bind the WD-Repeat/ bHLH/MYB complex to modulate gibberellin and jasmonate signaling synergy. Plant Cell **26**: 1118–1133.

Qi, T., Song, S., Ren, Q., Wu, D., Huang, H., Chen, Y., Fan, M., Peng, W., Ren, C., and Xiea, D. (2011). The jasmonate-ZIM-domain proteins interact with the WD-repeat/bHLH/MYB complexes to regulate jasmonate-mediated anthocyanin accumulation and trichome initiation in Arabidopsis thaliana. Plant Cell **23**: 1795–1814.

Qi, T., Wang, J., Huang, H., Song, S., and Xie, D. (2015). Regulation of jasmonate-mediated stamen development and seed production by a bHLH-MYB complex in Arabidopsis. Plant Cell **27**: 1620–1633.

Rahman, A., Hosokawa, S., Oono, Y., Amakawa, T., Goto, N., and Tsurumi, S. (2002). Auxin and ethylene response interactions during Arabidopsis root hair development dissected by auxin influx modulators. Plant Physiol. **130**: 1908–1917.

Reddy, S.M., Hitchin, S., Melayah, D., Pandey, A.K., Raffier, C., Henderson, J., Marmeisse, R., and Gay, G. (2006). The auxin-inducible GH3 homologue Pp-GH3.16 is downregulated in Pinus pinaster root systems on ectomycorrhizal symbiosis establishment. New Phytol. **170**: 391–400.

Redecker, D., Kodner, R., and Graham, L.E. (2000). Glomalean fungi from the Ordovician. Science (80-.). **289**: 1920–1921.

Reid, D.E., Ferguson, B.J., Hayashi, S., Lin, Y.-H., and Gresshoff, P.M. (2011). Molecular mechanisms controlling legume autoregulation of nodulation. Ann. Bot. **108**: 789–795.

Rekhter, D., Ding, Y., Lüdke, D., Feussner, K., and Wiermer, M. (2019). From isochorismate to salicylate : a new reaction mechanism for salicylic acid biosynthesis. bioRxiv: 600692.

Ren, C.M., Zhu, Q., Gao, B. Da, Ke, S.Y., Yu, W.C., Xie, D.X., and Peng, W. (2008). Transcription factor WRKY70 displays important but no indispensable roles in jasmonate and salicylic acid signaling. J. Integr. Plant Biol. **50**: 630–637.

Růžička, K., Ljung, K., Vanneste, S., Podhorská, R., Beeckman, T., Friml, J., and Benková, E. (2007). Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. Plant Cell **19**: 2197–2212.

Sánchez-Rodríguez, C., Rubio-Somoza, I., Sibout, R., and Persson, S. (2010). Phytohormones and the cell wall in Arabidopsis during seedling growth. Trends Plant Sci. **15**: 291–301.

Sasaki-Sekimoto, Y., Jikumaru, Y., Obayashi, T., Saito, H., Masuda, S., Kamiya, Y., Ohta, H., and Shirasu, K. (2013). Basic helix-loop-helix transcription factors JASMONATE-ASSOCIATED MYC2-LIKE1 (JAM1), JAM2, and JAM3 are negative regulators of jasmonate responses in Arabidopsis. Plant Physiol. **163**: 291–304.

Schulten, R. and Schnitzer, M. (1998). The chemistry of soil organic nitrogen : a review. Biol. Fertil. Soils **26**: 1–15.

Schweizer, F., Fernández-Calvo, P., Zander, M., Diez-Diaz, M., Fonseca, S., Glauser, G., Lewsey, M.G., Ecker, J.R., Solano, R., and Reymond, P. (2013). Arabidopsis Basic Helix-Loop-Helix Transcription Factors MYC2, MYC3, and MYC4 Regulate Glucosinolate Biosynthesis, Insect Performance, and Feeding Behavior. Plant Cell **25**: 3117–3132.

Sebastiana, M., Vieira, B., Lino-Neto, T., Monteiro, F., Figueiredo, A., Sousa, L., Salomé Pais, M., Tavares, R., and Paulo, O.S. (2014). Oak Root Response to Ectomycorrhizal Symbiosis Establishment : RNA-Seq Derived Transcript Identification and Expression Profiling. PLoS One **9**: e98376.

Selle, A., Willmann, M., Grunze, N., Geßler, A., Weiß, M., and Nehls, U. (2005). The high-affinity poplar ammonium importer PttAMT1.2 and its role in ectomycorrhizal symbiosis. New Phytol. **168**: 697–706.

Sels, J., Mathys, J., De Coninck, B.M.A., Cammue, B.P.A., and De Bolle, M.F.C. (2008). Plant pathogenesis-related (PR) proteins: A focus on PR peptides. Plant Physiol. Biochem. **46**: 941–950.

Seyfferth, C., Wessels, B., Jokipii-Lukkari, S., Sundberg, B., Delhomme, N., Felten, J., and Tuominen, H. (2018). Ethylene-related gene expression networks in wood formation. Front. Plant Sci. **9**: 272.

Shah, J. and Zeier, J. (2013). Long-distance communication and signal amplification in systemic acquired resistance. Front. Plant Sci. **4**: 30.

Shames, S.R. and Finlay, B.B. (2012). Bacterial effector interplay: A new way to view effector function. Trends Microbiol. **20**: 214–219.

Shin, K., Lee, S., Song, W.Y., Lee, R.A., Lee, I., Ha, K., Koo, J.C., Park, S.K., Nam, H.G., Lee, Y., and Soh, M.S. (2015). Genetic identification of ACC-RESISTANT2 reveals involvement of lysine histidine TRANSPORTER1 in the uptake of 1-Aminocyclopropane-1-Carboxylic acid in arabidopsis thaliana. Plant Cell Physiol. **56**: 572–582.

Shyu, C., Figueroa, P., DePew, C.L., Cooke, T.F., Sheard, L.B., Moreno, J.E., Katsir, L., Zheng, N., Browse, J., and Howe, G.A. (2012). JAZ8 Lacks a Canonical Degron and Has an EAR Motif That Mediates Transcriptional Repression of Jasmonate Responses in Arabidopsis. Plant Cell **24**: 536–550.

Sillo, F., Fangel, J.U., Henrissat, B., Faccio, A., Bonfante, P., Martin, F., Willats, W.G.T., and Balestrini, R. (2016). Understanding plant cell-wall remodelling during the symbiotic interaction between Tuber melanosporum and Corylus avellana using a carbohydrate microarray. Planta **244**: 347–359.

Simard, S.W., Perry, D.A., Jones, M.D., Myrold, D.D., Durall, D.M., and Molina, R. (1997). Net transfer of carbon between ectomycorrhizal tree species in the field. Nature **388**: 579–582.

De Smet, I. et al. (2007). Auxin-dependent regulation of lateral root positioning in the basal meristem of Arabidopsis. Development **134**: 681–690.

Song, J.T., Koo, Y.J., Seo, H.S., Kim, M.C., Choi, Y. Do, and Kim, J.H. (2008). Overexpression of AtSGT1, an Arabidopsis salicylic acid glucosyltransferase, leads to increased susceptibility to Pseudomonas syringae. Phytochemistry **69**: 1128–1134.

Song, L., Yu, H., Dong, J., Che, X., Jiao, Y., and Liu, D. (2016). The Molecular Mechanism of Ethylene-Mediated Root Hair Development Induced by Phosphate Starvation. PLoS Genet. **12**: e1006194.

Song, S., Qi, T., Fan, M., Zhang, X., Gao, H., Huang, H., Wu, D., Guo, H., and Xie, D. (2013). The bHLH Subgroup IIId Factors Negatively Regulate Jasmonate-Mediated Plant Defense and Development. PLoS Genet. **9**: e1003653.

Song, S., Qi, T., Huang, H., Ren, Q., Wu, D., Chang, C., Peng, W., Liu, Y., Peng, J., and Xie, D. (2011). The jasmonate-ZIM domain proteins interact with the R2R3-MYB transcription factors MYB21 and MYB24 to affect jasmonate-regulated stamen development in Arabidopsis. Plant Cell **23**: 1000–1013.

Splivallo, R., Fischer, U., Göbel, C., Feussner, I., and Karlovsky, P. (2009). Truffles Regulate Plant Root Morphogenesis via the Production of Auxin and Ethylene. Plant Physiol. **150**: 2018–2029.

Spoel, S.H. and Loake, G.J. (2011). Redox-based protein modifications: The missing link in plant immune signalling. Curr. Opin. Plant Biol. **14**: 358–364.

Spoel, S.H., Mou, Z., Tada, Y., Spivey, N.W., Genschik, P., and Dong, X. (2009). Proteasome-mediated turnover of the transcription co-activator NPR1 plays dual roles in regulating plant immunity. Cell **137**: 860–872.

Spoel, S. H., et al. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell **15**: 760–770.

Staswick, P.E., Serban, B., Rowe, M., Tiryaki, I., Maldonado, M.T., Maldonado, M., and Suza, W. (2005). Characterization of an Arabidopsis enzyme family that conjugates amino acids to Indole-3-Acetic Acid. Plant Cell **17**: 616–627.

Staswick, P.E. and Tiryaki, I. (2004). The Oxylipin Signal Jasmonic Acid is Activated by an Enzyme that Conjugates it to Isoleucine in Arabidopsis. Plant Cell **16**: 2117–2127.

Steidinger, B.S. et al. (2019). Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. Nature.

Stergiopoulos, I. and de Wit, P.J.G.M. (2009). Fungal Effector Proteins. Annu. Rev. Phytopathol. **47**: 233–263.

Stitz, M., Gase, K., Baldwin, I.T., and Gaquerel, E. (2011). Ectopic Expression of AtJMT in Nicotiana attenuata : Creating a Metabolic Sink Has Tissue-Specific Consequences for the Jasmonate Metabolic Network and Silences Downstream Gene Expression. Plant Physiol. **157**: 341–354.

Stotz, H.U., Mueller, S., Zoeller, M., Mueller, M.J., and Berger, S. (2013). TGA transcription factors and jasmonate-independent COI1 signalling regulate specific plant responses to reactive oxylipins. J. Exp. Bot. **64**: 963–975.

Strullu-Derrien, C., Kenrick, P., Pressel, S., Duckett, J.G., Rioult, J.P., and Strullu, D.G. (2014). Fungal associations in Horneophyton ligneri from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: Novel insights into ancestral plant-fungus symbioses. New Phytol. **203**: 964–979.

Stumpe, M., Carsjens, J.-G., Stenzel, I., Göbel, C., Lang, I., Pawlowski, K., Hause, B., and Feussner, I. (2005). Lipid metabolism in arbuscular mycorrhizal roots of Medicago truncatula. Phytochemistry **66**: 781–791.

Sun, J., Chen, Q., Qi, L., Jiang, H., Li, S., Xu, Y., Liu, F., Zhou, W., Pan, J., Li, X., Palme, K., and Li, C. (2011). Jasmonate modulates endocytosis and plasma membrane accumulation of the Arabidopsis PIN2 protein. New Phytol. **191**: 360–375.

Sun, T. and Gubler, F. (2004). Molecular Mechanism of Gibberellin Signaling in Plants. Annu. Rev. Plant Biol. **55**: 197–223.

Sundell, D., Mannapperuma, C., Netotea, S., Delhomme, N., Lin, Y.C., Sjödin, A., Van de Peer, Y., Jansson, S., Hvidsten, T.R., and Street, N.R. (2015). The Plant Genome Integrative Explorer Resource: PlantGenIE.org. New Phytol. **208**: 1149–1156.

Swarup, R. and Péret, B. (2012). AUX/LAX family of auxin influx carriers-An overview. Front. Plant Sci. **3**: 225.

Sylvia, D.M. and Sinclair, W.A. (1983). Phenolic Compounds and Resistance to Fungal Pathogens Induced in Primary Roots of Douglas-Fir Seedlings by the Ectomycorrhizal Fungus Laccaria laccata. Phytopathology **73**: 390.

Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zuo, J., and Dong, X. (2008). Plant immunity required conformational charges of NPR1 via S-nitrosylation and thioredoxins. Science (80-.). **321**: 5891.

Tagu, B.Y.D. and Martin, F. (1996). Molecular analysis of cell wall proteins expressed during the early steps of ectomycorrhiza development. New Phytol. **133**: 73–85.

Tagu, D., Bellis, R. De, Balestrini, R., Vries, O.M.H. De, Piccoli, G., Stocchi, V., Bonfante, P., and Martin, F. (2001). Immunolocalization of hydrophobin HYDPt-1 from the ectomycorrhizal basidiomycete Pisolithus tinctorius during colonization of Eucalyptus globulus roots. New Phytol. **149**: 127–135.

Tamaoki, D., Seo, S., Yamada, S., Kano, A., Miyamoto, A., Shishido, H., Miyoshi, S., Taniguchi, S., Akimitsu, K., and Gomi, K. (2013). Jasmonic acid and salicylic acid activate a common defense system in rice. Plant Signal. Behav. **8**: e24260.

Tedersoo, L. et al. (2012). Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Mol. Ecol.

Tedersoo, L., May, T.W., and Smith, M.E. (2010). Ectomycorrhizal lifestyle in fungi: Global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza.

Thaler, J.S., Humphrey, P.T., and Whiteman, N.K. (2012). Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci. **17**: 260–270.

Thatcher, L.F., Cevik, V., Grant, M., Zhai, B., Jones, J.D.G., Manners, J.M., and Kazan, K. (2016). Characterization of a JAZ7 activation-tagged Arabidopsis mutant with increased susceptibility to the fungal pathogen Fusarium oxysporum. J. Exp. Bot. **67**: 2367–2386.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A., and Browse, J. (2007). JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signalling. Nature **448**: 661–665.

Thireault, C., Shyu, C., Yoshida, Y., St. Aubin, B., Campos, M.L., and Howe, G.A. (2015). Repression of jasmonate signaling by a non-TIFY JAZ protein in Arabidopsis. Plant J. **82**: 669–679.

Tian, M., Caroline, C.V.D., Liu, P.P., Friso, G., Van Wijk, K.J., and Klessig, D.F. (2012). The combined use of photoaffinity labeling and surface plasmon resonance-based technology identifies multiple salicylic acid-binding proteins. Plant J. **72**: 1027–1038.

Tibbett, M. and Sanders, F.E. (2002). Ectomycorrhizal symbiosis can enhance plant nutrition through improved access to discrete organic nutrient patches of high resource quality. Ann. Bot.

Tisserant, E. et al. (2013). Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. Proc. Natl. Acad. Sci. **110**: 20117 LP – 20122.

Tisserant, E. et al. (2012). The transcriptome of the arbuscular mycorrhizal fungus Glomus intraradices (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. New Phytol. **193**: 755–769.

Torres-Aquino, M., Becquer, A., Le Guernevé, C., Louche, J., Amenc, L.K., Staunton, S., Quiquampoix, H., and Plassard, C. (2017). The host plant Pinus pinaster exerts specific effects on phosphate efflux and polyphosphate metabolism of the ectomycorrhizal fungus Hebeloma cylindrosporum: a radiotracer, cytological staining and 31P NMR spectroscopy study. Plant Cell Environ. **40**: 190–202.

de Torres Zabala, M., Zhai, B., Jayaraman, S., Eleftheriadou, G., Winsbury, R., Yang, R., Truman, W., Tang, S., Smirnoff, N., and Grant, M. (2016). Novel JAZ co-operativity and unexpected JA dynamics underpin Arabidopsis defence responses to Pseudomonas syringae infection. New Phytol. **209**: 1120–1134.

Tranvan, H., Habricot, Y., Jeannette, E., Gay, G., and Sotta, B. (2000). Dynamics of symbiotic establishment between an IAA-overproducing mutant of the ectomycorrhizal fungus Hebeloma cylindrosporum and Pinus pinaster. Tree Physiol. **20**: 123–129.

Trupiano, D., Yordanov, Y., Regan, S., Meilan, R., Tschaplinski, T., Scippa, G.S., and Busov, V. (2013). Identification, characterization of an AP2/ERF transcription factor that promotes adventitious, lateral root formation in Populus. Planta **238**: 271–282.

Tschaplinski, T.J., Plett, J.M., Engle, N.L., Deveau, A., Cushman, K.C., Martin, M.Z., Doktycz, M.J., Tuskan, G.A., Brun, A., Kohler, A., and Martin, F. (2014). Populus trichocarpa and Populus deltoides Exhibit Different Metabolomic Responses to Colonization by the Symbiotic Fungus Laccaria bicolor. Mol. Plant Microbe Interact. **27**: 546–556.

Tuskan, G.A. et al. (2006). The Genome of Black Cottonwood , Populus trichocarpa (Torr. & Gray). Science (80-.). **313**: 1596–1605.

Ullah, C., Tsai, C.J., Unsicker, S.B., Xue, L., Reichelt, M., Gershenzon, J., and Hammerbacher, A. (2019). Salicylic acid activates poplar defense against the biotrophic rust fungus Melampsora larici-populina via increased biosynthesis of catechin and proanthocyanidins. New Phytol. **221**: 960–975.

Vaid, N., Macovei, A., and Tuteja, N. (2013). Knights in action: Lectin receptor-like kinases in plant development and stress responses. Mol. Plant **6**: 1405–1418.

Vayssières, A., Pěnčík, A., Felten, J., Kohler, A., Ljung, K., Martin, F., and Legué, V. (2015). Development of the Poplar -*Laccaria bicolor* Ectomycorrhiza Modifies Root Auxin Metabolism, Signaling, and Response. Plant Physiol. **169**: 890–902.

Veneault-Fourrey, C., Commun, C., Kohler, A., Morin, E., Balestrini, R., Plett, J., Danchin, E., Coutinho, P., Wiebenga, A., de Vries, R.P., Henrissat, B., and Martin, F. (2014). Genomic and transcriptomic analysis of Laccaria bicolor CAZome reveals insights into polysaccharides remodelling during symbiosis establishment. Fungal Genet. Biol. **72**: 168–181.

Verhage, A., Vlaadingerbroek, I., Raaymakers, C., Van Dam, N.M., Dicke, M., Van Wees, S.C.M., and Pieterse, C.M.J. (2011). Rewiring of the jasmonate signaling pathway in Arabidopsis during insect herbivory. Front. Plant Sci. **2**: 47.

van Verk, M.C., Bol, J.F., and Linthorst, H.J.M. (2011). WRKY Transcription Factors Involved in Activation of SA Biosynthesis Genes. BMC Plant Biol. **11**: 89.

Vik, U., Logares, R., Blaalid, R., Halvorsen, R., Carlsen, T., Bakke, I., Kolstø, A.B., Økstad, O.A., and Kauserud, H. (2013). Different bacterial communities in ectomycorrhizae and surrounding soil. Sci. Rep.

De Vleesschauwer, D., Gheysen, G., and Höfte, M. (2013). Hormone defense networking in rice: Tales from a different world. Trends Plant Sci. **18**: 555–565.

Vlot, A.C., Dempsey, D.A., and Klessig, D.F. (2009). Salicylic Acid, a Multifaceted Hormone to Combat Disease. Annu. Rev. Phytopathol. **47**: 177–206.

Wang, D., Amornsiripanitch, N., and Dong, X. (2006). A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. PLoS Pathog. **2**: e123.

Wang, D., Weaver, N.D., Kesarwani, M., and Dong, X. (2005). Induction of protein secretory pathway is required for systemic acquired resistance. Science (80-.). **308**: 1036–1040.

Wang, K.L.C., Li, H., and Ecker, J.R. (2002). Ethylene biosynthesis and signaling networks. Plant Cell **Supplement**: S131–S152.

Wang, L., Kim, J., and Somers, D.E. (2013). Transcriptional corepressor TOPLESS complexes with pseudoresponse regulator proteins and histone deacetylases to regulate circadian transcription. Proc. Natl. Acad. Sci. **110**: 761–766.

Wang, L., Tsuda, K., Truman, W., Sato, M., Nguyen, L. V, Katagiri, F., and Glazebrook, J. (2011). CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling. Plant J. **67**: 1029–1041.

Wang, T., Tian, Z., Bengtson, P., Tunlid, A., and Persson, P. (2017). Mineral surface-reactive metabolites secreted during fungal decomposition contribute to the formation of soil organic matter. Environ. Microbiol.

Wang, Y. and Jiao, Y. (2018). Auxin and above-ground meristems. J. Exp. Bot. **69**: 147–154.

Wasternack, C. and Feussner, I. (2018). The Oxylipin Pathways: Biochemistry and Function. Annu. Rev. Plant Biol. **69**: 363–386.

Wasternack, C. and Hause, B. (2013). Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Ann. Bot. **111**: 1021–1058.

Wasternack, C. and Strnad, M. (2019). Jasmonates are signals in the biosynthesis of secondary metabolites — Pathways, transcription factors and applied aspects — A brief review. N. Biotechnol. **48**: 1–11.

Van Wees, S.C.M., De Swart, E.A.M., Van Pelt, J.A., Van Loon, L.C., and Pieterse, C.M.J. (2000). Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. **97**: 8711–8716.

Weigel, R.R., Pfitzner, U.M., and Gatz, C. (2005). Interaction of NIMIN1 with NPR1 modulates PR gene expression in Arabidopsis. Plant Cell **17**: 1279–1291.

Wiermer, M., Feys, B.J., and Parker, J.E. (2005). Plant immunity: The EDS1 regulatory node. Curr. Opin. Plant Biol. **8**: 383–389.

Wild, M., Davière, J., Cheminant, S., Regnault, T., Baumberger, N., Heintz, D., Baltz, R., Genschik, P., and Achard, P. (2012). The Arabidopsis DELLA RGA - LIKE3 Is a Direct Target of MYC2 and Modulates Jasmonate Signaling Responses. Plant Cell **24**: 3307–3319.

Wildermuth, M.C. (2006). Variations on a theme: synthesis and modification of plant benzoic acids. Curr. Opin. Plant Biol. **9**: 288–296.

Wildermuth, M.C., Dewdney, J., Wu, G., and Ausubel, F.M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature **417**: 562–571.

Willmann, A., Thomfohrde, S., Haensch, R., and Nehls, U. (2014). The poplar NRT2 gene family of high affinity nitrate importers: Impact of nitrogen nutrition and ectomycorrhiza formation. Environ. Exp. Bot. **108**: 79–88.

Wu, K., Zhang, L., Zhou, C., Yu, C.W., and Chaikam, V. (2008). HDA6 is required for jasmonate response, senescence and flowering in Arabidopsis. J. Exp. Bot. **59**: 225–234.

Wu, Y., Zhang, D., Chu, J.Y., Boyle, P., Wang, Y., Brindle, I.D., De Luca, V., and Després, C. (2012). The Arabidopsis NPR1 Protein Is a Receptor for the Plant Defense Hormone Salicylic Acid. Cell Rep. **1**: 639–647.

Xie, D.-X., Feys, B.F., James, S., Nieto-Rostro, M., and Turner, J.G. (1998). COI1: An Arabidopsis Gene Required for Jasmonate-Regulated Defense and Fertility. Science (80-.). **280**: 1091–1094.

Xu, H., Kemppainen, M., El Kayal, W., Lee, S.H., Pardo, A.G., Cooke, J.E.K., and Zwiazek, J.J. (2015). Overexpression of Laccaria bicolor aquaporin JQ585595 alters root water transport properties in ectomycorrhizal white spruce (Picea glauca) seedlings. New Phytol. **205**: 757–770.

Xu, L., Liu, F., Lechner, E., Genschik, P., Crosby, W.L., Ma, H., Peng, W., Huang, D., and Xie, D. (2002). The SCF COI1 Ubiquitin-Ligase Complexes Are Required for Jasmonate Response in Arabidopsis. Plant Cell **14**: 1919–1935.

Xue, L., Almario, J., Fabiańska, I., Saridis, G., and Bucher, M. (2019). Dysfunction in the arbuscular mycorrhizal symbiosis has consistent but small effects on the establishment of the fungal microbiota in Lotus japonicus . New Phytol.

Xue, L.J., Alabady, M.S., Mohebbi, M., and Tsai, C.J. (2015). Exploiting genome variation to improve next-generation sequencing data analysis and genome editing efficiency in Populus tremula × alba 717-1B4. Tree Genet. Genomes **11**: 82.

Xue, L.J., Guo, W., Yuan, Y., Anino, E.O., Nyamdari, B., Wilson, M.C., Frost, C.J., Chen, H.Y., Babst, B.A., Harding, S.A., and Tsai, C.J. (2013). Constitutively elevated salicylic acid levels alter photosynthesis and oxidative state but not growth in transgenic Populus. Plant Cell **25**: 2714–2730.

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. Annu. Rev. Plant Biol. **59**: 225–251.

Yan, C. and Xie, D. (2015). Jasmonate in plant defence: Sentinel or double agent? Plant Biotechnol. J. **13**: 1233–1240.

Yan, J., Li, S., Gu, M., Yao, R., Li, Y., Chen, J., Yang, M., Tong, J., Xiao, L., Nan, F., and Xie, D. (2016). Endogenous Bioactive Jasmonate Is Composed of a Set of (+)-7- iso- JA-Amino Acid Conjugates. Plant Physiol. **172**: 2154–2164.

Yan, Y., Stolz, S., Chételat, A., Reymond, P., Pagni, M., Dubugnon, L., and Farmer, E.E. (2007). A Downstream Mediator in the Growth Repression Limb of the Jasmonate Pathway. Plant Cell **19**: 2470–2483.

Yang, D., Yao, J., Mei, C., Tong, X., Zeng, L., Li, Q., and Xiao, L. (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc. Natl. Acad. Sci. U. S. A. **109**: E1192–E1200.

Yevtushenko, D.P. and Misra, S. (2010). Efficient Agrobacterium-mediated transformation of commercial hybrid poplar Populus nigra L. × P. maximowiczii A. Henry. Plant Cell Rep. **29**: 211–221.

Yuan, Y., Chung, J. Der, Fu, X., Johnson, V.E., Ranjan, P., Booth, S.L., Harding, S.A., and Tsai, C.J. (2009). Alternative splicing and gene duplication differentially shaped the regulation of isochorismate synthase in Populus and Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. **106**: 22020–22025.

Zander, M., Chen, S., Imkampe, J., Thurow, C., and Gatz, C. (2012). Repression of the Arabidopsis thaliana jasmonic acid/ethylene-induced defense pathway by TGA-interacting glutaredoxins depends on their c-terminal ALWL motif. Mol. Plant **5**: 831–840.

Zander, M., Thurow, C., and Gatz, C. (2014). TGA transcription factors activate the salicylic acid-suppressible branch of the ethylene-induced defense program by regulating ORA59 expression. Plant Physiol. **165**: 1671–1683.

Zawaski, C. and Busov, V.B. (2014). Roles of gibberellin catabolism and signaling in growth and physiological response to drought and short-day photoperiods in Populus trees. PLoS One **9**: e86217.

Zhai, Q., Zhang, X., Wu, F., Feng, H., Deng, L., Xu, L., Zhang, M., Wang, Q., and Li, C. (2015). Transcriptional mechanism of jasmonate receptor COI1-mediated delay of flowering time in arabidopsis. Plant Cell **27**: 2814–2828.

Zhang, F. et al. (2015). Structural basis of JAZ repression of MYC transcription factors in jasmonate signalling. Nature **525**: 269–273.

Zhang, F., et al. (2017). Structural insights into alternative splicing-mediated desensitization of jasmonate signaling. PNAS, **114**(7): 1720–1725.

Zhang, F. et al. (2018). The ectomycorrhizal basidiomycete Laccaria bicolor releases a secreted β-1,4 endoglucanase that plays a key role in symbiosis development. New Phytol. **220**: 1309–1321.

Zhang, J., Lin, J.E., Harris, C., Pereira, F.C.M., Wu, F., Blakeslee, J.J., and Peer, W.A. (2016). DAO1 catalyzes temporal and tissue-specific oxidative inactivation of auxin in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. **113**: 11010–11015.

Zhang, Y., Xu, S., Ding, P., Wang, D., Cheng, Y.T., He, J., Gao, M., Xu, F., Li, Y., Zhu, Z., Li, X., and Zhang, Y. (2010). Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proc. Natl. Acad. Sci. U. S. A. **107**: 18220–18225.

Zhang, Y., Zhang, Y., Tessaro, M.J., Tessaro, M.J., Lassner, M., Lassner, M., Li, X., and Li, X. (2003). Knockout Analysis of Arabidopsis Transcription Factors TGA2, TGA5, and TGA6 Reveals Their Redundant and Essential Roles in Systemic Acquired Resistance. Plant Cell **15**: 2647–2653.

Zhou, W., Lozano-Torres, J.L., Blilou, I., Zhang, X., Zhai, Q., Smant, G., Li, C., and Scheres, B. (2019). A Jasmonate Signaling Network Activates Root Stem Cells and Promotes Regeneration. Cell **177**: 1–15.

Zhu, Z. et al. (2011). Derepression of ethylene-stabilized transcription factors (EIN3/EIL1) mediates jasmonate and ethylene signaling synergy in Arabidopsis. Proc. Natl. Acad. Sci. **108**: 12539–12544.

6. Appendix: jasmonate in beneficial microbe-root interactions

In the following appendix we review the role of jasmonate in the establishment and development of different symbiotic associations between roots and microbes. This text has been accepted as a chapter in the book “Jasmonate in Plant Biology”, currently in press by the editor Springer.

The role of jasmonate in beneficial microbe-root interactions

Veronica Basso and Claire Veneault-Fourrey

UMR 1136 INRA-Université de Lorraine ‘Interactions Arbres/Microorganismes’, Laboratoire d’Excellence ARBRE, Centre INRA-Lorraine, 54280 Champenoux, France

Keywords: jasmonate, mutualism, root-microbe interactions, phytohormone, mycorrhiza, root nodule symbiosis, autoregulation, PGPR, PGPF, systemic induced resistance, mycorrhiza-induced resistance.

Running head: Jasmonate in beneficial microbe-root interactions

6.1. Abstract

The phytohormone jasmonate (JA) modulates various defense and developmental responses of plants, and is implied in integration of multiple environmental signals. Given its centrality in regulating plant physiology according to external stimuli, JA influences the establishment of interactions between plant roots and beneficial bacteria or fungi. In many cases, moderate JA signaling promotes the onset of mutualism, while massive JA signaling inhibits it. The output also depends on the compatibility between microbe and host plant and on nutritional or environmental cues. Also, JA biosynthesis and perception participate to the systemic regulation of mutualistic interactions and to microbe-induced resistance to biotic and abiotic stress. Here we review current knowledge of the role of JA biosynthesis, signaling and responses during mutualistic root-microbe interactions.

6.2. Introduction

Optimal plant nutrition and resistance to biotic and abiotic stresses result from an interplay between direct plant adaptation and the effects of beneficial plant-interacting microbes. Since root systems absorb water and mineral nutrients, the root-associated microbiome is of particular interest for the fitness of agricultural and silvicultural crops (1, 2). The root microbiome can improve plant nutrient uptake (3), resistance to abiotic stress like drought (4) and salinity (5) and to belowground and aboveground diseases and pests (6, 7, 8, 9).

Understanding the molecular mechanisms of beneficial plant-microbe interaction establishment is pivotal in order to engineer stress-resistant crops (1). In this respect, simplified models including one host and one microbial species have long been used to investigate beneficial interactions with high ecological relevance, like the arbuscular mycorrhizal (AM) symbiosis (10) and the root nodule symbiosis (RNS; 11). Phytohormones play a prominent role in modulating root-microbe interactions as well as in determining the development of symbiotic structures (11, 12, 13). They are also major regulators of the growth-defense balance. Plant defense is mainly orchestrated by a molecular crosstalk between jasmonate (JA), salicylate (SA) and ethylene (ET) signaling (14). SA and JA signaling mainly act antagonistically, inhibiting each other (14), and some pathogens hijack plant defense by manipulating the JA-SA crosstalk (15, 16). Since some symbiotic microbes have common ancestors with pathogens or saprotrophs (17, 13), it is interesting to investigate if tuning of host JA-mediated defense allows colonization by beneficial microbes while blocking excess invasion (18). Also, jasmonic acid and its derivatives, collectively known as jasmonates (JAs), are emerging as regulatory hubs of the plant development-to-defense trade-off (19, 20); therefore they likely integrate developmental and environmental signals in order to modulate interactions whose impact on plant fitness depends on genetic or environmental factors (21). Conversely, beneficial microbe-induced systemic resistance (ISR) to pathogens often implies enhanced sensitivity to JA/ET signaling (22), indicating multifaceted roles of JA in beneficial interactions. Indeed, JA is involved in both establishment and functional consequences of symbiotic interactions, even if contradictory findings have been reported. This review summarizes current knowledge on the diversified roles of JA in microbial recruitment, symbiosis development and physiological consequences on host plants throughout different types of root-microbe beneficial associations.

6.3. JA in arbuscular mycorrhiza development

Arbuscular mycorrhiza are symbiotic associations taking place between the roots of ~80% of land plants and some classes of fungi of the *Glomeromycota* order. Arbuscular mycorrhizal fungi (AMF) can increase plant uptake of water, nitrogen (N), phosphorus (P) and other mineral elements, in exchange for sugar and lipids (23), improving plant nutrition and health (24). Root infection by AMF begins with the formation of a hyphopodium at the root surface, followed by constitution of a pre-penetration apparatus (PPA) and hyphal intracellular penetration through epidermal and cortical cell layers. Eventually, AMF spread longitudinally through the root cortex and generate ramified structure called arbuscules that are sites of nutrient exchanges between the symbiotic partners (10).

6.3.1. JA biosynthesis and signaling during AM development

JA biosynthesis, secretion and associated responses modulate several stages of AM symbiosis development, with different outputs depending on phylogenetic, genetic and environmental factors.

Firstly, secretion of JA or of JA-induced compounds may induce fungal hyphae elongation for a quicker contact to the host root. In fact, root exudates of *Lotus japonicus* grown in high Red/Far Red (R/FR) light conditions, mimicking optimal light, as well as 0.5 µM exogenous JA, stimulated hyphal elongation of *Rhizophagus irregularis* (25). However, since R/FR light signaling represses JA signaling as part of the shade-avoidance syndrome (SAS; 26), it remains to be understood if light cues influence JA secretion in the early phase of AM symbiosis and/or if they participate to the systemic regulation of mycorrhization at the mature stage.

No JA biosynthesis was reported during early stages of AM development, i.e hyphopodium or prepenetration apparatus (PPA) formation. However, *Gigaspora margarita* inoculation of *Medicago truncatula* roots induced the expression of some JA-responsive genes during PPA formation (27). JA signaling at this stage might counteract the effect of transient SA accumulation upon first contact between the symbiotic partners (28, 29, 30).

On the other hand, JA biosynthesis and signaling pathway are active in late stages of AM colonization in several plant species (31, 32, 33, 34, 35). JA accumulation takes place in arbuscule-containing cells of barley (31) and *M. truncatula* (34). Local JA production in the roots has a positive effect on mycorrhization, as demonstrated by reduced colonization and/or arbuscule frequency in mutant *M. truncatula* and tomato roots impaired in JA biosynthesis (34, 35, 36). Conversely, constitutive high levels of JA resulted in enhanced arbuscule frequency in tomato (36) but slightly reduced it in *M. truncatula* (37), suggesting the involvement of regulatory feedback mechanisms in some plant species. Interestingly, the

positive effect of JA signaling in AM symbiosis is independent of the canonical JA-Ile receptor COI1 in tomato. In fact, *jai1-1* mutants, bereft of COI1, are more mycorrhized than wild type tomato plants (35, 38). Therefore, JA signaling might negatively influence arbuscule development through the COI1-dependent signaling pathway and positively influence it through an unknown, independent pathway (30). Also, it is still controversial whether JA biosynthesis is needed locally or systemically for its promoting action on AM symbiosis. In fact, moderate, systemic activation of JA signaling may also promote AM, since low dosage or sporadic application of JA/MeJA on leaves induced higher colonization and/or arbuscule frequency in garlic, tomato, cucumber and *M. truncatula* (36, 39, 40, 41).

Overall, the role of JA in AMF colonization depends on plant and fungal genotypes and on environmental conditions. In fact, JA biosynthesis and/or signaling are not necessary for the establishment of the semi-parasitical interaction between *Nicotiana attenuata* and *R. irregularis* (42), nor for AM symbiosis in rice (43), indicating the dispensability of JA-mediated regulation for some fungus-host plant pairs. Moreover, JAs differentially accumulated in soybean, tomato and maize inoculated with either *R. irregularis* or *Funneliformis mosseae*. Indeed, colonization by the less successful fungus, *F. mosseae*, promoted oxylipin biosynthesis and signaling to a greater extent than by *R. irregularis* (44). This suggests that JA accumulation suppresses colonization by poorly compatible AMF, possibly through induced systemic defense. This hypothesis is supported by the observation that, in several plant species, massive or repeated JA/MeJA application to leaves or roots reduced AM colonization with concomitant induction of plant defenses and root growth arrest (38, 40, 43, 45). Interestingly, exogenous JA-triggered promotion or delay of AM symbiosis depend on the P_i status of cucumber plants (40). Taken together, these results suggest that sustained JA signaling restricts AM colonization according to the fungus-host compatibility and to environmental conditions, as part of a defense-to-development trade-off.

6.3.2. Possible functions of JA signaling in mature AM

Several possible functions of JA signaling in mature mycorrhiza are emerging. We propose three non-mutually-exclusive hypotheses supported by several lines of evidence.

First, JA signaling might help reshape cortical cells for arbuscule accommodation. JA can induce microtubule depolymerization (46) and massive cytoskeletal remodeling occurs during arbuscule development (47, 48). Moreover, the *M. truncatula β-TUBULIN* gene is upregulated both during AM symbiosis (49) and in response to JA treatment (37). Also, JAs might modulate cell wall composition and vacuole osmotic pressure, similarly to their action during tuber development (50).

Secondly, JA accumulation in arbuscule-containing cells possibly enhances the carbon sink strength of arbuscules in order to sustain symbiotic nutrient exchanges. This

hypothesis is supported by several pieces of evidence: (i) low JA concentrations sprayed on a single leaf privilege carbon partitioning towards roots in *Populus tremuloides*, *Populus nigra* (51) and tomato (52); (ii) the JA biosynthetic genes *AOS* and *AOC* are induced by glucose in tomato flowers and barley seedlings (53, 54); (iii) AMF inoculation induces carbon accumulation in tomato roots (55); (iv) JA-responsive genes are simultaneously activated in arbusculated cells and in phloem companion cells in mycorrhizal barley, suggesting their involvement in nutrient transport (31); (v) the expression of the cell wall invertase gene *Lin6* is induced by both AM inoculation (56) and JA treatment (57); (vi) the structure and proteome of plastids of arbusculated *M. truncatula* cells display a nutrient starvation signature: therefore JA biosynthesis might be induced to increase shoot-to-root carbon flux in consequence of amyloplastic stress (58). We propose that, if the main function of JA signaling in arbusculated cells was to enhance carbon sink strength, overexpressing genes involved in symbiotic sugar transport and conversion in a JA-deficient background would compensate the reduced arbuscule frequency phenotype.

Finally, JA signaling may inhibit excessive *in planta* fungal colonization. This hypothesis is supported by the fact that treatment with a high dose of JA/MeJA reduces or delays AM colonization (38, 43, 45) and that JA-insensitive tomato mutants are more mycorrhized than wild type plants (38). JA signaling can lead to enhanced production of defense flavonoids in arbuscule-containing cells (59). In addition, the 9-LOX biosynthetic pathway, producing a range of antimicrobial oxylipins (60) is activated in mycorrhizal tomato roots (61), partially depending on functional JA biosynthesis and signaling (35). Alternatively, AM fungi may induce flavonoids or oxylipin production to prime the host plant for defense against pathogenic microbes (see paragraph 6.7.2).

In conclusion, JA biosynthesis and signaling may serve different purposes across the various developmental stages of AM symbiosis. In many plant species, moderate local or systemic JA accumulation promotes arbuscule formation. However, phylogenetic, genetic and environmental factors strongly influence the output of activated JA signaling on the extent of AM symbiosis, up to the point that sustained JA response counteracts mycorrhizal colonization. JA accumulation in arbuscule-containing cells may mediate cell reshaping, symbiotic nutrient exchange or regulation of excessive fungal invasion. Systematic studies on the genetic elements controlling JA production, conversion, signaling and downstream gene regulation will be necessary to draw a clear model of the role of JAs in the development of AM symbiosis (Figure 1).

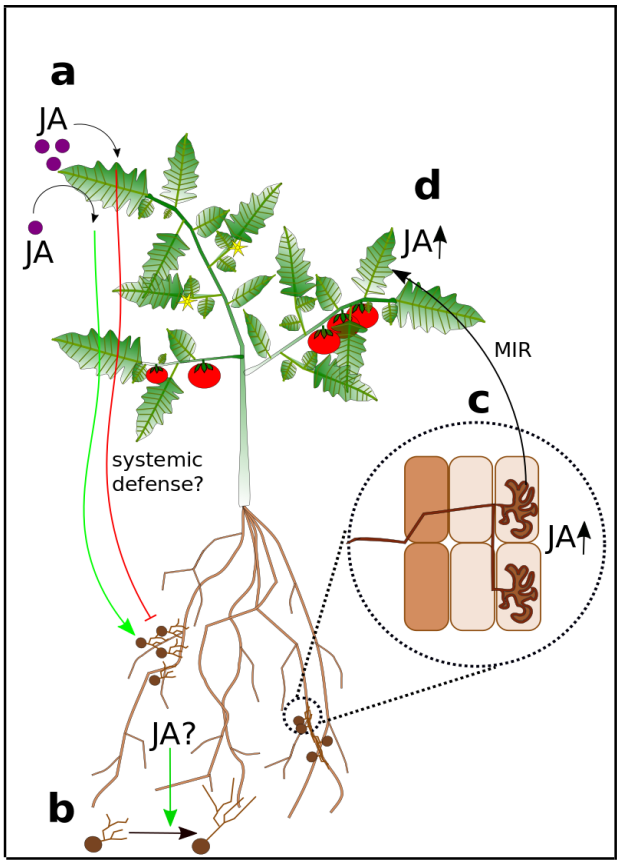


Figure 1: The role of JAs in development of AM symbiosis.

a) *High doses of exogenous JAs in leaves inhibit the establishment of AM symbiosis, possibly through activated systemic resistance; however, low doses promote it, through unknown mechanisms.* ***b)*** *JA may promote fungal hyphae elongation, thereby facilitating hyphae-root contact.* ***c)*** *The level of JA rises in arbusculated cells of model plant species, from tomato to* M. truncatula*. In the same species, JA biosynthesis or JA-mediated responses are important for the occurrence of arbuscules at high frequency. JA accumulation in arbuscule-containing cells may help (i) reshape the cell for arbuscule accommodation; (ii) induce a carbon sink status, reinforcing the flow of nutrients from shoot to arbuscules; (iii) suppress excessive fungal invasion.* ***d)*** *Some late-stage mycorrhizal plants increase their shoot JA level. This possibly mediates mycorrhiza-induced resistance (MIR) to abiotic and biotic stress.*

6.4. JA in root nodule symbiosis development

There are two types of root-nodule symbioses (RNS), the rhizobia-legume symbiosis and the actinorhizal symbiosis. Rhizobia-legume symbiosis is the mutually beneficial interaction between several species of the *Rhizobia* paraphyletic group of α- and β-proteobacteria and the roots of most legumes. The actinorhizal symbiosis results from the interaction between some members of the Cucurbitales, Fagales and Rosales orders and N-fixing actinorhizal bacteria of the *Frankia* genus. In both cases, the outcome of the interaction

is the generation of a novel organ, the N-fixing nodule, in which bacteria differentiate to convert atmospheric N into an organic form, available for the plant (62). Given the lack of evidence of any role of JA in the establishment of actinorhizal symbiosis, in the following paragraphs we will focus on JA-mediated modulation of the rhizobia-legume symbiosis.

6.4.1. JA biosynthesis and signaling during RNS

As in the case of AM symbiosis, there are reports of a positive (63), negative (64, 65, 66) and neutral (41) role of JA biosynthesis and signaling in RNS.

In legumes, JA regulates the early stages of bacterial colonization, i.e. root hair curling and infection thread (IT) formation, by influencing the plant-bacteria communication mechanism represented by bacterial Nod factor-triggered Ca^{2+}-spiking in root cortical cells (67). Exogenous JA treatment stimulated *Nod* genes expression and Nod factor production by isolated rhizobia (68, 69). Also, very moderate JA application (0.1 μM) to growth medium slightly increased IT and nodule number in *L. japonicus* (63). Therefore, secreted JA or JA-derived flavonoids may stimulate rhizobial expression of *Nod* genes and chemoattraction to the host root (70). However, increasing doses of exogenous MeJA inhibited root curling, IT formation and nodule primordia organogenesis of *L. japonicus* in a concentration-dependent manner (64) and progressively decreased *M. truncatula* sensitivity to Nod factor (65). Conversely, 1h treatment with Nod factor decreased the *M. truncatula* root content of oxylipin precursors (71). Altogether, these results suggest that active JA signaling in the pre-contact phases of RNS helps to activate the symbiotic molecular dialogue; and that subsequently, upon Nod factor signaling, reduced oxylipin biosynthesis allows bacterial infection. This hypothesis is supported by the finding that the *L. japonicus* JA biosynthetic genes *AOC* and *12-OPDA reductase* were strongly upregulated at 2-4 days post rhizobial inoculation, to almost revert to uninfected control expression levels later on (72). We thus propose that, similarly to AM symbiosis, moderate and transient JA signaling promotes RNS while massive or sustained JA signaling dampens RNS, by influencing the success of early colonization events.

In distinct N-fixing bacteria-plant interactions, oxylipin biosynthesis is enhanced in parenchymatous cells of mature nodules (73, 74, 75, 76, 77), although this phenomenon may be transient or species-specific (72, 78). Since bioactive JA-Ile levels do not vary upon nodulation (76, 79) and neither impaired nor enhanced root JA biosynthesis does impact RNS in *M. truncatula* (79), the production of other oxylipins may play a more significant role in nodulation. However, JA signaling seems to negatively affect bacteroid performance, since *Astragalus sinicus* hairy roots silencing the *JAZ1* gene, thus constitutively activating JA signaling (80), displayed reduced bacteroid differentiation and subsequent decrease in nitrogenase activity (81). AsJAZ1 interacts with the leghemoglobin AsB2510 (81).

Leghemoglobins generate the microaerobic environment necessary for nitrogenase activity (82) and are fundamental for RNS (83). Therefore, JA signaling may modulate symbiotic N fixation through tuning of leghemoglobin activity. The contradiction between absence of phenotype during constitutive JA biosynthesis (79) and severe nodulation phenotype when JA signaling is constitutively activated (81) hints at a modified JA sensitivity in nodules and needs further investigation.

6.4.2. JA signaling in the autoregulation of nodulation (AUT)

JA signaling contributes to the autoregulation of nodulation (AUT), a pathway suppressing nodule formation upon already established RNS (84). The fundamental genetic component of the AUT pathway is the LjHAR1/MtSUNN/GmNARK CLAVATA1-like leucine-rich repeat (LRR) receptor kinase (85, 86, 87, 88), acting in the shoot to perceive a RNS root-derived signal mediated by CLE peptides (89, 90). Subsequently, LjHAR1/MtSUNN/GmNARK codifies still partially unknown signals that suppress nodulation systemically. The corresponding mutants, the *L. japonicus har1*, the *M. truncatula sunn* and the soybean *nark* mutants, are hypernodulating (85, 86, 87, 88). Non-inoculated *nark*/*sunn* mutants show high JA levels and expression of JA biosynthetic/response genes (66, 91), whereas the same genes are downregulated in the leaves of nodulating wild type *M. truncatula* (91). This indicates that LjHAR1/MtSUNN/GmNARK-dependent systemic dampening of JA biosynthesis/signaling may restrict nodulation. Since decreased accumulation of the miR2111 microRNA has been proposed to be the AUT shoot-derived signal in *L. japonicus* (92), it is worth verifying if the expression of the *MIR2111* genes is JA-dependent. However, additional AUT mechanisms must exist, since shoot treatment with the JA biosynthesis inhibitor n-propyl gallate inhibited nodulation in the hypernodulating *nark* mutant, but failed to suppress it to wild type levels (91). Moreover, optimal JA homeostasis in shoots is crucial for RNS: in fact, both impaired JA biosynthesis and exogenous JA treatment reduce nodule number, both in wild type and hypernodulating AUT mutants (64, 66, 91, 93). Moderate JA signaling could counteract AUT through inhibited auxin synthesis and transport (94). Indeed, hypernodulating *nark* mutant roots failed to increase their auxin content upon rhizobial inoculation (95); and long-distance auxin transport is inhibited in *sunn* mutants (96), suggesting that auxin accumulation in roots inhibits excessive nodulation. To integrate all these results, we propose that: (i) moderate or transient JA systemic signaling, as in non-nodulating plants, alters auxin gradients to promote nodulation; (ii) very low levels of foliar JA, induced by action of LjHAR1/MtSUNN/GmNARK, fail to restrain auxin accumulation in roots, with a consequent inhibition of nodulation; (iii) massive and sustained JA signaling in the shoot, as obtained through exogenous application, induces a systemic immune response preventing rhizobial colonization. The latter assumption, however, contrasts with the

observation that attack by the foliar chewing insect *Spodoptera exigua*, supposed to trigger JA-dependent defenses, increases nodulation of *M. truncatula* plants (97). Nevertheless, JA-mediated responses to herbivore attack result from the integration of multiple environmental signals, for example light availability (19). Similarly, phytochrome signaling probably dampens JA signaling in order to repress RNS, as suggested by decreased nodulation, JA-Ile levels and/or JA marker gene expression in shade-grown wild type *L. japonicus* and in light-insensitive *phyB* roots (63). The interaction between LjHAR1/MtSUNN/GmNARK-mediated and PHYB-mediated inhibition of nodulation has not yet been investigated. We propose the restriction of JA biosynthesis to be downstream of both pathways. This hypothesis can be verified through phenotyping of hypernodulating, *phyB* and JA-deficient mutant legumes and their crossings.

In conclusion, moderate JA accumulation may promote the RNS molecular dialogue, while excessive JA accumulation inhibits RNS, probably through enhanced systemic defense. JA signaling, but not JA biosynthesis, might regulate symbiotic N fixation. Finally, the inhibition of JA biosynthesis, by either the AUT pathway or light signaling, contributes to the systemic restriction of nodulation, possibly through altered auxin homeostasis. Therefore, the correct balance of shoot oxylipins is crucial for optimal RNS (Figure 2).

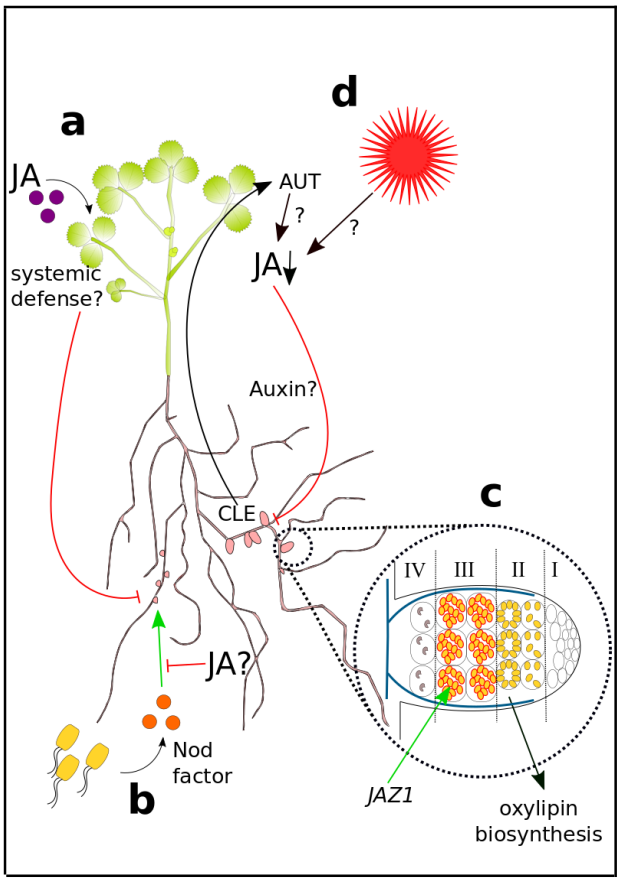


Figure 2: The role of JAs in RNS.

a) *Exogenous JAs treatment of leaves reduces the number of bacterial IT, nodule primordia and mature nodules in several plant species, possibly through enhanced systemic defense.* ***b)*** *JA may promote the production of rhizobial Nod factor, but impair its perception, thus counteracting the formation of nodule primordia.* ***c)*** *Oxylipin biosynthetic enzymes are not expressed in N-fixing cells and repression of JA signaling through JAZ1 is necessary for bacteroid differentiation and nitrogenase activity: this suggests a negative impact of JA signaling in bacteroid development and N fixation.* ***d)*** *AUT, triggered by CLE peptides, and SAS, triggered by low R/FR radiation, may systemically inhibit JA biosynthesis/signaling, thus releasing the repression over auxin biosynthesis and transport towards the roots; in turn, root auxin accumulation may repress RNS.*

6.5. JA in ectomycorrhizal symbiosis development

The ectomycorrhizal symbiosis, or ECM, is a mutually beneficial interaction occurring between the roots of about 6,000 tree species and more than 20,000 species of fungi of the *Basidiomycota* and *Ascomycota* phyla (13). ECM fungi are crucial for forest ecosystems, since (i) they enhance tree mineral nutrition (98, 99), (ii) they provide protection against biotic and abiotic stress (100) and (iii) they participate to the carbon and nitrogen cycles (101, 102). Fungal hyphae form a mantle around lateral roots and subsequently penetrate through the apoplastic space between epidermal and cortical cells to generate a ramified structure called the Hartig net, the probable site of nutrient exchange between the symbiotic partners (103). The molecular determinants of ECM development are much less known with respect to other intimate kinds of plant-microbe symbioses, and may vary considerably, since ECM evolved multiple times (104).

In the model system constituted by trees of the *Populus* genus and by the Basidiomycete *Laccaria bicolor*, suppression of JA signaling is pivotal for the establishment of the symbiosis. In fact, the effector Mycorrhiza-Induced Small Secreted Protein 7 (MiSSP7) from *L. bicolor,* secreted upon initiation of the symbiotic program (105), penetrates the nuclei of poplar root cortical cells and interacts with the poplar co-receptor of jasmonic acid JAZ6, stabilizing its repression over JA signaling (105, 106). Since both inoculation with *missp7* silenced strains of *L. bicolor* and very low concentrations of exogenous MeJA impaired Hartig net formation (105, 107), MiSSP7-mediated inhibition of the JA signaling pathway is necessary for ECM development.

However, JA signaling may have distinct functions at different mycorrhizal stages. At early mycorrhizal stage (1 week post inoculation) JA signaling may promote moderate immunity (Basso et al., in preparation). On the other hand, JA-downregulated genes involved in biosynthesis of and/or response to auxin, gibberellin, ethylene, cytokinin, strigolactone and brassinosteroid are induced in early ECM (Basso et al., in preparation), suggesting

suppression of the crosstalk between JA and other phytohormone signaling pathways during ECM development. In mature ECM (2 weeks post inoculation), JA signaling may play a role in root cell reshaping necessary for Hartig net formation (Basso et al., in preparation). Also, it has been suggested that active JA signaling triggers plant immunity in order to restrict fungal growth within the root at late stages (107). The use of poplar mutants defective in different branches of JA signaling will possibly highlight a multifaceted role of JA during ECM development.

6.6. JA in other beneficial rhizospheric/endophytic interactions

In the era of omics technology it has become easier and easier to tackle complex systems. It is thus possible to expand the field of plant-microbe interactions from the study of single host-single microbe systems to investigation of rhizospheric community dynamics. Indeed, N-fixing rhizobia and mycorrhizal fungi are just part of a highly diversified group of root-associated beneficial microbes called plant-growth promoting rhizobacteria (PGPR) and fungi (PGPF). Such microbes are attracted towards the rhizosphere, i.e. the area of soil occupied by roots, by plant exudates (108) and can become epiphytes colonizing the root surface, or endophytes populating inner root tissues (109). These microbes are a hidden reservoir of useful metabolic functions for plant nutrition and health (110, 111) and are being widely exploited as biocontrol agents in agriculture (112, 113). However, the output of such interactions on plant fitness depends on plant genotype (114, 115) and environment (55) and on the type of stress to which the host plant is subjected (116, 117). Therefore, to achieve optimal fitness, plants must integrate developmental and environmental cues prior to regulation of rhizospheric or endophytic interactions. JA signaling, the master switch between development and defense (19, 20), may contribute to both processes.

Firstly, secreted JA or JA-triggered secondary metabolites may remodel composition, diversity and richness of microbial communities (108). For example, various diseases due to necrotrophic plant pathogens induce the development of disease-suppressive soils (118, 119, 120), probably through increased abundance of particular microbial species upon plant-derived rhizospheric cues; the latter are possibly part of the JA-mediated defense against necrotrophic pathogens (14). In support of this hypothesis, Arabidopsis leaf treatment with low concentrations of MeJA increased rhizospheric abundance of bacteria closely related to pest-protective species (121). The same treatment in wheat provoked a shift in the endophytic bacterial community (122). In both cases, the consequences of microbial recruiting on plant fitness need to be investigated. In addition, JA perception seems to control the composition of root-associated microbial communities. Indeed, roots of JA-insensitive

Arabidopsis mutants reduced secretion of asparagine, tryptophan, glutamic acid, ornithine and galactinol, compounds implied in the chemotaxis of several beneficial bacterial species (123). These results suggest that JA-mediated production of secondary metabolites, rather than direct JA secretion, reshapes root-associated microbial communities.

Secondly, JA signaling may determine the level of compatibility between beneficial microbes and hosts, as suggested in case of mycorrhizal fungi (44) and N-fixing *Azoarcus* spp. bacteria (124). On the other hand, induction of JA signaling, counteracting SA signaling, is advantageous for some beneficial microbes. For example, shoot MeJA treatment promoted the colonization of Arabidopsis roots by the disease-protective endophyte *Trichoderma harzianum* T-78 (125). Also, the broad-host-range basidiomycete *Piriformospora indica*, conferring increased yield, abiotic stress and disease resistance to plants (126, 127), must escape SA-mediated hypersensitive response in the first, biotrophic phase of its root colonization and achieves so through induction of JA signaling (128), possibly through small secreted proteins (129). However, JA signaling seems to inhibit the subsequent, necrotrophic phase of colonization, since several Arabidopsis mutants impaired in JA biosynthesis and signaling were more colonized by *P. indica* at a later stage (128).

In conclusion, evidence suggests that endogenous JA production and its associated responses are implied in reshaping root-associated microbial communities in various plant species, especially in case of pathogen attack, with a putative protective outcome. Also, JA signaling may finely tune the level of colonization by beneficial microbes according to host-microbe compatibility or environmental cues.

6.7. JA in beneficial microbe-triggered systemic resistance

Since the first discoveries of disease-suppressive soils (130) it was speculated that plants under pathogen attack may attract root microorganisms capable of priming host defenses for better performance in case of subsequent biotic stress. Priming is the main mechanism of induced systemic resistance (ISR) by plant growth-promoting rhizobacteria (PGPR) or fungi (PGPF) (reviewed in 111, 131, 132). A similar phenomenon, triggered AMF, is mycorrhiza-induced systemic resistance (MIR).

6.7.1. Induced systemic resistance (ISR) by plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF)

Priming by ISR usually does not involve a direct induction of defense gene expression (132, 133) or alteration of hormonal levels (116, 134) upon presence of the beneficial microbe, even if, upon inoculation with some PGPFs, shoot JA accumulation or

enhanced expression of JA biosynthetic genes have been reported (135, 136). In any case, in several plant-microbe pairs ISR increases sensitivity to JA and ET upon subsequent pathogen challenge, enhancing defense mechanisms controlled by the JA/ET signaling pathway (22, 132). As a proof of principle, ISR is mostly effective against JA-sensitive chewing insects, rather than against SA-sensitive sap-sucking insects (117, 137, 138), even though the range of bacteria, oomycetes, fungi and viruses against which ISR is effective exceeds the variety of organisms usually contrasted through JA signaling (111, 139). For many PGPR and PGPF genetic evidence in Arabidopsis, tomato, cucumber and rice showed that JA and/or ET signaling, as well as the master switch of the SA-JA crosstalk, *NPR1*, are necessary for ISR (116, 132, 134, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152). In Arabidopsis, the root-specific transcription factor MYB72 is a central node for early ISR signaling upon *Pseudomonas simiae* WCS417r, *P. putida* WCS358r or *Trichoderma asperellum* T34 inoculation (153, 154). MYB72 interacts with the ethylene-responsive transcription factor EIL3 and acts upstream of ET and JA signaling (153). Therefore, a possible model of ISR signaling depicts activity of EIL3-MYB72 at the contact site with the beneficial microbe, followed by NPR1-dependent systemic priming of JA/ET-induced defenses (111, 139). However, the nature of the locally perceived signal, of the systemic priming signal and of the molecular mechanism of priming are still elusive.

In addition to the widespread JA/ET signaling priming, some beneficial rhizobacteria prime their host for enhanced SA-dependent defenses (155, 156, 157), while others, as well as several PGPFs of the *Trichoderma* genus, are able to prime both the SA and the JA/ET pathway (125, 158, 159, 160, 161). Other PGPR or PGPF, such as the endophytic fungus *P. indica*, prime plant defense through JA/ET and SA-independent mechanisms (127). Given the wide variety of priming mechanisms, inoculation with multiple beneficial microbes might enhance plant resistance in consequence of the activation of multiple pathways. For example, in cucumber, co-inoculation of PGPF *T. harzianum* and PGPR *Pseudomonas* sp. enhanced resistance to the stem pathogen *Fusarium oxysporum* by activating both JA- and SA-dependent defense responses (162). However, the molecular mechanism of the JA-SA crosstalk-induced systemic resistance has not been investigated yet.

6.7.2. Mycorrhiza-induced resistance (MIR)

A similar phenomenon to ISR is mycorrhiza-induced resistance (MIR) (reviewed in 163, 164, 165). Mycorrhized plants are more resistant to a wide range of soil-borne pathogens (166), while the impact on resistance to aboveground attackers is highly variable. In general, biotrophic pathogens and pierce-sucking insects perform better on leaves of mycorrhizal plants, even if increased tolerance was observed in terms of plant mass and yield (167). Conversely, AM promote resistance against necrotrophic pathogens (168, 169,

170) and leaf-chewing insects (171). One leading mechanism driving MIR is systemic priming of plant defenses, occurring both above and belowground (168, 172, 173, 174, 175, 176). Priming leads, upon pathogen challenge, to a more rapid and effective induction of defense responses, like expression of pathogenesis-related genes and callose deposition (168, 174, 175, 177). However, MIR is more effective in specific host-AMF pairs (172, 178, 179, 180, 181).

The role of JA signaling in MIR is supported by several pieces of evidence: (i) AM confer protection against necrotrophic pathogens and leaf-chewing insects, the immunity to which is mediated by JA (14); (ii) MIR-enhanced defense includes JA-dependent genes (Pozo et al., 2009); (iii) at late stages of AM colonization, JA levels increase in leaves of *M. truncatula* (Isayenkov et al., 2005) and tomato (Nair et al., 2015); (iv) AM colonization activates JA biosynthesis and responses in leaves of *M. truncatula* (55, 182, 183); (v) intact JA biosynthesis is required for MIR in tomato (175, 176); (vi) similarly to ISR, priming by MIR involves also enhanced JA sensitivity, since shoots of mycorrhizal tomato plants highly expressed *COI1* (176) and responded earlier to foliar JA application (182); (vii) tomato susceptibility to *Botrytis cinerea* was inversely correlated with the production of oxylipins and flavonoids in mycorrhizal, unchallenged plants (55). However, shoot JA accumulation is not required for MIR in all plant species (136) and depends on the N nutritional status in tomato (55). JA signaling may contribute to MIR also through partitioning of nutrient resources. In fact, herbivore attack (184) and MeJA application (51, 52) enhance carbon allocation towards roots, possibly helping the plant confront both nutritional and biotic stress (185). Thus, through priming of defense mechanisms and nutrient allocation, enhanced JA accumulation or sensitivity upon AM colonization might mediate resistance to both biotic and abiotic stress.

6.8. Conclusion

Jasmonate biosynthesis and signaling play very diversified roles in interactions between root-associated beneficial microbes and plants. A canonical defense phytohormone, JA often opposes the formation of root-microbe interactions, probably through the activation of systemic defense (38, 40, 43, 45, 64, 65, 66, 124). Indeed, endogenous JA levels seem to inversely correlate with the degree of compatibility between microbe and host (44, 124). To overcome plant immunity, similarly to pathogens, some beneficial organisms hijack JA signaling through the use of effectors to establish symbioses (105, 128). However, low levels of JA promote AM colonization (36, 39, 40, 41) and attract beneficial organisms through modification of root exudation (118, 119, 120, 121, 122). Overall, in many plant species the fine-tuning of JA accumulation and/or signaling determine the colonization success of beneficial microbes.

JA signaling is often involved in the maintenance of deeply connected interactions. For example, enhanced JA signaling in arbuscule-containing cells of some mycorrhizal species may support cell reshaping or fungal carbon nutrition, necessary for the maintenance of AM symbiosis (37). On the other hand, reduced shoot JA accumulation may be important for AUT in nodulating legumes (66, 91). However, sustained JA signaling is deleterious for many interactions. The interplay between JA-mediated stabilization or hampering of beneficial interactions needs to be further investigated. Finally, beneficial organisms can stimulate JA biosynthesis and/or signaling in order to prime the host plant for better resistance to biotic and abiotic stress (111, 164, 165).

Since genetic and environmental factors influence the output of JA accumulation and response in mutualistic plant-microbe interactions, JA signaling may integrate different kind of environmental stimuli and control the energetic defense-to-development trade-off (19, 20). Further research is necessary to investigate the multifaceted roles of this major regulator of plant physiology and beneficial interactions.

6.9. References

1. Lakshmanan, V., Selvaraj, G., & Bais, H. P. (2014). Functional Soil Microbiome: Belowground Solutions to an Aboveground Problem. *Plant Physiology*, *166*(2), 689–700. http://doi.org/10.1104/pp.114.245811
2. Mercado-Blanco, J., Abrantes, I., Caracciolo, A. B., Bevivino, A., Ciancio, A., Grenni, P., … Proença, D. N. (2018). Belowground microbiota and the health of tree crops. *Frontiers in Microbiology*, *9*(6), 1006. http://doi.org/10.3389/fmicb.2018.01006
3. Tkacz, A., & Poole, P. (2015). Role of root microbiota in plant productivity. *Journal of Experimental Botany*, *66*(8), 2167–2175. http://doi.org/10.1093/jxb/erv157
4. Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M. L., … Daffonchio, D. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environmental Microbiology*, *17*(2), 316–331. http://doi.org/10.1111/1462-2920.12439
5. Qin, Y., Druzhinina, I. S., Pan, X., & Yuan, Z. (2016). Microbially Mediated Plant Salt Tolerance and Microbiome-based Solutions for Saline Agriculture. *Biotechnology Advances*, *34*(7), 1245–1259. http://doi.org/10.1016/j.biotechadv.2016.08.005
6. Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, *17*(8), 478–486. http://doi.org/10.1016/j.tplants.2012.04.001
7. Raaijmakers, J. M., & Mazzola, M. (2016). Soil immune responses. *Science*, *352*(6292), 1392–1393. http://doi.org/10.1126/science.aaf3252

8. Shikano, I., Rosa, C., Tan, C.-W., & Felton, G. W. (2017). Tritrophic Interactions: Microbe-Mediated Plant Effects on Insect Herbivores. *Annual Review of Phytopathology*, *55*(1), 313–331. http://doi.org/10.1146/annurev-phyto-080516-035319
9. Bakker, P. A. H. M., Pieterse, C. M. J., de Jonge, R., & Berendsen, R. L. (2018). The Soil-Borne Legacy. *Cell*, *172*(6), 1178–1180. http://doi.org/10.1016/j.cell.2018.02.024
10. Gutjahr, C., & Parniske, M. (2013). Cell and Developmental Biology of Arbuscular Mycorrhiza Symbiosis. *Annual Review of Cell and Developmental Biology*, *29*(1), 593–617. http://doi.org/10.1146/annurev-cellbio-101512-122413
11. Oldroyd, G. E. D., Murray, J. D., Poole, P. S., & Downie, J. A. (2011). The Rules of Engagement in the Legume-Rhizobial Symbiosis. *Annual Review of Genetics*, *45*(1), 119–144. http://doi.org/10.1146/annurev-genet-110410-132549
12. Gutjahr, C. (2014). Phytohormone signaling in arbuscular mycorhiza development. *Current Opinion in Plant Biology*, *20*, 26–34. http://doi.org/10.1016/j.pbi.2014.04.003
13. Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., & Hibbett, D. S. (2016). Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology*, *14*(12), 760–773. http://doi.org/10.1038/nrmicro.2016.149
14. Pieterse, C. M. J., Leon-Reyes, A., Van Der Ent, S., & Van Wees, S. C. M. (2009). Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology*, *5*(5), 308–316. http://doi.org/10.1038/nchembio.164
15. Nomura, K., Melotto, M., & He, S.-Y. (2005). Suppression of host defense in compatible plant–*Pseudomonas syringae* interactions. *Current Opinion in Plant Biology*, *8*(4), 361–368. https://doi.org/10.1016/j.pbi.2005.05.005
16. El Oirdi, M., El Rahman, T. A., Rigano, L., El Hadrami, A., Rodriguez, M. C., Daayf, F., … Bouarab, K. (2011). *Botrytis cinerea* Manipulates the Antagonistic Effects between Immune Pathways to Promote Disease Development in Tomato. *The Plant Cell*, *23*(6), 2405-2421. https://doi.org/10.1105/tpc.111.083394
17. Marchetti, M., Capela, D., Glew, M., Cruveiller, S., Chane-Woon-Ming, B., Gris, C., … Masson-Boivin, C. (2010). Experimental evolution of a plant pathogen into a legume symbiont. *PLoS Biology*, *8*(1), e1000280. http://doi.org/10.1371/journal.pbio.1000280
18. Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal Modulation of Plant Immunity. *Annual Review of Cell and Developmental Biology*, *28*(1), 489–521. http://doi.org/10.1146/annurev-cellbio-092910-154055
19. Kazan, K., & Manners, J. M. (2011). The interplay between light and jasmonate signalling during defence and development. *Journal of Experimental Botany*, *62*(12), 4087–4100. http://doi.org/10.1093/jxb/err142
20. Campos, M. L., Yoshida, Y., Major, I. T., De Oliveira Ferreira, D., Weraduwage, S. M., Froehlich, J. E., … Howe, G. A. (2016). Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. *Nature Communications*, *7*, 12570. http://doi.org/10.1038/ncomms12570

21. Friesen, M. L., Porter, S. S., Stark, S. C., von Wettberg, E. J., Sachs, J. L., & Martinez-Romero, E. (2011). Microbially Mediated Plant Functional Traits. *Annual Review of Ecology, Evolution, and Systematics*, *42*(1), 23–46. http://doi.org/10.1146/annurev-ecolsys-102710-145039
22. Verhagen, B. W. M., Glazebrook, J., Zhu, T., Chang, H.-S., van Loon, L. C., & Pieterse, C. M. J. (2004). The Transcriptome of Rhizobacteria-Induced Systemic Resistance in *Arabidopsis*. *Molecular Plant-Microbe Interactions*, *17*(8), 895–908. http://doi.org/10.1094/MPMI.2004.17.8.895
23. Wang, W., Shi, J., Xie, Q., Jiang, Y., Yu, N., & Wang, E. (2017). Nutrient Exchange and Regulation in Arbuscular Mycorrhizal Symbiosis. *Molecular Plant*, *10*(9), 1147–1158. http://doi.org/10.1016/j.molp.2017.07.012
24. Berruti, A., Lumini, E., Balestrini, R., & Bianciotto, V. (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes. *Frontiers in Microbiology*, *6*(1), 1559. http://doi.org/10.3389/fmicb.2015.01559
25. Nagata, M., Yamamoto, N., Miyamoto, T., Shimomura, A., Arima, S., Hirsch, A. M., & Suzuki, A. (2016). Enhanced hyphal growth of arbuscular mycorrhizae by root exudates derived from high R/FR treated *Lotus japonicus*. *Plant Signaling and Behavior*, *11*(6), e1187356. http://doi.org/10.1080/15592324.2016.1187356
26. Ballaré, C. L. (2014). Light Regulation of Plant Defense. *Annual Review of Plant Biology*, *65*(1), 335–363. http://doi.org/10.1146/annurev-arplant-050213-040145
27. Siciliano, V., Genre, A., Balestrini, R., Cappellazzo, G., deWit, P. J. G. M., & Bonfante, P. (2007). Transcriptome Analysis of Arbuscular Mycorrhizal Roots during Development of the Prepenetration Apparatus. *Plant Physiology*, *144*(3), 1455–1466. http://doi.org/10.1104/pp.107.097980
28. Blilou, I., Ocampo, J. A., & García-Garrido, J. M. (1999). Resistance of pea roots to endomycorrhizal fungus or Rhizobium correlates with enhanced levels of endogenous salicylic acid. *Journal of Experimental Botany*, *50*(340), 1663–1668. http://dx.doi.org/10.1093/jxb/50.340.1663
29. Blilou, I., Ocampo, J. a., García-Garrido, J. M., & Garcia-Garrido, J. M. (2000). Induction of *Ltp* (lipid transfer protein) and *Pal* (phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*. *Journal of Experimental Botany*, *51*(353), 1969–1977. http://doi.org/10.1093/jexbot/51.353.1969
30. Gutjahr, C., & Paszkowski, U. (2009). Weights in the Balance: Jasmonic Acid and Salicylic Acid Signaling in Root-Biotroph Interactions. *Molecular Plant-Microbe Interactions*, *22*(7), 763–772. http://doi.org/10.1094/MPMI-22-7-0763
31. Hause, B., Maier, W., Miersch, O., Kramell, R., & Strach, D. (2002). Induction of Jasmonate Biosynthesis in Arbuscular Mycorrhizal Barley Roots. *Plant Physiology*, *130*(3), 1213–1220. https://doi.org/10.1104/pp.006007
32. Stumpe, M., Carsjens, J.-G., Stenzel, I., Göbel, C., Lang, I., Pawlowski, K., … Feussner, I. (2005). Lipid metabolism in arbuscular mycorrhizal roots of *Medicago truncatula*.

Phytochemistry, *66*(7), 781–791. http://doi.org/https://doi.org/10.1016/j.phytochem.2005.01.020

33. Meixner, C., Ludwig-Müller, J., Miersch, O., Gresshoff, P., Staehelin, C., & Vierheilig, H. (2005). Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutant *nts1007*. *Planta*, *222*(4), 709–715. http://doi.org/10.1007/s00425-005-0003-4
34. Isayenkov, S., Mrosk, C., Stenzel, I., Strack, D., & Hause, B. (2005). Suppression of Allene Oxide Cyclase in Hairy Roots of *Medicago truncatula* Reduces Jasmonate Levels and the Degree of Mycorrhization with *Glomus intraradices*. *Plant Physiology*, *139*(3), 1401–1410. http://doi.org/10.1104/pp.105.069054
35. León-Morcillo, R. J., Ángel, J., Martín-Rodríguez, Vierheilig, H., Ocampo, J. A., & García-Garrido, J. M. (2012). Late activation of the 9-oxylipin pathway during arbuscular mycorrhiza formation in tomato and its regulation by jasmonate signalling. *Journal of Experimental Botany*, *63*(10), 3545–3558. http://doi.org/10.1093/jxb/ers010
36. Tejeda-Sartorius, M., Martínez De La Vega, O., & Délano-Frier, J. P. (2008). Jasmonic acid influences mycorrhizal colonization in tomato plants by modifying the expression of genes involved in carbohydrate partitioning. *Physiologia Plantarum*, *133*(2), 339–353. http://doi.org/10.1111/j.1399-3054.2008.01081.x
37. Hause, B., Mrosk, C., Isayenkov, S., & Strack, D. (2007). Jasmonates in arbuscular mycorrhizal interactions. *Phytochemistry*, *68*(1), 101–110. http://doi.org/10.1016/j.phytochem.2006.09.025
38. Herrera-Medina, M. J., Tamayo, M. I., Vierheilig, H., Ocampo, J. A., & García-Garrido, J. M. (2008). The jasmonic acid signalling pathway restricts the development of the arbuscular mycorrhizal association in tomato. *Journal of Plant Growth Regulation*, *27*(3), 221–230. http://doi.org/10.1007/s00344-008-9049-4
39. Regvar, M., Gogala, N., & Zalar, P. (1996). Effect of jasmonic acid on mycorrhizal *Allium sativum*. *New Phytologist*, *134*(134), 703–707. https://nph.onlinelibrary.wiley.com/doi/pdf/10.1111/j.1469-8137.1996.tb04936.x
40. Kiers, E.T., Adler, L. S., Grman, E. L., & van der Heijden, M. G. A. (2010). Manipulating the jasmonate response: How do methyl jasmonate additions mediate characteristics of aboveground and belowground mutualisms? *Functional Ecology*, *24*(2), 434–443. https://doi.org/10.1111/j.1365-2435.2009.01625.x
41. Landgraf, R., Schaarschmidt, S., & Hause, B. (2012). Repeated leaf wounding alters the colonization of *Medicago truncatula* roots by beneficial and pathogenic microorganisms. *Plant, Cell and Environment*, *35*(7), 1344–1357. http://doi.org/10.1111/j.1365-3040.2012.02495.x
42. Riedel, T., Groten, K., & Baldwin, I. T. (2008). Symbiosis between *Nicotiana attenuata* and *Glomus intraradices*: Ethylene plays a role, jasmonic acid does not. *Plant, Cell and Environment*, *31*(9), 1203–1213. http://doi.org/10.1111/j.1365-3040.2008.01827.x
43. Gutjahr, C., Siegler, H., Haga, K., Iino, M., & Paszkowski, U. (2015). Full establishment of arbuscular mycorrhizal symbiosis in rice occurs independently of enzymatic jasmonate biosynthesis. *PLoS ONE*, *10*(4), e0123422. http://doi.org/10.1371/journal.pone.0123422

44. Fernández, I., Merlos, M., López-Ráez, J. A., Martínez-Medina, A., Ferrol, N., Azcón, C., … Pozo, M. J. (2014). Defense Related Phytohormones Regulation in Arbuscular Mycorrhizal Symbioses Depends on the Partner Genotypes. *Journal of Chemical Ecology*, *40*(7), 791–803. http://doi.org/10.1007/s10886-014-0473-6
45. Ludwig-Müller, J., Bennett, R. N., García-Garrido, J. M., Piché, Y., & Vierheilig, H. (2002). Reduced arbuscular mycorrhizal root colonization in *Tropaeolum majus* and *Carica papaya* after jasmonic acid application can not be attributed to increased glucosinolate levels. *Journal of Plant Physiology*, *159*(5), 517–523. http://doi.org/10.1078/0176-1617-00731
46. Yang, Z.-B., He, C., Ma, Y., Herde, M., & Ding, Z. (2017). Jasmonic Acid Enhances Al-Induced Root Growth Inhibition. *Plant Physiology*, *173*(2), 1420–1433. http://doi.org/10.1104/pp.16.01756
47. Genre, A., & Bonfante, P. (1998). Actin versus tubulin configuration in arbuscule-containing cells from mycorrhizal tobacco roots. *New Phytologist*, *140*(4), 745–752. http://doi.org/10.1046/j.1469-8137.1998.00314.x
48. Blancaflor, E. B., Zhao, L., & Harrison, M. J. (2001). Microtubule organization in root cells of *Medicago truncatula* during development of an arbuscular mycorrhizal symbiosis with *Glomus versiforme*. *Protoplasma*, *217*(4), 154–165. http://doi.org/10.1007/BF01283396
49. Manthey, K., Krajinski, F., Hohnjec, N., Firnhaber, C., Pühler, A., Perlick, A. M., & Küster, H. (2004). Transcriptome Profiling in Root Nodules and Arbuscular Mycorrhiza Identifies a Collection of Novel Genes Induced During *Medicago truncatula* Root Endosymbioses. *Molecular Plant-Microbe Interactions*, *17*(10), 1063–1077. http://doi.org/10.1094/MPMI.2004.17.10.1063
50. Koda, Y. (1997). Possible involvement of jasmonates in various morphogenic events. *Physiologia Plantarum*, *100*(3), 639–646. http://doi.org/10.1111/j.1399-3054.1997.tb03070.x
51. Babst, B. A., Ferrieri, R. a, Gray, D. W., Lerdau, M., Schlyer, D. J., Schueller, M., … Orians, C. M. (2005). Jasmonic acid induces rapid changes in carbon transport and partitioning in *Populus*. *New Phytologist*, *167*, 63–72. http://doi.org/10.1111/j.1469-8137.2005.01388.x
52. Gómez, S., Ferrieri, R. A., Schueller, M., & Orians, C. M. (2010). Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. *New Phytologist*, *188*(3), 835–844. http://doi.org/10.1111/j.1469-8137.2010.03414.x
53. Hause, B., Stenzel, I., Miersch, O., Maucher, H., Kramell, R., Ziegler, J., & Wasternack, C. (2000). Tissue-specific oxylipin signature of tomato flowers: Allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles. *Plant Journal*, *24*(1), 113–126. https://doi.org/10.1046/j.1365-313X.2000.00861.x
54. Maucher, H., Hause, B., Feussner, I., Ziegler, J., & Wasternack, C. (2001). Allene oxide synthases of barley (*Hordeum vulgare* cv. Salome): tissue specific regulation in seedling development. *The Plant Journal*, *21*(2), 199–213. http://doi.org/10.1046/j.1365-313x.2000.00669.x
55. Sánchez-Bel, P., Sanmartín, N., Pastor, V., Mateu, D., Cerezo, M., Vidal-Albalat, A., … Flors, V. (2018). Mycorrhizal tomato plants fine tunes the growth-defence balance upon N depleted

root environments. *Plant Cell and Environment*, *41*(2), 406–420. http://doi.org/10.1111/pce.13105

56. Schaarschmidt, S., Roitsch, T., & Hause, B. (2006). Arbuscular mycorrhiza induces gene expression of the apoplastic invertase *LIN6* in tomato (*Lycopersicon esculentum*) roots. *Journal of Experimental Botany*, *57*(15), 4015–4023. http://dx.doi.org/10.1093/jxb/erl172
57. Thoma, I., Loeffler, C., Sinha, A. K., Gupta, M., Krischke, M., Steffan, B., … Mueller, M. J. (2003). Cyclopentenone isoprostanes induced by reactive oxygen species trigger defense gene activation and phytoalexin accumulation in plants. *Plant Journal*, *34*(3), 363–375. http://doi.org/10.1046/j.1365-313X.2003.01730.x
58. Daher, Z., Recorbet, G., Solymosi, K., Wienkoop, S., Mounier, A., Morandi, D., … Schoefs, B. (2017). Changes in plastid proteome and structure in arbuscular mycorrhizal roots display a nutrient starvation signature. *Physiologia Plantarum*, *159*(1), 13–29. http://doi.org/10.1111/ppl.12505
59. Harrison, M. J., & Dixon, R. A. (1994). Spatial patterns of expression of flavonoid/isoflavonoid pathway genes during interactions between roots of *Medicago truncatula* and the mycorrhizal fungus *Glomus versiforme*. *The Plant Journal*, *6*(1), 9–20. http://doi.org/10.1046/j.1365-313X.1994.6010009.x
60. Vellosillo, T., Martinez, M., Lopez, M. A., Vicente, J., Cascon, T., Dolan, L., … Castresana, C. (2007). Oxylipins Produced by the 9-Lipoxygenase Pathway in Arabidopsis Regulate Lateral Root Development and Defense Responses through a Specific Signaling Cascade. *The Plant Cell*, *19*(3), 831–846. http://doi.org/10.1105/tpc.106.046052
61. López-Ráez, J. A., Verhage, A., Fernández, I., García, J. M., Azcón-Aguilar, C., Flors, V., & Pozo, M. J. (2010). Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. *Journal of Experimental Botany*, *61*(10), 2589–2601. http://dx.doi.org/10.1093/jxb/erq089
62. Brewin, N. J. (1991). Development of the Legume Root Nodule. *Annual Review of Cell Biology*, *7*(1), 191–226. http://doi.org/10.1146/annurev.cb.07.110191.001203
63. Suzuki, A., Suriyagoda, L., Shigeyama, T., Tominaga, A., Sasaki, M., Hiratsuka, Y., … Hirsch, A. M. (2011). *Lotus japonicus* nodulation is photomorphogenetically controlled by sensing the red/far red (R/FR) ratio through jasmonic acid (JA) signaling. *Proceedings of the National Academy of Sciences*, *108*(40), 16837–16842. http://doi.org/10.1073/pnas.1105892108
64. Nakagawa, T., & Kawaguchi, M. (2006). Shoot-applied MeJA suppresses root nodulation in *Lotus japonicus*. *Plant and Cell Physiology*, *47*(1), 176–180. http://doi.org/10.1093/pcp/pci222
65. Sun, J., Cardoza, V., Mitchell, D. M., Bright, L., Oldroyd, G., & Harris, J. M. (2006). Crosstalk between jasmonic acid, ethylene and Nod factor signaling allows integration of diverse inputs for regulation of nodulation. *Plant Journal*, *46*(6), 961–970. http://doi.org/10.1111/j.1365-313X.2006.02751.x
66. Seo, H. S., Li, J., Lee, S.-Y., Yu, J.-W., Kim, K.-H., Lee, S.-H., & Paek, I.-J. L. and N.-C. (2007). The Hypernodulating *nts* Mutation Induces Jasmonate Synthetic Pathway in Soybean Leaves. *Molecules and Cells*, *24*(2), 185–193.

67. Oldroyd, G. E. D., & Downie, J. A. (2008). Coordinating Nodule Morphogenesis with Rhizobial Infection in Legumes. *Annual Review of Plant Biology*, *59*(1), 519–546. http://doi.org/10.1146/annurev.arplant.59.032607.092839
68. Rosas, S., Soria, R., Correa, N., & Abdala, G. (1998). Jasmonic acid stimulates the expression of *nod* genes in *Rhizobium*. *Plant Molecular Biology*, *38*, 1161–1168. https://doi.org/10.1023/A:1006064807870
69. Mabood, F., Souleimanov, A., Khan, W., & Smith, D. L. (2006). Jasmonates induce Nod factor production by *Bradyrhizobium japonicum*. *Plant Physiology and Biochemistry*, *44*(11), 759–765. http://doi.org/10.1016/j.plaphy.2006.10.025
70. Cooper, J. E. (2004). Multiple Responses of Rhizobia to Flavonoids During Legume Root Infection. *Advances in Botanical Research*, *41*, 1–62. http://doi:10.1016/S0065-2296(04)41001-5
71. Zhang, N., Venkateshwaran, M., Boersma, M., Harms, A., Howes-Podoll, M., Den Os, D., … Sussman, M. R. (2012). Metabolomic profiling reveals suppression of oxylipin biosynthesis during the early stages of legume-rhizobia symbiosis. *FEBS Letters*, *586*(19), 3150–3158. http://doi.org/10.1016/j.febslet.2012.06.046
72. Kouchi, H., Shimomura, K., Hata, S., Hirota, A., Wu, G. J., Kumagai, H., … Tabata, S. (2004). Large-scale analysis of gene expression profiles during early stages of root nodule formation in a model legume, Lotus japonicus. *DNA Research*, *11*(4), 263–274. https://doi.org/10.1093/dnares/11.4.263
73. Perlick, A. M., Albus, U., Stavridis, T., Frühling, M., Küster, H., & Pühler, A. (1996). The *Vicia faba* Lipoxygenase Gene *VfLOX1* is Expressed in the Root Nodule Parenchyma. *Molecular Plant-Microbe Interactions*, *9*(9), 860–863. http://doi.org/10.1094/MPMI-9-0860
74. Bueno, P., Soto, M. J., Rodríguez-Rosales, M. P., Sanjuan, J., Olivares, J., & Donaire, J. P. (2001). Time-course of lipoxygenase, antioxidant enzyme activities and H_2O_2 accumulation during the early stages of *Rhizobium*–legume symbiosis. *New Phytologist*, *152*(1), 91–96. http://doi.org/10.1046/j.0028-646x.2001.00246.x
75. Mohammadi, M., & Karr, A. L. (2003). Induced lipoxygenases in soybean root nodules. *Plant Science*, *164*(4), 471–479. https://doi.org/10.1016/S0168-9452(02)00431-4
76. Costanzo, M. E., Andrade, A., Del Carmen Tordable, M., Cassán, F., & Abdala, G. (2012). Production and function of jasmonates in nodulated roots of soybean plants inoculated with *Bradyrhizobium japonicum*. *Archives of Microbiology*, *194*(10), 837–845. http://doi.org/10.1007/s00203-012-0817-y
77. Demchenko, K., Zdyb, A., Feussner, I., & Pawlowski, K. (2012). Analysis of the subcellular localisation of lipoxygenase in legume and actinorhizal nodules. *Plant Biology*, *14*(1), 56–63. http://doi.org/10.1111/j.1438-8677.2011.00480.x
78. Zdyb, A., Salgado, M. G., Demchenko, K. N., Brenner, W. G., Płaszczyca, M., Stumpe, M., … Pawlowski, K. (2018). Allene oxide synthase, allene oxide cyclase and jasmonic acid levels in *Lotus japonicus* nodules. *PLoS ONE*, *13*(1), 1–16. http://doi.org/10.1371/journal.pone.0190884

79. Zdyb, A., Demchenko, K., Heumann, J., Mrosk, C., Grzeganek, P., Göbel, C., … Hause, B. (2011). Jasmonate biosynthesis in legume and actinorhizal nodules. *New Phytologist*, *189*(2), 568–579. http://doi.org/10.1111/j.1469-8137.2010.03504.x
80. Pauwels, L., & Goossens, A. (2011). The JAZ Proteins: A Crucial Interface in the Jasmonate Signaling Cascade. *The Plant Cell*, *23*(9), 3089–3100. http://doi.org/10.1105/tpc.111.089300
81. Li, Y., Xu, M., Wang, N., & Li, Y. (2015). A JAZ protein in *Astragalus sinicus* interacts with a leghemoglobin through the TIFY domain and is involved in nodule development and nitrogen fixation. *PLoS ONE*, *10*(10), e0139964. http://doi.org/10.1371/journal.pone.0139964
82. Wittenberg, J. B., Bergersen, F. J., Appleby, C. A., & Turner, G. L. (1974). The role of leghemoglobin in nitrogen fixation by bacteroids isolated from soybean root nodules. *The Journal of Biological Chemistry*, *29*(13), 4057–4066. http://www.jbc.org/content/249/13/4057.abstract
83. Ott, T., van Dongen, J. T., Günther, C., Krusell, L., Desbrosses, G., Vigeolas, H., … Udvardi, M. K. (2005). Symbiotic Leghemoglobins Are Crucial for Nitrogen Fixation in Legume Root Nodules but Not for General Plant Growth and Development. *Current Biology*, *15*(6), 531–535. http://doi.org/https://doi.org/10.1016/j.cub.2005.01.042
84. Reid, D. E., Ferguson, B. J., Hayashi, S., Lin, Y.-H., & Gresshoff, P. M. (2011). Molecular mechanisms controlling legume autoregulation of nodulation. *Annals of Botany*, *108*(5), 789–795. http://dx.doi.org/10.1093/aob/mcr205
85. Krusell, L., Madsen, L. H., Sato, S., Aubert, G., Genua, A., Szczyglowski, K., … Stougaard, J. (2002). Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature*, *420*, 422-426. http://dx.doi.org/10.1038/nature01207
86. Nishimura, R., Hayashi, M., Wu, G.-J., Kouchi, H., Imaizumi-Anraku, H., Murakami, Y., … Kawaguchi, M. (2002). HAR1 mediates systemic regulation of symbiotic organ development. *Nature*, *420*, 426. http://dx.doi.org/10.1038/nature01231
87. Searle, I. R., Men, A. E., Laniya, T. S., Buzas, D. M., Iturbe-Ormaetxe, I., Carroll, B. J., & Gresshoff, P. M. (2003). Long-Distance Signaling in Nodulation Directed by a CLAVATA1-Like Receptor Kinase. *Science*, *299*(5603), 109-112. https://doi.org/10.1126/science.1077937
88. Schnabel, E., Journet, E. P., De Carvalho-Niebel, F., Duc, G., & Frugoli, J. (2005). The *Medicago truncatula SUNN* gene encodes a CLV1-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Molecular Biology*, *58*(6), 809–822. http://doi.org/10.1007/s11103-005-8102-y
89. Okamoto, S., Ohnishi, E., Sato, S., Takahashi, H., Nakazono, M., Tabata, S., & Kawaguchi, M. (2009). Nod factor/nitrate-induced *CLE* genes that drive HAR1-mediated systemic regulation of nodulation. *Plant and Cell Physiology*, *50*(1), 67–77. http://doi.org/10.1093/pcp/pcn194
90. Mortier, V., Den Herder, G., Whitford, R., Van de Velde, W., Rombauts, S., D'haeseleer, K., … Goormachtig, S. (2010). CLE Peptides Control *Medicago truncatula* Nodulation Locally and Systemically. *Plant Physiology*, *153*(1), 222–237. http://doi.org/10.1104/pp.110.153718

91. Kinkema, M., & Gresshoff, P. M. (2008). Investigation of Downstream Signals of the Soybean Autoregulation of Nodulation Receptor Kinase GmNARK. *Molecular Plant-Microbe Interactions*, *21*(10), 1337–1348. http://doi.org/10.1094/MPMI-21-10-1337
92. Tsikou, D., Yan, Z., Holt, D. B., Abel, N. B., Reid, D. E., Madsen, L. H., … Markmann, K. (2018). Systemic control of legume susceptibility to rhizobial infection by a mobile microRNA. *Science*, *6907*(8), eaat6907. http://doi.org/10.1126/science.aat6907
93. Magori, S., & Kawaguchi, M. (2010). Analysis of two potential long-distance signaling molecules, LjCLE-RS1/2 and jasmonic acid, in a hypernodulating mutant *too much love*. *Plant Signaling & Behavior*, *5*(4), 403–405. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2958591/
94. Dombrecht, B., Xue, G. P., Sprague, S. J., Kirkegaard, J. A., Ross, J. J., Reid, J. B., … Kazan, K. (2007). MYC2 Differentially Modulates Diverse Jasmonate-Dependent Functions in *Arabidopsis*. *The Plant Cell*, *19*(7), 2225–2245. http://doi.org/10.1105/tpc.106.048017
95. Caba, J. M., Centeno, M. L., Fernández, B., Gresshoff, P. M., & Ligero, F. (2000). Inoculation and nitrate alter phytohormone levels in soybean roots: differences between a supernodulating mutant and the wild type. *Planta*, *211*(1), 98–104. http://doi.org/10.1007/s004250000265
96. Van Noorden, G. E., Ross, J. J., Reid, J. B., Rolfe, B. G., & Mathesius, U. (2006). Defective Long-Distance Auxin Transport Regulation in the *Medicago truncatula super numeric nodules* Mutant. *Plant Physiology*, *140*(4), 1494 LP-1506. http://www.plantphysiol.org/content/140/4/1494.abstract
97. Heath, K. D., & Lau, J. A. (2011). Herbivores alter the fitness benefits of a plant–rhizobium mutualism. *Acta Oecologica*, *37*(2), 87–92. http://doi.org/https://doi.org/10.1016/j.actao.2010.12.002
98. Perez-Moreno, J., & Read, D. J. (2000). Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. *New Phytologist*, *145*(2), 301–309. http://doi.org/10.1046/j.1469-8137.2000.00569.x
99. Tibbett, M., & Sanders, F. E. (2002). Ectomycorrhizal Symbiosis can Enhance Plant Nutrition through Improved Access to Discrete Organic Nutrient Patches of High Resource Quality. *Annals of Botany*, *89*(6), 783–789. http://www.jstor.org/stable/42771516
100. Luo, Z.-B., Janz, D., Jiang, X., Gobel, C., Wildhagen, H., Tan, Y., … Polle, A. (2009). Upgrading Root Physiology for Stress Tolerance by Ectomycorrhizas: Insights from Metabolite and Transcriptional Profiling into Reprogramming for Stress Anticipation. *Plant Physiology*, *151*(4), 1902–1917. http://doi.org/10.1104/pp.109.143735
101. Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., … Lindahl, B. D. (2013). Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest. *Science*, *339*(6127), 1615-1618. http://doi.org/10.1126/science.1231923
102. Lindahl, B. D., & Tunlid, A. (2014). Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytologist*, *205*(4), 1443–1447. http://doi.org/10.1111/nph.13201

103. Peterson, R. L., & Massicotte, H. B. (2004). Exploring structural definitions of mycorrhizas, with emphasis on nutrient-exchange interfaces. *Canadian Journal of Botany*, *82*(8), 1074–1088. http://doi.org/10.1139/b04-071
104. Kohler, A., Kuo, A., Nagy, L. G., Morin, E., Barry, K. W., Buscot, F., … Martin, F. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics*, *47*, 410. http://dx.doi.org/10.1038/ng.3223
105. Plett, J. M., Kemppainen, M., Kale, S. D., Kohler, A., Legué, V., Brun, A., … Martin, F. (2011). A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Current Biology*, *21*(14), 1197–1203. http://doi.org/10.1016/j.cub.2011.05.033
106. Plett, J. M., Daguerre, Y., Wittulsky, S., Vayssieres, A., Deveau, A., Melton, S. J., … Martin, F. (2014a). 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 Sciences*, *111*(22), 8299–8304. http://doi.org/10.1073/pnas.1322671111
107. Plett, J. M., Khachane, A., Ouassou, M., Sundberg, B., Kohler, A., & Martin, F. (2014b). Ethylene and jasmonic acid act as negative modulators during mutualistic symbiosis between *Laccaria bicolor* and *Populus* roots. *New Phytologist*, *202*(1), 270–286. http://doi.org/10.1111/nph.12655
108. Sasse, J., Martinoia, E., & Northen, T. (2018). Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in Plant Science*, *23*(1), 25–41. http://doi.org/10.1016/j.tplants.2017.09.003
109. Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., & Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytologist*, *206*(4), 1196–1206. http://doi.org/10.1111/nph.13312
110. Goh, C.-H., Veliz Vallejos, D. F., Nicotra, A. B., & Mathesius, U. (2013). The Impact of Beneficial Plant-Associated Microbes on Plant Phenotypic Plasticity. *Journal of Chemical Ecology*, *39*(7), 826–839. http://doi.org/10.1007/s10886-013-0326-8
111. Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., & Bakker, P. A. H. M. (2014). Induced Systemic Resistance by Beneficial Microbes. *Annual Review of Phytopathology*, *52*(1), 347–375. http://doi.org/10.1146/annurev-phyto-082712-102340
112. Shoresh, M., Harman, G. E., & Mastouri, F. (2010). Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. *Annual Review of Phytopathology*, *48*(1), 21–43. http://doi.org/10.1146/annurev-phyto-073009-114450
113. Walters, D. R., Ratsep, J., & Havis, N. D. (2013). Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany*, *64*(5), 1263–1280. http://dx.doi.org/10.1093/jxb/ert026
114. Haney, C. H., Samuel, B. S., Bush, J., Ausubel, F. M., & Hospital, M. G. (2015). Associations with rhizosphere bacteria can confer an adaptive advantage to plants. *Nature Plants*, *1*(6), 1–22. http://doi.org/10.1038/nplants.2015.51

115. Wintermans, P. C. A., Bakker, P. A. H. M., & Pieterse, C. M. J. (2016). Natural genetic variation in *Arabidopsis* for responsiveness to plant growth-promoting rhizobacteria. *Plant Molecular Biology*, *90*(6), 623–634. http://doi.org/10.1007/s11103-016-0442-2

116. Iavicoli, A., Boutet, E., Buchala, A., & Métraux, J.-P. (2003). Induced Systemic Resistance in *Arabidopsis thaliana* in Response to Root Inoculation with *Pseudomonas fluorescens* CHA0. *Molecular Plant-Microbe Interactions*, *16*(10), 851–858. http://doi.org/10.1094/MPMI.2003.16.10.851

117. Pineda, A., Soler, R., Weldegergis, B. T., Shimwela, M. M., Van Loon, J. J. A., & Dicke, M. (2013). Non-pathogenic rhizobacteria interfere with the attraction of parasitoids to aphid-induced plant volatiles via jasmonic acid signalling. *Plant, Cell and Environment*, *36*(2), 393–404. http://doi.org/10.1111/j.1365-3040.2012.02581.x

118. Weller, D. M., Raaijmakers, J. M., Gardener, B. B. M., & Thomashow, L. S. (2002). Microbial Populations Responsible for Specific Soil Suppressiveness to Plant Pathogens. *Annual Review of Phytopathology*, *40*(1), 309–348. http://doi.org/10.1146/annurev.phyto.40.030402.110010

119. Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, *321*(1), 341–361. https://doi.org/10.1007/s11104-008-9568-6

120. Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H. M., Piceno, Y. M., DeSantis, T.Z., Andersen, G. L., Bakker, P., Raaijmakers, J. (2012). Deciphering the Rhizosphere Microbiome. *Science*, *1097*(2011), 1097–1100. http://doi.org/10.1126/science.1203980

121. Carvalhais, L. C., Dennis, P. G., Badri, D. V., Tyson, G. W., Vivanco, J. M., & Schenk, P. M. (2013). Activation of the Jasmonic Acid Plant Defence Pathway Alters the Composition of Rhizosphere Bacterial Communities. *PLoS ONE*, *8*(2), e56457. http://doi.org/10.1371/journal.pone.0056457

122. Liu, H., Carvalhais, L. C., Schenk, P. M., & Dennis, P. G. (2017). Effects of jasmonic acid signalling on the wheat microbiome differ between body sites. *Scientific Reports*, *7*, 41766. http://doi.org/10.1038/srep41766

123. Carvalhais, L. C., Dennis, P. G., Badri, D. V., Kidd, B. N., Vivanco, J. M., & Schenk, P. M. (2015). Linking Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. *Molecular Plant-Microbe Interactions*, *28*(9), 1049–1058. http://doi.org/10.1094/MPMI-01-15-0016-R

124. Miché, L., Battistoni, F., Gemmer, S., Belghazi, M., & Reinhold-Hurek, B. (2006). Upregulation of jasmonate-inducible defense proteins and differential colonization of roots of *Oryza sativa* cultivars with the endophyte *Azoarcus* sp. *Molecular Plant-Microbe Interactions* , *19*(5), 502–511. http://doi.org/10.1094/MPMI-19-0502

125. Martínez-Medina, A., Appels, F. V. W., & van Wees, S. C. M. (2017). Impact of salicylic acid- and jasmonic acid-regulated defences on root colonization by *Trichoderma harzianum* T-78. *Plant Signaling and Behavior*, *12*(8). http://doi.org/10.1080/15592324.2017.1345404

126. Varma, A., Verma, S., Sudha, Sahay, N., Bütehorn, B., & Franken, P. (1999). *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Applied and Environmental Microbiology*, *65*(6), 2741–2744. http://doi.org/0099-2240/99/$04.00?0
127. Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., … Kogel, K.-H. (2005). The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the National Academy of Sciences*, *102*(38), 13386–13391. http://doi.org/10.1073/pnas.0504423102
128. Jacobs, S., Zechmann, B., Molitor, A., Trujillo, M., Petutschnig, E., Lipka, V., … Schafer, P. (2011). Broad-Spectrum Suppression of Innate Immunity Is Required for Colonization of *Arabidopsis* Roots by the Fungus *Piriformospora indica*. *Plant Physiology*, *156*(2), 726–740. http://doi.org/10.1104/pp.111.176446
129. Zuccaro, A., Lahrmann, U., Güldener, U., Langen, G., Pfiffi, S., Biedenkopf, D., … Kogel, K. H. (2011). Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica*. *PLoS Pathogens*, *7*(10). http://doi.org/10.1371/journal.ppat.1002290
130. Baker, K., & Cook, R. J. (1974). *Biological control of plant pathogens.* San Francisco: W.H. Freeman and Company.
131. Conrath, U., Beckers, G. J. M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., … Mauch-Mani, B. (2006). Priming: Getting Ready for Battle. *Molecular Plant-Microbe Interactions*, *19*(10), 1062–1071. http://doi.org/10.1094/MPMI-19-1062
132. Van Wees, S. C. M., Luijendijk, M., Smoorenburg, I., van Loon, L. C., & Pieterse, C. M. J. (1999). Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. *Plant Molecular Biology*, *41*(4), 537–549. http://doi.org/10.1023/A:1006319216982
133. Pieterse, C. M. J., Van Wees, S. C. M., Ton, J., Van Pelt, J. A., & Van Loon, L. C. (2002). Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *Plant Biology*, *4*(5), 535–544. http://doi.org/10.1055/s-2002-35441
134. Pieterse, C. M. J., Van Pelt, J. A., Ton, J., Parchmann, S., Mueller, M. J., Buchala, A. J., … Van Loon, L. C. (2000). Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. *Physiological and Molecular Plant Pathology*, *57*(3), 123–134. http://doi.org/10.1006/pmpp.2000.0291
135. Djonović, S., Vargas, W. A., Kolomiets, M. V., Horndeski, M., Wiest, A., & Kenerley, C. M. (2007). A Proteinaceous Elicitor Sm1 from the Beneficial Fungus *Trichoderma virens* Is Required for Induced Systemic Resistance in Maize. *Plant Physiology*, *145*(3), 875–889. http://doi.org/10.1104/pp.107.103689
136. Martínez-Medina, A., Roldán, A., Albacete, A., & Pascual, J. A. (2011). The interaction with arbuscular mycorrhizal fungi or *Trichoderma harzianum* alters the shoot hormonal profile in

melon plants. *Phytochemistry*, *72*(2–3), 223–229. http://doi.org/10.1016/j.phytochem.2010.11.008

137. Shavit, R., Ofek-Lalzar, M., Burdman, S., & Morin, S. (2013). Inoculation of tomato plants with rhizobacteria enhances the performance of the phloem-feeding insect *Bemisia tabaci*. *Frontiers in Plant Science*, *4*(8), 306. http://doi.org/10.3389/fpls.2013.00306
138. Dean, J. M., Mescher, M. C., & De Moraes, C. M. (2014). Plant dependence on rhizobia for nitrogen influences induced plant defenses and herbivore performance. *International Journal of Molecular Sciences*, *15*(1), 1466–1480. http://doi.org/10.3390/ijms15011466
139. Van Wees, S. C., Van der Ent, S., & Pieterse, C. M. (2008). Plant immune responses triggered by beneficial microbes. *Current Opinion in Plant Biology*, *11*(4), 443–448. http://doi.org/10.1016/j.pbi.2008.05.005
140. Pieterse, C. M. J., Van Wees, S. C. M., Van Pelt, J. A., Knoester, M., Laan, R., Gerrits, H., … Van Loon, L. C. (1998). A Novel Signaling Pathway Controlling Induced Systemic Resistance in *Arabidopsis*. *The Plant Cell*, *10*(9), 1571–1580. http://doi.org/10.1105/tpc.10.9.1571
141. Knoester, M., Pieterse, C. M. J., Bol, J. F., & Van Loon, L. C. (1999). Systemic Resistance in Arabidopsis Induced by Rhizobacteria Requires Ethylene-Dependent Signaling at the Site of Application. *Molecular Plant-Microbe Interactions*, *12*(8), 720–727. http://doi.org/10.1094/MPMI.1999.12.8.720
142. Yan, Z., Reddy, M. S., Ryu, C.-M., McInroy, J. A., Wilson, M., & Kloepper, J. W. (2002). Induced Systemic Protection Against Tomato Late Blight Elicited by Plant Growth-Promoting Rhizobacteria. *Phytopathology*, *92*(12), 1329–1333. http://doi.org/10.1094/PHYTO.2002.92.12.1329
143. Ryu, C.-M., Murphy, J. F., Mysore, K. S., & Kloepper, J. W. (2004). Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic virus* by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. *The Plant Journal*, *39*(3), 381–392. http://doi.org/10.1111/j.1365-313X.2004.02142.x
144. Shoresh, M., Yedidia, I., & Chet, I. (2005). Involvement of Jasmonic Acid/Ethylene Signaling Pathway in the Systemic Resistance Induced in Cucumber by *Trichoderma asperellum* T203. *Phytopathology*, *95*(1), 76–84. http://doi.org/10.1094/PHYTO-95-0076
145. Ahn, I.-P., Lee, S.-W., & Suh, S.-C. (2007). Rhizobacteria-Induced Priming in *Arabidopsis* Is Dependent on Ethylene, Jasmonic Acid, and *NPR1*. *Molecular Plant-Microbe Interactions*, *20*(7), 759–768. http://doi.org/10.1094/MPMI-20-7-0759
146. De Vleesschauwer, D., Bakker, P. A. H. M., Djavaheri, M., & Hofte, M. (2008). *Pseudomonas fluorescens* WCS374r-induced systemic resistance in rice against *Magnaporthe oryzae* is based on pseudobactin-mediated priming for a salicylic acid-repressible multifaceted defense response. *Plant Physiology*, *148*, 1996–2012. https://doi.org/10.1104/pp.108.127878
147. Hase, S., Takahashi, S., Takenaka, S., Nakaho, K., Arie, T., Seo, S., … Takahashi, H. (2008). Involvement of jasmonic acid signalling in bacterial wilt disease resistance induced by biocontrol agent *Pythium oligandrum* in tomato. *Plant Pathology*, *57*(5), 870–876. http://doi.org/10.1111/j.1365-3059.2008.01858.x

148. Hossain, M. M., Sultana, F., Kubota, M., & Hyakumachi, M. (2008). Differential inducible defense mechanisms against bacterial speck pathogen in Arabidopsis thaliana by plant-growth-promoting-fungus *Penicillium sp*. GP16-2 and its cell free filtrate. *Plant and Soil*, *304*(1), 227–239. http://doi.org/10.1007/s11104-008-9542-3

149. Korolev, N., Rav David, D., & Elad, Y. (2008). The role of phytohormones in basal resistance and *Trichoderma*-induced systemic resistance to *Botrytis cinerea* in *Arabidopsis thaliana*. *Biological Control*, *53*(4), 667–683. http://doi.org/10.1007/s10526-007-9103-3

150. Stein, E., Molitor, A., Kogel, K.-H., & Waller, F. (2008). Systemic Resistance in *Arabidopsis* Conferred by the Mycorrhizal Fungus *Piriformospora indica* Requires Jasmonic Acid Signaling and the Cytoplasmic Function of NPR1. *Plant and Cell Physiology*, *49*(11), 1747–1751. http://dx.doi.org/10.1093/pcp/pcn147

151. Weller, D. M., Mavrodi, D. V, Pelt, J. a Van, Pieterse, C. M. J., Loon, L. C. Van, & Bakker, P. a H. M. (2012). Induced Systemic Resistance in *Arabidopsis thaliana* against *Pseudomonas syringae* pv. tomato by 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens*. *Phytopathology*, *102*(4), 403–412. http://doi.org/10.1094/PHYTO-08-11-0222

152. Pangesti, N., Reichelt, M., van de Mortel, J. E., Kapsomenou, E., Gershenzon, J., van Loon, J. J. A., … Pineda, A. (2016). Jasmonic Acid and Ethylene Signaling Pathways Regulate Glucosinolate Levels in Plants During Rhizobacteria-Induced Systemic Resistance Against a Leaf-Chewing Herbivore. *Journal of Chemical Ecology*, *42*(12), 1212–1225. http://doi.org/10.1007/s10886-016-0787-7

153. Van der Ent, S., Verhagen, B. W. M., Van Doorn, R., Bakker, D., Verlaan, M. G., Pel, M. J. C., … Pieterse, C. M. J. (2008). MYB72 Is Required in Early Signaling Steps of Rhizobacteria-Induced Systemic Resistance in *Arabidopsis*. *Plant Physiology*, *146*(3), 1293–1304. http://doi.org/10.1104/pp.107.113829

154. Segarra, G., Van Der Ent, S., Trillas, I., & Pieterse, C. M. J. (2009). MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. *Plant Biology*, *11*(1), 90–96. http://doi.org/10.1111/j.1438-8677.2008.00162.x

155. Audenaert, K., Pattery, T., Cornelis, P., & Höfte, M. (2002). Induction of Systemic Resistance to *Botrytis cinerea* in Tomato by *Pseudomonas aeruginosa* 7NSK2: Role of Salicylic Acid, Pyochelin, and Pyocyanin. *Molecular Plant-Microbe Interactions*, *15*(11), 1147–1156. http://doi.org/10.1094/MPMI.2002.15.11.1147

156. Tjamos, S. E., Flemetakis, E., Paplomatas, E. J., & Katinakis, P. (2005). Induction of Resistance to *Verticillium dahliae* in *Arabidopsis thaliana* by the Biocontrol Agent K-165 and Pathogenesis-Related Proteins Gene Expression. *Molecular Plant-Microbe Interactions*, *18*(6), 555–561. http://doi.org/10.1094/MPMI-18-0555

157. Van de Mortel, J. E., de Vos, R. C. H., Dekkers, E., Pineda, A., Guillod, L., Bouwmeester, K., … Raaijmakers, J. M. (2012). Metabolic and Transcriptomic Changes Induced in *Arabidopsis* by the Rhizobacterium *Pseudomonas fluorescens* SS101. *Plant Physiology*, *160*(4), 2173–2188. http://doi.org/10.1104/pp.112.207324

158. Conn, V.M., Walker, A. R., & Franco, C. M. M. (2008). Endophytic Actinobacteria Induce Defense Pathways in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions*, *21*(2), 208–218. http://doi.org/10.1094/MPMI-21-2-0208
159. Contreras-Cornejo, H. A., Macías-Rodríguez, L., Beltrán-Peña, E., Herrera-Estrella, A., & López-Bucio, J. (2011). *Trichoderma*-induced plant immunity likely involves both hormonal- and camalexin-dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungus *Botrytis cinerea*. *Plant Signaling and Behavior*, *6*(10), 1554–1563. http://doi.org/10.4161/psb.6.10.17443
160. Mathys, J., De Cremer, K., Timmermans, P., Van Kerkhove, S., Lievens, B., Vanhaecke, M., … De Coninck, B. (2012). Genome-Wide Characterization of ISR Induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 Against *Botrytis cinerea* Infection. *Frontiers in Plant Science*, *3*, 108. http://doi.org/10.3389/fpls.2012.00108
161. Martínez-Medina, A., Fernández, I., Sánchez-Guzmán, M. J., Jung, S. C., Pascual, J. A., & Pozo, M. J. (2013). Deciphering the hormonal signalling network behind the systemic resistance induced by *Trichoderma harzianum* in tomato. *Frontiers in Plant Science*, *4*(6), 206. http://doi.org/10.3389/fpls.2013.00206
162. Alizadeh, H., Behboudi, K., Ahmadzadeh, M., Javan-Nikkhah, M., Zamioudis, C., Pieterse, C. M. J., & Bakker, P. A. H. M. (2013). Induced systemic resistance in cucumber and *Arabidopsis thaliana* by the combination of *Trichoderma harzianum* Tr6 and *Pseudomonas* sp. Ps14. *Biological Control*, *65*(1), 14–23. http://doi.org/10.1016/j.biocontrol.2013.01.009
163. Pozo, M. J., & Azcón-Aguilar, C. (2007). Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology*, *10*(4), 393–398. http://doi.org/10.1016/j.pbi.2007.05.004
164. Pozo, M. J., Jung, S. C., López-Ráez, J. A., & Azcón-Aguilar, C. (2010). Impact of Arbuscular Mycorrhizal Symbiosis on Plant Response to Biotic Stress: The Role of Plant Defence Mechanisms. In H. Koltai & Y. Kapulnik (Eds.), *Arbuscular Mycorrhizas: Physiology and Function* (pp. 193–207). Dordrecht: Springer. http://doi.org/10.1007/978-90-481-9489-6
165. Jung, S. C., Martínez-Medina, A., López-Ráez, J. A., & Pozo, M. J. (2012). Mycorrhiza-Induced Resistance and Priming of Plant Defenses. *Journal of Chemical Ecology*, *38*(6), 651–664. http://doi.org/10.1007/s10886-012-0134-6
166. Whipps, J. M. (2004). Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany*, *82*(8), 1198–1227. http://doi.org/10.1139/b04-082
167. Gernns, H., Alten, H., & Poehling, H.-M. (2001). Arbuscular mycorrhiza increased the activity of a biotrophic leaf pathogen – is a compensation possible? *Mycorrhiza*, *11*(5), 237–243. http://doi.org/10.1007/s005720100128
168. Liu, J., Maldonado-Mendoza, I., Lopez-Meyer, M., Cheung, F., Town, C. D., & Harrison, M. J. (2007). Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. *The Plant Journal*, *50*(3), 529–544. http://doi.org/10.1111/j.1365-313X.2007.03069.x

169. Fritz, M., Jakobsen, I., Lyngkjær, M. F., Thordal-Christensen, H., & Pons-Kühnemann, J. (2006). Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. *Mycorrhiza*, *16*(6), 413–419. http://doi.org/10.1007/s00572-006-0051-z
170. De La Noval, B., Pérez, E., Martínez, B., León, O., Martínez-Gallardo, N., & Délano-Frier, J. (2007). Exogenous systemin has a contrasting effect on disease resistance in mycorrhizal tomato (*Solanum lycopersicum*) plants infected with necrotrophic or hemibiotrophic pathogens. *Mycorrhiza*, *17*(5), 449–460. http://doi.org/10.1007/s00572-007-0122-9
171. Hartley, S. E., & Gange, A. C. (2009). Impacts of Plant Symbiotic Fungi on Insect Herbivores: Mutualism in a Multitrophic Context. *Annual Review of Entomology*, *54*(1), 323–342. http://doi.org/10.1146/annurev.ento.54.110807.090614
172. Pozo, M. J., Cordier, C., Dumas-Gaudot, E., Gianinazzi, S., Barea, J. M., & Azcón-Aguilar, C. (2002). Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. *Journal of Experimental Botany*, *53*(368), 525–534. http://dx.doi.org/10.1093/jexbot/53.368.525
173. Gange, A. C. (2007). Insect-mycorrhizal interactions: patterns, processes, and consequences. In T. Ohgushi, T. P. Craig, & P. W. Price (Eds.), *Ecological Communities: Plant Mediation in Indirect Interaction Webs* (pp. 124–144). Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511542701.007
174. Campos-Soriano, L., García-Martínez, J., & Segundo, B. S. (2012). The arbuscular mycorrhizal symbiosis promotes the systemic induction of regulatory defence-related genes in rice leaves and confers resistance to pathogen infection. *Molecular Plant Pathology*, *13*(6), 579–592. http://doi.org/10.1111/j.1364-3703.2011.00773.x
175. Song, Y., Chen, D., Lu, K., Sun, Z., & Zeng, R. (2015). Enhanced tomato disease resistance primed by arbuscular mycorrhizal fungus. *Frontiers in Plant Science*, *6*(9), 786. http://doi.org/10.3389/fpls.2015.00786
176. Nair, A., Kolet, S. P., Thulasiram, H. V., & Bhargava, S. (2015). Systemic jasmonic acid modulation in mycorrhizal tomato plants and its role in induced resistance against *Alternaria alternata*. *Plant Biology*, *17*(3), 625–631. http://doi.org/10.1111/plb.12277
177. Cordier, C., Pozo, M. J., Barea, J. M., Gianinazzi, S., & Gianinazzi-Pearson, V. (1998). Cell Defense Responses Associated with Localized and Systemic Resistance to *Phytophthora parasitica* Induced in Tomato by an Arbuscular Mycorrhizal Fungus. *Molecular Plant-Microbe Interactions*, *11*(10), 1017–1028. http://doi.org/10.1094/MPMI.1998.11.10.1017
178. Garmendia, I., Goicoechea, N., & Aguirreolea, J. (2004). Effectiveness of three *Glomus* species in protecting pepper (*Capsicum annuum L.*) against verticillium wilt. *Biological Control*, *31*(3), 296–305. http://doi.org/https://doi.org/10.1016/j.biocontrol.2004.04.015
179. Kobra, N., Jalil, K., & Youbert, G. (2009). Effects of three *Glomus* species as biocontrol agents against verticillium-induced wilt in cotton. *Journal of Plant Protection Research*, *49*(2), 185–189. http://doi.org/10.2478/v10045-009-0027-z

180. Sikes, B. A., Cottenie, K., & Klironomos, J. N. (2009). Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology*, *97*(6), 1274–1280. http://doi.org/10.1111/j.1365-2745.2009.01557.x

181. Bernaola, L., Cosme, M., Schneider, R. W., & Stout, M. (2018). Belowground Inoculation With Arbuscular Mycorrhizal Fungi Increases Local and Systemic Susceptibility of Rice Plants to Different Pest Organisms. *Frontiers in Plant Science*, *9*(6), 747. http://doi.org/10.3389/fpls.2018.00747

182. Pozo, M. J., Verhage, A., García-Andrade, J., García, J. M., & Azcón-Aguilar, C. (2009). Priming Plant Defence Against Pathogens by Arbuscular Mycorrhizal Fungi. In C. Azcón-Aguilar, J. M. Barea, S. Gianinazzi, & V. Gianinazzi-Pearson (Eds.), *Mycorrhizas- Functional Processes and Ecological Impact* (pp. 123–135). Berlin Heidelberg: Springer. https://doi.org/10.1007/978-90-481-9489-6_9

183. Adolfsson, L., Nziengui, H., Abreu, I. N., Šimura, J., Beebo, A., Herdean, A., … Spetea, C. (2017). Enhanced Secondary- and Hormone Metabolism in Leaves of Arbuscular Mycorrhizal *Medicago truncatula*. *Plant Physiology*, *175*(9), 392-411. http://doi.org/10.1104/pp.16.01509

184. Holland, J. N., Cheng, W., & Crossley, D. A. (1996). Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. *Oecologia*, *107*(1), 87–94. http://doi.org/10.1007/BF00582238

185. Schultz, J. C., Appel, H. M., Ferrieri, A. P., & Arnold, T. M. (2013). Flexible resource allocation during plant defense responses. *Frontiers in Plant Science*, *4*(8), 324. http://doi.org/10.3389/fpls.2013.00324

Chapter 2: MiSSP7 alters the interactome of PtJAZ6

Chapter 2: MiSSP7 alters the interactome of PtJAZ6

1. Preface

The mutualistic effector protein MiSSP7, secreted by the mycelium of the ectomycorrhizal fungus *Laccaria bicolor* upon perception of host root exudates (Plett et al., 2011), can enter nuclei of western balsam poplar (*Populus trichocarpa*) roots, interact with the poplar co-receptor of jasmonic acid (JA) and dampen the expression of JA-regulated genes (Plett et al., 2014a). The functionality of the *LbMiSSP7* gene has been proven crucial for the development of the putative surface of symbiotic nutrient exchange, the Hartig net (Plett et al., 2014a). In Arabidopsis JAZ proteins repress the activity of JA-dependent transcription factors (TFs) and are targeted for 26S-proteasome-mediated degradation upon JA perception, releasing such TFs and allowing JA-dependent transcription (Howe et al., 2018). Since MiSSP7 stabilizes PtJAZ6 in a dose-dependent manner, it has been suggested that this effector protein hampers JA-dependent gene expression by maintaining the repression over JA-responsive TFs (Plett et al., 2014a).

However, the influence of the MiSSP7-PtJAZ6 interaction over the transcriptomic network of poplar root cells is still unknown. For instance, the composition of the PtJAZ6-associated protein complex in poplar roots was never investigated. Consequently, it is not clear whether the stabilization of PtJAZ6 impacts the activity of downstream TFs. In addition, different PtJAZ6-interacting TFs may specifically regulate the expression of a determined group of target genes. Indeed, in Arabidopsis, JAZ proteins interact with a wide array of TFs, each of which is responsible for a hierarchical cascade of gene expression leading to the activation of specific JA-dependent responses (see Chapter 1, Paragraph 2.1.4; Chini et al., 2016; Howe et al., 2018). The identification of PtJAZ6-associated TFs in poplar roots is therefore crucial for the dissection of the role of JA signaling in ectomycorrhizal development. Different TFs might regulate distinct branches of JA signaling, some of which could be perturbed in presence of fungal effector proteins.

To gain insight into the alteration of JA perception and responses during ectomycorrhizal development, we first set out to determine the composition of the PtJAZ6-associated protein complex in poplar roots. To this aim, we performed a yeast-two-hybrid screen, and identified a poplar bHLH TF interacting with PtJAZ6. Subsequently, we tested further candidate PtJAZ6-interacting poplar proteins, based on their homology to Arabidopsis, in yeast and tobacco. We found that PtJAZ6 interacts with the poplar homologs of the main regulators of JA-dependent defense responses and root development in Arabidopsis: the transcriptional activators PtMYC2.1 (Potri.001G142200) and PtMYC2.2 (Potri.003G092200) and the transcriptional repressors PtJAM1.1 (Potri.014G099700) and PtJAM1.2 (Potri.002G172100).

We confirmed these interactions *in planta*. Subsequently, we tested via yeast-three-hybrid whether MiSSP7 altered the binding specificities of PtJAZ6 to its associated proteins. We found that the establishment of PtJAZ homo- and heterodimers is impaired in the presence of the fungal effector. Interestingly, we observed that MiSSP7 strengthens the interaction between PtJAZ6 and PtMYC2.1, while releasing PtMYC2.2 from this transcriptional inhibitor. These interaction dynamics suggest the presence of multiple branches of JA signaling in poplar roots, and that MiSSP7 preferentially represses the PtMYC2.1-regulated branch. During the course of this project, the existence of distinct branches of JA signaling with different functional roles in the establishment of symbiosis has also been explored via phenotyping of poplar roots with altered expression of *PtaMYC2* and *PtaJAM1* (Chapter 3), as well as via transcriptomic analysis of JA-treated poplar-*L. bicolor* ECM (Chapter 4). The present chapter is under review for publication in the journal Scientific Reports (16th of September 2019).

2. Article (submitted)

TITLE: The mutualism effector MiSSP7 of *Laccaria bicolor* alters poplar JAZ6 protein containing complex.

Authors: Yohann Daguerre[*1†], Veronica Basso[*1], Sebastian Wittulski[1], Romain Schellenberger[1], Laura Meyer[1], Justine Bailly[1], Annegret Kohler[1], Jonathan M. Plett[1, §], Francis Martin[1], Claire Veneault-Fourrey[$1]

* co-first authors

Yohann.Daguerre@slu.se, veronica.basso@inra.fr, S.Wittulsky@gmx.de, schellenberger.romain@orange.fr, laurameyer68140@gmail.com, justine.bailly4@outlook.fr, annegret.kohler@inra.fr, J.Plett@uws.edu.au, francis.martin@inra.fr, claire.veneault-fourrey@inra.fr

[1] Université de Lorraine/INRA, UMR 1136, Interactions Arbres/Microorganismes (IAM), Centre INRA de Nancy, Champenoux, France

[§] Current address: Hawkesbury Institute for the Environment; Western Sydney University; Penrith, NSW, Australia, 2751

↟ Current address: Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden

[$] **Corresponding Author**: Claire Veneault-Fourrey

claire.veneault-fourrey@inra.fr; +33 (0)383394081

Running title: MiSSP7 alters PtJAZ6 interactome.

Date of submission: 13 June, 2019

Number of tables: 0

Number of figures: 6

Number of Supplemental figures: 5

Number of Supplemental tables: 2

Word Count: 6,097

Highlight: A fungal mutualistic effector altered dynamics of JA-signaling-associated protein-protein interaction networks and likely maintains the repression of MYC2.1-regulated genes.

2.1. Abstract

Despite the pivotal role jasmonic acid in the outcome of plant-microorganism interactions, JA-signaling components in roots of perennial trees like western balsam poplar (*Populus trichocarpa*) are poorly characterized. Here we decipher the composition of the poplar-root JA-perception complex centered on PtJAZ6, a co-repressor of JA-signaling targeted by the effector protein MiSSP7 from the ectomycorrhizal basidiomycete *Laccaria bicolor* during symbiotic development. Through protein-protein interaction studies in yeast and *in planta* we determined the poplar root-specific interactome of PtJAZ6. We assessed via yeast triple-hybrid how the mutualistic effector MiSSP7 reshapes the PtJAZ6-associated protein complex. In the absence of the symbiotic effector, PtJAZ6 interacts with the transcription factors PtMYC2 and PtJAM1.1, with one TOPLESS-related protein and two NINJA proteins. PtJAZ6 interacts with it-self and with other *Populus* JAZ proteins. MiSSP7 strengthens binding of PtJAZ6 to PtMYC2.1 and antagonizes PtJAZ6 homo-/heterodimerization. We conclude that a symbiotic effector secreted by a mutualistic fungus may promote the symbiotic interaction through altered dynamics of JA-signaling-associated protein-protein interaction networks and maintain the repression of PtMYC2.1-regulated genes.

Keywords: Ectomycorrhiza; Effector; Jasmonate; JAZ; protein-protein interaction; Symbiosis

2.2. Introduction

As an adaptation to nutrient (N,P)-limiting conditions, the root systems of forest trees interact with the hyphae of mutualistic ectomycorrhizal (ECM) fungi to form a hybrid organ called ectomycorrhizal root tip. To build this tissue, ECM fungal hyphae first grow towards lateral roots and aggregate on the root surface forming a hyphal sheath called the mantle around root tips. In a second step, fungal hyphae grow within the apoplastic space of root cortical cells to form the biotrophic interface named the Hartig net. This interface is the site of bi-directional nutrient exchanges between root cells and fungal cells whereby water and growth-limiting nutrients are sourced by the soil-borne extraradical mycelium are exchanged with photosynthetic carbon from the host tree (Bücking et al., 2007; Nehls, 2007; Nehls et al., 2008). In addition to improving tree nutrition, ectomycorrhizal fungi stimulate the plant immune system, leading to induced systemic resistance (ISR) against root and shoot fungal pathogens and aboveground herbivores (Gange et al., 2005; Kaling et al., 2018). Furthermore, ECM fungi enhance tree tolerance to salinity, drought and heavy metals (Beniwal et al., 2010; Li et al., 2012; Pena et al., 2013; Ma et al., 2014; Danielsen and Polle,

2014; Luo et al., 2014 and references therein). Despite the crucial importance of ECM fungi for the nutrition and health of trees, identification of master regulators involved in the molecular dialogue between plant and fungal cells is still in its infancy. Establishment, development and maintenance of functional ectomycorrhizal root tips require a strict coordination of development, immunity and physiology of both fungal and plant cells. The control of these processes is likely to occur, in part, through phytohormones. Exploiting the mutualistic interaction occurring between *Laccaria bicolor* and *Populus* spp. as a model to study ECM symbiosis at the molecular level, we have previously demonstrated that hyphae of the fungus *L. bicolor* secrete Mycorrhiza-induced Small Secreted Proteins (MiSSPs) in the vicinity of poplar roots (Martin et al., 2008; Martin et al., 2016). MiSSP7, a symbiotic effector required for ECM development (Plett et al., 2011a), hinders jasmonate (JA)-mediated responses in poplar root cells (Plett et al., 2014a). To do so, MiSSP7 physically interacts with the *Populus* JAZ6 (PtJAZ6) protein in nuclei of host cells. This interaction inhibits degradation of PtJAZ6 in presence of jasmonic acid and consequently suppresses JA-dependent responses allowing *in planta* fungal colonization. In addition, exogenous treatment with MeJA inhibits the development of ectomycorrhizal root tips; in particular the formation of the Hartig net (Plett et al., 2014b). These studies highlight that the mutualistic fungus *L. bicolor* hijacks the host JA-signaling pathway, but in an opposite manner as compared to biotrophic plant-pathogenic microbes (Thatcher et al., 2009; Jacobs et al., 2011; Zheng et al., 2012).

Jasmonates (JAs) are well-known plant defense hormones involved in responses to wounding and herbivory (Bari and Jones, 2009; De Geyter et al., 2012; Wasternack and Strnad, 2019). Furthermore, JAs regulate plant developmental processes such as root development (Huang et al., 2017), fertility (Song et al., 2013), senescence (Qi et al., 2015a), secondary metabolites production (De Geyter et al., 2012) and response to abiotic stress (Dar et al., 2015; Riemann et al., 2015; Kazan, 2015; Sharma and Laxmi, 2016; Goossens et al., 2016). During the last decade, considerable effort has been devoted to decipher the JA-signaling pathway, JA-triggered responses and how the JA-signaling pathway is integrated with other hormone-mediated responses (Pieterse et al., 2012; Wasternack and Hause, 2013; Ahmad et al., 2016; Huang et al., 2017; Howe et al., 2018). The majority of studies on JA pathways have mainly been performed in the leaves of *Arabidopsis*, tomato or rice.

In these well-studied model systems, the bioactive JA-hormone (i.e. (+)-7- iso-jasmonoyl-L-Ile (Fonseca et al., 2009), is perceived by a co-receptor complex consisting of dimerized JASMONATE ZIM-DOMAIN (JAZ) proteins, which form part of a transcription factor (TF) repression complex, and the F-box protein CORONATIN INSENSITIVE1 (COI1), which is part of a Skp1/Cullin/F-box E3 ubiquitin ligase (Feys et al., 1994; Xie et al., 1998). JA perception leads to ubiquitination of the JAZ proteins, tagging them to 26S-proteasome-

mediated degradation, thereby releasing inhibition of downstream TFs and initiating activation of JA-mediated responses. Therefore, members of the JAZ family are key repressors of JA-mediated hormone responses. JAZ proteins interact with a wide range of transcription factors (TFs) and are thus major hubs integrating environmental and developmental cues in order to modulate several developmental and physiological processes on the basis of a growth-to-defense energetic trade-off (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Howe et al., 2018).

The *JAZ* family in *Arabidopsis* is composed of 12 canonical members and the atypical *JAZ13* repressor (Chico et al., 2008; Thireault et al., 2015). Canonical JAZ proteins show three structural domains mediating protein-protein interactions: a variable N-terminal (NT) domain, a highly conserved ZIM domain and a conserved C-terminal JA-associated (Jas) domain. This last domain is required for binding to COI1 and TFs (Katsir et al., 2008; Pauwels and Goossens, 2011; Chini et al., 2016). The central ZIM domain contains a highly conserved TIFY motif (TIF(F/Y)XG) required for homo- and heterodimerization of JAZ proteins as well as for the recruitment of TOPLESS (TPL) or TOPLESS-RELATED (TPR) transcriptional repressors, either directly bound through an ETHYLENE RESPONSIVE FACTORS-associated amphiphilic repression (EAR) motif or indirectly bound through the NOVEL INTERACTOR OF JAZ (NINJA) bridge protein (Chini et al., 2009; Chung and Howe, 2009, Pauwels et al., 2010).

In *Arabidopsis* JAZ-interacting TFs include both transcriptional activators and repressors modulating several developmental and physiological processes, e.g. suppression of root elongation, biosynthesis of secondary metabolites, trichome development and pollen maturation (Pauwels and Goossens, 2011; Chini et al., 2016; Wasternack and Song, 2017; Howe et al., 2018). JA-signaling activators include basic helix-loop-helix (bHLH) proteins such as MYC2, which are involved in plant defense, glucosinolates biosynthesis, pollen development and growth inhibition (Chini et al., 2007; Fernandez-Calvo et al., 2011; Qi et al., 2015a,b; Figueroa and Browse, 2015; Chini et al., 2016; Howe et al., 2018). JAZ proteins also interact with the transcriptional repressors JAM1/2/3 and bHLH14, which are hypothesized to inhibit excessive defense responses to avoid stunted growth (Nakata et al., 2013; Sasaki-Sekimoto et al., 2013; Fonseca et al., 2014; Song et al., 2013; Qi et al., 2015a,b).

A large component of the knowledge generated on JA-signaling, and JA-signaling-related proteins, involves the use of the model plant *Arabidopsis*. The diversity of JA-dependent responses in other organs such as roots or in perennial plants, however, is less well understood (Acosta et al., 2013; Lu et al., 2015). More importantly, to our knowledge, there are no detailed reports of JA-signaling components in roots of perennial trees that form mycorrhizal interactions within its root system. This latter point is emphasized a critical hole

in our knowledge of this pathway as plant-associated organisms are known to manipulate JA-Ile signaling through the use of effectors, which modify JAZ stability (Jiang et al., 2013; Ma et al., 2015; Gimenez-Ibanez et al., 2014; Plett et al., 2014a; Yang et al., 2017), interfere with the activity of jasmonate-regulated TFs (Li et al., 2014; Yang et al., 2017) dependent or interfere with JA-Ile biosynthesis (Sugio et al., 2011; Geng et al., 2014; Katsir et al., 2008; Sheard et al., 2010). Therefore, to understand the significance of JA-signaling during the interaction of *Populus* with its ECM basidiomycete *L. bicolor* partner, and to expand our understanding of the role of the secreted symbiotic effector MiSSP7 (Plett et al., 2014a), we aimed in the present paper to decipher the JA-perception complex containing PtJAZ6 in poplar ectomycorrhizal roots. We intend to answer the three following questions: Which poplar proteins are interacting with PtJAZ6 in poplar root cells? Which domains of PtJAZ6 are required for such interactions? How does the stabilization of PtJAZ6 by MiSSP7 symbiotic effector MiSSP7 impact the composition and stability of the complex?

2.3. Materials and methods

Gene cloning and editing of PtJAZ6

PtJAZs, *PtMYC2s*, *PtJAM1s*, *PtTPLs*, *PtNINJAs*, *PtCOI1* as well as full-length and domain-deleted versions of *PtJAZ6* were PCR-amplified from *Populus trichocarpa* root cDNA and cloned into entry (pDONR222/pDONR207) and destination vectors (pDEST22/ pDEST32/ pB7WGF2.0 or pK7WGR2.0) using Gateway recombination (Life Technologies). Point mutations in *PtJAZ6* were generated with QuickChange II XL Site-Directed Mutagenesis Kit (Agilent) on the entry vector pDONR222:PtJAZ6. A list of the primers used in this study is available in Table S2. The *MiSSP7* gene (Lacbi2 protein ID: 298595) lacking its N-terminal signal peptide was PCR-amplified from *Populus trichocarpa* root cDNA and cloned into entry (pDONR222) and destination (pZMU-Dest; Grefen, 2014) vectors. The latter vector bears the *URA3* gene as auxotrophic genetic marker.

Yeast two–hybrid screen and assays

Yeast two-hybrid screens with PtJAZ6 as bait and yeast two-hybrid assays were carried out as described in Plett et al. (2011b) and Walhout and Vidal (2001), using cDNA library constructed from *L. bicolor-Populus* ectomycorrhiza root tips. *Populus* gene and protein IDs used in this study are listed in Table S1. MaV203 *(MATα, leu2-3,112, trp1-901, his3Δ200, ade2-101, gal4Δ, gal80Δ,SPAL10::URA3, GAL1::lacZ, HIS3UAS GAL1::HIS3@LYS2, can1R, cyh2R)* and MaV103 *(MATa, leu2-3,112, trp1-901, his3Δ200, ade2-101, gal4Δ,*

gal80Δ, SPAL10::URA3, GAL1::lacZ, HIS3UAS GAL1::HIS3@LYS2, can1R, cyh2R) yeast strains were used. Three control yeasts colonies are included : Krev1/RalGDS-*wt* as a strong positive interaction, Krev1/RalGDS-*m1* as a weak interaction, and Krev1/RalGDS-*m2* as a no interaction control (ProQuest YTH system, Invitrogen). The three reporter genes available within the system are *URA3*, *HIS3* and *LacZ*, allowing yeast growth on medium lacking uracil or histidine or the production of the β-galactosidase enzyme, respectively. Amino-1, 2, 4-triazole (3-AT) at 25mM was used to suppress self-activation at the *HIS3* gene.

Yeast triple hybrid assays

MaV203 yeast containing the vectors pDEST22 and pDEST32 with various constructs were grown in 5 mL of liquid SD-LW medium in shaker at 28°C and 200 rpm for 16h. Cultures were then diluted to an OD_{600} of 0.6 in a five mL volume of liquid SD-LW medium and grown in a shaker using the same conditions until they reached an OD_{600} of 2. Cultures were subsequently centrifuged for five minutes at 4°C and 3000 rpm for five minutes and washed three times with sterile water. 29 µL of the washed yeast culture were added to the transformation mix (240 µL of 50% PEG3350, 36 µL of 1M LiAC and 50 µL of 2 mg/mL salmon sperm ssDNA) and in presence of 200 ng of pZMU-Dest plasmid in which *MiSSP7* was cloned. Tubes were mixed, then incubated in a water bath at 42°C for 45 min. Tubes were then centrifuged rpm for five minutes at 9000 rpm and the supernatant discarded. The pellet was resuspended in 50 µL of liquid SD-LW medium and plated on SD-LWU plates. Plates were sealed with parafilm and incubated in static oven at 28°C for 72h. Transformed colonies were tested via PCR for presence of *MiSSP7* and then stored in glycerol in freezer at -80°C. The Drop test was similarly performed for yeast-triple hybrid assays as for the yeast-two hybrid assays, with the difference that all solid and liquid media also lacked Uracil, to select for the *MiSSP7*- containing vector pZMU-Dest.

β-galactosidase reporter assay

The activity of the yeast β-galactosidase, a subunit which is coded by the *GAL*1::*lacZ* reporter, was tested according to a previously published method (Walhout and Vidal, 2001). Briefly, yeast patches were replica-plated onto a YAPD plates covered by 0.45 µM nitrocellulose filters (Hybond™-C, Amersham Biosciences) and incubated overnight in static incubator at 30°C. Two Whatman filter papers were placed in an empty 15-cm Petri dish and soaked with 3 mL of Z-buffer X-Gal solution (16.1 g/L $Na_2HPO_4*7H_2O$, 5.5 g/L $NaH_2PO_4*H_2O$, 0.75 g/L KCl, 0.246 g/L $MgSO_4*7H_2O$, pH 7.0) supplemented with 1.83 µL/mL of 2-mercaptoethanol and 16.66 µL/mL of 4% X-Gal (4-Methylumbelliferyl-beta-D-galactopyranoside, BIOSINTH®). The nitrocellulose membranes with the yeast streaks were then detached from the YAPD plates, submitted to two freeze-thaw cycles in liquid nitrogen

and placed on the Z-buffer-soaked Whatman filter papers. Plates were incubated at 37°C for ~6h, after which pictures were taken. The white color indicates lack of β-galactosidase activity and thus lack of expression of the reporter construct *GAL*1::*lacZ*, while progressively darker blue color indicates stronger β-galactosidase activity and thus stronger interaction between AD- and DBD-fused proteins (ProQuest2hybrid manual, Invitrogen).

Plant material and growth conditions

Nicotiana benthamiana seeds were germinated and grown for 3 weeks in a climate chamber at 24°C, 16h photoperiod, 70% relative humidity.

Agroinfiltration of tobacco leaves and localization of the fusion proteins in epidermal cells

Agrobacterium tumefaciens GV3101 transformed with pB7WGF2.0 or pK7WGR2.0 harboring each gene of interest or empty (as control) were cultivated overnight at 28°C in Luria-Bertani medium supplemented with gentamycin 10μg/mL, spectinomycin 50μg/mL and rifampicin 50μg/mL. After centrifugation, bacterial pellets were resuspended in infiltration buffer (10 mM $MgCl_2$, 10 mM MES, 200 μM Acetosyringone, pH 5,6) and incubated for 2h at 28°C with gentle shaking. Bacterial cultures were then mixed at a ratio 1:1 to a final OD_{600} of 0.6 before infiltration of 3-week-old tobacco leaves. In order to increase the expression of the transgene and counteract its potential silencing, an *A. tumefaciens* GV3101 strain expressing the tomato bushy virus (TBSV) suppressor of post-transcriptional gene silencing – P19 was added to the mix at a final OD_{600} of 0.1. Infiltration buffer was used as negative control. After 72h-incubation in a climate chamber (24°C, 16h photoperiod, 70% relative humidity), leaf discs were harvested, mounted between slide and coverslip (glycerol 20%). Live-cell imaging was performed using 10× (air) and 40× (water immersion) objectives of the confocal microscope Zeiss LSM780,. The GFP was excited at 488 nm, whereas the mCherry was excited at 561 nm. Specific emission signals corresponding to the GFP and the mCherry were collected between 505–525 and 580–620 nm, respectively. Alternatively, leaves were harvested and flash-frozen for *in planta* co-immunoprecipitation.

***In planta* co-immunoprecipitation**

Three days after bacterial infiltration, four leaves per transformation were harvested and ground using liquid nitrogen. The resulting powder was resuspended in 10 mL of extraction buffer (10% glycerol, 25 mM Tris pH 7,5, 1mM EDTA, 150 mM NaCl, 5 mM DTT, 0,1% Tergitol NP40S, 200 mg PVPP and 100 μL protease inhibitor [Stock 100x, Sigma, P9599]. PVPP was added in the buffer the night before the extraction while DTT, tergitol and protease inhibitors were added just prior to extraction. Samples were incubated 10 min at

4°C in a rotator and then centrifuged 5 min at 4°C at 9000 rpm. The supernatant was filtered using a cell strainer with 70 μm-wide pores. About 500 μL of supernatant was TCA-precipitated and pellet was resuspended with 60μL of buffer Laemmli + DTT (5mM) and conserved on ice (Total protein fraction or input). Magnetic beads coupled with antiGFP antibodies (GFP-Trap®_M, Chromotek) were used for CoIP. Before use, they were washed twice using washing buffer (10 mM Tris-HCl pH7,5, 150 mM NaCl, 0,5 mM EDTA and protease inhibitors). Each supernatant was mixed with 30 μL of magnetic beads and incubated for 20 min at 4°C in a rotator with slight agitation. Then beads were collected, washed 5 times with 1 ml of washing buffer and resuspended in 75 μL of buffer Laemmli 1x + DTT (5mM) (coimmunoprecipitated fraction).

Western Blot

The input and coimmunoprecipitated fractions were incubated for five minutes at 95°C and centrifuged for one minute at 9,000 rpm. Aliquots of 15 μL were separated on Polyacrylamide gels (Mini-Protean TGX Precast Gels 4-15%., Biorad), followed by a transfer onto PVDF membranes (Bio-Rad Transblot Turbo, Trans-Blot® Turbo™ Transfer System). After transfer, membranes were stained with Ponceau red to control for total protein loading, then de-stained with NaOH 0.1 M and washed with distilled water. Membranes were blocked for 2h at 4°C and 50 rpm in blocking buffer (PBS 1x, 1% casein) followed by incubation with the primary antibody (anti-GFP (GFP Antibody (B-2) HRP, Santa Cruz Biotechnology, 200 μg/mL) or anti-RFP (RFP Tag Antibody (RF5R), Thermo Fisher scientific, 1mg/mL)) diluted 1/1000 in blocking buffer overnight at 4°C and 50 rpm. The antibody anti-GFP is conjugated to HRP allowing a direct detection on the membranes. Membranes incubated with the primary antibody anti-RFP were washed four times for five minutes with PBS + Tween 20 0.05% and incubated with a secondary antibody conjugated to HRP (ab6789 2mg/mL) diluted 1/10000. After immunolabelling, membranes were washed four times for five minutes with PBS + Tween 20 0.05% and imaged five-seven minutes after addition of five mL of substrate (Clarity™ Western ECL Substrate, Biorad). Pictures were taken using Chemidoc (Biorad Molecular Imager).

Phylogenetic analysis

Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016). In all cases, the evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones et al., 1992). The tree with the highest log likelihood is shown. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The trees were drawn to scale,

with branch lengths measured in the number of substitutions per site. 500 bootstraps were performed to assess robustness of the data (Felsenstein J., 1985). FigTree (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize and annotate the tree.

2.4. Results

The transcription factors PtJAM1 and PtMYC2 interact with PtJAZ6

In order to identify PtJAZ6-interacting proteins, we conducted a yeast two-hybrid screen. The full-length coding sequence of PtJAZ6 was cloned in fusion with the DNA-binding domain of GAL4. We first confirmed that this fusion protein is not able to activate the three reporter genes in the absence of an interacting protein (Supplemental Figure S1). Among positive clones, we identified PtJAZ6 (Potri.003G068900), MiSSP7 and a basic-helix-loop-helix (bHLH) transcription factors with similarities with *Arabidopsis* JAM1/2 (orhologous to At1g01260 and At2g46510), that we thus named PtJAM1.1. As the *Populus* genome contains two co-orthologous genes to *AtJAM1* (Figure 1A), we cloned the full-length sequence of the two paralogs *PtJAM1.1* and *PtJAM1.2* in fusion with the activation domain (AD) of *GAL4* and co-expressed them with *PtJAZ6* fused to the DNA-binding (DBD) domain of *GAL4*. In *Arabidopsis* AtJAM1 is a transcriptional repressor and AtJAZ proteins are well known to interact with AtJAMs as well as with the transcriptional activators AtMYC2-5 (Chini et al., 2016). In *Populus* genome, we found only two orthologous genes to *AtMYC2-5.* We named them *PtMYC2.1* and *PtMYC2.2* and we cloned their full-length sequences fused to the AD of GAL4. Yeast strains co-expressing PtJAM1.1/PtJAZ6 or PtMYC2.1/PtJAZ6 or PtMYC2.2/PtJAZ6 were able to grow on medium lacking histidine or uracil and activate □-galactosidase reporter gene, whereas yeasts co-expressing PtJAM1.2/PtJAZ6 activate only one reporter gene (Figure 1B). These data demonstrate that the 2 PtMYC2 (Potri.001G142200 and Potri.003G092200) and PtJAM1.1 protein (Potri.014G099700.1) strongly interact with PtJAZ6 in yeasts, whereas PtJAM1.2 (Potri.002G172100.1) weakly interacts with PtJAZ6.

To validate these interactions *in planta,* and to determine the sub-cellular localization of these proteins, we transiently co-expressed *PtMYC2:RFP/PtJAZ6:GFP* or *PtJAM1:RFP/PtJAZ6:GFP* in tobacco leaves and performed co-immunoprecipitation and confocal microscopy. Transient expression of these constructs in tobacco epidermal cells indicated that PtJAZ6:GFP, PtMYC2s:RFP and PtJAM1s:RFP fusion proteins accumulated in nuclei of *N. benthamiana* leaf epidermal cells (Figure 1C). Proteins were extracted from the agroinfiltrated leaves and both fusion proteins were detected by Western blots (Figure 1D).

The CoIP experiments revealed that PtMYC2s:RFP and PtJAM1s:RFP associate with PtJAZ6:GFP in the same protein complex *in planta* (Figure 1D). Neither free GFP nor free RFP were able to immunoprecipitate PtMYC2s/PtJAM1s:RFP or PtJAZ6:GFP, respectively, indicating that the associations between PtMYC2s and PtJAZ6 or PtJAM1s and PtJAZ6 are specific to the proteins of interest and not due to interactions between the fluorophore tags (Figure 1D). These results suggest that PtMYC2s and PtJAZ6 as well as PtJAM1s and PtJAZ6, physically interact within plant nuclei. Poplar JAM1 and MYC2 proteins exhibit between 33% to 38% sequence identity and nearly identical bHLH domains with respect to their *Arabidopsis* counterparts (Supplemental Figure S2A,B). In a yeast one-hybrid assay, while yeasts expressing PtMYC2.1 or PtMYC2.2 fused to DBD of GAL4 were growing on medium lacking histidine, on the contrary for yeast expressing PtJAM1.1 fused to DBD of GAL4 activated the expression of the reporter genes *His3* and *LacZ*, yeast expressing DBD:PtJAM1.1 did not (Supplemental Figure S2C). Because PtJAZ6 interacts weakly with PtJAM1.2, we decided to exclude it for further analysis. Our data, suggest that PtMYC2s and PtJAM1 might be transcriptional activators and repressors, respectively.

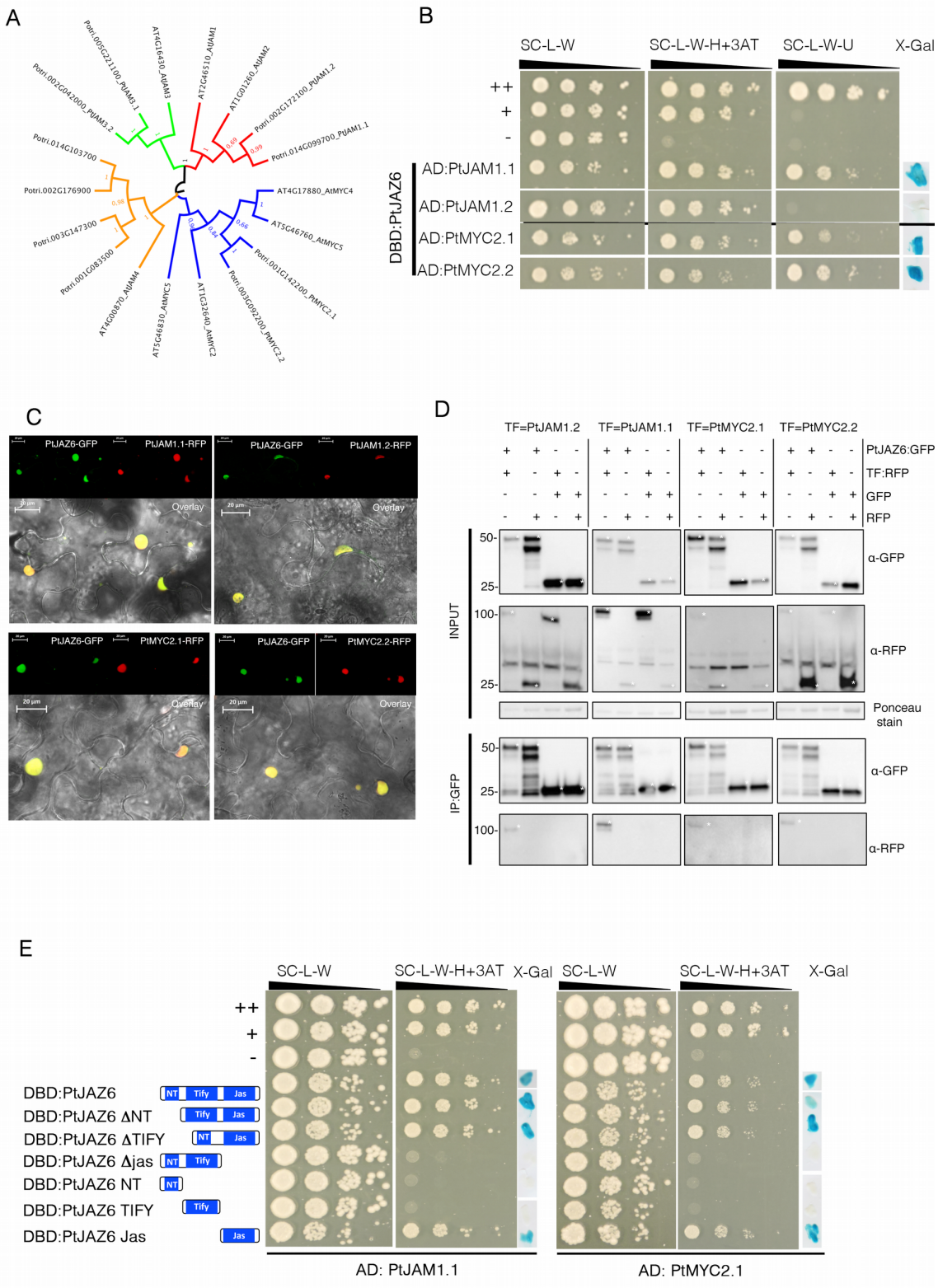

A
Potri.005G221100_PtJAM3.1
AT4G16430_AtJAM3
AT2G46510_AtJAM1
AT1G01260_AtJAM2
Potri.002G172100_PtJAM1.2
Potri.014G099700_PtJAM1.1
Potri.002G042000_PtJAM3.2
Potri.014G103700
Potri.002G176900
Potri.003G147300
Potri.001G083500
AT4G00870_AtJAM4
AT5G46830_AtMYC5
AT1G32640_AtMYC2
Potri.003G092200_PtMYC2.2
Potri.001G142200_PtMYC2.1
AT5G46760_AtMYC5
AT4G17880_AtMYC4
B
SC-L-W
SC-L-W-H+3AT
SC-L-W-U
X-Gal
++
+
-
DBD:PtJAZ6
AD:PtJAM1.1
AD:PtJAM1.2
AD:PtMYC2.1
AD:PtMYC2.2
C
PtJAZ6-GFP
PtJAM1.1-RFP
PtJAM1.2-RFP
PtMYC2.1-RFP
PtMYC2.2-RFP
Overlay
D
TF=PtJAM1.2
TF=PtJAM1.1
TF=PtMYC2.1
TF=PtMYC2.2
PtJAZ6:GFP
TF:RFP
GFP
RFP
INPUT
IP:GFP
50-
25-
100-
α-GFP
α-RFP
Ponceau stain
E
SC-L-W
SC-L-W-H+3AT
X-Gal
DBD:PtJAZ6
DBD:PtJAZ6 ΔNT
DBD:PtJAZ6 ΔTIFY
DBD:PtJAZ6 Δjas
DBD:PtJAZ6 NT
DBD:PtJAZ6 TIFY
DBD:PtJAZ6 Jas
NT
Tify
Jas
AD: PtJAM1.1
AD: PtMYC2.1

Figure 1. PtJAZ6 interacts with PtJAM1 and PtMYC2 transcription factors in yeast and* in planta*.

(A) *Phylogenetic analysis of the bHLH transcription factors orthologous to* A. thaliana *MYC and JAM. The evolutionary history was inferred by using the Maximum Likelihood method and conducted in MEGA7. 1000 bootstraps were performed and their values are indicated.*

(B) *Yeast-two hybrid (Y2H) assays to reveal interactions of PtJAZ6 with the PtJAM1.1, PtJAM1.2, PtMYC2.1 or PtMYC2.2. PtJAZ6 was fused to GAL4 DNA-binding domain (DBD) whereas the diverse transcription factors (TFs) were fused to the GAL4-activation domain (AD).* TRP1 *and* LEU2 *serve as transformation markers for vectors and* HIS3*,* URA3 *and* □-galactosidase LacZ *(X-Gal) were used as reporter genes. The control colonies are shown in the upper part of the panel: ++ is a strong positive interaction, + is a weak interacting pair, and - is a negative control (no interaction). 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene.*

(C) *Live-cell imaging of PtJAZ6::GFP and TF::RFP transiently expressed in* Nicotiana benthamiana *epidermal leaf cells transformed by agroinfiltration. Live-cell imaging was performed with a laser-scanning confocal microscope in a sequential scanning mode. Scale bar: 20µm.*

(D) *In planta co-immunoprecipitation of PtJAZ6 and PtJAM1 or PtMYC2 proteins.*

Fusion proteins (PtJAZ6:GFP and TF:RFP) were isolated from agroinfiltrated leaves and were immediately used for anti-GFP immunoprecipitation. Both initial proteins (Input) and immunoprecipitated protein (IP:GFP) were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were electro-transferred onto PVDF membranes. Immunodetection was performed with anti-GFP or anti-RFP antibodies, and immunoblots were revealed with a chemiluminescent imager. Ponceau staining of the PVDF membrane was used as a loading control. White asterisks indicate specific protein bands. Numbers on the left side of the blots indicate protein size in kilodaltons.

(E) *Yeast-two hybrid (Y2H) assays to assess which domains of PtJAZ6 are required for its interaction with PtMYC2.1 and PtJAM1.1. PtMYC2.1, PtJAM1.1 were fused to GAL4-activation domain (AD), whereas full length or mutated version of PtJAZ6 were fused with the GAL4-DNA-binding domain (DBD).* TRP1 *and* LEU2 *served as transformation markers for vectors and* HIS3 *and* □-galactosidase LacZ *(X-Gal) were used as reporter genes. 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene. The control colonies are shown in the upper part of the panel: ++ is a strong positive interaction, + is a weak interacting pair, - is negative control (no interaction).*

The PtJAZ6 Jas domain is required and sufficient for the interaction with PtJAM1.1 and PtMYC2.1

PtJAZ6 contains a variable N-terminal (NT) domain, a central ZIM domain (TIFY) and a C-terminal Jas domain (Supplemental Figure S3). To dissect the role of the different domains in the assembly of the PtJAZ6 protein complex, we constructed different deletion mutants of PtJAZ6, namely PtJAZ6 □NT, PtJAZ6 □TIFY, PtJAZ6 □Jas deleted respectively for the NT, TIFY or Jas domains. We also made constructions harboring a single domain and named them: PtJAZ6 NT, PtJAZ6 TIFY, PtJAZ6 Jas. All these constructions were fused to

GAL4 DNA-binding domain and expressed in yeast strains harboring different members of the protein complex fused to the GAL4 activation domain. The yeast strains co-expressing AD:PtJAM1.1/DBD:PtJAZ6 or PtJAZ6 ΔNT or PtJAZ6 ΔTIFY were able to grow on medium lacking Histidine, whereas yeasts co-expressing AD:PtJAM1s/DBD:PtJAZ6 Jas were not (Figure 1E). In addition, yeasts co-expressing AD:PtJAM1.1/DBD:PtJAZ6 Jas were able to grow. The same results for yeast growth were obtained with yeast harboring AD:PtMYC2.1 and the various forms of PtJAZ6 (Figure 1E). We conclude that the Jas domain of PtJAZ6 is necessary and sufficient for the interaction between PtJAZ6 and the four transcription factors PtMYC2.1 and PtJAM1.1.

PtJAZ6 physically interacts with other poplar JAZ proteins through its TIFY domain

To explore the possible interactions between PtJAZ6 and other PtJAZ proteins, we tested AD:PtJAZ6 and DBD:PtJAZs interaction in the yeast two-hybrid system described above. The *Populus* genome contains 12 canonical PtJAZ-encoding genes, the same number as the canonical *Arabidopsis* JAZ protein complement. We classified and named them according to their phylogenetic position (Figure 2). We successfully cloned nine of these *PtJAZs* genes from a cDNA library of poplar-*L. bicolor* ECM root tips (Table S1). We thus conducted yeast-two-hybrid (Y2H) studies using all pairs of candidate interactors to identify possible PtJAZ6 homo- or heterodimers. In addition, we exploited the PtJAZ6 ΔTIFY and the PtJAZ6 TIFY versions of PtJAZ6 to test whether the highly conserved TIF(F/Y)XG motif within the ZIM domain of PtJAZ6 (Supplemental Figure S3) is required for PtJAZ6 homo/heterodimerization. We found that PtJAZ6 is able to form homodimers with itself and heterodimers with PtJAZ5, PtJAZ1/2, PtJAZ7/8, PtJAZ10.2 and PtJAZ12. In all cases, the deletion of the TIFY domain of PtJAZ6 inhibited the dimerization in yeast-two hybrid system. In addition, the TIFY domain of PtJAZ6 alone ensured PtJAZ6 homo- and heterodimerization with PtJAZ5, PtJAZ10.2 and PtJAZ12 (Figure 2B). From our results we conclude that PtJAZ6 interacts with itself and the other JAZ proteins PtJAZ5, PtJAZ10.2 and PtJAZ12. We also show that the TIFY domain is required for the homo- and heterodimerization of PtJAZ6 (Figure 2B).

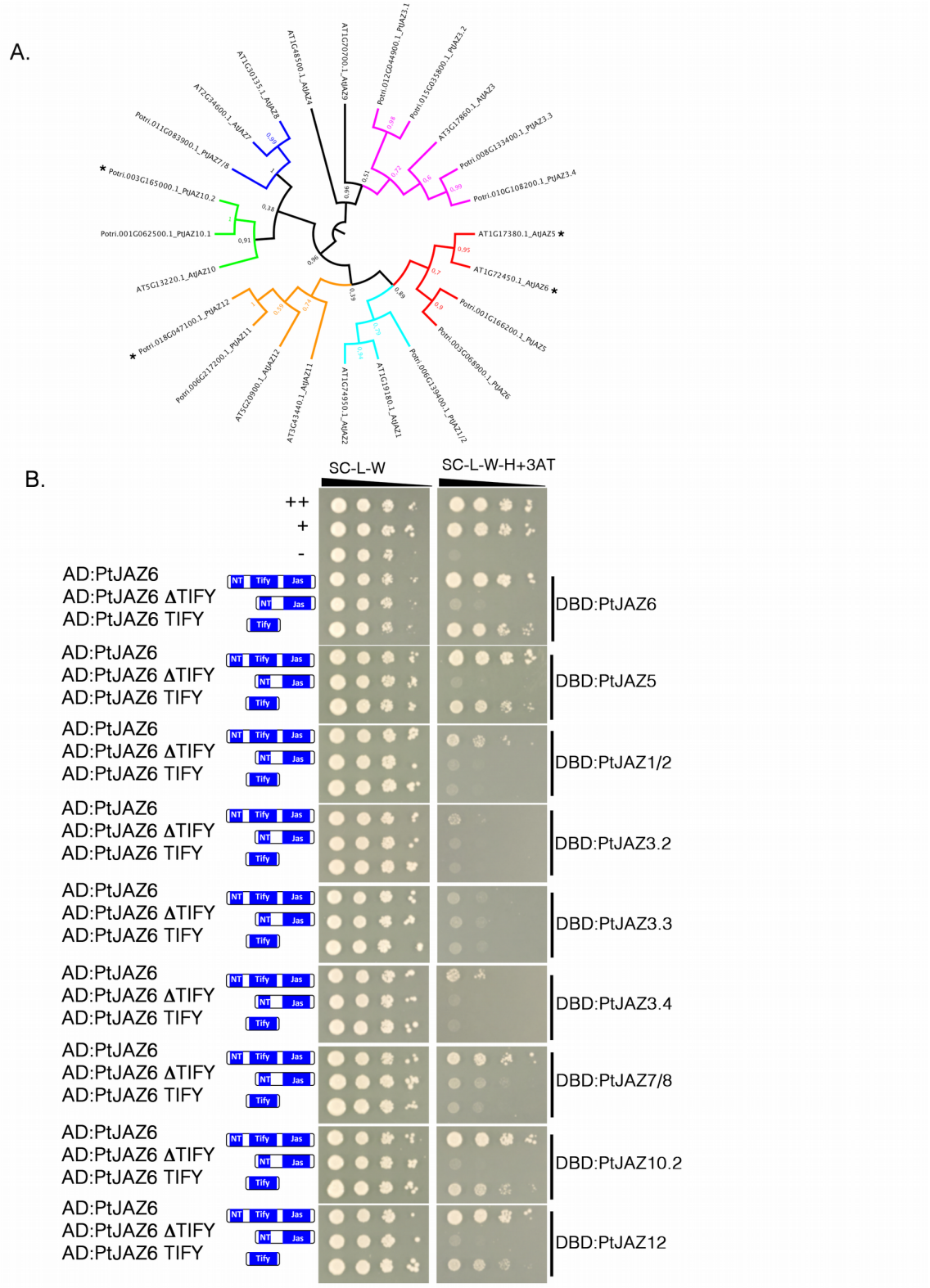

A.
AT1G48500.1_AtJAZ4
AT1G70700.1_AtJAZ9
Potri.012G044900.1_PtJAZ3.1
Potri.015G035800.1_PtJAZ3.2
AT3G17860.1_AtJAZ3
Potri.008G133400.1_PtJAZ3.3
Potri.010G108200.1_PtJAZ3.4
AT1G17380.1_AtJAZ5
AT1G72450.1_AtJAZ6
Potri.001G166200.1_PtJAZ5
Potri.003G085000.1_PtJAZ6
Potri.006G139400.1_PtJAZ1/2
AT1G19180.1_AtJAZ1
AT1G74950.1_AtJAZ2
AT3G43440.1_AtJAZ11
AT5G20900.1_AtJAZ12
Potri.006G217200.1_PtJAZ11
Potri.018G047100.1_PtJAZ12
AT5G13220.1_AtJAZ10
Potri.001G062500.1_PtJAZ10.1
Potri.003G165000.1_PtJAZ10.2
Potri.011G083900.1_PtJAZ7/8
AT2G34600.1_AtJAZ7
AT1G30135.1_AtJAZ8
B.
SC-L-W
SC-L-W-H+3AT
++
+
-
AD:PtJAZ6
AD:PtJAZ6 ΔTIFY
AD:PtJAZ6 TIFY
NT
Tify
Jas
DBD:PtJAZ6
DBD:PtJAZ5
DBD:PtJAZ1/2
DBD:PtJAZ3.2
DBD:PtJAZ3.3
DBD:PtJAZ3.4
DBD:PtJAZ7/8
DBD:PtJAZ10.2
DBD:PtJAZ12

Figure 2. PtJAZ6 is able to form homodimers and heterodimers with PtJAZ proteins through its TIFY domain.

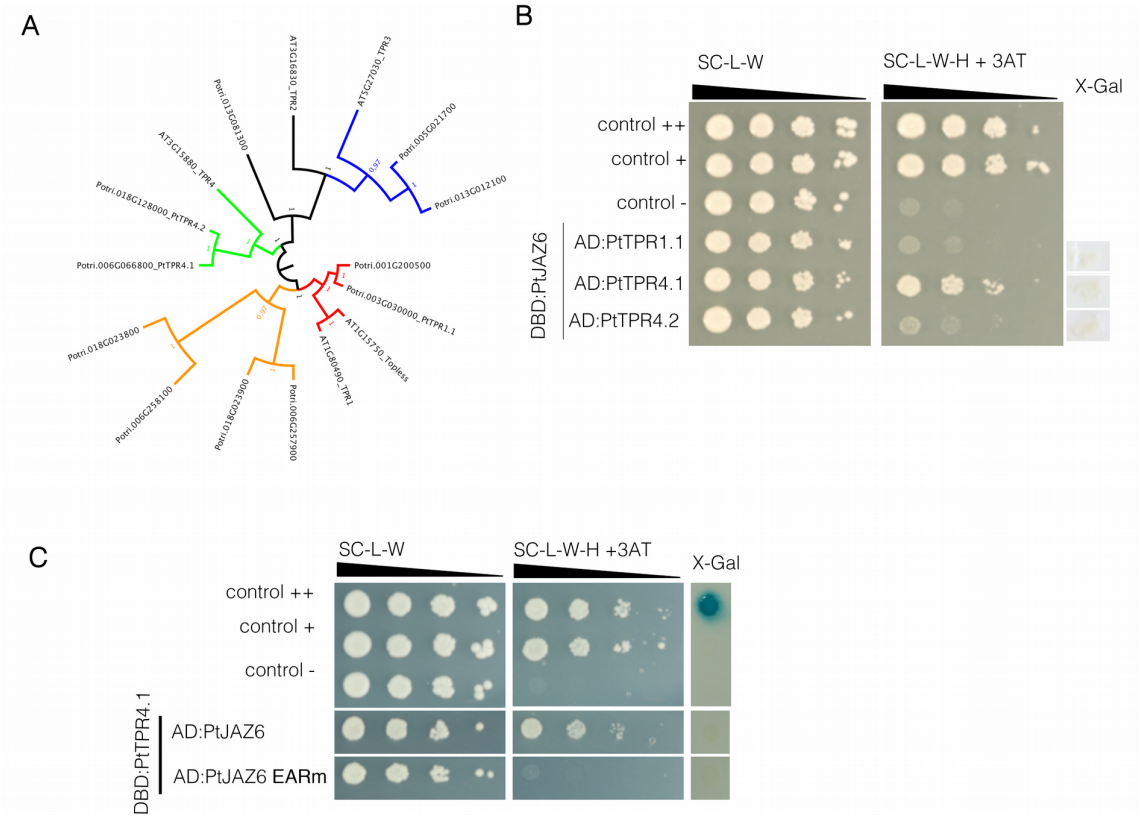
(A) *Phylogenetic tree of canonical JAZ proteins from* Arabidopsis *and* Populus*. JAZ proteins interacting with PtJAZ6 are highlighted in red. The evolutionary history was inferred by using the Maximum Likelihood method and conducted in MEGA7. 1000 bootstraps were performed and their values are indicated. * indicate PtJAZ proteins interacting with PtJAZ6 forming homo- and heterodimers.*

(B) *Yeast-two hybrid (Y2H) assays to detect interactions of PtJAZ6 with itself and with other PtJAZ protein expressed in ECM root tips. PtJAZ proteins were fused to GAL4 DNA-binding domain (DBD). PtJAZ6 or PtJAZ6 deleted for its TIFY domain (PtJAZ6□TIFY) or containing the TIFY domain only (PtJAZ6 TIFY) were fused with the GAL4-activation domain (AD).* TRP1 *and* LEU2 *serve as transformation markers for vectors and* HIS3 *was used as reporter gene. The control colonies are shown in the upper part of the panel: ++ is a strong positive interaction, + is a weak interacting pair, - is negative control (no interaction). 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene.*

PtJAZ6 interacts directly with two poplar NINJA proteins and one TOPLESS protein

Arabidopsis JAZ proteins form a repressor complex through recruitment of the general repressor TOPLESS (TPL) and TPL-related (TPR) proteins either directly or indirectly through interaction with the bridge protein NINJA (Pauwels et al., 2010). To first test the direct interaction between PtJAZ6 and TOPLESS, we successfully cloned three poplar *TPR* genes expressed in ectomycorrhizal root tips (*Potri.006G066800* alias *PtTPR4.1*; *Potri.018G128000* alias *PtTPR4.2* and *Potri.003G030000* alias *PtTPR1.1*) (Table S1; Figure 3A). Exclusively PtTPR4.1 physically interacted with PtJAZ6 in yeast cells similarly to the weak-interaction control (Figure 3B). Since *Arabidopsis* TOPLESS-related proteins interact with the EAR motif found of some AtJAZ proteins (Shyu et al., 2012; Thatcher et al., 2016), we mutated the EAR motif located at the C-terminus of PtJAZ6 (Supplemental Figure S3). Yeasts co-expressing PtJAZ6 EARm and PtTPR4.1 were not able to grow on the selective medium (Figure 3C), suggesting that the EAR motif of PtJAZ6 is required for its weak interaction with PtTPR4.1. In addition to the direct interaction of PtJAZ6 with PtTPR4.1, we tested whether PtJAZ6 is able to recruit any of the PtNINJA proteins. The *P. trichocarpa* genome contains three co-orthologues (*Potri.006G156100.1*; *Potri.006G162900.1*; *Potri.018G085100.1*) of the *Arabidopsis NINJA* gene (*AT4G28910.1*), all expressed in ectomycorrhizal root tips (Table S1). We were able to clone both *Potri.018G085100.1*, encoding PtNINJA3, and *Potri.006G162900.1*, encoding PtNINJA1 from a cDNA libray of *L. bicolor*-poplar ectomycorrhizal root tips. PtJAZ6 physically interacts with both PtNINJA1 and PtNINJA3 in yeast cells (Figure 3D). The yeast strains co-expressing PtNINJA3 or PtNINJA1 together with full-length PtJAZ6, PtJAZ6 ΔNT, PtJAZ6 ΔJas or PtJAZ6 TIFY were able to

grow on medium lacking histidine and displayed □-galactosidase activity (Figure 3D). This indicates that the TIFY domain of PtJAZ6 is required and sufficient to mediate the interaction with PtNINJA3 and PtNINJA1 in yeast cells. From these data, we conclude that PtJAZ6 weakly interacts with PtTPR4.1 TOPLESS-related protein and strongly interacts with two PtNINJA proteins.



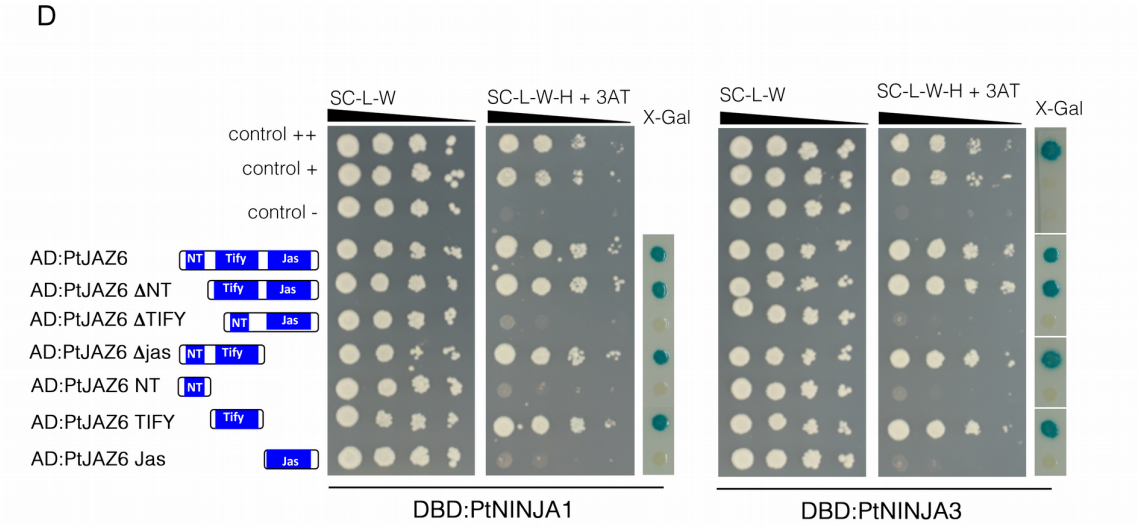


Figure 3. PtJAZ6 interacts directly with one PtTOPLESS-related protein and two NINJA

proteins.

(A) *Phylogenetic tree of* Populus trichocarpa *TOPLESS proteins expressed in poplar roots. The evolutionary history was inferred by using the Maximum Likelihood method and conducted in MEGA7. 1000 bootstraps were performed and their values are indicated. The three poplar TOPLESS proteins cloned from poplar ECM cDNA library are mentioned as PtTPR1.1, PtTPR4.1 and PtTPR4.2.*

(B) *Yeast-two hybrid (Y2H) assays to reveal interactions of PtJAZ6 with one TOPLESS-related protein PtTPR4.1 expressed in ECM root tips. PtJAZ6 was fused to GAL4 DNA-binding domain (DBD) whereas the three TOPLESS-related proteins were fused to the GAL4-activation domain (AD).*

(C) *The C terminus EAR motif of PtJAZ6 is required for its interaction with PtTPR4.1 in yeasts. Yeast-two hybrid (Y2H) assays were performed with yeast co-expressing DBD:PtTPR4.1 and AD:PtJAZ6 or AD:PtJAZ6 mutated for its EAR motif (EARm).*

(D) *PtJAZ6 interacts with PtNINJA1 and PtNINJ3 and the TIFY domain of PtJAZ6 is necessary and sufficient for the interaction. Yeast-two hybrid (Y2H) assays were performed with yeast co-expressing DBD:PtNINJA1 or DBD:PtNINJA3 and full length or mutated version of PtJAZ6 were fused with the GAL4-activation domain (AD).*

For all yeast-two hybrid assays (B,C,D), TRP1 *and* LEU2 *served as transformation markers for vectors and* HIS3 *and* □-galactosidase LacZ *(X-Gal) were used as reporter genes. 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene. The control colonies are shown in the upper part of the panel: ++ is a strong positive interaction, + is a weak interacting pair, - is negative control (no interaction).*

MiSSP7 physically interacts with PtJAZ6 through its NT and TIFY domains and indirectly inhibits the PtJAZ6-PtCOI1 interaction

We previously demonstrated that the fungal effector MiSSP7 from the ectomycorrhizal basidiomycete *Laccaria bicolor* binds to PtJAZ6 and alters its stability (Plett et al., 2014a). To determine which domain of PtJAZ6 is required for the interaction with MiSSP7, we co-expressed in yeast MiSSP7 and the different variants of PtJAZ6 in yeast cells. Yeasts harboring full-length or Jas domain-deleted *PtJAZ6* together with MiSSP7 were able to grow on the selective medium lacking histidine, whereas none of the other deleted forms of *PtJAZ6* did (Figure 4). From these data we conclude that both NT and TIFY domains are required for the interaction of PtJAZ6 with MiSSP7. In a previous work, we showed that PtJAZ6 interacts with PtCOI1 in a JA (coronatine)-dependent fashion (Plett et al., 2014a). Within the PtJAZ6-Jas domain, two arginine (R) residues at position 208 and 209 are likely required for interaction with PtCOI1, similarly to *Arabidopsis* (Melotto et al., 2008). To assess this, we engineered mutated PtJAZ6 proteins in which arginine is replaced by alanine in either one position (R208A or R209A) or in both positions (R208A R209A). Only in presence of coronatine did yeast strains expressing AD:PtJAZ6-R208A and DBD:PtCOI1 grow on the selective medium without histidine, whether none of the yeasts transformed with

other versions of PtJAZ6 fused to the AD of GAL4 did. In addition, only yeast expressing wild-type PtJAZ6 and PtCOI1 faintly activated the β-galactosidase reporter gene in presence of coronatine (Supplemental Figure S4). These data show that the R209 residue of PtJAZ6 is required for the interaction between PtCOI1 and PtJAZ6 in presence of coronatine. However, the Jas domain, containing these arginine residues, is not required for PtJAZ6-MiSSP7 interaction in yeasts (Figure 4). MiSSP7 is thus unlikely to directly impede PtCOI1 binding to PtJAZ6. However, we cannot exclude that the interaction between MiSSP7 and PtJAZ6 sterically inhibits the PtJAZ6-PtCOI1 interaction. To test this possibility, we used a yeast-three-hybrid assay. Yeasts co-expressing AD:PtJAZ6 and DBD:PtCOI1 were transformed with the *MiSSP7* gene. We tested the expression of the two reporter genes *HIS3* and *lacZ* with these triple-hybrid strains, in presence of coronatine to trigger the interaction between PtJAZ6 and PtCOI1. The growth of double- and triple-hybrid clones was similar on the selective medium without histidine, whereas the triple-hybrid clones displayed no detectable -galactosidase activity in presence of coronatine (Supplemental Figure S4). These results suggest that MiSSP7 does not directly compete with PtCOI1 for binding to PtJAZ6 but disturbs their interaction through steric hindrance or induced conformational change of PtJAZ6.

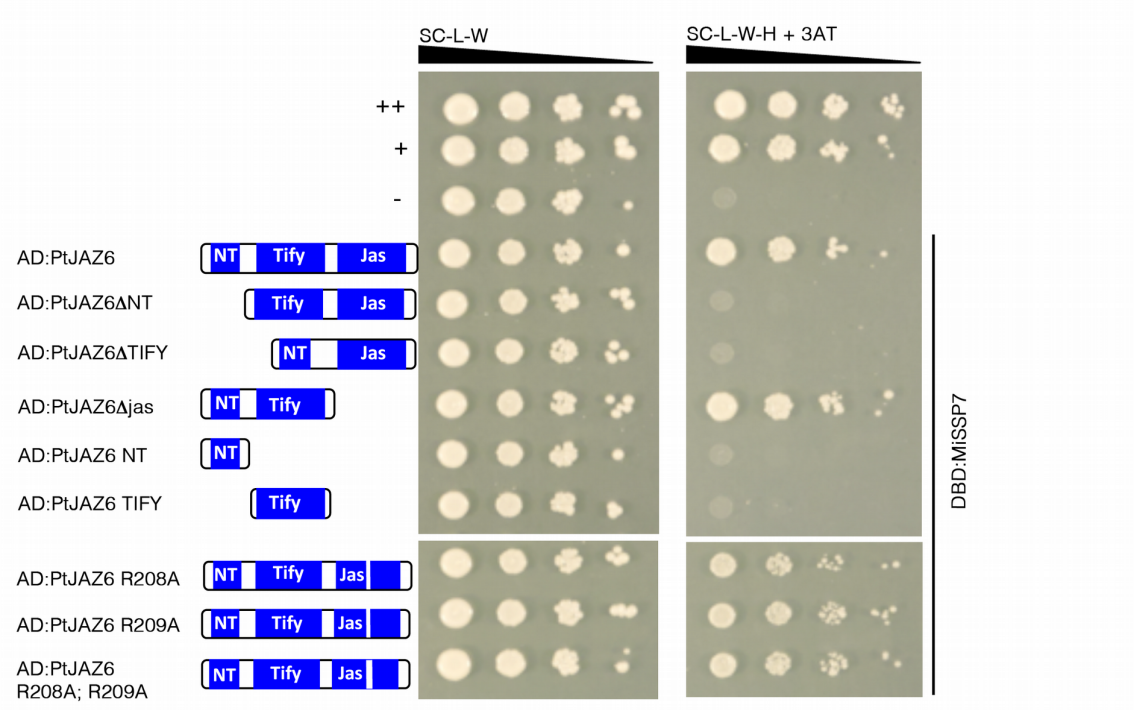


Figure 4. Both PtJAZ6 NT and TIFY domains are necessary for PtJAZ6 interaction with MiSSP7.

Yeast-two hybrid (Y2H) assays to assess which domains of PtJAZ6 are required for its interaction with MiSSP7. MiSSP7 was fused to GAL4 DNA-binding domain (DBD), whereas full length or mutated versions of PtJAZ6 were fused with the GAL4-activation domain (AD). TRP1 *and* LEU2 *served as transformation markers for vectors and* HIS3 *was used as reporter gene. The control colonies are shown in the upper part of the panel: ++ is a strong positive interaction, + is a weak interacting pair, - is negative control (no interaction). 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene.*

MiSSP7 affects PtJAZ6 homo- and heterodimerization

To assess the role of the MiSSP7 protein in the assembly of the PtJAZ6 complex, we investigated the impact of MiSSP7 on the interactions between PtJAZ6 and other proteins of the complex via a yeast three-hybrid assay. Yeast strains expressing DBD:PtJAZ6 together with PtMYC2s, PtJAM1.2, PtTPR4.1, PtNINJA3 or other PtJAZ proteins fused to GAL4 AD were transformed with the *MiSSP7* gene and the expression of the reporter genes *HIS3* and *lacZ* was assessed. We were unable to recover a triple hybrids expressing AD:PtNINJA1 and AD:PtJAM1.1 despite three tries; therefore, we could not test if MiSSP7 influenced the PtJAZ6-PtJAM1.1or PtJAZ6-PtNINJA1 interaction. Yeast colonies expressing AD:PtMYC2.1, DBD:PtJAZ6 and MiSSP7 grew better on medium lacking histidine and showed a darker blue color in the β-galactosidase activity assay with respect to yeasts expressing AD:PtMYC2.1 and DBD:PtJAZ6 only. An opposite result was observed in case of colonies expressing AD:PtMYC2.2 or AD:PtNINJA3, while no difference was seen in case of yeasts expressing AD:PtJAM1.2 or AD:PtTPR4.1 (Figure 5A). These results indicate that the presence of MiSSP7 appears to improve/strengthen the interaction between PtJAZ6 and PtMYC2.1 and impede/weaken the interaction between PtJAZ6 and PtMYC2.2. Since MiSSP7 cannot bind neither MYC2.1 nor PtMYC2.2 alone (Supplemental Figure S5A), this effect is not due to MiSSP7-mediated activation of these TFs. In addition, MiSSP7 weakens the interaction between PtJAZ6 and PtNINJA3.

As the MiSSP7 interaction with PtJAZ6 weakened the interaction between PtJAZ6-PtNINJA3, but neither the PtJAZ6-PtJAM1.2 nor the PtJAZ6-PtTRP4.1 interaction, we tested whether the expression of *MiSSP7* affected the formation of PtJAZ6 homo- and heterodimers. We detected diminished β-galactosidase activity upon MiSSP7 expression in yeasts harboring DBD:PtJAZ6, DBD:PtJAZ5, DBD:PtJAZ12 or DBD:PtJAZ1/2 together with AD:PtJAZ6. Moreover, yeasts expressing AD:PtJAZ6, DBD:PtJAZ6 and MiSSP7 displayed reduced growth on selective medium lacking histidine with respect to AD:PtJAZ6/DBD:PtJAZ6-harboring yeasts (Figure 5B). However, MiSSP7 did not promote

PtJAZ6 interaction with non-interacting PtJAZ proteins (Supplemental Figure S5B). These results suggests that MiSSP7 specifically antagonizes the formation of PtJAZ6-PtJAZ6 homodimers and of PtJAZ6-PtJAZ5, PtJAZ6-PtJAZ12 and PtJAZ6-PtJAZ1/2 heterodimers.

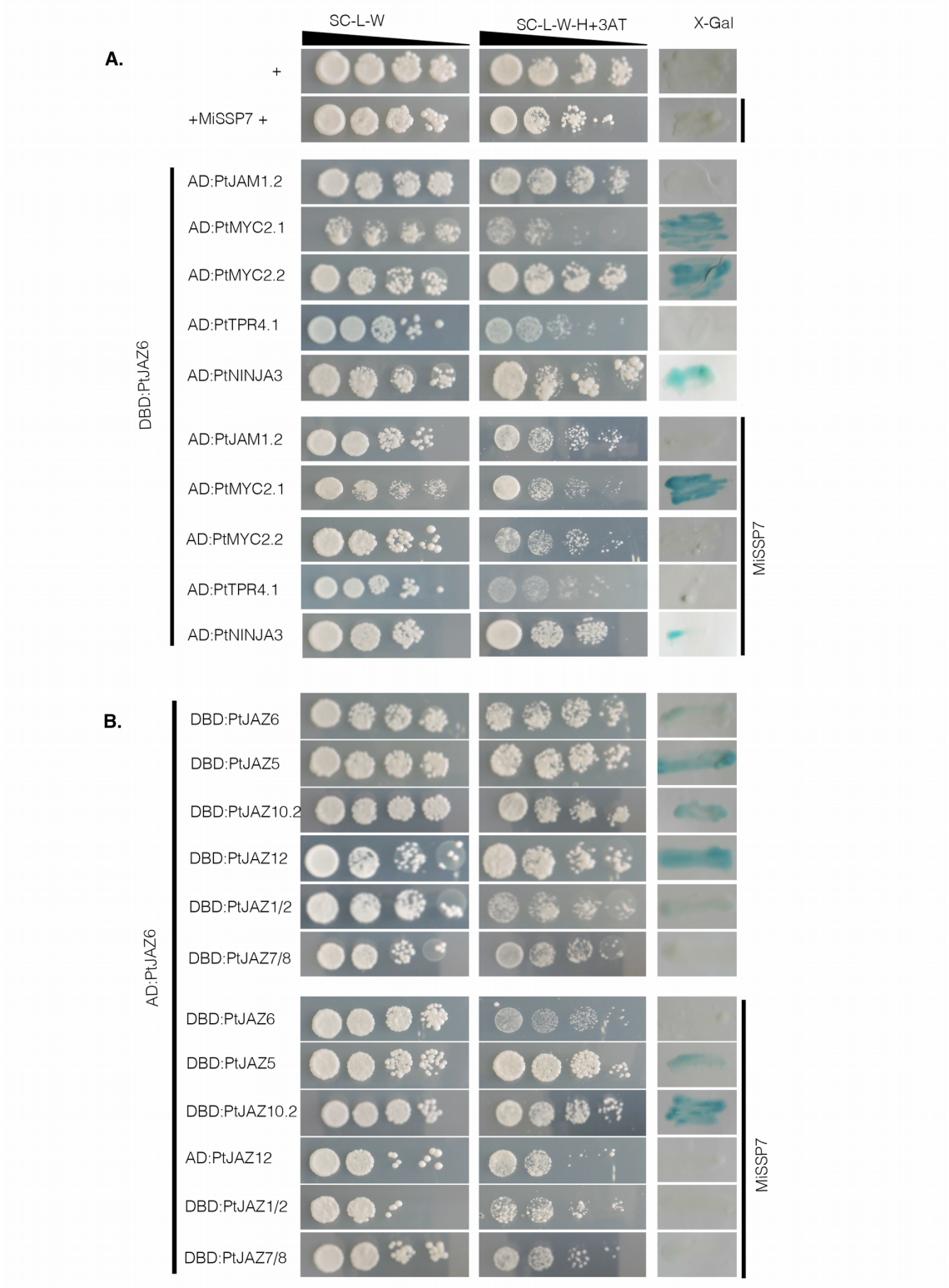


Figure 5. MiSSP7 impacts the structure of the jasmonate perception complex.

(A) *MiSSP7 differentially impacts the interaction between PtJAZ6 and the two isoforms of PtMYC2 but does not alter PtJAZ6-PtJAM1.2 or PtJAZ6-PtTPR4.1 interactions. PtJAZ6 was fused to GAL4 DNA-binding domain (DBD). PtMYC2s, PtJAM1.2, PtNINJA3 and PtTPR4.1 were fused with the GAL4-activation domain (AD).*

(B) *MiSSP7 antagonizes dimerization between PtJAZ6 and other PtJAZ proteins. PtJAZ6 was fused to the GAL4-activation domain (AD) and PtJAZ proteins were fused with the GAL4 DNA-binding domain (DBD).*

For A and B, yeasts were transformed with MiSSP7 *to give triple hybrids.* TRP1*,* LEU2 *and* URA3 *served as transformation markers (the latter in case of triple hybrid colonies) and* HIS3 *and* □-galactosidase LacZ *(X-Gal) were used as reporter genes. 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene. The control colonies are shown in the upper part of the panel: + is a weak interacting pair.*

2.5. Discussion

Comparative genomic and phylogenetic analyses of genes encoding the core components of the JA-Ile signaling pathway have highlighted its conservation throughout evolution from the last common ancestor of land plants (Wang et al., 2015). However, the presence of orthologs of JA signaling components in a plant genome does not determine the composition of the JA-signaling complex in a precise biological context and environment. Furthermore, studies of plant immunity demonstrate that plant-associated microorganisms manipulate JA-signaling through the use of effectors; some interfere with JA-Ile biosynthesis (Sugio et al., 2011; Geng et al., 2014; Katsir et al., 2008; Sheard et al., 2010), others modify the stability of the repressor JAZ (Jiang et al., 2013; Gimenez-Ibanez et al., 2014, Plett et al. 2014a; Ma et al., 2015) while yet others hamper the activity of jasmonate-regulated TFs (Yang et al., 2017; Li et al., 2014). The molecular mechanisms behind these outcomes vary considerably. For instance, *Pseudomonas syringae* effectors HopZ1 and HopX1 promote JAZ degradation, thus activating JA-dependent responses to antagonize SA-induced responses and promoting bacterial infection. These effectors achieve so through distinct molecular mechanism: HopX1 is a cysteine protease cleaving the central ZIM domain of JAZ proteins (Gimenez-Ibanez et al., 2014), whereas HopZ1 acetylates soybean and *Arabidopsis* JAZ proteins (Jiang et al., 2013). Recent studies demonstrated that effectors can also target transcription-factors mediating JA-responses. For example, the tomato yellow leaf curl China virus □C1 effector interacts with MYC2 to inhibit the activation of terpene synthase genes (Li et al., 2014). The *P. syringae* HopBB1 effector also has a dual function. It dissociates the *Arabidopsis* JAZ3-MYC2 complex and connects JAZ3 to the TF named TCP14, leading to

TCP14 degradation (Yang et al., 2017). HopBB1 effector thus modulates two distinct JA-dependent transcriptional modules. MiSSP7 from the mutualistic fungus *L. bicolor* prevents PtJAZ6 degradation (Plett et al., 2014), but the molecular mechanism has not been deciphered.

In this study, we identify the protein-complex interacting with the repressor PtJAZ6. Further, we studied how this complex might be affected in ectomycorrhizal poplar roots by studying the impact that the effector MiSSP7 secreted by the mutualistic fungus *Laccaria bicolor* had on PtJAZ6 protein-protein interactions (Figure 6). We reasoned that MiSSP7 might dampen or enhance the strength of the interaction between PtJAZ6 and other poplar proteins through competition or bridging, respectively. We found that, similarly to *Arabidopsis*, PtJAZ6 interacts with PtMYC2 and PtJAM1 transcription factors, as well as with one TOPLESS-related protein, PtTPR4.1, two NINJA proteins, PtNINJA1 and PtNINJA3, and other PtJAZ proteins (Figure 6A). In addition, the presence of MiSSP7 qualitatively enhances the interaction between PtJAZ6 and PtMYC2.1, while negatively impacting the interactions between PtJAZ6 and PtMYC2.2, PtNINJA3 and several PtJAZ proteins (Figure 6B).

The interaction between PtJAZ6 and PtMYC2.1, PtMYC2.2 or PtJAM1.1 requires the PtJAZ6 Jas domain, similarly to *Arabidopsis* (Chini et al., 2007). AtJAMs are repressors of transcription whereas AtMYC2 is a transcriptional activator (Goossens et al., 2016). These proteins compete for binding to the G-box in the promoter of target genes (Qi et al., 2015b) and the activity of both is inhibited by AtJAZ proteins (Chini et al., 2007; Fonseca et al., 2014; Song et al., 2013). Since the DNA-binding domains of PtMYC2s and PtJAM1s are highly similar (Supplemental Figure S2A-B), we can speculate that PtMYC2s and PtJAM1s directly compete for DNA-binding sites. We also hypothesize that these transcription factors compete for binding to PtJAZ6, leading to either attenuation of JA-signaling or release of repression. It will be interesting to investigate whether cell-specific or mycorrhizal stage-specific modulation of PtMYC2s and PtJAM1s activity contributes to the attenuation of JA-signaling during mutualistic tree-microbe interactions (Plett et al., 2014a). The expression of *PtMYC2s* and *PtJAM1s* was unaltered during the development of ectomycorrhizae *in vitro* (Felten et al., 2009; Basso et al., unpublished). However, post-translational modifications, adapted protein turnover rates or enhanced interactions with repressors proteins like PtJAZ6 may regulate DNA binding and activity of these transcription factors (Yan et al., 2007; Chung and Howe, 2009; Chung et al., 2010; Moreno et al., 2013; Zhai et al., 2013). For example, our data suggest that MiSSP7 induces preferential binding of PtJAZ6 to PtMYC2.1 rather than to its paralogous protein PtMYC2.2. These results suggest neo-functionalization of the two paralogues PtMYC2.1 and PtMYC2.2 in poplar roots and hint at differential regulation of distinct branches of JA-signaling upon ECM establishment, with preferential repression of the PtMYC2.1-regulated branch (Figure 6B). We previously demonstrated that inoculation of

poplar roots with *L. bicolor* reduces the expression of some JA-marker genes (Plett et al., 2014a) and dampens root sensitivity to exogenous JA (Basso et al., unpublished). On the other hand, moderate activation of JA-signaling may contribute to ectomycorrhizal development (Basso et al., unpublished). Specific activation or dampening of JA-responsive genes during ectomycorrhizae formation may rely on differential regulation of PtMYC2.1 and PtMYC2.2 activity. Identifying genes specifically regulated by PtMYC2.1 or PtMYC2.2 in ectomycorrhizae will allow testing this hypothesis.

Based on the results of yeast two-hybrid assays, we also demonstrate that PtJAZ6 interacts directly with one poplar TOPLESS-related protein through its EAR motif and with two NINJA proteins through its TIFY domain (Figure 3C-D), similarly to the *Arabidopsis* AtJAZ6 (Causier et al., 2012) and to four additional AtJAZ proteins (Shyu et al., 2012; Thireault et al., 2015; Thatcher et al., 2016). In addition, PtJAZ6 interacts with itself and other PtJAZs proteins. The strongest interactions occurred within the homodimer PtJAZ6-PtJAZ6 and the heterodimers PtJAZ6-PtJAZ5, PtJAZ6-PtJAZ10.2 and PtJAZ6-PtJAZ12. Contrasting reports have been published on the interactome of AtJAZ6, ranging from no dimerization (Chini et al., 2009) to weak dimerization with AtJAZ5 and AtJAZ6, medium-strength dimerization with JAZ10.1 and JAZ12 and strong dimerization with JAZ2 (Chini et al., 2009; Chung and Howe, 2009). Interaction of MiSSP7 with PtJAZ6 antagonizes its homo- and heterodimerization. Whether this dynamics has a stabilizing effect on the repressor protein PtJAZ6 remains to be investigated, since the *in vivo* function of JAZs dimerization is unknown. However, since protein dimerization is a common regulatory mechanism in signal transduction (Klemm et al., 1998), we could speculate that different PtJAZ dimers might generate diverse JA-signalling outputs. Alternatively, the formation of PtJAZ complexes with a specific range of stability may modulate the amplitude and the duration of JA-dependent responses (Chung and Howe, 2009).

In a previous study, we showed that the *L. bicolor* symbiotic effector MiSSP7 prevents PtJAZ6 degradation in presence of jasmonates (Plett et al., 2014a). Here, we provide evidence that this mutualistic effector modifies the dynamics of PtJAZ6 protein-protein interaction networks. More precisely, this effector upon interaction with PtJAZ6 may maintain the repression over PtMYC2.1-regulated genes, one of the JA-dependent transcriptional modules of poplar roots. It remains to be understood how such changes are required for successful root colonization by *L. bicolor*. Moreover, it remains to be demonstrated whether this mechanism is conserved for other species of ectomycorrhizal fungi.

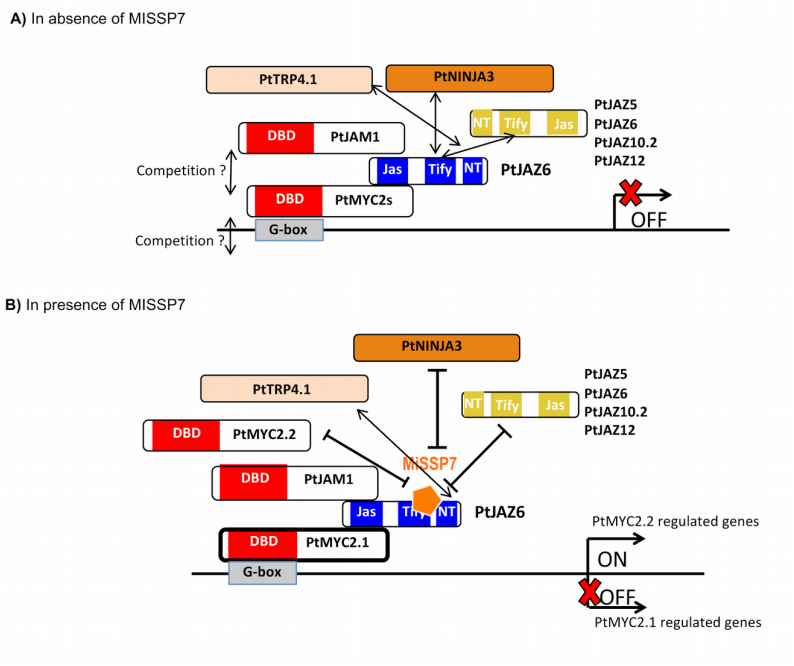


Figure 6. PtJAZ6 interactome in* Populus *ectomycorrhizal root tips.

(A) *PtJAZ6 physically interacts with the transcription factors PtMYC2s and PtJAM1s, as well as with PtTPR4.1. It also forms homodimers and heterodimers with PtJAZ5, PtJAZ10.2 and PtJAZ12. PtJAZ6 probably suppresses the transcriptional activity of PtMYC2s and PtJAM1s by recruiting the general repressor PtTPR4.1 to the promoter of MYC2-responsive genes.*

(B) *The fungal symbiotic effector MiSSP7 interacts with PtJAZ6 (Plett et al, 2014a) inhibiting homo- and heterodimerization of PtJAZ6. Such inhibition may alter the stability of PtJAZ6. Also, MiSSP7 loosens the interaction between PtJAZ6 and PtMYC2.2, while strengthening the interaction between PtJAZ6 and PtMYC2.1. MiSSP7-mediated enhancement of the PtJAZ6-PtMYC2.1 interaction probably dampens the transcriptional activity of PtMYC2.1. On the other hand, the release of PtMYC2.2 from PtJAZ6-mediated repression may activate gene expression dependent on this transcription factor.*

2.6. Acknowledgments

This research was sponsored by the Genomic Science Program, US Department of Energy, Office of Science, Biological and Environmental Research as part of the Plant-Microbe Interfaces Scientific Focus Area (http://pmi.ornl.gov) and the Laboratory of Excellence ARBRE (grant no. ANR-11-LABX-0002_ARBRE). The research is supported by the Institut National de la Recherche Agronomique and the University of Lorraine. Both Région Lorraine Research council and the European Fund for Regional Development give funding for the Functional Genomics Facilities at Institut National de la Recherche Agronomique-Grand Est.

2.7. References

Acosta, Ivan F., Gasperini, Debora, Chetelat, Aurore, Stolz, Stephanie, Santuari, Luca, Farmer, Edward E. 2013 Role of NINJA in root jasmonate signaling. Proc. Natl. Acad. Sci. USA. **110**:15473 15478

Ahmad, P., Rasool, S., Gul, A., Sheikh, S.A., Akram, N.A., Ashraf, M., Kazi, A. M., Gucel, S. 2016 Jasmonates: Multifunctional Roles in Stress Tolerance. Frontiers in Plant Sci. **7**: 813

Bari, R. and Jones, J.D.G. 2009 Role of plant hormones in plant defence responses Plant. Mol. Biol. **69**: 473 488

Beniwal, R.S., Langenfeld-Heyser, R., Polle, A. 2010 Ectomycorrhiza and hydrogel protect hybrid poplar from water deficit and unravel plastic responses of xylem anatomy Env. Exp. Botany **69**:189 197

Bücking, H., Hans, R., Heyser, W. 2007 The apoplast of ectomycorrhizal roots – site of nutrient uptake and nutrient exchange between the symbiotic partners. In: Sattelmacher B., Horst W.J. eds The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions. Springer, Dordrecht.

Causier, B., Ashworth, M., Guo, W., Davies, B. 2012 The TOPLESS Interactome: A Framework for Gene Repression in *Arabidopsis*. Plant Physiol. **158**: 423 438

Chico, J.M., Chini, A., Fonseca, S., Solano, R. 2008 JAZ repressors set the rhythm in jasmonate signaling. Curr. Opin. Plant. Biol. **11**: 486 494

Chini, A., Fonseca, S., Fernandez, G., Adie, B., Chico, J. M., Lorenzo, O., Garcia-Casado, G., Lopez-Vidriero, I., Lozano, F. M., Ponce, M. R., Micol, J. L., Solano, R. 2007 The JAZ family of repressors is the missing link in jasmonate signaling. Nature **448**: 666-670

Chini, A., Fonseca, S., Chico, J.M., Fernandez-Calvo, P., Solano, R. 2009 The ZIM domain mediates homo- and heteromeric interactions between Arabidopsis JAZ proteins. Plant J. **59:** 77-87

Chini, A., Gimenez-Ibanez, S., Goossens, A., Solano, R. 2016 Redundancy and specificity in jasmonate signaling. Curr. Opinion Plant Biol. **33**:147-156

Chung, H.S. and Howe, G.A. 2009 A Critical Role for the TIFY Motif in Repression of Jasmonate Signaling by a Stabilized Splice Variant of the JASMONATE ZIM-Domain Protein JAZ10 in *Arabidopsis.* Plant Cell **21:**131 145

Chung HS, Cooke TF, Depew CL, Patel LC, Ogawa N, Kobayashi Y, Howe GA. 2010 Alternative splicing expands the repertoire of dominant JAZ repressors of jasmonate signaling. Plant J. **63**:613-22**.**

Danielsen, L. and Polle, A. 2014 Poplar nutrition under drought as affected by ectomycorrhizal colonization. Env. Exp. Botany **108**:89 98

Dar, T.A., Uddin, M., Khan, M.M.A., Hakeem, K.R., Jaleel, H. 2015 Jasmonates counter plant stress: A Review. Env. Exp. Botany **115**:49 57

De Geyter, N., Gholami, A., Goormachtig, S., Goossens, A. 2012 Transcriptional machineries in jasmonate-elicited plant secondary metabolism. Trends in Plant Sci. **17**:349 359

Felten, J., Kohler, A., Morin, E., Bhalerao, R.P., Palme, K., Martin, F., Ditengou, F.A., Legue, V. 2009 The Ectomycorrhizal Fungus *Laccaria bicolor* Stimulates Lateral Root Formation in Poplar and Arabidopsis through Auxin Transport and Signaling. Plant Physiol. **151**:1991 2005

Fernandez-Calvo, P., Chini, A., Fernandez-Barbero, G., Chico, J-M., Gimenez-Ibanez, S., Geerinck, J., Eeckhout, D., Schweizer, F., Godoy, M., Manuel Franco-Zorrilla, J., Pauwels, L., Witters, E., Isabel Puga, M., Paz-Ares, J., Goossens, A., Reymond, P., De Jaeger, G., Solano, R. 2011 The Arabidopsis bHLH Transcription Factors MYC3 and MYC4 Are Targets of JAZ Repressors and Act Additively with MYC2 in the Activation of Jasmonate Responses. Plant Cell **23**:701 715

Feys, B.J.F., Benedetti, C.E., Penfold, C.N., Turner, J.G. 1994 *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male-sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Plant Cell **6**:751-759

Figueroa, P., Browse, J. 2015 Male sterility in *Arabidopsis* induced by overexpression of a MYC5-SRDX chimeric repressor. Plant J. **81**:849 860

Fonseca, S., Chini, A., Hamberg, M., Adie, B., Porzel, A., Kramell, R., Miersch, O., Wasternack, C., Solano, R. 2009 (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. Nat. Chemical Biol. **5:**344 350

Fonseca, S., Fernandez-Calvo, P., Fernandez, G.M., Diez-Diaz, M. Gimenez-Ibanez, S., Lopez-Vidriero, I., Godoy, M., Fernandez-Barbero, G., Van Leene, J., De Jaeger, G., Manuel Franco-Zorrilla, J., Solano, R. 2014 bHLH003, bHLH013 and bHLH017 Are New Targets of JAZ Repressors Negatively Regulating JA Responses. PLOS ONE **9**:e86182

Gange, A.C., Gane, D.R.J., Chen, Y.L., Gong, M.Q. 2005 Dual colonization of *Eucalyptus urophylla* ST Blake by arbuscular and ectomycorrhizal fungi affects levels of insect herbivore attack. Agricultural and Forest Entomology. **7**:253 263

Geng, X., Jin, L., Shimada, M., Kim, M.G., Mackey, D. 2014 The phytotoxin coronatine is a multifunctional component of the virulence armament of *Pseudomonas syringae*. Planta **240**:1149 1165

Gimenez-Ibanez, S., Boter, M., Fernandez-Barbero, G., Chini, A., Rathjen, J.P., Solano, R. 2014 The Bacterial Effector HopX1 Targets JAZ Transcriptional Repressors to Activate Jasmonate Signaling and Promote Infection in *Arabidopsis*. PLOS BIOLOGY **12**:e1001792

Goossens, J., Fernandez-Calvo, P., Schweizer, F., Goossens, A. 2016 Jasmonates: signal transduction components and their roles in environmental stress responses. Plant Mol. Biol. **91**:673-689

Grefen C. 2014 The Split-Ubiquitin System for the Analysis of Three-Component Interactions. In: Sanchez-Serrano J., Salinas J. eds Arabidopsis Protocols. Methods in Molecular Biology Methods and Protocols, **1062**. Humana Press, Totowa, NJ

Howe, G.A., Major, I.T., Koo, A.J., Merchant, SS 2018 Modularity in Jasmonate Signaling for Multistress Resilience. Ann. Rev. Plant Biol. **69:**387 415

Huang, H., Liu, B., Liu, L., Song, S. 2017 Jasmonate action in plant growth and development. J. Exp. Bot. **68:**1349 1359

Jacobs, S., Zechmann, B., Molitor, A., Trujillo, M., Petutschnig, E., Likpa, V., Kogel, K-H., Schaefer, P. 2011 Broad-Spectrum Suppression of Innate Immunity Is Required for Colonization of Arabidopsis Roots by the Fungus *Piriformospora indica*. Plant Physiol. **156**:726 740

Jiang, S., Yao, J., Ma, K-W., Zhou, H., Song, J., He, S.Y., Ma, W. 2013 Bacterial Effector Activates Jasmonate Signaling by Directly Targeting JAZ Transcriptional Repressors. PLOS Pathogens **9**: e1003715

Jones D.T., Taylor W.R., and Thornton J.M. 1992. The rapid generation of mutation data matrices from protein sequences. Computer Applications in the Biosciences **8**: 275-282

Kaling, M., Schmidt, A., Moritz, F., Rosenkranz, M., Witting, M., Kasper, K., Janz, D., Schmitt-Kopplin, P., Schnitzler, J-P., Polle, A. 2018 Mycorrhiza-Triggered Transcriptomic and Metabolomic Networks Impinge on Herbivore Fitness. Plant Physiology, **176** :2639-2656

Katsir, L., Schilmiller, A.L., Staswick, P.E., He, S.Y., Howe, G.A. 2008 COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine Proc. Natl. Acad. Sci. USA. **105:**7100 7105

Kazan, K. 2015 Diverse roles of jasmonates and ethylene in abiotic stress tolerance. Trends in Plant Sci. **20**:219 229

Klemm, J.D., Schreiber, S.L., Crabtree, G.R. 1998 Dimerization as a regulatory mechanism in signal transduction. Ann. Rev. Immunology **16**:569 592

Kumar S., Stecher G., and Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution **33**:1870-1874.

Li, J., Bao, S., Zhang, Y., Ma, X., Mishra-Knyrim, M., Sun, J., Sa, G., Shen, X. Polle, A., Chen, S. 2012 *Paxillus involutus* Strains MAJ and NAU Mediate K+/Na+ Homeostasis in Ectomycorrhizal *Populus x canescens* under Sodium Chloride Stress. Plant Physiol. **159**:1771 1786

Li, R., Weldegergis, B.T., Li, J., Jung, C., Qu, J., Sun, Y., Qian, H., Tee, C., van Loon, J.J.A., Dicke, M., Chua, N-H., Liu, S-S., Ye, J. 2014 Virulence Factors of Geminivirus Interact with MYC2 to Subvert Plant Resistance and Promote Vector Performance. Plant Cell **26**:4991-5008

Lu, J., Robert, C.A.M., Riemann, M., Cosme, M., Mene-Saffrane, L., Massana, J., Stout, M.J., Lou, Y., Gershenzon, J., Erb, M. 2015 Induced Jasmonate Signaling Leads to Contrasting Effects on Root Damage and Herbivore Performance. Plant Physiol. 167: 1100

Luo, Z-B., Wu, C., Zhang, C., Li, H.g, Lipka, U., Polle, A. 2014 The role of ectomycorrhizas in heavy metal stress tolerance of host plants. Env. Exp. Botany **108**:47 62

Ma, K-W., Jiang, S., Hawara, E., Lee, D-H., Pan, S., Coaker, G., Song, Ji., Ma, W. (2015 Two serine residues in *Pseudomonas syringae* effector HopZ1a are required for acetyltransferase activity and association with the host co-factor. New Phytol. **208**:1157-1168

Ma, Y., He, J., Ma, C., Luo, J., Li, H., Liu, T., Polle, A., Peng, C., Luo, Z-B. 2014. Ectomycorrhizas with *Paxillus involutus* enhance cadmium uptake and tolerance in *Populus x canescens*. Plant Cell and Env. **37**:627-642

Martin, F., Aerts, A., Ahren, D., et al.. 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature **452:**88

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., Hibbett, D.S. 2016. Unearthing the roots of ectomycorrhizal symbioses. Nat. Reviews Microbiol. **14**:760-773

Melotto, M., Mecey, C., Niu, Y., Chung, H.S., Katsir, L., Yao, J., Zeng, W., Thines, B., Staswick, P., Browse, J., Howe, G. A., He, S.Y. 2008. A critical role of two positively charged amino acids in the

Jas motif of *Arabidopsis* JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1F-box protein. Plant J. **55:**979-988

Moreno JE, Shyu C, Campos ML, Patel LC, Chung HS, Yao J, He SY, Howe GA. 2013. Negative feedback control of jasmonate signaling by an alternative splice variant of JAZ10. Plant Physiol. **162**:10016-17

Nakata, M., Mitsuda, N., Herde, M., Koo, A.J.K., Moreno, J.E., Suzuki, K., Howe, G.A., Ohme-Takagi, M. 2013. A bHLH-Type Transcription Factor, ABA-INDUCIBLE BHLH-TYPE TRANSCRIPTION FACTOR/JA-ASSOCIATED MYC2-LIKE1, Acts as a Repressor to Negatively Regulate Jasmonate Signaling in *Arabidopsis*. Plant Cell **25**:1641-1656

Nehls, U. 2008. Mastering ectomycorrhizal symbiosis: the impact of carbohydrates. Journal of Experimental Botany. **59**:1097–1108.

Nehls, U., Grunze, N., Willmann, M., Reich M. and Küsterb, H. 2007. Sugar for my honey: Carbohydrate partitioning in ectomycorrhizal symbiosis. Phytochemistry. **68**: 82-91

Pauwels, L., Barbero, G.F., Geerinck, J., Tilleman, S., Grunewald, W., Perez, A.C., Chico, J.M., Vanden Bossche, R., Sewell, J., Gil, E., Garcia-Casado, G., Witters, E., Inze, D., Long, J.A., De Jaeger, G., Solano, R., Goossens, A. 2010. NINJA connects the co-repressor TOPLESS to jasmonate signaling. Nature **464:**788

Pauwels, L., Goossens, A. 2011. The JAZ Proteins: A Crucial Interface in the Jasmonate Signaling Cascade. Plant Cell **23**:3089 3100

Pena, R., Simon, J., Rennenberg, H., Polle, A. 2013. Ectomycorrhiza affect architecture and nitrogen partitioning of beech *Fagus sylvatica* L.. seedlings under shade and drought. Env. Exp. Botany **87**:207-217

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. 2012.. Hormonal Modulation of Plant Immunity. Annual Review of Cell and Developmental Biology **28**:489–521.

Plett, J.M., Kemppainen, M., Kale, S.D., Kohler, A., Legue, V., Brun, A., Tyler, B.M., Pardo, A.G., Martin, F. 2011a. A Secreted Effector Protein of Laccaria bicolor Is Required for Symbiosis Development. Curr. Biology **21**:1197 1203

Plett, J.M., Montanini, B., Kohler, A., Ottonello, S., Martin, F. 2011b. Tapping Genomics to Unravel Ectomycorrhizal Symbiosis. Fungal Genomics: Methods and Protocols. **722**:249-281

Plett, J.M., Daguerre, Y., Wittulsky, S., Vayssieres, A., Deveau, A., Melton, Sa.J., Kohler, A., Morrell-Falvey, J.L., Brun, A., Veneault-Fourrey, C., Martin, F. 2014a. Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes Proc. Natl. Acad. Sci. USA. **111:** 8299 8304

Plett, J.M., Khachane, A., Ouassou, M., Sundberg, B., Kohler, A., Martin, F. 2014b. Ethylene and jasmonic acid act as negative modulators during mutualistic symbiosis between *Laccaria bicolor* and *Populus* roots. New Phytologist **202**:270-286

Qi, T., Huang, H., Song, S., Xie, D. 2015a. Regulation of Jasmonate-Mediated Stamen Development and Seed Production by a bHLH-MYB Complex in *Arabidopsis*. Plant Cell **27**:1620-1633

Qi, T., Wang, J., Huang, H., Liu, B.i, Gao, H., Liu, Y., Song, S., Xie, D. 2015b. Regulation of Jasmonate-Induced Leaf Senescence by Antagonism between bHLH Subgroup IIIe and IIId Factors in *Arabidopsis*. Plant Cell **27**:1634 1649

Riemann, M., Dhakarey, R., Hazman, M., Miro, B., Kohli, A., Nick, P. 2015. Exploring Jasmonates in the Hormonal Network of Drought and Salinity Responses. Frontiers in Plant Science **6**:1077

Sasaki-Sekimoto, Y., Jikumaru, Y., Obayashi, T., Saito, H., Masuda, S., Kamiya, Y., Ohta, H., Shirasu, K. 2013. Basic Helix-Loop-Helix Transcription Factors JASMONATE-ASSOCIATED MYC2-LIKE1 (JAM1), JAM2, and JAM3 Are Negative Regulators of Jasmonate Responses in *Arabidopsis*. Plant Physiol. **163**:291-304

Sharma, M., Laxmi, A. 2016. Jasmonates: Emerging Players in Controlling Temperature Stress Tolerance. Frontiers in Plant Sci. **6**: 1129

Sheard, L.B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T.R., Kobayashi, Y., Hsu, F-F., Sharon, M., Browse, J., He, S.Y., Rizo, J., Howe, G.A., Zheng, N. 2010. Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature **468**:400-

Shyu, C., Figueroa, P., DePew, C.L., Cooke, T.F., Sheard, L.B., Moreno, J.E., Katsir, L., Zheng, N., Browse, J., Howe, G.A. 2012. JAZ8 Lacks a Canonical Degron and Has an EAR Motif That Mediates Transcriptional Repression of Jasmonate Responses in *Arabidopsis*. Plant Cell **24**:536 550

Song, S., Qi, Tiancong, F., Meng, Z., Xing, Gao, H., Huang, H., Wu, D., Guo, H., Xie, D. 2013. The bHLH Subgroup IIId Factors Negatively Regulate Jasmonate-Mediated Plant Defense and Development. PLOS GENETICS **9**:e1003653

Sugio, A., Kingdom, H.N., MacLean, A.M., Grieve, V.M., Hogenhout, S.A. 2011. Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis Proc. Natl. Acad. Sci. USA. **108**:E1254 E1263

Thatcher, L.F., Manners, J.M., Kazan, K. 2009. *Fusarium oxysporum* hijacks COI1-mediated jasmonate signaling to promote disease development in Arabidopsis. **Plant J.** 58:927-939

Thatcher LF, Cevik V, Grant M, Zhai B, Jones JD, et al. 2016.. Characterization of a JAZ7 activation- tagged *Arabidopsis* mutant with increased susceptibility to the fungal pathogen *Fusarium oxysporum*.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A., Browse, J. 2007. JAZ repressor proteins are targets of the SCFCO11 complex during jasmonate signaling. Nature **448**:661

Thireault, C., Shyu, C., Yoshida, Y., St Aubin, B., Campos, M.L., Howe, G.A. 2015. Repression of jasmonate signaling by a non-TIFY JAZ protein in *Arabidopsis*. Plant J. **82**:669-679

Walhout, AJM, Vidal, M 2001. High-throughput yeast two-hybrid assays for large-scale protein interaction manning. Methods **24**:297-306

Wang, C., Liu, Y., Li, S-S., Han, G-Z. 2015. Insights into the Origin and Evolution of the Plant Hormone Signaling Machinery. Plant Physiol.**167:**872

Wasternack, C., Hause, B. 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Annals of Botany. **111**:1021 1058

Wasternack, C., Song, S. 2017. Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. J. Exp. Bot. **68**:1303 1321

Wasternack, C., Strnad, M. 2019. Jasmonates are signals in the biosynthesis of secondary metabolites - Pathways, transcription factors and applied aspects - A brief review. New Biotech. **48:** 1-11

Xie, DX, Feys, BF, James, S, Nieto-Rostro, M, Turner, JG 1998. COI1: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science **280:**1091 1094

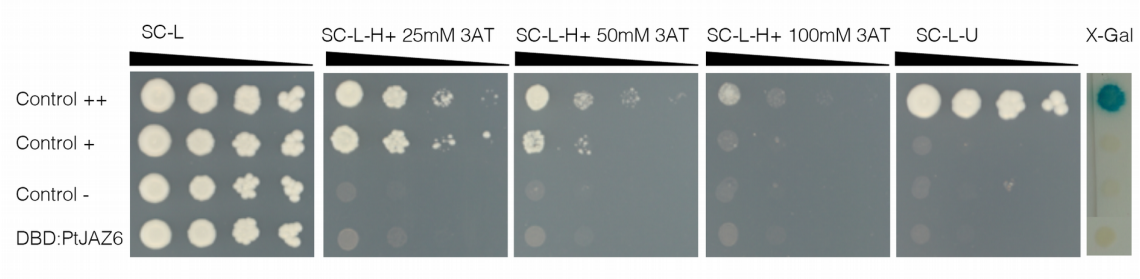
Yan, Y., Stolz, S., Chetelat, A., Reymond, P., Pagni, M., Dubugnon, L., Farmer, E.E. 2007. A downstream mediator in the growth repression limb of the jasmonate pathway. Plant Cell **19**:2470 2483

Yang, L., Teixeira, P.J., Biswas, S., Finkel, O.M., He, Y., Salas-Gonzalez, I., English, M.E., Epple, P., Mieczkowski, P., Dangl, J.L. 2017. *Pseudomonas syringae* Type III Effector HopBB1 Promotes Host Transcriptional Repressor Degradation to Regulate Phytohormone Responses and Virulence. **21**:156-168.

Zhai Q, Yan L, Tan D, Chen R, Sun J, et al. 2013. Phosphorylation-Coupled Proteolysis of the Transcription Factor MYC2 Is Important for Jasmonate-Signaled Plant Immunity. PLOS Genetics **9**: e1003422.

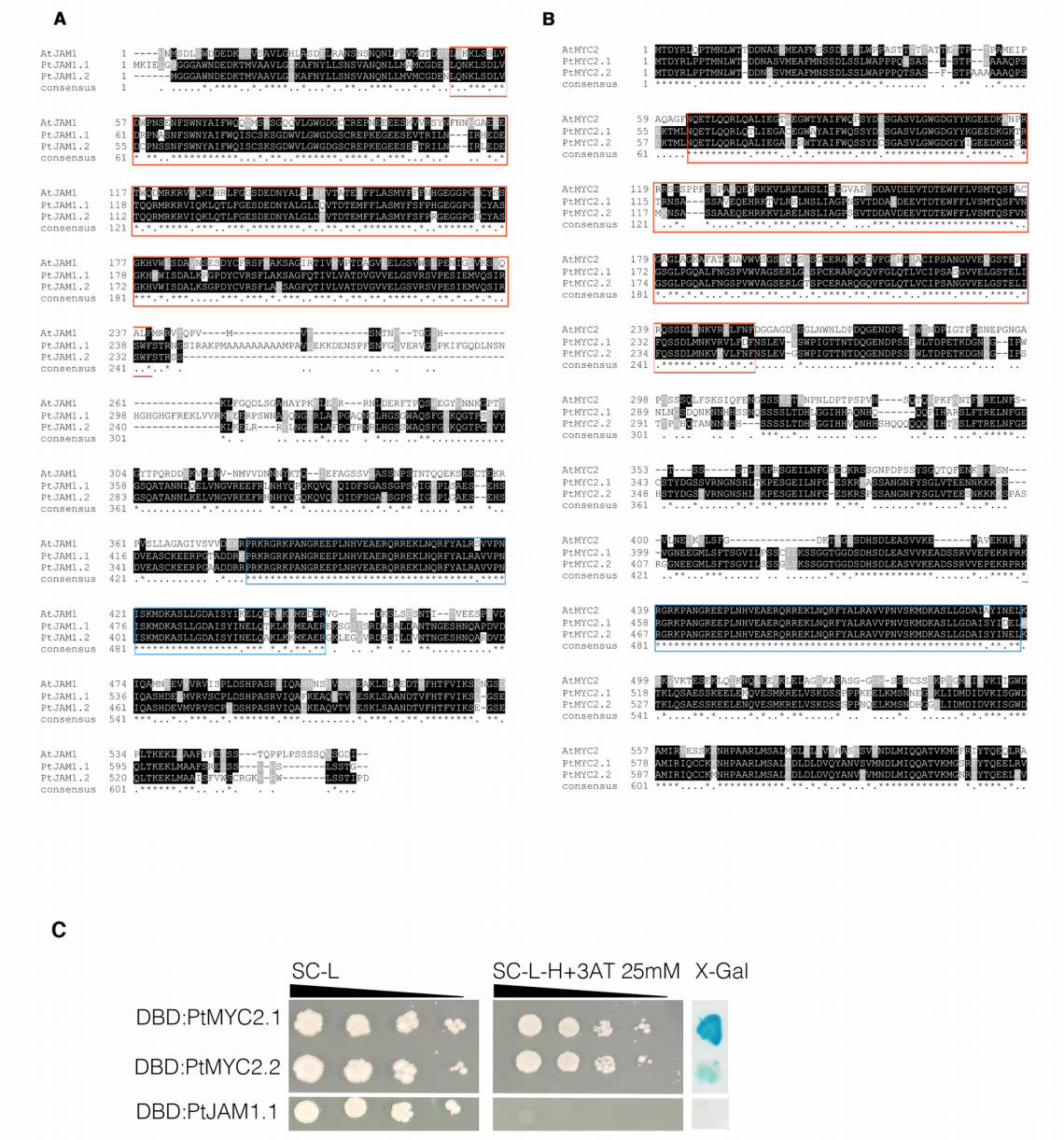
Zheng, X-Y., Spivey, N.W., Zeng, W., Liu, P-P., Fu, Z.Q., Klessig, D.F., He, S.Y., Dong, X. 2012. Coronatine Promotes *Pseudomonas syringae* Virulence in Plants by Activating a Signaling Cascade that Inhibits Salicylic Acid Accumulation. Cell Host & Microbe **11:**587 596

2.8. Supplemental material



Supplemental Figure S1. PtJAZ6 does not auto-activate the three reporter genes used in the yeast-two hybrid system.

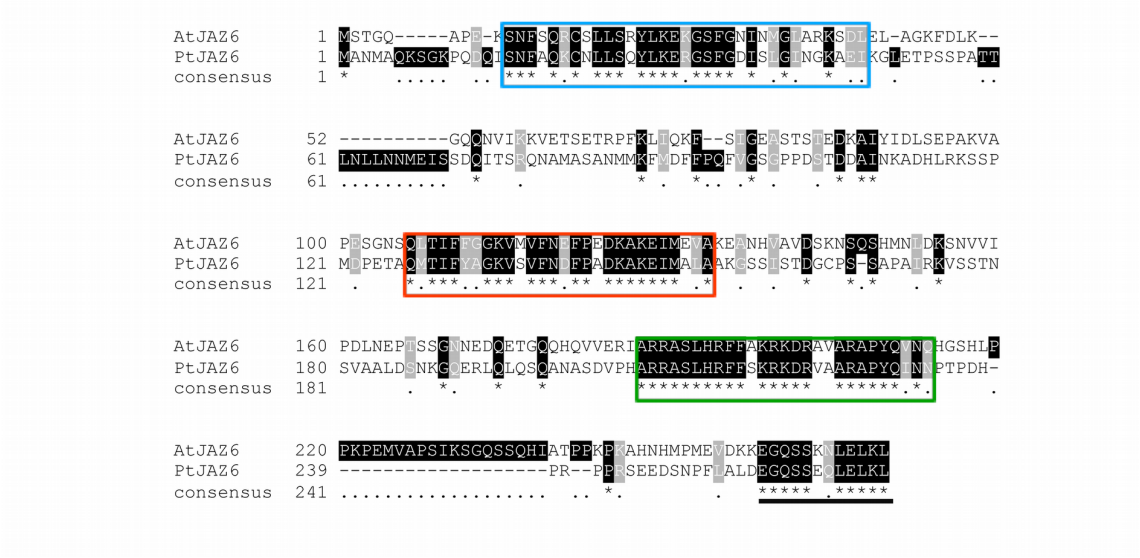
PtJAZ6 was fused to the DNA-binding domain (DBD) of GAL4-yeast transcription factor. LEU2 *served as transformation marker for vector.* HIS3, URA3 *and* LacZ *(X-Gal) were used as reporter genes. 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene.*



Supplemental Figure S2. Features of Populus MYC2 and JAM1 proteins.

(A-B) *Alignment of aminoacid sequences of Populus and Arabidopsis JAM1 (A) and MYC2s (B) proteins. Alignments were performed using T-Coffee and Boxshade for publication-quality output. DNA-binding domain and bHLH-MYC N-terminal domain are indicated in blue and red, respectively.*

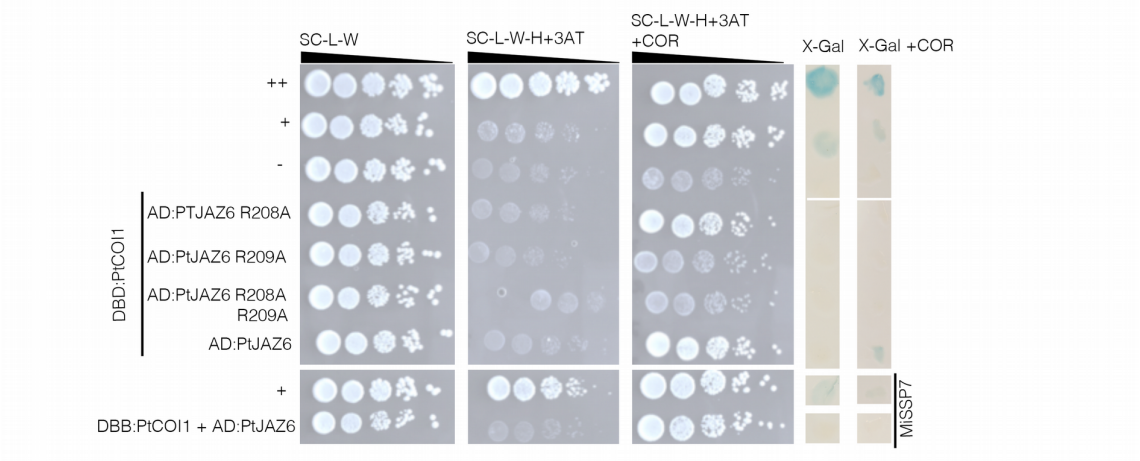
(C) *Autoactivation tests in yeast using PtMYC2.1, PtMYC2.2 or PtJAM1.1 fused to the DNA-binding domain of GAL4-yeast transcription factor.* LEU2 *served as transformation marker for vector and* HIS3 *was used as reporter gene. 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene.*



Supplemental Figure S3. PtJAZ6 protein contains three typical domains of JAZ proteins.

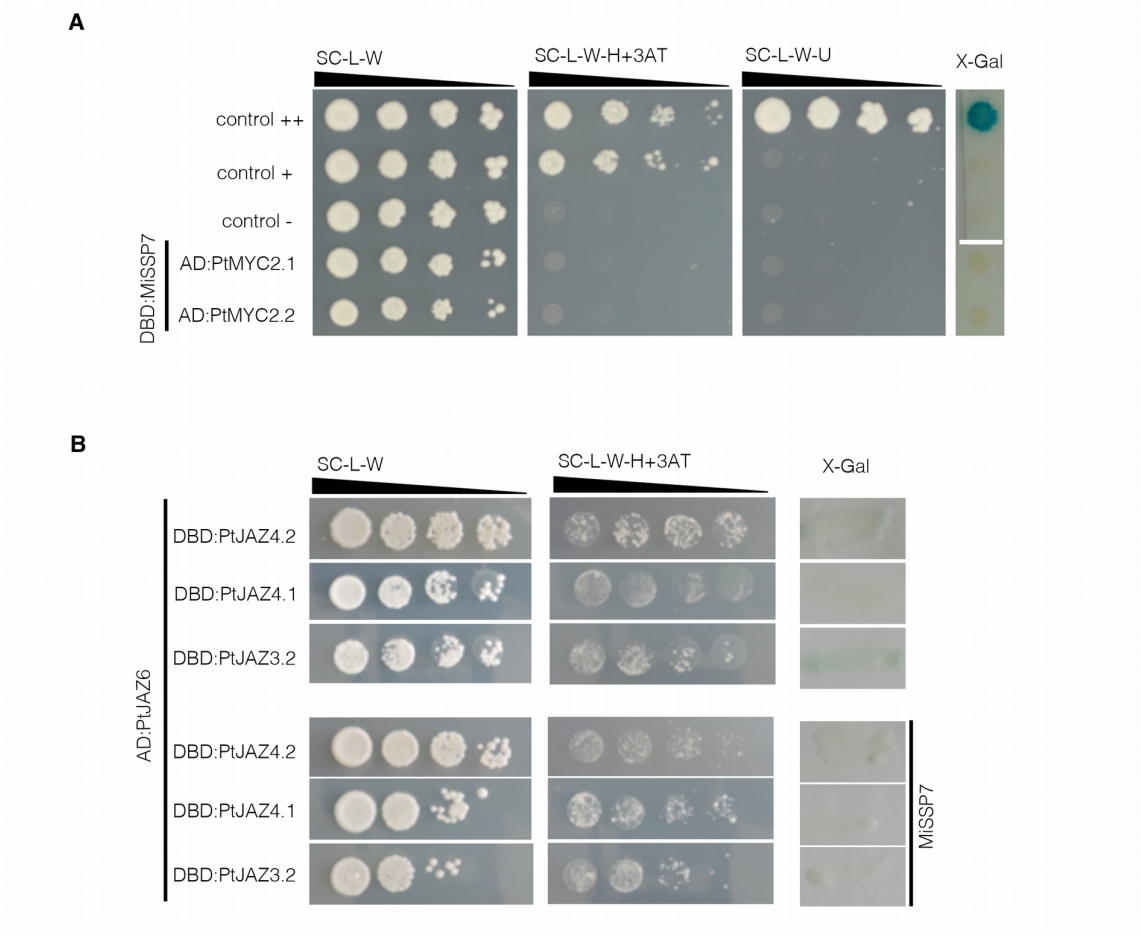
The variable NT domain (blue) is required for interaction with several distinct signal transductions factors such as DELLA protein or HDA6. The TIFY (ZIM) domain (red) in the middle is required for the dimerization of JAZs. For most Arabidopisis JAZ proteins, it is also required to recruit TPL or TPRL proteins indirectly through the EAR motif-containing in NINJA. The C-terminal Jas domain (green) is highly conserved, contains the degron and is required for binding to COI1 and TFs. The aminoacid sequences of PtJAZ6 and AtJAZ6 were aligned using T-Coffee and the alignment was formatted with Boxshade.

EAR (Ethylene-responsive element binding factor-associated amphiphilic repression) is underlined.



Supplemental Figure S4. R209 residue of PtJAZ6 is required for its coronatine-dependent interaction with PtCOI1.

PtJAZ6 was fused to GAL4 DNA-binding domain (DBD). PtJAZ proteins were fused with the GAL4-activation domain (AD). Yeasts were transformed with MiSSP7 *to give triple hybrids.* TRP1, LEU2 *and* URA3 *served as transformation marker genes and* HIS3 *and* □-galactosidase LacZ *(X-Gal) were used as reporter genes. 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene.*



Supplemental Figure S5.

(A) MiSSP7 does not interact with either PtMYC2.1 or PtMYC2.2*. PtMYC2.1 and PtMYC2.2 were fused to GAL4-activation domain (AD) and MiSSP7 to GAL4-DNA binding domain (DBD).* LEU2 *and* TRP1 *served as transformation markers.* HIS3, URA3 *and* □-galactosidase LacZ *(XGal) were used as reporter genes.*

(B) MiSSP7 does not affect the weak interactions between PtJAZ6 and other PtJAZ proteins. *PtJAZ6 was fused to with the GAL4-activation domain (AD). Other PtJAZ proteins were fused to GAL4 DNA-binding domain (DBD). Yeasts were transformed with MiSSP7 to give triple hybrids.* TRP1, LEU2 *and* URA3 *served as transformation markers and* HIS3 *and* □-galactosidase LacZ *(XGal) were used as reporter genes. 3-Amino-1, 2, 4-triazole (3-AT) was used to suppress self-activation at the* HIS3 *gene.*

Protein ID	best arabidopsis TAIR10 hit defline	Name (phylogeny)	In vitro-grown poplar and L. bicolor							
MYC2			**FPKM ULR 1w**	**FPKM CLR 1w**	**FPKM ULR 2w**	**FPKM CLR 2w**	**log2FC CLR vs ULR 1w**	**p-value CLR vs ULR 1w**	**log2FC CLR vs ULR 2w**	**p-value CLR vs ULR 2w**
Potri.001G142200.1	Basic helix-loop-helix (bHLH) DNA-binding family protein	PtMYC2.1	11.88	6.68	11.96	7.46	-0.66	0.1	-0.53	0.25
Potri.003G092200.1	Basic helix-loop-helix (bHLH) DNA-binding family protein	PtMYC2.2	10.74	5.35	10.62	6.94	-0.83	0.035	-0.46	0.31
JAM			**FPKM ULR 1w**	**FPKM CLR 1w**	**FPKM ULR 2w**	**FPKM CLR 2w**	**log2FC CLR vs ULR 1w**	**p-value CLR vs ULR 1w**	**log2FC CLR vs ULR 2w**	**p-value CLR vs ULR 2w**
Potri.002G172100.1	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	PtJAM1.2	5.83	7.00	7.49	8.57	0.49	0.022	0.39	0.11
Potri.014G099700.1	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	PtJAM1.1	8.40	7.01	8.80	8.79	-0.088	0.75	0.15	0.61
JAZ			**FPKM ULR 1w**	**FPKM CLR 1w**	**FPKM ULR 2w**	**FPKM CLR 2w**	**log2FC CLR vs ULR 1w**	**p-value CLR vs ULR 1w**	**log2FC CLR vs ULR 2w**	**p-value CLR vs ULR 2w**
Potri.001G062500.1	jasmonate-zim-domain protein 10	PtJAZ10.1	3.26	3.91	2.26	2.29	0.47	0.12	0.23	0.55
Potri.003G165000.1	jasmonate-zim-domain protein 10	PtJAZ10.2	1.49	1.94	2.82	2.64	0.51	0.16	0.09	0.85
Potri.006G139400.1	jasmonate-zim-domain protein 1	PtJAZ1/2	7.93	8.10	9.28	3.74	0.2	0.66	-1.1	0.012
Potri.006G217200.1	jasmonate-zim-domain protein 12	PtJAZ11	32.35	28.14	35.29	37.89	0.0045	0.99	0.28	0.23
Potri.018G047100.1	jasmonate-zim-domain protein 12	PtJAZ12	0.78	0.82	0.79	0.79	0.29	0.49	0.16	0.75
Potri.001G166200.1	jasmonate-zim-domain protein 5	PtJAZ5	0.72	0.61	0.79	0.25	0.067	0.94	-1.2	0.027
Potri.003G068900.1	jasmonate-zim-domain protein 6	PtJAZ6	3.37	3.67	3.62	1.63	0.27	0.54	-0.95	0.039
Potri.011G083900.1	jasmonate-zim-domain protein 8	PtJAZ7/8	0.15	0.50	0.39	0.09	1.5	0.039	-1.3	0.11
Potri.008G133400.1	jasmonate-zim-domain protein 3	PtJAZ3.3	15.46	17.83	18.34	15.55	0.41	0.079	-0.057	0.86
Potri.010G108200.1	jasmonate-zim-domain protein 3	PtJAZ3.4	12.38	12.88	16.53	19.11	0.25	0.35	0.39	0.19
Potri.012G044900.1	jasmonate-zim-domain protein 3	PtJAZ3.1	15.32	15.18	16.12	13.68	0.2	0.59	-0.045	0.92
Potri.015G035800.1	jasmonate-zim-domain protein 3	PtJAZ3.2	8.63	6.17	7.18	3.49	-0.28	0.49	-0.85	0.054
NINJA			**FPKM ULR 1w**	**FPKM CLR 1w**	**FPKM ULR 2w**	**FPKM CLR 2w**	**log2FC CLR vs ULR 1w**	**p-value CLR vs ULR 1w**	**log2FC CLR vs ULR 2w**	**p-value CLR vs ULR 2w**
Potri.006G156100.1	novel interactor of JAZ	PtNINJA2	0.47	0.76	0.75	1.09	0.86	0.067	0.7	0.18
Potri.006G162900.1	novel interactor of JAZ	PtNINJA1	0.34	0.28	0.53	0.51	0.0058	1	0.073	0.92
Potri.018G085100.1	novel interactor of JAZ	PtNINJA3	21.41	33.80	23.90	31.35	0.88	0.00097	0.56	0.069

TOPLESS			FPKM ULR 1w	FPKM CLR 1w	FPKM ULR 2w	FPKM CLR 2w	log2FC CLR vs ULR 1w	p-value CLR vs ULR 1w	log2FC CLR vs ULR 2w	p-value CLR vs ULR 2w
Potri.006G257900.1	TOPLESS-related 1		8.68	3.62	8.04	5.17	-1.1	0.035	-0.49	0.4
Potri.018G023900.1	TOPLESS-related 1		7.00	3.09	6.29	3.93	-0.93	0.14	-0.52	0.49
Potri.001G200500.1	TOPLESS-related 1	PtTPR1.2	10.50	7.17	9.76	7.81	-0.33	0.097	-0.16	0.5
Potri.003G030000.1	TOPLESS-related 1	PtTPR1.1	12.86	7.03	11.11	7.08	-0.66	0.038	-0.48	0.19
Potri.006G258100.1	TOPLESS-related 1		0.60	0.24	0.80	0.38	-1	0.22	-0.81	0.39
Potri.005G021700.1	TOPLESS-related 3	PtTPR3.1	17.30	9.25	17.99	13.50	-0.69	0.14	-0.27	0.64
Potri.013G012100.1	TOPLESS-related 3	PtTPR3.2	9.65	5.67	10.57	7.56	-0.57	0.15	-0.33	0.49
Potri.005G019500.1	TOPLESS-related 3		0.00	0.00	0.00	0.00	NA	NA	NA	NA
Potri.006G066800.1	WUS-interacting protein 2	PtTPR4.1	13.71	4.87	12.49	5.46	-1.2	0.11	-0.94	0.28
Potri.018G128000.1	WUS-interacting protein 2	PtTPR4.2	12.32	4.33	11.72	6.67	-1.3	0.03	-0.65	0.34

Table S1. Expression level of poplar proteins involved in JA-signaling pathway in in vitro grown poplar-L. bicolor ectomycorrhiza and in poplar root tips.

Data were extracted from the NCBI Bioproject PRJNA444231, PRJNA444232, PRJNA444233, PRJNA443960, PRJNA443961, PRJNA443962, PRJNA444765, PRJNA444766, PRJNA444185, PRJNA444207, PRJNA444208, PRJNA444209. ULR: Uncolonized Lateral Roots. CLR: Colonized Lateral Roots.

Amplified sequence	Targeted gene	Primer names	Sequence 5'-3'
MiSSP7	298595	MiSSP7F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGAAGCTT TTAGTCCCTCTCAA
		MiSSP7R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAACGATGC AAATCACCC
PtJAZ6	Potri.003G068900.1	J6attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGCGAATATGGCACAG
		J6attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACAATTTAAGCTCGAGCTG
PtJAZ6 TIFY domain	Potri.003G068900.1	J6TIFYattB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGTTTGTGGGTTCTGGTCCTC
		J6TIFYattB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAAGCAGAGCTGGGACAC
PtJAZ6 JAS domain	Potri.003G068900.1	J6JASattB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGAGAAAAGTCAGCTCCACC
		J6JASattB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAAGGCCTAGGATGGTCC
PtJAZ6 NT domain	Potri.003G068900.1	J6attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGCGAATATGGCACAG
		J6NTattB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAAGCGTTTTGCCTTGAAG
PtJAZ6ΔNT	Potri.003G068900.1	J6TIFYattB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGTTTGTGGGTTCTGGTCCTC
		J6attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACAATTTAAGCTCGAGCTG
PtJAZ6ΔTIFY	Potri.003G068900.1	J6del121-159fw	CTTGAGGAAATCGTCTCCAATTAGCACAGATGGGTGTC
		J6del121-159rv	GACACCCATCTGTGCTAATTGGAGACGATTTCCTCAAG
PtJAZ6ΔJAS	Potri.003G068900.1	J6attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGCGAATATGGCACAG
		J6TIFYattB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAAGCAGAGCTGGGACAC
PtJAZ6R208A	Potri.003G068900.1	J6R208Afw	TCAGATGTGCCCCATGCTGCAAGAGCTTCACTTCATCG
		J6R208Arv	CGATGAAGTGAAGCTCTTGCAGCATGGGGCACATCTGA
PtJAZ6R209A	Potri.003G068900.1	J6R209Afw	TCAGATGTGCCCCATGCTAGAGCAGCTTCACTTCATCG
		J6R209Arv	CGATGAAGTGAAGCTGCTCTAGCATGGGGCACATCTGA
PtJAZ6R208,209A	Potri.003G068900.1	J6R208AR209Af w	TCAGATGTGCCCCATGCTGCAGCAGCTTCACTTCATCG
		J6R208AR209Arv	CGATGAAGTGAAGCTGCTGCAGCATGGGGCACATCTGA
PtMYC2.1	Potri.001G142200.1	MYC2.1attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGACTGATTACCGACTACCG
		MYC2.1attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATGGACGCCACCAAC
PtMYC2.2	Potri.003G092200.1	MYC2.2attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGACTGATTACCGTCTACC
		MYC2.2attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATCGGGCATCACCAAC
PtJAM1.1	Potri.014G099700.1	JAM1.1attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGAAGATTGAGCTGGGTGT G
		JAM1.1attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACCCAGTTGATGATAATG
PtJAM1.2	Potri.002G172100.1	JAM1.2attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGGAGGTGGGGCATGG
		JAM1.2attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAGTCGGGAATTGTTGATG
PtJAM3.1	Potri.005G221100.1	JAM3.1attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGGTGATAAATTTTGGGTG
		JAM3.1attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCATTTAGAAAGTGCGGCCTC
PtJAM3.2	Potri.002G042000.1	JAM3.2attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGGTGAGAAATTTTGGGT G
		JAM3.2attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCATTTAGAAAGTGCAGCCTC
PtNINJA1/PtNINJA3	Potri.006G162900.1/ Potri.018G085100.1	NINJAattB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGAGGATGAGAATGGGC
		NINJAattB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAGCTCTGAGTAGAGGCAGC
PtTPR1.1	Potri.003G030000.1	TPR1.1attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGGGATTTCTGATGAG
		TPR1.1attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCGTATGATACCTTCAAGCA
PtTPR4.1	Potri.006G066800.1	TPR4.1attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGTCTTCGCTTAGCCGAG
		TPR4.1attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCGCCACGAATATCTTCTG
PtTPR4.2	Potri.018G128000.1	TPR4.2attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGTCTTCGCTTAGTAGAG
		TPR4.2attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCACGAGAGTCAGAAGCCA

Table S2. List of primers used in the study.

Chapter 3: The JAZ6-associated transcription factor MYC2.1 inhibits Hartig net development

Chapter 3: The JAZ6-associated transcription factor MYC2.1 inhibits Hartig net development

1. Preface

Along the course of this PhD project we found that four *Populus trichocarpa* bHLH transcription factors (TFs), PtMYC2.1, PtMYC2.2, PtJAM1.1 and PtJAM1.2, interact with the jasmonate (JA) co-receptor PtJAZ6. Further, we demonstrated that the effector protein LbMiSSP7 from *Laccaria bicolor* enhances the binding of PtJAZ6 to PtMYC2.1, while negatively affecting the interaction between PtJAZ6 and PtMYC2.2. Since PtJAZ6 is a repressor of JA signaling, these dynamics suggest preferential repression of the branch of JA signaling regulated by PtMYC2.1 during ectomycorrhizal development (Chapter 2). We consequently investigate whether distinct branches of JA signaling, specifically controlled by one PtMYC2 or PtJAM1 TF, play different roles in the development of ectomycorrhizae (ECM) between roots of grey poplar (*Populus tremula x alba*) and hyphae of *L. bicolor*.

To this end, we genetically manipulated the expression of three *P. tremula x alba* bHLH-type TFs: *PtaMYC2.1* (Potri.001G142200), *PtaMYC2.2* (Potri.003G092200), and *PtaJAM1.1* (Potri.014G099700). In this chapter we show the phenotype of poplar transgenic lines overexpressing *PtaMYC2.1* and *PtaJAM1.1*, as well as chimeric plants with roots silenced for *PtaMYC2s* or *PtaJAM1s*. We measured ectomycorrhizal colonization rate, Hartig net frequency and depth, shoot weight, pigment content, and root architecture. We also demonstrate, via RT-qPCR, that fungal inoculation affects the expression of *PtaMYC2.1* in roots of both control and *PtaMYC2.1*-overexpressing poplar lines, and that similar trends are visible for the expression of *PtaMYC2.2* and *PtaJAM1.1*. These results suggest that multiple regulatory mechanisms ensure the suppression of the PtaMYC2.1-dependent branch of JA signaling, hinting at its crucial importance for apoplastic colonization of fungal hyphae.

Although this chapter is structured as a research article, the presented data is still preliminary and some results need to be strengthened in order to be published.

2. Article (in preparation)

Title: The poplar JAZ6-associated transcription factor MYC2.1 inhibits Hartig net development in poplar-*L. bicolor* ectomycorrhizae

Authors: Veronica Basso[1], Francis Martin[1,2], Claire Veneault-Fourrey[1]

Contact information: [1]Université de Lorraine, INRA, UMR Interactions Arbres/Microorganismes (IAM), Laboratoire d'excellence Recherches Avancés sur la Biologie de l'Arbre et les Ecosystèmes Forestiers (LabEx ARBRE), Centre INRA Grand-Est, 54280 Champenoux, France.
[2]Beijing Forestry University, 35 Qinghua E Rd, Haidian Qu, Beijing Shi, China, 100083.

2.1. Abstract

Active suppression of plant responses to the phytohormone jasmonate (JA) is crucial for successful host colonization by the mutualistic ectomycorrhizal fungus *Laccaria bicolor*. Indeed, the fungal effector MiSSP7, stabilizing the poplar co-receptor of JA signaling PtJAZ6, is required for the development of ectomycorrhizae (ECM) between roots of *Populus* spp. and *L. bicolor* mycelium. We previously found that PtJAZ6 interacts with PtMYC2 and PtJAM1 transcription factors (TFs), respectively homologous to Arabidopsis activators and repressors of JA signaling. Here, via altered expression of *PtaMYC2s* and *PtaJAM1s* through genetic engineering of poplar roots, we investigated the role of JAZ6-associated TFs in the development of ECM between roots of *Populus tremula x alba* and *L. bicolor*. We found that PtaMYC2.1 and PtaJAM1.1 contribute to the regulation of Hartig net depth and frequency, and that the expression of the correspondent genes is influenced by fungal colonization. These results confirm the pivotal importance of repressed JA signaling for the establishment of mutualism and hint at the existence of multiple regulatory mechanism for the suppression of the PtaMYC2.1-dependent branch of JA signaling during ECM formation.

2.2. Introduction

The phytohormone jasmonate (JA) is a pivotal signal driving plant defenses against herbivores and necrotrophic pathogens, as well as a regulator of physiological processes such as pollen maturation, primary root growth inhibition, and trichome development (Glazebrook, 2005; Wasternack & Hause, 2013). In Arabidopsis the main bioactive form of

JA, JA-Ile, is bound by a co-receptor complex formed by the F-box protein CORONATIN INSENSITIVE1 (COI1) and JAZ proteins (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007). Since COI1 is part of the E3 ubiquitin ligase complex SCF^{COI1}, JAZ proteins are degraded through 26S proteasome upon JA perception, releasing the repression over JAZ-interacting TFs and allowing the initiation of responses to JA (Melotto et al., 2008; Katsir et al., 2008). The variety of responses to JA thus depends on a wide array of TFs transducing JA-dependent signals (Chini et al., 2016; Howe et al., 2018). In Arabidopsis the bHLH family TFs MYC2-4 play partially redundant roles in the regulation of root growth, biosynthesis of secondary metabolites, defense against herbivores, and leaf senescence (Fernandez-Calvo et al., 2011; Schweizer et al., 2013; Chico et al., 2014; Qi et al., 2015; Major et al., 2017). In particular, MYC2, binding to G-boxes of its target promoters, orchestrates a hierarchical transcriptional cascade regulating responses to wounding and necrotrophic pathogens (Kazan & Manners, 2013; Dombrecht et al., 2007; Du et al., 2017). On the other hand, the bHLH subgroup IIId TFs bHLH17/JASMONATE ASSOCIATED MYC2-LIKE1(JAM1), bHLH13/JAM2, bHLH3/JAM3 and bHLH14 are transcriptional repressors implied in the termination of JA signaling (Song et al., 2013; Nakata et al., 2013; Sasaki-Sekimoto et al., 2013; Fonseca et al., 2014; Liu et al., 2019). JAM-like TFs are expressed in a COI1- and MYC2-dependent manner and antagonize transcriptional activators by competitively binding G-boxes in the promoters of target genes (Sasaki-Sekimoto et al., 2013; Fonseca et al., 2014; Song et al., 2013; Nakata et al., 2013; Qi et al., 2015b). Interestingly, the activity of both MYC- and JAM-like TFs can be suppressed by JAZ proteins (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Song et al., 2013). Therefore, the output of activated JA signaling depends on the repertoire of TFs expressed in determined conditions.

The modulation of JA signaling is often connected to the success of plant-microbe pathogenic or mutualistic interactions (Gimenez-Ibanez et al., 2016; Zhang et al., 2017; Basso & Veneault-Fourrey, 2019). Indeed, the antagonism between JA and salicylic acid (SA) signaling is central in the regulation of plant responses to microbes with different trophic strategies (Glazebrook, 2005). The signaling components of JA and SA transduction pathways interact at multiple levels ensuring mutually exclusive activation of targeted defenses (Pieterse et al., 2009; 2012). Consequently, many plant pathogens evolved the ability to exploit the SA-JA antagonism to ensure their own growth *in planta*, either through the production of phytohormones and phytohormone-mimics or upon secretion of specialized small secreted proteins, termed effectors, targeting plant signaling components (Gimenez-Ibanez et al., 2016; Zhang et al., 2017). Sustained activation of JA signaling is also detrimental for the establishment of several mutualistic interactions between plants and microbes, possibly because of an untimely activation of plant defenses (Basso & Veneault-Fourrey, 2019).

To suppress JA signaling in *Populus* roots, the ectomycorrhizal fungus *Laccaria bicolor* secretes the mutualistic effector Mycorrhiza-induced Small Secreted Protein of 7 kDa (MiSSP7), which stabilizes the poplar PtJAZ6 protein (Plett et al., 2011; 2014a). This ensures the growth of fungal hyphae in the root apoplast to form the Hartig net, the structure of nutrient exchange between the symbiotic partners. However, transcriptomic analysis of MeJA-treated *L. bicolor-Populus tremula x alba* co-cultures revealed that a subset of JA-responsive genes is regulated during the establishment of ectomycorrhizae (ECM; Basso et al., submitted). Therefore, it is possible that different branches of JA signaling play contrasting roles in ECM development. In addition, we showed in a previous study that PtJAZ6 interacts with two poplar paralogs of MYC2, which we named PtMYC2.1 (Potri.001G142200) and PtMYC2.2 (Potri.003G092200), as well as with two poplar paralogs of JAM1, that we named PtJAM1.1 (Potri.014G099700) and PtJAM1.2 (Potri.002G172100). We also demonstrated that MiSSP7 enhances the interaction between PtJAZ6 and PtMYC2.1, while negatively affecting the interaction between PtJAZ6 and PtMYC2.2. Since PtJAZ6 is a repressor of JA signaling, these findings allow to speculate that *L. bicolor* induces PtMYC2.2-dependent gene expression in poplar roots, while suppressing PtMYC2.1-mediated responses. We advanced the hypothesis that PtMYC2.1 and PtMYC2.2 control different branches of JA signaling, with putative different target genes (Daguerre et al., submitted).

To test this hypothesis and further investigate whether poplar MYC2 or JAM1 TFs play different roles in the establishment of mutualism with *L. bicolor*, we genetically engineered the expression level of genes coding for these TFs in the easily transformable *P. tremula x alba* INRA 717-1B4 clone. We created poplar transgenic lines overexpressing *PtaMYC2.1*, *PtaMYC2.2*, and *PtaJAM1.1* via *Rhizobium*-mediated transformation. In addition, we generated chimeric plants with roots silenced for the two *PtaMYC2* or the two *PtaJAM1* genes via double-stranded RNA-interference (dsRNAi). In this chapter we analyze the phenotypes of *L. bicolor*-colonized and uncolonized poplar transgenic lines overexpressing *PtaMYC2.1* and *PtaJAM1.1*, as well as chimeric plants silencing *PtaMYC2s* or *PtaJAM1s* in their root system. Here we show the impact of such genetic manipulation on mycorrhizal colonization rate, ECM development, shoot weight and pigment content, root architecture, and expression of JAZ6-associated TFs.

2.3. Results

Generation of poplar transgenic lines and chimeric plants overexpressing or silencing *PtaMYC2s* or *PtaJAM1s*

We isolated the *P. tremula x alba* full-length coding sequence (CDS) of *PtaMYC2.1*, *PtaMYC2.2* and *PtaJAM1.1* via PCR on a cDNA library obtained from poplar-*L. bicolor* ECM. However, we failed to isolate *PtaJAM1.2*, possibly because of an erroneous assembly of the gene model in the genome of *P. trichocarpa* v.3.0 and, consequently, an erroneous design of cloning primers. Indeed, the gene model of *PtJAM1.2* differs significantly between the versions 3.0 and 3.1 of the *P. trichocarpa* genome with the 56 bases at the 3' terminus of the CDS displaying only ~45% sequence identity between the two gene models (Supplemental Figure 1), suggesting that *PtJAM1.2* is highly polymorphic in its 3' terminus or that the gene model is imprecise in one of the two genome assemblies. We cloned the CDS of the three genes under the control of the Cauliflower Mosaic Virus 35S promoter onto a binary vector for plant transformation. We generated poplar transgenic lines overexpressing *PtaMYC2.1*, *PtaMYC2.2*, and *PtaJAM1.1* (named MYC2.1, MYC2.2, and JAM1.1 OE lines), as well as control transgenic lines overexpressing a fragment of the *GUS* gene (named GUS lines). Our method is based on *Rhizobium rhizogenes* (formerly *Agrobacterium rhizogenes*)-mediated transformation of poplar leaves, selection of transformed roots and shoot regeneration via exposure to the cytokinin (CK) thidiazuron (TDZ) (Cope et al., 2019). In addition, we isolated ~300 bp-long CDS fragments of *PtaMYC2* and of *PtaJAM1*, conserved between the two paralogs, and cloned them into a vector for double-stranded RNA-interference (dsRNAi)-mediated gene silencing. We attempted to generate dsRNAi-silenced lines for *PtaMYC2* and *PtaJAM1* (named MYC2 and JAM1 dsRNAi lines), as well as control lines transformed with the empty vector (named pRedRoot lines). In addition to the genes or gene fragments of interest under the control of the 35S promoter, transgenic roots constitutively expressed a red fluorescent protein. Thus transformed roots and regenerated shoots could be selected via emission of red fluorescence when excited by a laser at 561 nm (Supplemental Figure 2A).

We selected three GUS lines, three MYC2.1 OE lines, one MYC2.2 OE line, and one JAM1.1 OE line based on the level of expression of *PtaMYC2.1*, *PtaMYC2.2*, and *PtaJAM1.1* in shoots (Supplemental Figure 2C). However, we were incapable of recovering MYC2 and JAM1 dsRNAi transgenic poplar lines, since the transformed roots never generated shoots. GUS and pRedRoot transgenic lines were phenotypically similar to each other and *L. bicolor* colonized their roots to the same extent as wild-type roots (Supplemental Figure 2B). However, MYC2.1 and MYC2.2 OE plants displayed ectopic root growth at several internodes. The MYC2.2 OE line 2 also showed enhanced shoot branching, resulting

in a bush-like vegetative growth. On the other hand, transgenic roots silenced for *PtaMYC2* and *PtaJAM1*, as well as overexpressing *PtaJAM1.1*, hardly produced any shoots, even in presence of TDZ (Supplemental Figure 2D). The sole poplar JAM1.1 OE line able to regenerate shoots expressed *PtaJAM1.1* only ~22 times more than GUS lines (Supplemental Figure 2C). These results suggest that JAM1.1 OE roots could produce shoots only when expressing *PtaJAM1.1* within a limited fold-change with respect to control.

To compensate for the lack of MYC2 and JAM1 dsRNAi lines, we produced composite plants with roots silenced for *PtaMYC2s* and *PtaJAM1s* via *Rhizobium rhizogenes*-mediated transformation. Through this approach we obtained 10 to 16 chimeric plants per construct composed of a wild-type shoot and transformed roots. Transformed roots were selected according to the expression of the *p35S::dsRED* fluorescent marker, under an epifluorescence microscope.

In the following paragraphs we will present the results relative to one GUS line, one MYC2.1 OE line, one JAM1.1 OE line, and chimeric silenced plants. For lack of time, we did not test any MYC2.2 OE lines.

The overexpression of *PtaMYC2.1* and *PtaJAM1.1* impacts ectomycorrhizal development

To investigate the role of PtaMYC2.1 and PtaJAM1.1 in ECM development, we assessed the mycorrhization phenotype of the poplar lines GUS 6, MYC2.1 OE 2 and JAM1.1 OE 3 grown in contact with *L. bicolor* mycelium under axenic conditions. After two weeks of co-culture, the rate of mycorrhizal colonization was quantified and mature ECM were harvested for microscopic analysis. We found that the frequency of mycorrhizal colonization was higher in the OE lines with respect to the control line (Figure 1A). However, whereas the overexpression of *PtaJAM1.1* resulted in a higher frequency of Hartig net penetration through the root apoplastic space, and in a tendency towards an increased Hartig net depth (Figure 1A-B), the overexpression of *PtaMYC2.1* induced a decrease in mantle thickness, a diminished frequency of Hartig net penetration, and a tendency towards a diminished Hartig net depth (Figure 1A-B). These phenotypes were independent from the root diameter, the average number of apoplastic spaces in the colonized cell layer, and the frequency of Hartig net initiation, which did not differ among lines (Supplemental Figure 3).

In conclusion, the overexpression of the transcriptional activator PtaMYC2.1 impaired normal ECM development, while the overexpression of the transcriptional repressor PtaJAM1.1 enhanced intercellular fungal colonization.

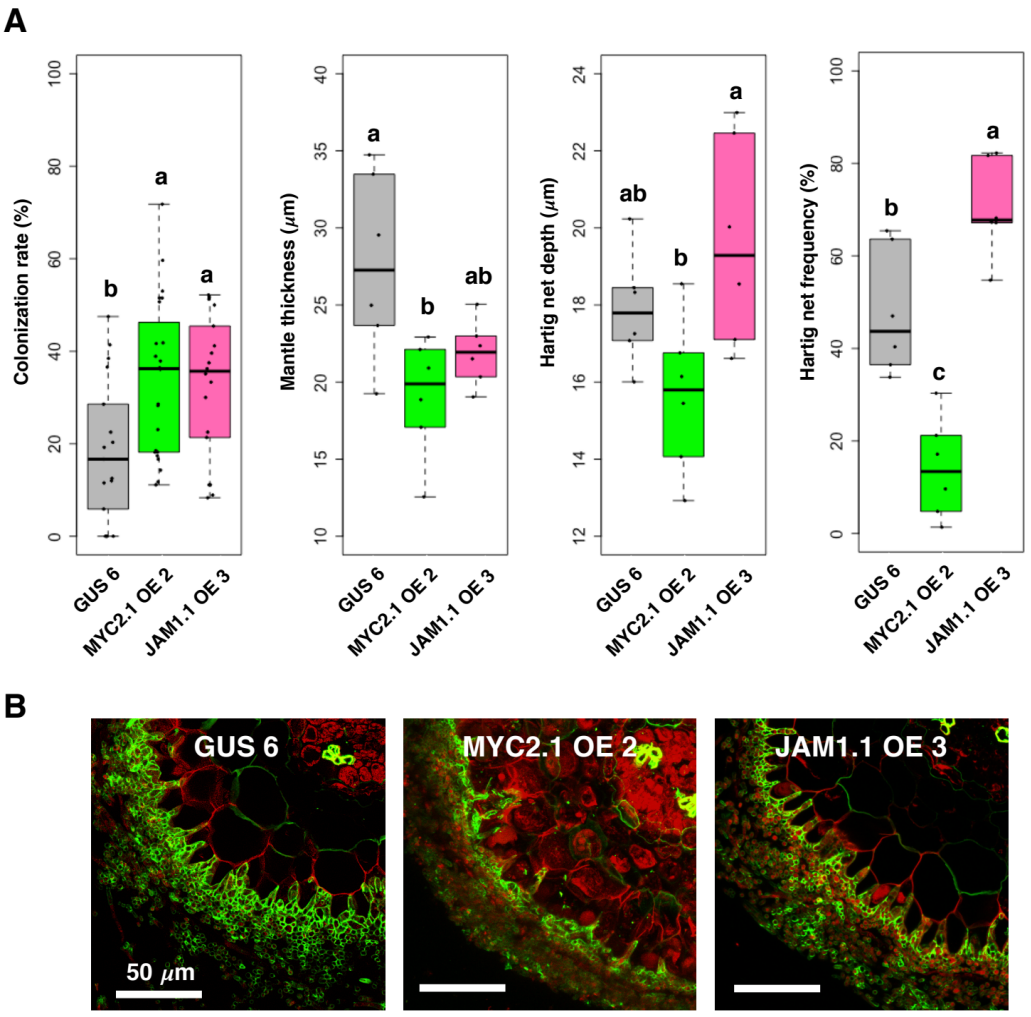


Figure 1. The overexpression of PtaMYC2.1 and PtaJAM1.1 in poplar roots alters Hartig net development during L. bicolor colonization.

A. Boxplots showing the distribution of colonization rate, mantle thickness, Hartig net depth and Hartig net frequency of colonized transgenic lines overexpressing a fragment of the GUS *gene (GUS line 6) or the poplar genes* PtaMYC2.1 *(MYC2.1 OE line 2) and* PtaJAM1.1 *(JAM1.1 OE line 3). Whiskers represent the limits of the 1.5 interquartile range. Letters indicate significant differences based on the results of Kruskal-Wallis one-way analysis of variance and post-hoc Fischer's LSD test with Bonferroni correction ($p < 0.05$). B. Confocal microscopy images of representative sections of ECM harvested from colonized GUS 6, MYC2.1 OE 2, and JAM1.1 OE 3 lines. Scale bar: 50 µm.*

The overexpression of *PtaMYC2.1* and *PtaJAM1.1* affects root architecture

To determine the possible impact of MYC2.1 or JAM1.1 overexpression on poplar development, we measured phenotypic traits, such as shoot weight, pigment content, and root architecture of the transgenic lines (GUS 6, MYC2.1 OE 2 and JAM1.1 OE 3) colonized or not with *L. bicolor* hyphae (Supplemental Figure 4). Overexpressing lines were similar to control lines in terms of shoot weight, anthocyanin and chlorophyll contents in both mycorrhizal and non-mycorrhizal conditions (Supplemental Figure 4A). However, we found that uncolonized MYC2.1 OE 2 cuttings displayed more adventitious roots and a tendency towards reduced lateral root density with respect to control GUS 6 plants (Supplemental Figure 4B). On the other hand, uncolonized JAM1.1 OE 3 cuttings displayed a tendency towards higher lateral root density with respect to the control. In mycorrhizal conditions all lines were almost identical, with the exception of a higher secondary lateral root density and a lower adventitious root length of JAM1.1 OE 3 cuttings.

Overall, these results suggest that the overexpression of *PtaMYC2.1* and *PtaJAM1.1* differentially affects root architecture.

***L. bicolor* colonization represses *PtaMYC2.1*, *PtaMYC2.2* and *PtaJAM1.1* expression in transgenic poplar roots**

As controls for our experiment, we monitored the expression of *PtaMYC2.1*, *PtaMYC2.2*, and *PtaJAM1.1* in roots of transgenic GUS 6, MYC2.1 OE 2 and JAM1.1 OE 3 lines, normalized by the expression of the housekeeping gene *Ubiquitin* (Figure 2). We found that roots of uncolonized MYC2.1 OE 2 plants expressed *PtaMYC2.1* ~11 times more with respect to roots of the GUS 6 line. Interestingly, the expression of *PtaMYC2.1* was significantly reduced (two-sided Welch t-test, $p < 0.05$) in colonized roots of both GUS 6 (7-fold) and MYC2.1 OE 2 (6.6-fold) plants with respect to their uncolonized counterparts, eliminating the difference between the two lines. A similar pattern was detected for the expression of *PtaJAM1.1* in GUS 6 and JAM1.1 OE 3 colonized and non-colonized lines, even though the high variance among replicates of JAM1.1 OE 3 roots did not allow to highlight significant differences between the control and OE line. Finally, a non-statistically significant tendency towards diminished *PtaMYC2.2* expression was detected in roots of GUS 6 control plants (Figure 2A). These results were unexpected, since a previous study, employing a high-throughput analysis of wild-type *P. tremula x alba-L. bicolor* metatranscriptome, did not reveal any significant transcriptional regulation of *PtaMYC2.1*, *PtaMYC2.2*, and *PtaJAM1.1* during ECM development (Basso et al., submitted).

To exclude that results may be due to cross-amplification of *L. bicolor* transcripts within the composite ectomycorrhizal tissue, we verified whether the primers employed for detection of the target transcripts, as well as *Ubiquitin*, amplified fragments using a *L. bicolor*

free-living mycelium (FLM) cDNA library as template. We found that in some cases FLM samples produced amplicons; however, such amplification was inconsistent across technical and biological replicates, and occurred, if ever, after 30 or more PCR cycles. In other cases, the melting curves from FLM samples coincided with those of negative controls without any cDNA, suggesting the formation of primer dimers in absence of template (Supplemental Figure 5A). Altogether, these results indicate that the employed primers are unlikely to amplify off-target sequences in presence of the correct template. Thus, the presence of fungal mycelium in ECM is unlikely to impair the detection of poplar target transcripts with the employed primers. We also assessed that colonized transgenic roots still expressed the fluorescent marker RFP, thereby excluding the possibility that these cuttings had lost the transgenic construct (Supplemental Figure 5B). Finally, quality control of the extracted RNAs ensured that colonized and uncolonized roots displayed similar levels of RNA integrity (Supplemental Figure 5C).

To assess whether the observed results are genotype-specific, or dependent on the sampling strategy, we checked the relative expression level of *PtaMYC2.1*, *PtaMYC2.2* and *PtaJAM1.1* in colonized and uncolonized (i) wild-type poplar lateral roots, (ii) whole root systems of poplar GUS 6 transgenic lines, and (iii) lateral roots of GUS 2 transgenic lines (Figure 2B). We found that in uncolonized conditions the expression of *PtaMYC2.1* and *PtaMYC2.2* tended to be higher in GUS 2 lateral roots than in wild-type lateral roots, while high variability between biological replicates impeded to detect differences in *PtaJAM1.1* expression. Also, mycorrhizal colonization significantly suppressed the expression of *PtaMYC2.2* and *PtaJAM1.1* only in GUS 2 lateral roots, but a tendency towards the same trend was visible for GUS 6 whole root systems. The expression of *PtaMYC2.1* tended to be lower in colonized conditions, especially in the GUS lines, but high variability impeded to detect significant differences.

Altogether, these results suggest that slight suppression of *PtaMYC2.1*, *PtaMYC2.2* and *PtaJAM1.1* expression occurs in poplar transgenic lines upon mycorrhizal colonization, especially when only lateral roots have been sampled.

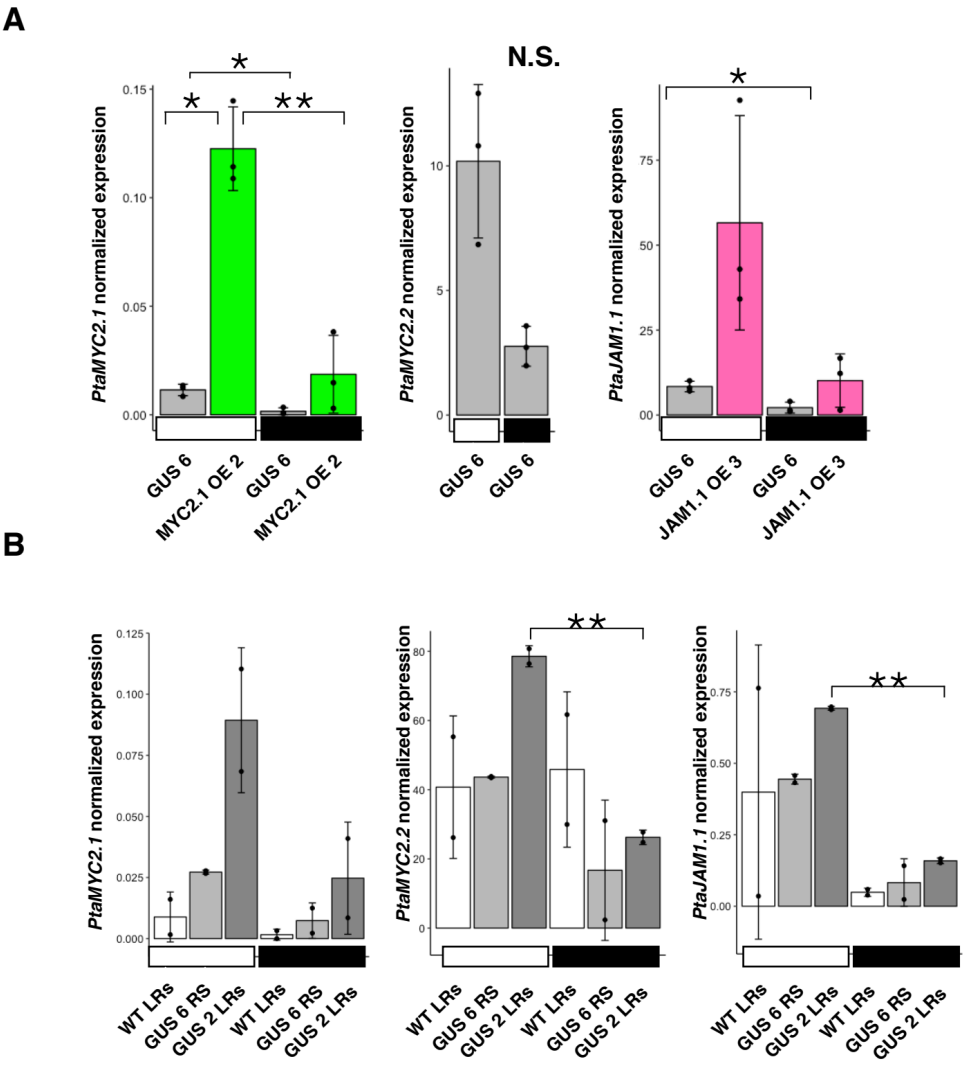


Figure 2. L. bicolor colonization represses PtaMYC2.1, PtaMYC2.2, and PtaJAM1.1 expression in transgenic roots.

A. Bar plots representing differences between whole root systems of GUS 6, MYC2.1 OE 2, and JAM1.1 OE 3 transgenic lines, in colonized and uncolonized conditions, in terms of relative PtaMYC2.1, PtaMYC2.2, *and* PtJAM1.1 *expression, as detected by RT-qPCR. B. Bar plots representing differences between lateral roots of wild-type poplar cuttings, whole root systems of GUS 6 transgenic lines, and lateral roots of GUS 2 transgenic lines, in colonized and uncolonized conditions, in terms of relative* PtaMYC2.1, PtaMYC2.2, *and* PtJAM1.1 *expression, as detected by RT-qPCR. Gene expression was normalized by the expression level of the housekeeping gene* Ubiquitin. *The error bars represent the standard deviation of three (A) or two (B) biological replicates. White boxes indicate uncolonized poplar roots, while black boxes indicate colonized poplar roots. Asterisks indicate significant differences between samples, as shown by two-sided Welch t-test. ** = p < 0.01; * = p < 0.05; N.S. = non-significant. WT = wild type. LRs = lateral roots. RS = root system.*

Silencing of *PtaMYC2s* or *PtaJAM1s* affects ectomycorrhizal development

To assess whether silencing of the genes of interest affected ectomycorrhizal development, we inoculated 10 to 16 chimeric plants silenced for *PtaMYC2s* and *PtaJAM1s* in their root system or transformed with an empty vector (pRedRoot) with mycelium of *L. bicolor*. After two weeks of co-culture, we estimated the mycorrhizal colonization rate and collected mature ECM for microscopic inspection (Figure 3). We found that the silencing of neither *PtaMYC2s* nor *PtaJAM1s* did affect mycorrhizal colonization, nor the total number of lateral roots (Figure 3A). The observation of 6-7 ECM from a preliminary experiment allowed to detect an increased thickness of fungal mantle in MYC2 dsRNAi mycorrhizae (Figure 3A). However, the absence of Hartig net development in our empty vector control indicates that the experimental setup was non optimal and prevents meaningful interpretation of results (Figure 3B).

These preliminary results suggest that suppressed expression of these PtJAZ6-associated TFs does not affect fungal colonization, but might impact mantle thickness. Further experiments are required to confirm these results.

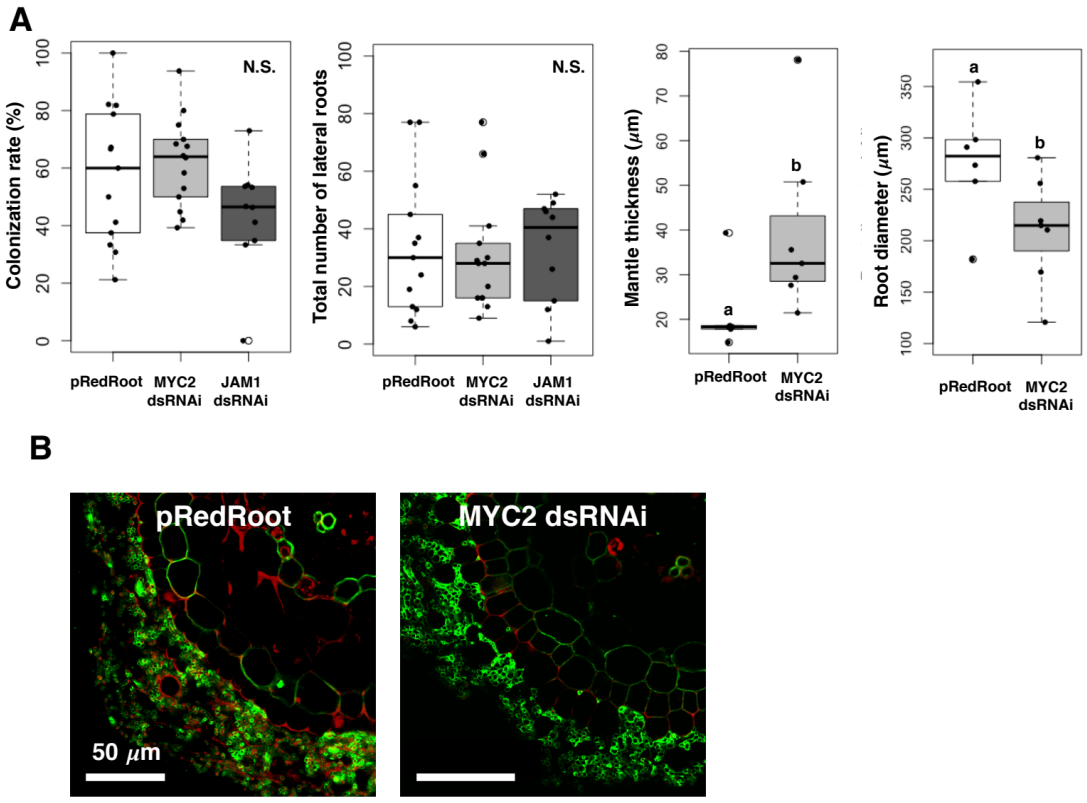


Figure 3. Silencing of PtaMYC2s or PtaJAM1s does not affect the mycorrhizal colonization rate.

A. Boxplots showing the distribution of colonization rate, total number of roots, mantle thickness, and root diameter of colonized roots transformed with an empty vector (pRedRoot) or silenced for PtaMYC2s *(MYC2 dsRNAi) or* PtaJAM1s *(JAM1 dsRNAi). Whiskers represent the limits of the 1.5 interquartile range. Letters indicate significant differences based on the results of Kruskal-Wallis one-way analysis of variance and post-hoc Fischer's LSD test with Bonferroni correction ($p < 0.05$). N.S. = non-significant. B. Confocal microscopy images of representative sections of ECM harvested from colonized pRedRoot and MYC2 dsRNAi roots. Scale bar: 50 µm.*

2.4. Discussion

PtaMYC2 and PtaJAM1 transcription factors are essential for shoot development in transgenic poplar lines

We attempted to generate poplar transgenic lines silenced for *PtaMYC2s* or *PtaJAM1s*, but failed to recover shoots from the transformed roots, even after prolonged treatment with TDZ (Supplemental Figure 2D). A similar effect was observed for most of the JAM1.1 OE lines, with the exception of JAM1.1 OE 3, which expressed the transgene only ~22 times more than the average expression in control GUS lines (Supplemental Figure 2C). These results suggest that both significant activation and suppression of JA signaling negatively impact shoot growth of poplar transgenic lines.

Shoot growth inhibition upon silencing of *PtaJAM1s*, encoding transcriptional repressors of JA signaling, may be attributed to the deleterious effects of constitutively active JA signaling. Indeed, in Arabidopsis active JA signaling promotes defense and suppresses growth via its negative crosstalk with GA signaling (Guo et al., 2018a and references therein). For instance, a decuple *jaz* mutant, displaying constitutive JA responses, presents a severe dwarf phenotype (Guo et al., 2018b). Besides, several reports highlighted the presence of a negative crosstalk between JA and CK signaling in Arabidopsis, acting in xylem development, adaptation to circadian stress, and activation of plant defenses (Jang et al., 2017; Nitschke et al., 2016; Naseem et al., 2013). In particular, MeJA treatment inhibited CK-induced callus growth in soybean (Ueda & Kato, 1982). Therefore, constitutively active JA signaling in poplar transgenic roots may have hampered the perception of the supplied CK TDZ and, consequently, shoot development. Since in Arabidopsis MYC2 induces the expression of the transcriptional repressors JAZ, JAM1-3, and bHLH14, thereby limiting the duration and the amplitude of JA-dependent responses (Sasaki-Sekimoto et al., 2013; Fonseca et al., 2014; Song et al., 2013; Nakata et al., 2013; Qi et al., 2015b), we propose that shoot regeneration by MYC2.1 and MYC2.2 OE poplar lines reflects the presence of

regulatory feedback loops that impeded constitutive expression of MYC2-dependent genes. It remains to be demonstrated if such feedback mechanism also occurs in poplar.

Shoot growth inhibition by *PtaJAM1.1* overexpression or *PtaMYC2s* silencing indicates that constitutive suppression of the MYC2-branch of JA signaling may be deleterious for aerial development of transgenic poplars. This could be linked to the fact that MYC2-dependent JA signaling promotes secondary growth in Arabidopsis (Sehr et al., 2010). Also, a moderate concentration of exogenous MeJA (0.1 µM) was shown to promote the elongation of 4-days-old wheat seedlings through increased CK accumulation (Avalbaev et al., 2016). Therefore, moderate activation of JA signaling may be essential to poplar shoot development.

In conclusion, the complex interplay between JA, GA and CK signaling in poplar might have determined our difficulty to recover shoots for PtaJAM1.1 OE, PtaMYC2s dsRNAi, and PtaJAM1s dsRNAi transgenic lines. Such JA-GA-CK crosstalk in poplar aerial development deserves further attention.

PtaJAM1.1 promotes Hartig net development, while PtaMYC2.1 inhibits it

The inoculation of JAM1.1 OE poplar plants with mycelium of *L. bicolor* resulted in an increased Hartig net depth and frequency with respect to the control GUS line. Conversely, mycorrhizae formed with roots of MYC2.1 OE poplar plants displayed thinner mantles and less deep Hartig nets (Figure 1). These results confirm previous reports indicating that the activation of JA signaling is deleterious for poplar-*L. bicolor* ECM development, while the suppression of JA-dependent gene expression promotes Hartig net formation. Indeed, the *L. bicolor* effector MiSSP7, a repressor of JA responses via the stabilization of the poplar JA co-receptor PtJAZ6, is required for normal Hartig net depth (Plett et al., 2011; 2014a), while exogenous JA treatment or suppressed expression of *PtJAZ6* in poplar roots inhibits fungal apoplastic penetration (Plett et al., 2014a,b; Basso et al., submitted). Nonetheless, we demonstrate here that the two PtJAZ6-interacting TFs MYC2.1 and JAM1.1 are responsible of this effect. Since ECM from MYC2.1 OE roots still displayed ~17% Hartig net frequency, it is possible that additional TFs or signals independent from JA contribute to the regulation of fungal root colonization. For lack of time, we could not test whether the overexpression of *PtaMYC2.2* affects ECM development, but it will be interesting to know if these two paralogous TFs act redundantly in the regulation of Hartig net formation.

Future research should also focus on the transcriptional effect of *PtaMYC2* and *PtaJAM1* overexpression. Considering our recent report that a number of JA-responsive genes putatively coding for pectin lyases, pectinesterases, xyloglucan endotransglucosylases, expansins, and glycosyl hydrolases are regulated in developing ECM (Basso et al., submitted), we expect that some of the genes regulated by these TFs are

implied in cell wall remodeling. Since MYC2, together with MYC4, promotes secondary cell wall thickening of Arabidopsis stems via the activation of a transcriptional cascade enhancing the biosynthesis of cellulose, lignin, and non-cellulosic polysaccharides (Zhang et al., 2018), it is possible that PtaMYC2s affect fungal penetration of the poplar root apoplast through an increased cell wall stiffening.

Despite their effects on Hartig net development, *PtaMYC2.1* and *PtaJAM1.1* overexpression did not affect the mycorrhizal colonization rate (Figure 1). The slightly higher rate of the MYC2.1 OE and JAM1.1 OE lines with respect to the control GUS line can be attributed to a suboptimal colonization of the control line, which does not reach the ~40% colonization rate typical of normal experiments (Plett et al., 2015; Basso et al., submitted). Also poplar roots silenced for *PtaMYC2s* and *PtaJAM1s* did not show a colonization phenotype (Figure 3). However, exogenous JA treatment also impaired the fungal colonization rate (Basso et al., submitted). Therefore, additional JA-dependent mechanisms must regulate the extent of mycorrhizal colonization, in terms of percentage of colonized lateral roots. In alternative, the level of overexpression of these JA-related TFs was not sufficient to reproduce all the phenotypes observed upon 50 µM exogenous JA application.

PtaMYC2.1 and PtaJAM1.1 have opposite effects on lateral root development

We found that in uncolonized conditions, MYC2.1 OE poplar plants displayed a tendency towards reduced lateral root density, while JAM1.1 OE cuttings tended to show an increased lateral root density (Supplemental Figure 4B). These observations confirm that activated JA signaling is deleterious for lateral root development in poplar cuttings (Basso et al., submitted).

In Arabidopsis low concentrations of JA (5 µM) induce lateral root formation in a COI1-dependent manner by influencing auxin biosynthesis and transport (Sun et al., 2009; 2011), while strong concentrations (50 µM) inhibit auxin-induced lateral rooting in a COI1-independent fashion (Ishimaru et al., 2018). The fact that *L. bicolor* produces elevated amounts of auxin, which promote lateral root development in the host (Felten et al., 2009), might explain why the lateral root development of GUS and MYC2.1 OE poplar transgenic lines does not differ upon fungal colonization. Indeed, fungus-induced auxin signaling might have overridden the effect of enhanced JA signaling in lateral root development of colonized poplar roots.

Interestingly, though, colonized JAM1.1 OE plants displayed increased secondary lateral root density with respect to colonized GUS plants. This suggests that the combinatory effect of fungal signals and suppressed JA signaling may enhance lateral root development. Transcriptomic analysis will be required to confirm these hypotheses. In any case, differences in lateral root development between poplar transgenic lines did not determine

differences in the mycorrhizal colonization rate, indicating that the number of available lateral roots is not a limiting factor for colonization by *L. bicolor*.

The suppression of MYC2.1 activity in colonized poplar roots may rely on both MiSSP7 and yet unknown sRNAs

Routine controls for our experimental setup revealed a surprising result: the expression of *PtaMYC2.1* was significantly repressed in colonized poplar roots with respect to their uncolonized counterparts, in both GUS and MYC2.1 OE lines. Similar trends, albeit often non-significant, were observed for the expression of *PtaMYC2.2* and *PtaJAM1.1* (Figure 2A). These results were unexpected, since a previous transcriptomic analysis did not reveal any significant regulation of *PtaMYC2.1, PtaMYC2.2* or *PtaJAM1.1* expression in wild-type poplar roots during ECM development (Basso et al., submitted). However, we excluded that the observed results were due to a bias in the specificity of the RT-qPCR primers, in the quality of the cDNA templates, or in the integration of the transgenic construct (Supplemental Figure 5). We found that uncolonized lateral roots of transgenic lines tended to accumulate more *PtaMYC2.1* and *PtaMYC2.2* transcripts with respect to wild-type uncolonized poplar lateral roots. Also, the downregulation of *PtaMYC2.1*, *PtaMYC2.2*, and *PtaJAM1.1* in colonized conditions was more pronounced in transgenic than in wild-type poplar roots, especially in the case when only lateral roots were sampled for RNA extraction (Figure 2B). To explain these observations, we propose that genetic manipulation rendered poplar roots more sensitive to JA, and that the initiation of a response to wounding upon sampling of lateral roots led to increased expression of JA-responsive genes, such as *PtaMYC2.1* and *PtaMYC2.2,* in uncolonized conditions. On the other hand, we recently found that *L. bicolor* inoculation suppresses the sensitivity to JA of poplar roots (Basso et al., submitted). Therefore, ECM-dependent suppression of JA sensitivity in colonized roots might have determined the downregulation of *PtaMYC2.1*, *PtaMYC2.2*, and *PtaJAM1.1* in the more JA-sensitive transgenic wounded roots. This hypothesis implies that *L. bicolor* inoculation does not suppress the expression of JA-responsive genes constitutively, but only in case these genes are induced by additional environmental stimuli. Further experimental evidence is needed to establish whether poplar transgenic lines are more sensitive to wounding and JA signaling.

The suppressed expression of *PtaMYC2.1* in colonized poplar roots even when this gene is under the control of a constitutive promoter suggests that such repression occurs via an RNAi mechanism. Non-coding small RNAs (sRNAs) and the RNAi machinery have emerged as an important mechanism to regulate gene expression in plants, used to hamper the expression of viral infecting RNA, as well as for swift regulation of plant immunity and development (Huang et al., 2019; Song et al., 2019; Katiyar-Agarwal & Jin, 2010; Mallory &

Vaucheret, 2006; Baulcombe, 2004). Microbes also possess RNAi machineries that they exploit for the regulation of their own development and infection potential, but in recent years it was discovered that cross-kingdom RNA transfer between plants and microbes can occur. (Weiberg et al., 2013; Cai et al., 2018; Ren et al., 2019). To our knowledge, symbiotic RNA transfer between plants and mycorrhizal fungi has not yet been demonstrated, even though the exchange of extracellular vesicles between arbuscular mycorrhizal fungi and rice seems important for the maintenance of symbiosis (Roth et al., 2019). In the case of the downregulation of *PtaMYC2.1* during the poplar-*L. bicolor* interaction, we do not know yet whether the putative sRNA used for RNAi derives from the plant or has been transferred by the fungus. However, it will be interesting to assess if cross-kingdom RNA transfer occurs also in the establishment of this mutualistic interaction.

In any case, this finding demonstrates the crucial importance of suppressed PtaMYC2.1-dependent gene expression for Hartig net development. In a previous study, we found that the fungal effector MiSSP7 enhances the strength of interaction between the transcriptional repressor PtJAZ6 and PtMYC2.1 (Daguerre et al., submitted). PtJAZ6, therefore, might affect PtMYC2.1 transcriptional activity in mycorrhizal poplar roots. Altogether, these results suggest that *L. bicolor* exploits both transcriptional regulation and post-translational mechanisms to suppress the activity of PtMYC2.1. The fact that MYC2.1 OE ECM still displayed a Hartig net phenotype indicates that leaky suppression of the activity of this TF is sufficient to counteract apoplastic penetration of fungal hyphae. It will be of extreme interest to assess which PtaMYC2.1-activated genes are specifically downregulated during symbiotic development to understand why a mutualistic fungus requires multiple mechanisms to impede their expression. It will be also crucial to assess, through the use of additional poplar transgenic lines, if similar suppression mechanisms are employed for *PtaMYC2.2* and *PtaJAM1.1*.

Conclusion

In this study we assessed the role of the JA-responsive, PtJAZ6-associated TFs PtaMCY2.1 and PtaJAM1.1 in the development of ECM between poplar roots and *L. bicolor*. We found that PtaMYC2.1 inhibits Hartig net development, while PtaJAM1.1 promotes it. A similar effect was found for lateral root development in absence of the fungus. Strikingly, the expression of *PtaMYC2.1* was suppressed in colonized transgenic poplar roots likely through an RNA-dependent mechanism. Altogether, these results suggest that the suppression of the PtaMYC2.1-driven branch of JA signaling is fundamental to ensure apoplastic colonization of fungal hyphae. The molecular mechanisms at the origin of such suppression, and the specific targets activated by PtaMYC2.1, deserve further attention in the future.

2.5. Methods

Gene cloning and plasmid generation

We isolated the *P. tremula x alba* full-length coding sequence (CDS) of *PtaMYC2.1* (Potri.001G142200), *PtaMYC2.2* (Potri.003G092200), and *PtaJAM1.1* (Potri.014G099700) via PCR using as template a cDNA library obtained from poplar-*L. bicolor* ECM. However, we failed to clone *PtaJAM1.2* (Potri.002G172100), possibly because of an erroneous reconstruction of the gene model in the version 3.0 of the *P. trichocarpa* genome (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Ptrichocarpa), against which the cloning primers were designed. The PCR-amplified fragments were cloned under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter allowing gene overexpression into the binary vector pJCV53 (OE vector; VIB-Plant Systems biology; Ghent University) through sequential Gateway® BP and LR recombination. Cloning into the OE vector also allowed to fuse a HA-tag to the C terminus of the protein of interest. As negative control, we cloned a fragment of the *GUS* gene (bases 345-1034) in pJCV53. We did not use the empty vector in order to avoid the expression of the *ccdB* cassette of pJCV53 in transgenic plants.

In addition, we selected a ~300 bp-long CDS fragment of *PtaMYC2* (bases 141-480 of *PtaMYC2.1*) and a ~300 bp-long CDS fragment of *PtaJAM1* (bases 21-315 of *PtaJAM1.1*) conserved between the two paralogs and cloned them via Gateway® BP and LR recombination into the binary vector pK7GWIWG2_II-RedRoot (VIB-Plant System Biology; Ghent University) for double-stranded RNA-interference (dsRNAi)-mediated gene silencing (dsRNAi vector). As negative control, we used the empty vector (pRedRoot). As plant transformation markers, pJCV53 carried a *p35S::mRFP* construct, while pK7GWIWG2_II-RedRoot vector carried a *pAtUbq10::dsRED* construct. Both vectors displayed a kanamycin resistance gene for transgenic plant selection. The list of primers used for cloning is provided in Supplemental Table 1.

Generation of poplar transgenic lines

We generated poplar transgenic lines according to Cope et al. (2019). Briefly, *Rhizobium rhizogenes* (formerly *Agrobacterium rhizogenes*) Arqua 4 competent cells were transformed by electroporation with OE vectors or dsRNAi vectors. After selection of transformed colonies, *R. rhizogenes* strains were cultivated on 5 mL of liquid MG/L broth (5 $g.L^{-1}$ tryptone, 2.5 $g.L^{-1}$ yeast extract, 5.2 $g.L^{-1}$ NaCl, 2 $g.L^{-1}$ glutamic acid, 10 $g.L^{-1}$ mannitol, 0.2 $g.L^{-1}$ $MgSO_4$-7 H_2O, 0.5 $g.L^{-1}$ K_2HPO_4, pH 7.0. Autoclave, add 4 $\mu g.L^{-1}$ biotin, 50 $\mu g.mL^{-1}$ rifampicin and 50 $\mu g.mL^{-1}$ spectinomycin) in shaker O/N at 28°C and 200 rpm. The following day, the culture was brought up to 50 mL by addition of fresh MG/L broth and grown in shaker O/N at 28°C and 200 rpm. Cultures were spinned at 3500 rpm for 20 minutes and the

bacterial pellet was resuspended in 50 mL of induction broth (modified Murashige & Skoog with 1X micronutrients (Sigma-Aldrich), 1X macronutrients (Sigma-Aldrich), 0.25 $g.L^{-1}$ MES (Sigma-Aldrich), 0.1 $g.L^{-1}$ *myo*-inositol, 30 $g.L^{-1}$ sucrose, pH 5.8, with addition of 100 mM acetosyringone (Sigma-Aldrich) after autoclaving). Bacterial cultures were diluted in induction broth to an OD_{600} of 0.8. Leaves from *in vitro* poplar plants were excised with scissors and incisions in prominent veins on the underside of the leaf were practiced with a scalpel. Wounded leaves were kept in sterile water to prevent desiccation. Leaves were removed from the water, immersed in the bacterial culture for 5 minutes and placed on co-cultivation solid medium (modified Murashige & Skoog with 1X micronutrients (Sigma-Aldrich), 1X macronutrients (Sigma-Aldrich), 0.25 $g.L^{-1}$ MES (Sigma-Aldrich), 0.1 $g.L^{-1}$ *myo*-inositol, 30 $g.L^{-1}$ sucrose, 7 $g.L^{-1}$ agar agar, pH 5.8, with addition of 100 mM acetosyringone (Sigma-Aldrich) after autoclaving) and incubated in the dark at 24°C for 2 days. Afterwards, leaves were transferred to antibiotics medium (co-cultivation medium, without acetosyringone but with cefotaxime and carbenicillin at 250 $\mu g.mL^{-1}$ each (Duchefa Biochemie)) to eliminate excess bacteria. Leaves were kept at 24°C, 60% humidity, 50 $\mu mol.m^{-2}.s^{-1}$ light intensity under a 16-h photoperiod and transferred onto new antibiotics medium every 2 weeks until emergence of roots from the veins of the leaves. Leaves were then placed on selection medium (antibiotics medium, with addition of 50 $\mu g.mL^{-1}$ kanamycin (Sigma-Aldrich)) until the roots reached at least 2-3 cm of length. Roots were observed under a fluorescent stereomicroscope to detect the red fluorescence of the mRFP or dsRED transformation markers. Transformed roots were excised from the leaves and placed on regeneration medium (selection medium, with addition of 0.055 $\mu g.mL^{-1}$ thidiazuron (Sigma-Aldrich)) and cultivated in the same conditions until emergence of shoots. Shoots derived from the same transformed root were considered as belonging to the same transgenic line and further propagated in the same conditions as wild-type poplar plants.

***Rhizobium rhizogenes*-mediated root transformation**

We generated chimeric poplar plants with transformed roots according to Neb et al. (2017), with modifications. Briefly, *Rhizobium rhizogenes* Arqua 4 competent cells were transformed by electroporation with dsRNAi vectors. After selection of transformed colonies, *R. rhizogenes* strains were cultivated on liquid YEB (5$g.L^{-1}$ beef extract, 1 $g.L^{-1}$ yeast extract, 5 $g.L^{-1}$ peptone, 5 $g.L^{-1}$ sucrose, 0.5 $g.L^{-1}$ $MgCl_2$, pH 7.2, add 50 $\mu g.mL^{-1}$ of rifampicin (Sigma-Aldrich) and 50 $\mu g.mL^{-1}$ of spectinomycin (Sigma-Aldrich) after autoclaving) O/N at 28°C and 200 rpm. Afterwards, 250 µL of culture were plated on solid YEB plates and kept in static oven at 28°C for 3 days. The thick layer of cells was collected with the help of a scalpel and suspended in 3 mL of sterile water in order to generate foam. Axenically grown wild-type poplar cuttings were briefly dipped in the bacterial foam and transferred to co-cultivation

plates (modified Murashige & Skoog with 1X micronutrients (Sigma-Aldrich), 0.5X macronutrients (Sigma-Aldrich), 3% sucrose, 1 $g.L^{-1}$ MES (Sigma-Aldrich), 20 $mg.L^{-1}$ L-glutamine, 0.1 $mg.L^{-1}$ Ca-panthotenate, 0.1 $mg.L^{-1}$ L-cysteine, 0.01 $mg.L^{-1}$ biotine, and 1X Gamborgs vitamins, pH 5.8). Plates were sealed and kept in the dark at 24°C 60% humidity for 3 days. Subsequently, plants were transferred onto co-cultivation plates with addition of cefotaxime and carbenicillin at 250 $\mu g.mL^{-1}$ each (Duchefa Biochemie) in order to eliminate excess growth of *R. rhizogenes*. Plants were kept at 24°C 60% humidity and 50 $\mu mol.m^{-2}.s^{-1}$ light intensity under a 16-h photoperiod. Plants were transferred onto new co-cultivation plates with antibiotics until the emergence of roots. Then, plants were transferred onto selection plates (co-cultivation plates with antibiotics and the addition of 50 $\mu g.mL^{-1}$ kanamycin (Sigma-Aldrich)). Chimeric plants displaying transformed roots were selected via monitoring of the fluorescence generated upon expression of the *pAtUbq10::dsRED* construct present in the dsRNAi vector.

Plant and fungal materials, growth conditions and poplar- *L. bicolor* co-cultivation method

Plant and fungal materials were cultured as described by Felten et al. (2009). Briefly, transgenic grey poplar (*Populus tremula x Populus alba* line INRA 717-1-B4) clones were micropropagated in vitro and grown in half-strength Murashige and Skoog (MS/2) medium in glass culture tubes in a growth chamber at 24°C and 150 $\mu mol.m^{-2}.s^{-1}$ light intensity under a 16-h photoperiod. Light came from OSRAM Fluorescent tubes (50/50 Fluora / Cool white) placed 15 cm from poplar plants. The dikaryotic vegetative mycelia of strain S238N of the ectomycorrhizal fungus *Laccaria bicolor* were maintained on modified Pachlewski agar medium P5 at 25°C in the dark (Deveau et al., 2007). For *in vitro* co-culture of poplar with *L. bicolor*, we used the sandwich system described by Felten et al. (2009). Briefly, ~10-mm-long rooted stem cuttings from *in vitro*-grown poplar plants were transferred to Petri dishes containing Pachlewski agar medium with reduced sugar (P20) covered with a cellophane membrane and a second, mycelium-covered cellophane membrane was placed on the roots (colonized plants). For single cultures, poplar plants were grown in absence of fungal mycelium (uncolonized plants). The Pachlewski agar medium was supplemented with MES Na to maintain the pH at 5.8, and, in case of the chimeric poplar plants, with 250 $\mu g.mL^{-1}$ cefotaxime and carbenicillin (Duchefa Biochemie), as well as with 50 $\mu g.mL^{-1}$ kanamycin (Sigma-Aldrich). The Petri dishes were positioned vertically and incubated for 2 weeks in a growth chamber at 20°C and 50 $\mu mol.m^{-2}.s^{-1}$ light intensity under a 16-h photoperiod. For the transgenic poplar lines, the whole root systems from three colonized or uncolonized poplar plants were pooled, snap-frozen in liquid nitrogen and stored at -80°C for subsequent RNA

extraction. Three replicates per condition were produced. For the chimeric plants, individual root systems were collected for RNA extraction.

Analysis of mycorrhizal phenotypes

The quantification of ectomycorrhizal colonization and the observation of ectomycorrhizal structures were performed as described by Felten et al. (2009). Briefly, colonized plants were observed under a Discovery V.8 stereomicroscope (Zeiss), and short, rounded lateral roots ensheathed by fungal mantle were considered to be colonized. The rate of ectomycorrhizal colonization was defined as the ratio of colonized lateral roots to the total number of lateral roots (expressed as a percentage). Between 18 and 24 plants per transgenic line were observed. To confirm the development of intraradical Hartig nets, six ECM per transgenic line, and five ECM from chimeric plants were fixed with 4% paraformaldehyde in 1X phosphate-buffered saline and embedded in 4% (w/v) agarose. Transverse 30-µm-thick sections of ECM obtained 200 to 400 µm from the lateral root tip were produced with a Leica VT1200 S vibratome and stained with propidium iodide (1:100 dilution; Sigma-Aldrich) and WGA-488 (1:100 dilution; Wheat Germ Agglutinin, Alexa Fluor™ 488 Conjugate, Thermo Fischer Scientific) to highlight plant and fungal structures, respectively. The sections were observed under a Zeiss LSM 700 laser scanning microscope and the images analyzed with Fiji software (Schindelin et al., 2012). Root diameter, fungal mantle thickness, and Hartig net depth were measured. Hartig net frequency (expressed as the percentage of root apoplastic spaces occupied by fungal hyphae) and frequency of Hartig net initiation (expressed as the percentage of root apoplastic spaces starting to be occupied by fungal hyphae) were also calculated. At least three sections per sample were analyzed.

Analysis of root architecture and pigment content

The root architectures of 12 to 18 plants per condition (colonized and uncolonized transgenic lines) were assessed by analyzing scanned (Epson Perfection V700 PHOTO) plates with Fiji software (Schindelin et al., 2012). Lateral root density was defined as the number of lateral roots over the length (mm) of the respective adventitious root. To measure shoot weight, 4 shoots were pooled per biological replicate and in 4 to 7 replicates were generated. Plants were weighted immediately after sampling (fresh weight, FW) or after lyophilization for 48 h in a Univapo 100H evaporator centrifuge (dry weight, DW). Lyophilized shoots were subsequently used for total chlorophyll (Hall and Rao, 1999) or anthocyanin measurements (Ticconi et al., 2001). In brief, shoots were ground into a fine powder with a tissue lyser (MM 400, Retsch). Chlorophyll was extracted in 80% acetone, while anthocyanin was extracted by boiling the sample for 3 min in 15% (v/v) isopropanol 9.96 N HCl buffer. The supernatants were diluted and their absorbance (A) measured with a Tecan Infinite

M200 PRO plate reader. The quantity of chlorophyll was determined using the following formula:

$$Totalchlorophyll\left(mg/g\,DW\right)=\frac{A_{652}*dilutionfactor*1000}{DW\left(mg\right)*34.5}$$

Anthocyanin was quantified using the following formula:

$$Anthocyanin\left(A_{535}/g\,DW\right)=\frac{\left(A_{535}-A_{650}\right)*1000}{DW\left(mg\right)}$$

RNA extraction and RT-qPCR

RNA was extracted with the plant RNeasy Mini Kit (Qiagen), following manufacturer's instructions. RNA quality was assessed with a Fragment Analyzer Automated CE System (Agilent). 500 ng of RNA were used to synthesize cDNA with the iSCRIPT™ cDNA Synthesis Kit (BioRad) and the resulting cDNA was diluted to 2.5 ng/mL in RNase-free water. 2 µL of diluted cDNA were added to 5 µL of iQ SYBR Green Supermix (BioRad) and 0.5 µL of 10 nM forward and reverse primers in a 10 µL reaction. Real-time qPCR was performed in a Rotor-Gene Q thermocycler (Qiagen) with two technical replicates. Threshold cycles were calculated for each pair of tested primers and the number of cDNA molecules for the gene of interest present at the beginning of the reaction was estimated with the following formula:

$$Numberofmolecules=\left(E_p^{-C_t}\right)$$

with E_p = coefficient of primer pair efficiency (equals 2 in case of 100 % efficiency)
C_t = threshold cycle

The expression level of each gene of interest was normalized by the expression level of the housekeeping gene *Ubiquitin* (*UbiQ*) for each sample. For the screening of transgenic poplar lines, single shoot replicates have been used. To test colonized and uncolonized poplar roots belonging to the transgenic lines, two or three biological replicates have been produced. The list of primers used for RT-qPCR is provided in Supplemental Table 2.

Statistical analyses

Differences among samples in terms of mycorrhizal development, root architecture, weight and pigment content, and gene expression were tested via Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction (R package

agricolae, https://CRAN.R-project.org/package=agricolae). Differences in gene expression were tested via two-sided Welch t-test.

2.6. Acknowledgements

The authors acknowledge Raphaël Bichni and Frédéric Guinet for their help with the propagation and maintenance of the transgenic lines, and Félix Fracchia for the mycorrhization assay of wild-type poplars and control transgenic lines. This research was sponsored by the Genomic Science Program, US Department of Energy, Office of Science, Biological and Environmental Research as part of the Plant-Microbe Interfaces Scientific Focus Area (http://pmi.ornl.gov) and the Laboratory of Excellence ARBRE (grant no. ANR-11-LABX-0002_ARBRE). The research is supported by the Institut National de la Recherche Agronomique and the University of Lorraine. Both Région Lorraine Research council and the European Fund for Regional Development give funding for the Functional Genomics Facilities at Institut National de la Recherche Agronomique-Grand Est.

2.7. References

Avalbaev, A., Yuldashev, R., Fedorova, K., Somov, K., Vysotskaya, L., Allagulova, C., and Shakirova, F. (2016). Exogenous methyl jasmonate regulates cytokinin content by modulating cytokinin oxidase activity in wheat seedlings under salinity. J. Plant Physiol. **191**: 101–110.

Basso, V. et al. An ectomycorrhizal fungus alters sensitivity to jasmonate, salicylate, gibberellin, and ethylene in host roots. submitted.

Basso, V. and Veneault-Fourrey, C. The role of jasmonates in beneficial microbe-root interactions. In Jasmonate in Plant Biology.

Baulcombe, D. (2004). RNA silencing in plants David Baulcombe. Nature **431**: 356–363.

Cai, Q., Qiao, L., Wang, M., He, B., Lin, F., Palmquist, J., and Jin, H. (2018). Pathogen To Silence Virulence Genes. Science (80-.). **360**: 1126–1129.

Chico, J.M., Fernández-Barbero, G., Chini, A., Fernández-Calvo, P., Díez-Díaz, M., and Solano, R. (2014). Repression of jasmonate-dependent defenses by shade involves differential regulation of protein stability of MYC transcription factors and their JAZ repressors in Arabidopsis. Plant Cell **26**: 1967–1980.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J.M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L., and Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. Nature **448**: 666–671.

Chini, A., Gimenez-Ibanez, S., Goossens, A., and Solano, R. (2016). Redundancy and specificity in jasmonate signalling. Curr. Opin. Plant Biol. **33**: 147–156.

Cope, K.R. et al. (2019). The Ectomycorrhizal Fungus Laccaria bicolor Produces Lipochitooligosaccharides and Uses the Common Symbiosis Pathway to Colonize Populus

Roots. Plant Cell: tpc.00676.2018.

Corpet, F. (1988). Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. **16**: 10881–10890.

Daguerre, Y., Basso, V., Wittulsky, S., Schellenberger, R., Meyer, L., Bailly, J., Kohler, A., Plett, J.M., Martin, F., and Veneault-Fourrey, C. The mutualism effector MiSSP7 of Laccaria bicolor alters poplar JAZ6 protein containing complex. submitted.

Deveau, A., Palin, B., Delaruelle, C., Peter, M., Kohler, A., Pierrat, J.C., Sarniguet, A., Garbaye, J., Martin, F., and Frey-Klett, P. (2007). The mycorrhiza helper Pseudomonas fluorescens BBc6R8 has a specific priming effect on the growth, morphology and gene expression of the ectomycorrhizal fungus Laccaria bicolor S238N. New Phytol. **175**: 743–755.

Dombrecht, B., Xue, G.P., Sprague, S.J., Kirkegaard, J.A., Ross, J.J., Reid, J.B., Fitt, G.P., Sewelam, N., Schenk, P.M., Manners, J.M., and Kazan, K. (2007). MYC2 Differentially Modulates Diverse Jasmonate-Dependent Functions in Arabidopsis. Plant Cell Online **19**: 2225–2245.

Du, M. et al. (2017). MYC2 Orchestrates a Hierarchical Transcriptional Cascade That Regulates Jasmonate-Mediated Plant Immunity in Tomato. Plant Cell **29**: 1883–1906.

Felten, J., Kohler, A., Morin, E., Bhalerao, R.P., Palme, K., Martin, F., Ditengou, F.A., and Legue, V. (2009). The Ectomycorrhizal Fungus Laccaria bicolor Stimulates Lateral Root Formation in Poplar and Arabidopsis through Auxin Transport and Signaling. Plant Physiol. **151**: 1991–2005.

Fernández-Calvo, P. et al. (2011). The Arabidopsis bHLH Transcription Factors MYC3 and MYC4 Are Targets of JAZ Repressors and Act Additively with MYC2 in the Activation of Jasmonate Responses. Plant Cell **23**: 701–715.

Fonseca, S., Fernández-Calvo, P., Fernández, G.M., Díez-Díaz, M., Gimenez-Ibanez, S., López-Vidriero, I., Godoy, M., Fernández-Barbero, G., Van Leene, J., De Jaeger, G., Franco-Zorrilla, J.M., and Solano, R. (2014). bHLH003, bHLH013 and bHLH017 are new targets of JAZ repressors negatively regulating JA responses. PLoS One **9**: e86182.

Gimenez-Ibanez, S., Chini, A., and Solano, R. (2016). How microbes twist jasmonate signaling around their little fingers. Plants **5**: 323–329.

Glazebrook, J. (2005). Contrasting Mechanisms of Defense Against Biotrophic and Necrotrophic Pathogens. Annu. Rev. Phytopathol. **43**: 205–227.

Guo, Q., Major, I.T., and Howe, G.A. (2018a). Resolution of growth – defense conflict : mechanistic insights from jasmonate signaling. Curr. Opin. Plant Biol. **44**: 72–81.

Guo, Q., Yoshida, Y., Major, I.T., Wang, K., Sugimoto, K., Kapali, G., Havko, N.E., Benning, C., and Howe, G.A. (2018b). JAZ repressors of metabolic defense promote growth and reproductive fitness in Arabidopsis . Proc. Natl. Acad. Sci. **115**: E10768–E10777.

Hall, D.O. and Rao, K. (1999). Photosynthesis 6th edition. (Cambridge University Press).

Howe, G.A., Major, I.T., and Koo, A.J. (2018). Annual Review of Plant Biology Modularity in Jasmonate Signaling for Multistress Resilience.

Huang, C.-Y., Wang, H., Hu, P., Hamby, R., and Jin, H. (2019). Small RNAs – Big Players in Plant-

Microbe Interactions. Cell Host Microbe **26**: 173–182.

Ishimaru, Y. et al. (2018). Jasmonic acid inhibits auxin-induced lateral rooting independently of the CORONATINE INSENSITIVE1 receptor. Plant Physiol. **177**.

Jang, G., Chang, S.H., Um, T.Y., Lee, S., Kim, J.K., and Choi, Y. Do (2017). Antagonistic interaction between jasmonic acid and cytokinin in xylem development. Sci. Rep. **7**: 1–13.

Katiyar-Agarwal, S. and Jin, H. (2010). Role of Small RNAs in Host-Microbe Interactions. Annu. Rev. Phytopathol. **48**: 225–246.

Katsir, L., Schilmiller, A.L., Howe, G.A., He, S.Y., and Staswick, P.E. (2008). COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Proc. Natl. Acad. Sci.

Kazan, K. and Manners, J.M. (2013). MYC2: The master in action. Mol. Plant **6**: 686–703.

Liu, Y., Du, M., Deng, L., Shen, J., Fang, M., Chen, Q., Lu, Y., Wang, Q., Li, C., and Zhai, Q. (2019). MYC2 regulates the termination of jasmonate signaling via an autoregulatory negative feedback loop. Plant Cell **31**: 106–127.

Major, I.T., Yoshida, Y., Campos, M.L., Kapali, G., Xin, X.F., Sugimoto, K., de Oliveira Ferreira, D., He, S.Y., and Howe, G.A. (2017). Regulation of growth–defense balance by the JASMONATE ZIM-DOMAIN (JAZ)-MYC transcriptional module. New Phytol. **215**: 1533–1547.

Mallory, A.C. and Vaucheret, H. (2006). Functions of micrornas and related small a rnas in plants. Nat. Genet. **38**: S31–S36.

Melotto, M., Mecey, C., Niu, Y., Chung, H.S., Katsir, L., Yao, J., Zeng, W., Thines, B., Staswick, P., Browse, J., and Howe, G. (2008). Critical role of two positively charged amino acids in the Jas motif of Arabidopsis JAZ proteins. Plant J. **55**: 979–988.

Nakata, M., Mitsuda, N., Herde, M., Koo, A.J.K., Moreno, J.E., Suzuki, K., Howe, G.A., and Ohme-Takagi, M. (2013). A bHLH-type transcription factor, ABA-INDUCIBLE BHLH-TYPE TRANSCRIPTION FACTOR/JA-ASSOCIATED MYC2-LIKE1, acts as a repressor to negatively regulate jasmonate signaling in Arabidopsis. Plant Cell **25**: 1641–1656.

Naseem, M., Kaltdorf, M., Hussain, A., and Dandekar, T. (2013). The impact of cytokinin on jasmonate-salicylate antagonism in Arabidopsis immunity against infection with Pst DC3000. Plant Signal. Behav. **8**: e26791.

Neb, D., Das, A., Hintelmann, A., and Nehls, U. (2017). Composite poplars: a novel tool for ectomycorrhizal research. Plant Cell Rep. **36**: 1959–1970.

Nitschke, S., Cortleven, A., Iven, T., Feussner, I., Havaux, M., Riefler, M., and Schmülling, T. (2016). Circadian stress regimes affect the circadian clock and cause jasmonic acid-dependent cell death in cytokinin-deficient arabidopsis plants. Plant Cell **28**: 1616–1639.

Pieterse, C.M.J., Leon-Reyes, A., Van Der Ent, S., and Van Wees, S.C.M. (2009). Networking by small-molecule hormones in plant immunity. Nat. Chem. Biol. **5**: 308–316.

Pieterse, M.J., Van Der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C.M. (2012). Hormonal Modulation of Plant Immunity. Annu. Rev. Cell Dev. Biol. **28**: 489–521.

Plett, J.M., Daguerre, Y., Wittulsky, S., Vayssieres, A., Deveau, A., Melton, S.J., Kohler, A., Morrell-Falvey, J.L., Brun, A., Veneault-Fourrey, C., and Martin, F. (2014a). Effector MiSSP7

of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes. Proc. Natl. Acad. Sci. **111**: 8299–8304.

Plett, J.M., Kemppainen, M., Kale, S.D., Kohler, A., Legué, V., Brun, A., Tyler, B.M., Pardo, A.G., and Martin, F. (2011). A secreted effector protein of Laccaria bicolor is required for symbiosis development. Curr. Biol. **21**: 1197–1203.

Plett, J.M., Khachane, A., Ouassou, M., Kohler, A., and Martin, F. (2014b). Ethylene and jasmonic acid act as negative modulators during mutualistic symbiosis between Laccaria bicolor and Populus roots. New Phytol. **202**: 270–286.

Plett, J.M., Tisserant, E., Brun, A., Morin, E., Grigoriev, I. V, Kuo, A., Martin, F., and Kohler, A. (2015). The Mutualist Laccaria bicolor Expresses a Core Gene Regulon During the Colonization of Diverse Host Plants and a Variable Regulon to Counteract Host-Specific Defenses. Mol. Plant Microbe Interact. **28**: 261–273.

Qi, T., Huang, H., Song, S., and Xie, D. (2015a). Regulation of Jasmonate-Mediated Stamen Development and Seed Production by a bHLH-MYB Complex in Arabidopsis. Plant Cell.

Qi, T., Wang, J., Huang, H., Liu, B., Gao, H., Liu, Y., Song, S., and Xie, D. (2015b). Regulation of jasmonate-induced leaf senescence by antagonism between bHLH subgroup IIIe and IIId factors in arabidopsis. Plant Cell **27**: 1634–1649.

Ren, B., Wang, X., Duan, J., and Ma, J. (2019). Rhizobial tRNA-derived small RNAs are signal molecules regulating plant nodulation. Science (80-.). **365**: 919–922.

Roth, R., Hillmer, S., Funaya, C., Chiapello, M., Schumacher, K., Lo Presti, L., Kahmann, R., and Paszkowski, U. (2019). Arbuscular cell invasion coincides with extracellular vesicles and membrane tubules. Nat. Plants **5**: 204–211.

Sasaki-Sekimoto, Y., Jikumaru, Y., Obayashi, T., Saito, H., Masuda, S., Kamiya, Y., Ohta, H., and Shirasu, K. (2013). Basic helix-loop-helix transcription factors JASMONATE-ASSOCIATED MYC2-LIKE1 (JAM1), JAM2, and JAM3 are negative regulators of jasmonate responses in Arabidopsis. Plant Physiol. **163**: 291–304.

Schindelin, J. et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods **9**: 676–682.

Schweizer, F., Fernández-Calvo, P., Zander, M., Diez-Diaz, M., Fonseca, S., Glauser, G., Lewsey, M.G., Ecker, J.R., Solano, R., and Reymond, P. (2013). Arabidopsis Basic Helix-Loop-Helix Transcription Factors MYC2, MYC3, and MYC4 Regulate Glucosinolate Biosynthesis, Insect Performance, and Feeding Behavior. Plant Cell **25**: 3117–3132.

Sehr, E.M., Agusti, J., Lehner, R., Farmer, E.E., Schwarz, M., and Greb, T. (2010). Analysis of secondary growth in the Arabidopsis shoot reveals a positive role of jasmonate signalling in cambium formation. Plant J. **63**: 811–822.

Song, S., Qi, T., Fan, M., Zhang, X., Gao, H., Huang, H., Wu, D., Guo, H., and Xie, D. (2013). The bHLH Subgroup IIId Factors Negatively Regulate Jasmonate-Mediated Plant Defense and Development. PLoS Genet. **9**: 1–19.

Song, X., Li, Y., Cao, X., and Qi, Y. (2019). MicroRNAs and Their Regulatory Roles in Plant–Environment Interactions. Annu. Rev. Plant Biol. **70**: 489–525.

Sun, J. et al. (2009). Arabidopsis ASA1 is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. Plant Cell **21**: 1495–1511.

Sun, J., Chen, Q., Qi, L., Jiang, H., Li, S., Xu, Y., Liu, F., Zhou, W., Pan, J., Li, X., Palme, K., and Li, C. (2011). Jasmonate modulates endocytosis and plasma membrane accumulation of the Arabidopsis PIN2 protein. New Phytol. **191**: 360–375.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A., and Browse, J. (2007). JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signalling. Nature **448**: 661–665.

Ticconi, C.A., Delatorre, C.A., and Abel, S. (2001). Attenuation of Phosphate Starvation Responses by Phosphite in Arabidopsis. Plant Physiol. **127**: 963–972.

Ueda, J. and Kato, J. (1982). Inhibition of cytokinin‐induced plant growth by jasmonic acid and its methyl ester. Physiol. Plant. **54**: 249–252.

Wasternack, C. and Hause, B. (2013). Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Ann. Bot. **111**: 1021–1058.

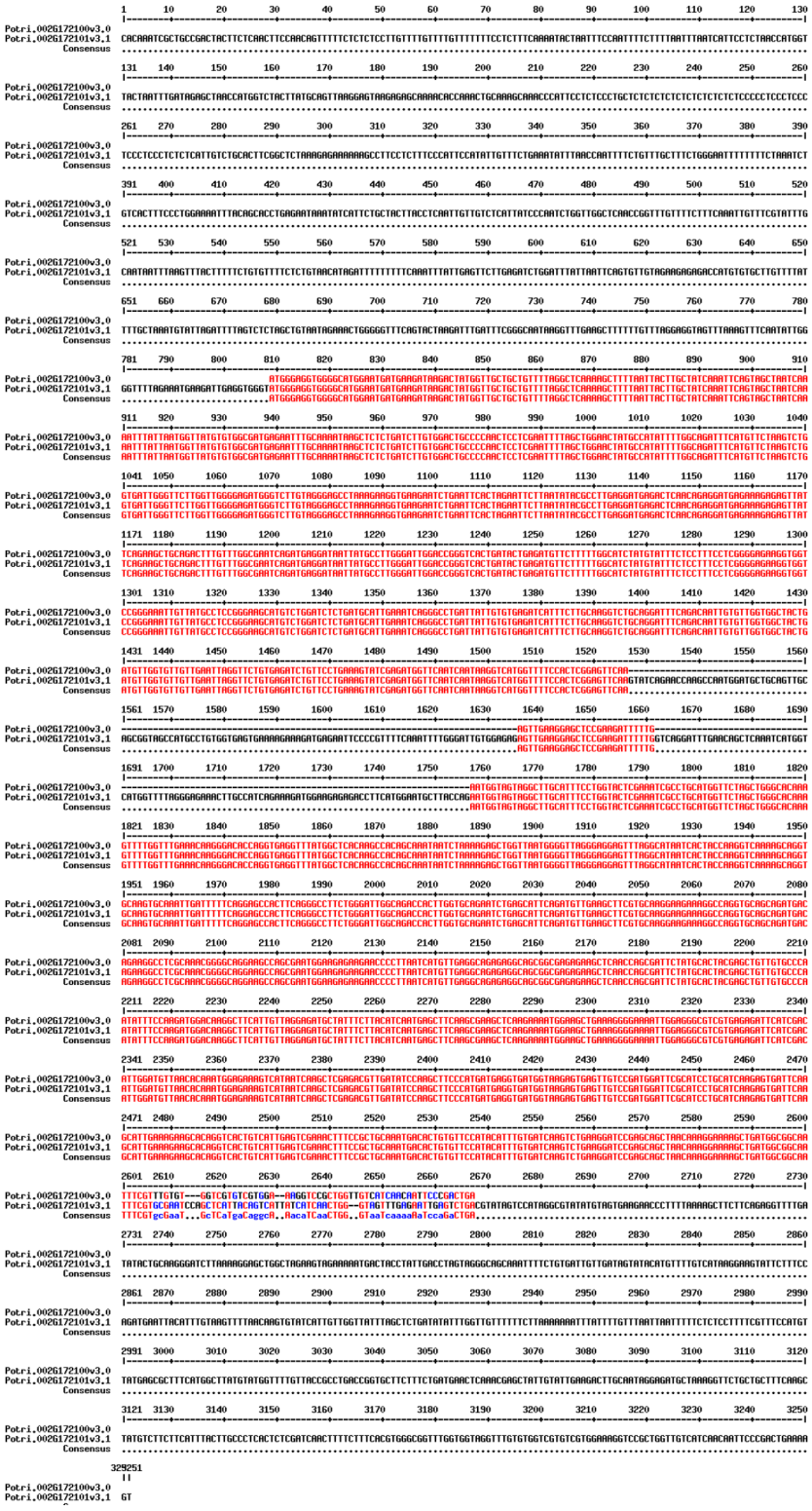
Weiberg, A., Wang, M., Lin, F.-M., Zhao, H., Zhang, Z., Kaloshian, I., Huang, H.-D., Jin, H., Khaloshian, I., Huang, H.-D., and Jin, H. (2014). Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways. Science. **342**: 118–123.

Yan, Y., Stolz, S., Chételat, A., Reymond, P., Pagni, M., Dubugnon, L., and Farmer, E.E. (2007). A Downstream Mediator in the Growth Repression Limb of the Jasmonate Pathway. Plant Cell **19**: 2470–2483.

Zhang, L., Zhang, F., Melotto, M., Yao, J., and He, S.Y. (2017). Jasmonate signaling and manipulation by pathogens and insects. J. Exp. Bot. **6**: 1371–1385.

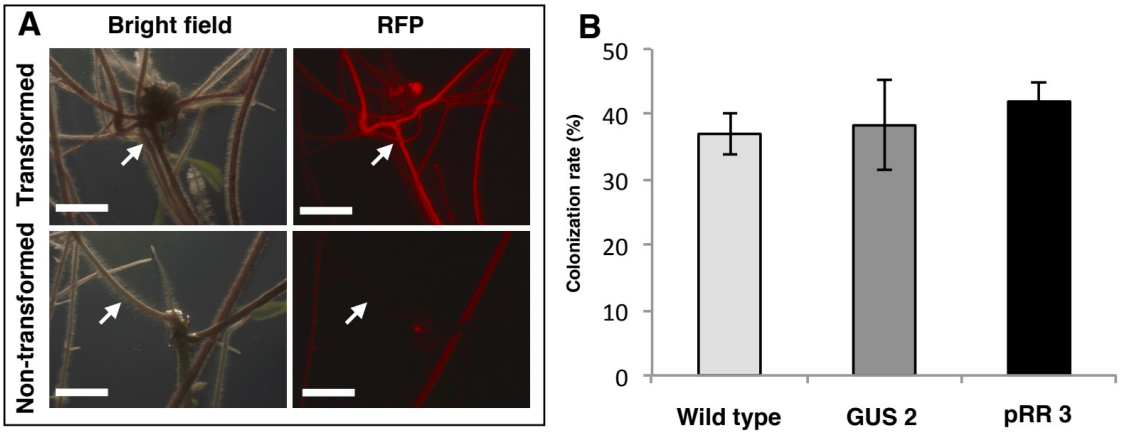
Zhang, Q., Xie, Z., Zhang, R., Xu, P., Liu, H., Yang, H., Doblin, M.S., Bacic, A., and Li, L. (2018). Blue Light Regulates Secondary Cell Wall Thickening via MYC2/MYC4 Activation of the NST1-Directed Transcriptional Network in Arabidopsis. Plant Cell **30**: 2512–2528.

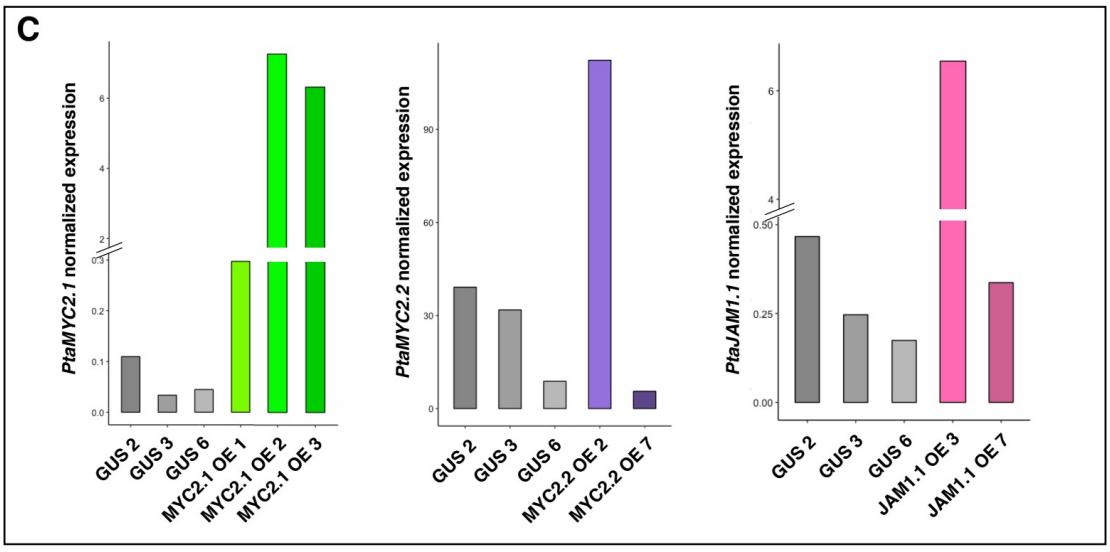
2.8. Supplemental material

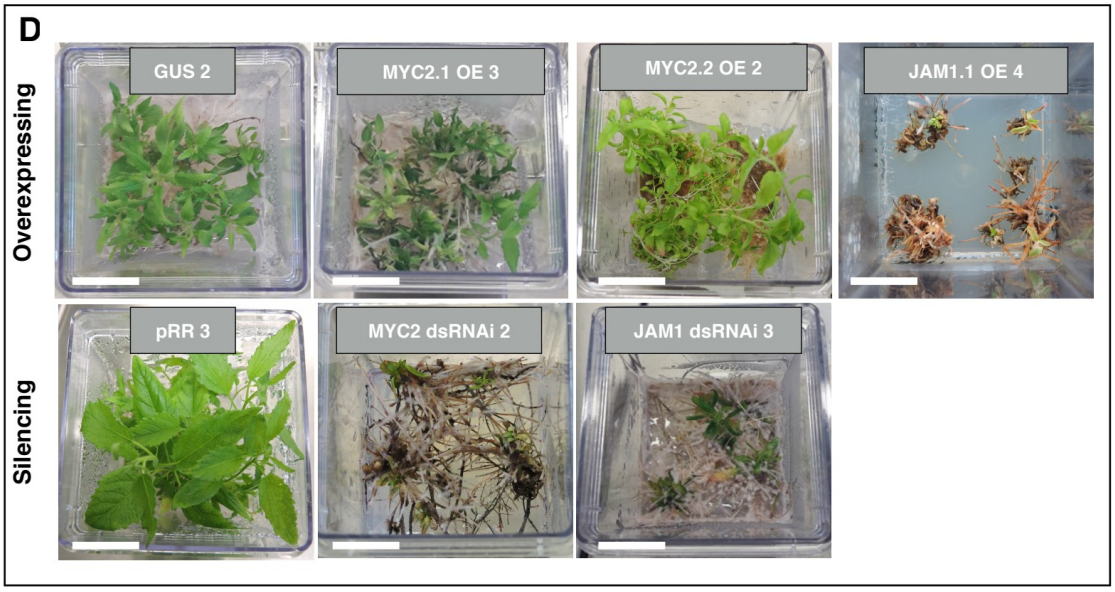


Supplemental Figure 1. The gene model of PtaJAM1.2 differs in its 3' between two versions of the* Populus trichocarpa *genome.

Nucleotide alignment of the gene models of PtaJAM1.2*, as present in the database of the* Populus trichocarpa *genome version 3.0 (*Potri.002G172100v3.0*) and 3.1 (*Potri.002G172101v3.1*), obtained through the web software MultAlin (Corpet, 1998). Nucleotides reported in red are conserved between the two gene models, while nucleotides reported in blue and black differ between the two gene models. Long stretches of nucleotides present only in the* Potri.002G172101v3.1 *gene model represent previously unreported 5'-UTR, 3'-UTR, and intronic regions.*

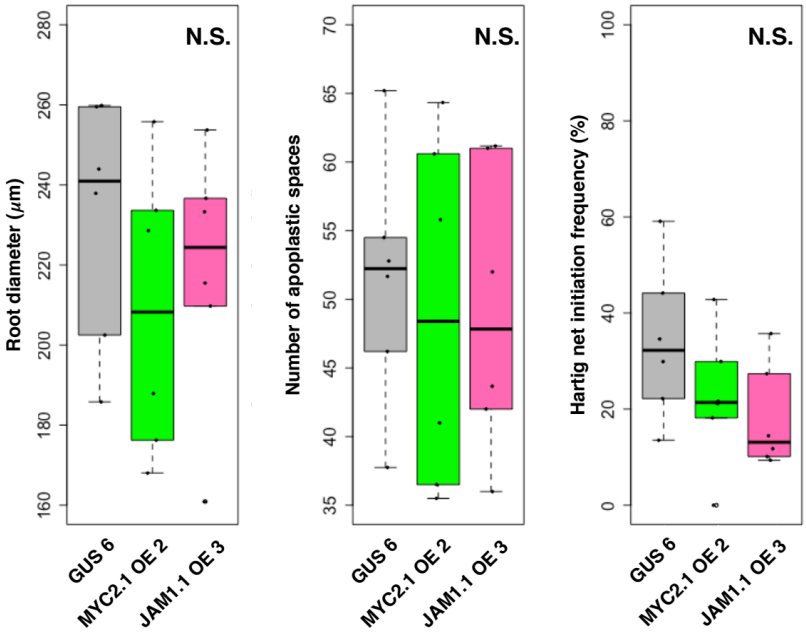

A
Bright field
RFP
Transformed
Non-transformed
B
Colonization rate (%)
50
40
30
20
10
0
Wild type
GUS 2
pRR 3


C
PtaMYC2.1 normalized expression
GUS 2
GUS 3
GUS 6
MYC2.1 OE 1
MYC2.1 OE 2
MYC2.1 OE 3
PtaMYC2.2 normalized expression
GUS 2
GUS 3
GUS 6
MYC2.2 OE 2
MYC2.2 OE 7
PtaJAM1.1 normalized expression
GUS 2
GUS 3
GUS 6
JAM1.1 OE 3
JAM1.1 OE 7


D
Overexpressing
GUS 2
MYC2.1 OE 3
MYC2.2 OE 2
JAM1.1 OE 4
Silencing
pRR 3
MYC2 dsRNAi 2
JAM1 dsRNAi 3

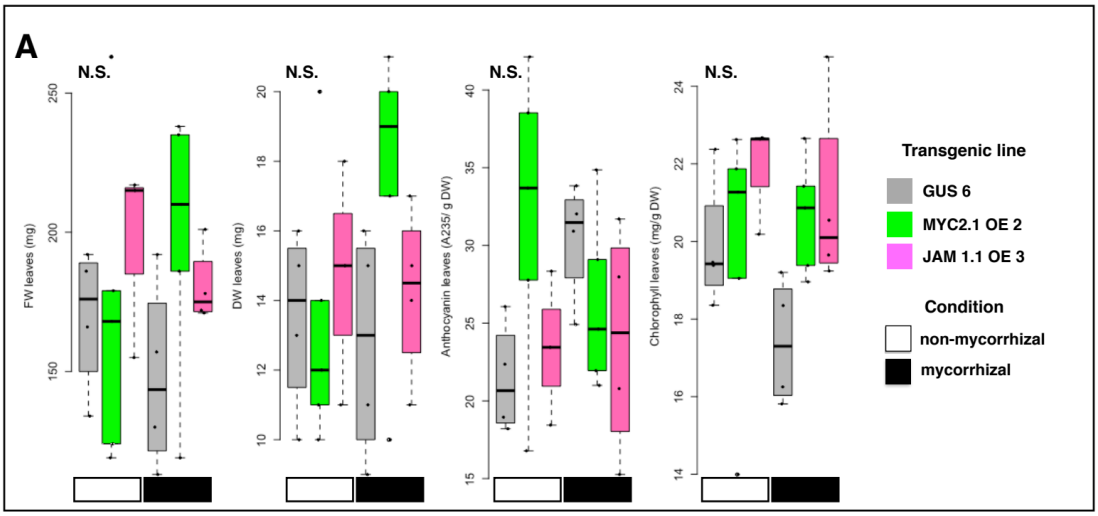
Supplemental Figure 2. Generation of poplar transgenic lines overexpressing PtaMYC2.1, PtaMYC2.2, and PtaJAM1.1.

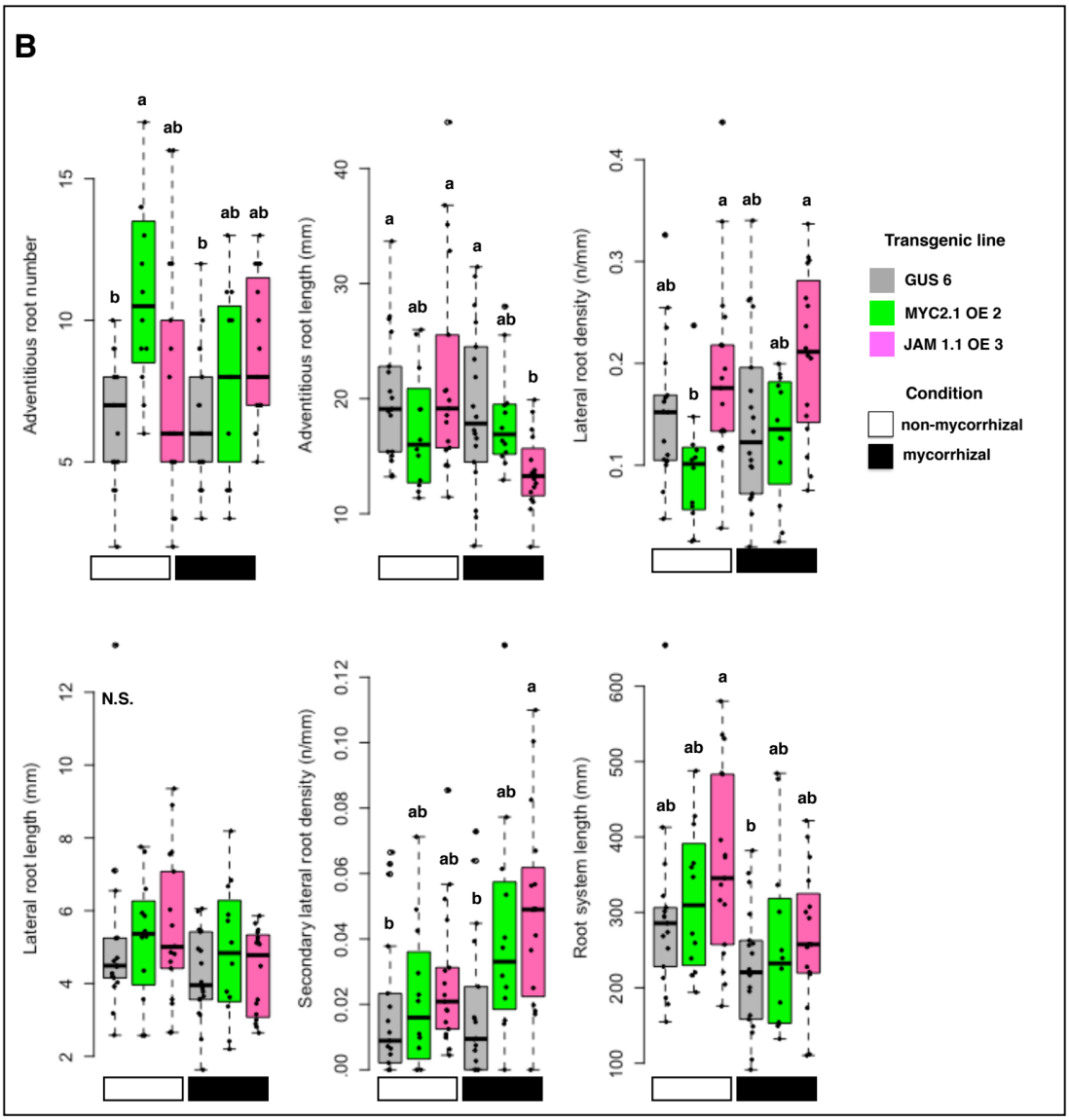
A. Stereomicroscope images of transformed and non-transformed poplar roots after R. rhizogenes *infection of poplar leaves according to Cope et al. (2019). Left panels: bright field; right panels: RFP filter. Arrows indicate one transformed (upper panels) and one non-transformed (lower panel) root. Scale bar: 1 cm. B. Bar plot representing the colonization rate of* L. bicolor*-inoculated roots from wild-type poplar cuttings, or GUS 2 and pRR 3 transgenic poplar lines. Error bars represent the standard error of six biological replicates. C. Bar plots representing the differences between shoots of control (GUS), MYC2.1 OE, MYC2.2 OE, and JAM1.1 OE transgenic lines in terms of relative* PtaMYC2.1*,* PtaMYC2.2*, and* PtJAM1.1 *expression, as detected by RT-qPCR. Gene expression was normalized by the expression level of the housekeeping gene* Ubiquitin*. Single biological replicates were used for the screening of transgenic lines. D. Representative pictures of growth phenotype of transgenic lines overexpressing (upper panels) or silencing (lower panels) the genes of interest after CK-induced shoot development. Scale bar: 2 cm.*



Supplemental Figure 3. The Hartig net phenotype of MYC2.1 OE 2 and JAM1.1 OE 3 plants does not depend on root diameter, number of apoplastic spaces or frequency of Hartig net initiation.

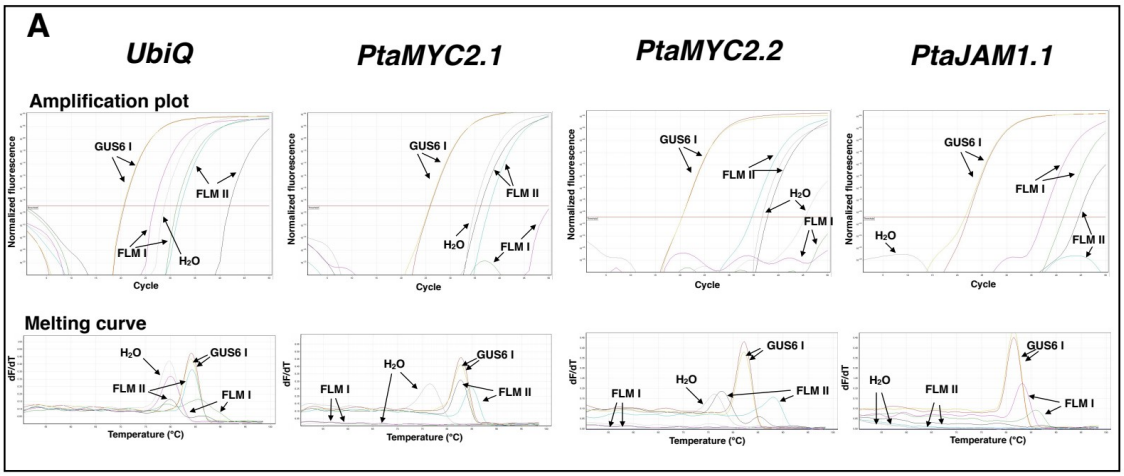
Boxplots showing the distribution of root diameter, number of apoplastic spaces, and frequency of Hartig net initiation of colonized GUS 6, MYC2.1 OE 2, and JAM1.1 OE 3 transgenic lines. Whiskers represent the limits of the 1.5 interquartile range. N.S. indicate absence of significant differences based on the results of Kruskal-Wallis one-way analysis of variance and post-hoc Fischer's LSD test with Bonferroni correction ($p < 0.05$).

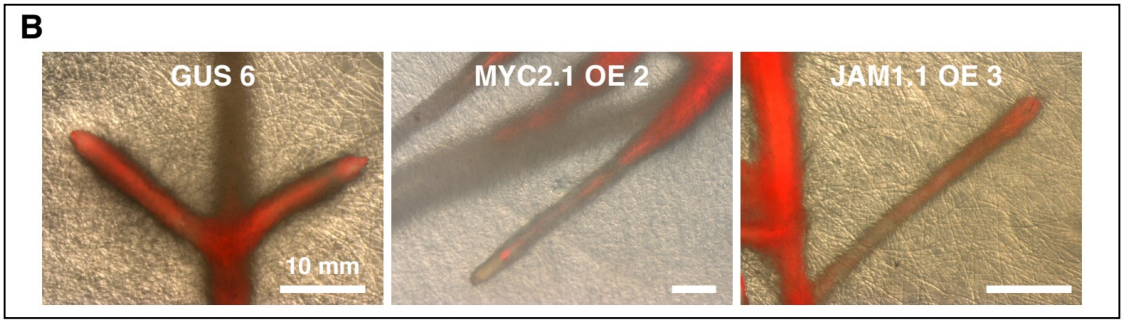

A
N.S.
FW leaves (mg)
DW leaves (mg)
Anthocyanin leaves (A235/ g DW)
Chlorophyll leaves (mg/g DW)
Transgenic line
GUS 6
MYC2.1 OE 2
JAM 1.1 OE 3
Condition
non-mycorrhizal
mycorrhizal

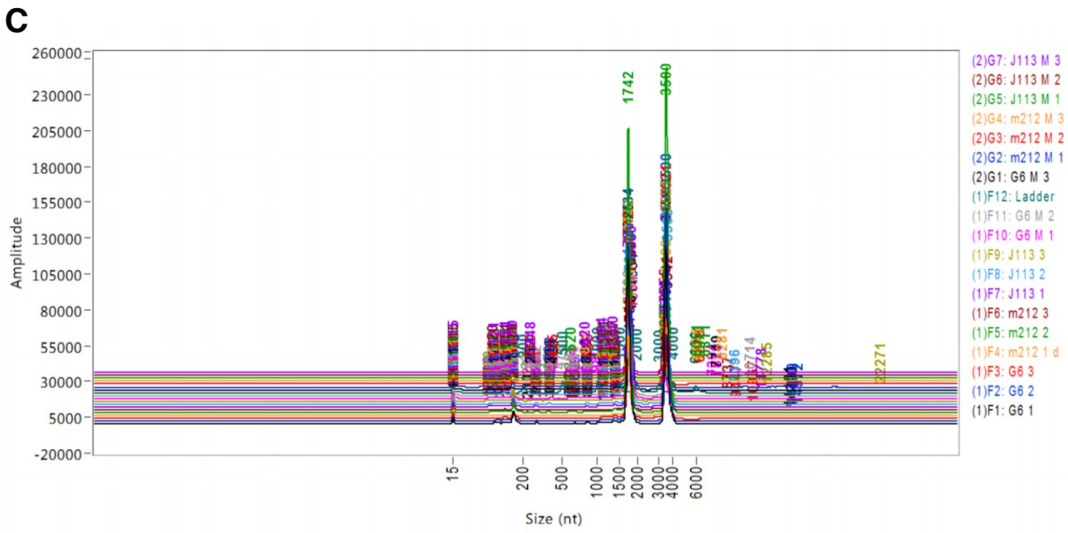

B
Adventitious root number
Adventitious root length (mm)
Lateral root density (n/mm)
Lateral root length (mm)
Secondary lateral root density (n/mm)
Root system length (mm)
N.S.
Transgenic line
GUS 6
MYC2.1 OE 2
JAM 1.1 OE 3
Condition
non-mycorrhizal
mycorrhizal

Supplemental Figure 4. The overexpression of PtaMYC2.1 and PtaJAM1.1 in poplar roots does not alter shoot physiology but affects root architecture.

A. Boxplots showing the distribution of fresh weight (FW), dry weight (DW), and anthocyanin and chlorophyll content of colonized and uncolonized GUS 6, MYC2.1 OE 2, and JAM1.1 OE 3 transgenic lines. B. Boxplots showing the distribution of adventitious root number, adventitious root length, lateral root density, lateral root length, secondary lateral root density, and root system length of colonized and uncolonized GUS 6, MYC2.1 OE 2, and JAM1.1 OE 3 transgenic lines. White boxes indicate uncolonized poplar roots, while black boxes indicate colonized poplar roots. Whiskers represent the limits of the 1.5 interquartile range. Letters indicate significant differences based on the results of Kruskal-Wallis one-way analysis of variance and post-hoc Fischer's LSD test with Bonferroni correction ($p < 0.05$). N.S.= non-significant.


A
UbiQ
PtaMYC2.1
PtaMYC2.2
PtaJAM1.1
Amplification plot
Normalized fluorescence
Cycle
GUS6 I
FLM II
FLM I
H2O
Melting curve
dF/dT
Temperature (°C)


B
GUS 6
MYC2.1 OE 2
JAM1.1 OE 3
10 mm


C
Amplitude
Size (nt)
(2)G7: J113 M 3
(2)G6: J113 M 2
(2)G5: J113 M 1
(2)G4: m212 M 3
(2)G3: m212 M 2
(2)G2: m212 M 1
(2)G1: G6 M 3
(1)F12: Ladder
(1)F11: G6 M 2
(1)F10: G6 M 1
(1)F9: J113 3
(1)F8: J113 2
(1)F7: J113 1
(1)F6: m212 3
(1)F5: m212 2
(1)F4: m212 1 d
(1)F3: G6 3
(1)F2: G6 2
(1)F1: G6 1

Supplemental Figure 5. The suppression of PtaMYC2.1 expression in colonized GUS 6 and MYC2.1 OE 2 transgenic roots is not due to technical bias.

A. Amplification plots and melting curve plots of uncolonized GUS 6 roots and L. bicolor *free-living mycelium (FLM) cDNA samples tested via RT-qPCR with the primers reported in Supplemental Table 2. dF/dT = variation of normalized fluorescence through time. B. Stereomicroscope images of* L. bicolor*-colonized poplar lateral roots from GUS 6, MYC2.1 OE 2, and JAM1.1 OE 3 transgenic lines. The red color indicates the expression of the fluorescent marker dsRED contained in the OE vector. Scale bar: 10 mm. C. Fragment Analyzer data output showing the observed sizes in the RNA samples extracted from roots of colonized and uncolonized GUS 6, MYC2.1 OE 2, and JAM1.1 OE 3 transgenic lines. The presence of two main peaks, representing 18S and 28S rRNA, and the absence of a pool of small-sized fragments indicate good quality of the RNA extractions. The RNA Quality Number of the samples ranged between 8.9 and 10. G6: GUS 6; m212: MYC2.1 OE 2; J113: JAM1.1 OE 3; M: colonized.*

Primer	Sequence	Target	Destination	Use

name			vector	
VB_53F	GGGGACAAGTTTGTACAAAA AAGCAGGCTGGCCAGCGTAT CGTGCTGCG	*GUS* fragment	pJCV53	Control for overexpression
VB_54R	GGGGACCACTTTGTACAAGA AAGCTGGGTGGTCGTGCACC ATCAGCACG	*GUS* fragment	pJCV53	Control for overexpression
VB_8F	GGGGACAAGTTTGTACAAAA AAGCAGGCTATGACTGATTA CCGACTGCCG	*PtaMYC2.1* full-length without STOP	pJCV53	Overexpression
VB_9R	GGGGACCACTTTGTACAAGA AAGCTGGGTCTCGGACGCCA CCAACTTTTGTTG	*PtaMYC2.1* full-length without STOP	pJCV53	Overexpression
VB_10F	GGGGACAAGTTTGTACAAAA AAGCAGGCTATGACTGATTA CCGTCTACCACCG	*PtaMYC2.2* full-length without STOP	pJCV53	Overexpression
VB_11R	GGGGACCACTTTGTACAAGA AAGCTGGGTCTCGGGCATCA CCAACTTTTG	*PtaMYC2.2* full-length without STOP	pJCV53	Overexpression
VB_12F	GGGGACAAGTTTGTACAAAA AAGCAGGCTATGAAGATTGA GCTGGGTGTGG	*PtaJAM1.1* full-length without STOP	pJCV53	Overexpression
VB_67R	GGGGACCACTTTGTACAAGA AAGCTGGGTCCCCAGTCGAT GATAATGAGTG	*PtaJAM1.1* full-length without STOP	pJCV53	Overexpression
VB_1F	GGGGACAAGTTTGTACAAAA AAGCAGGCTTGAGAAAACTA TGTTGAACCAAGA	*PtaMYC2* RNAi fragment	pK7GWIWG2_ II-RedRoot	Silencing
VB_2R	GGGGACCACTTTGTACAAGA AAGCTGGGTAAAACCACTCA GTATCAGTAACCTC	*PtaMYC2* RNAi fragment	pK7GWIWG2_ II-RedRoot	Silencing
VB_3F	GGGGACAAGTTTGTACAAAA AAGCAGGCTGTGGGAGGTG GGGCATGGAACGAT	*PtaJAM1* RNAi fragment	pK7GWIWG2_ II-RedRoot	Silencing
VB_4R	GGGGACCACTTTGTACAAGA AAGCTGGGTGACCTCAGATT CTTCACCTTCTTTA	*PtaJAM1* RNAi fragment	pK7GWIWG2_ II-RedRoot	Silencing

Supplemental Table 1. Primers used for cloning the genes or gene fragments employed in this study.

attB1 (forward primers) and attB2 (reverse primers) sequences, used to generate Gateway-compatible PCR fragments, are highlighted in red.

Primer	Sequence	Target	Amplicon size

name			
UbiQ_F	GCAGGGAAACAGTGAGGAAGG	*Ubiquitin*	155 bp
UbiQ_R	GCTCTGGACTCACGAGGACAG		
qVB_22F	ATCATCAACAGCAGCCGATT	*PtaMYC2.1*	206 bp
qVB_23R	CTCTGTCACCAAACCCGAAT		
qVB_81F	ATTCCGTCAACTACTCCGG	*PtaMYC2.2*	125 bp
qVB_82R	TGCTGTTGATGGCTGTGATG		
qVB_79F	CCATGCCTGCTGTGAGTGAA	*PtaJAM1.1*	135 bp
qVB_80R	CCCTAAATCCATGACCATGACCA		

Supplemental Table 2. Primers used for RT-qPCR.

Chapter 4: *L. bicolor* alters poplar root sensitivity to jasmonate, gibberellin, salicylate, and ethylene

Chapter 4: *L. bicolor* alters poplar root sensitivity to jasmonate, gibberellin, salicylate, and ethylene

1. Preface

Phytohormones are master regulators of plant development and of its responses to environmental cues. Since the establishment of symbiotic interactions between roots and microbes requires tight control of root morphology, metabolism and defense, it is not surprising that plant hormones heavily influence the development and the functionality of several beneficial root-microbe interactions. In the case of the ectomycorrhizal symbiosis, fungal manipulation of the tree's jasmonate (JA) signaling has been proven crucial for the development of the Hartig net (Plett et al., 2014a,b). In model plants such as Arabidopsis JA signaling is at the centre of an intricate regulatory network involving the interaction with other hormone signaling pathways. For instance, the crosstalk between JA and salicylate (SA) signaling regulates the balance between plant resistance against necrotrophic and biotrophic pathogens (Pieterse et al., 2012). Active ethylene (ET) signaling can amplify JA-mediated responses against pathogens, but counteracts JA-derived responses against wounding (Lorenzo et al., 2003). Additionally, mutual suppression between JA and gibberellin (GA) signaling is implied in the balance between growth and defense, preventing plants from deploying resources into vegetative growth upon challenging environmental conditions (Guo et al., 2018). During the poplar-*L. bicolor* interaction, constitutive ET accumulation is detrimental for Hartig net development, impairing the expression of genes involved in cell-cell recognition and cell wall synthesis (Plett et al., 2014b). In the same study, the existence of a positive crosstalk between JA and ET signaling was suggested, due to the similar transcriptional signature of JA- and ET-treated poplar roots. On the other hand, exogenous SA did not impact the ectomycorrhizal phenotype (Plett et al., 2014b).

However, many questions on the role of JA signaling and its interaction with other hormone signaling pathways during the ectomycorrhizal symbiosis remain unanswered. Indeed, the lack of hormone-responsive marker genes in poplar roots makes it difficult to determine whether fungal inoculation affects phytohormone signaling at particular developmental stages. In addition, the abundance of endogenous JA, SA, ET and GA in colonized and uncolonized poplar roots has not been examined. Finally, the evidence over the existence of phytohormone crosstalk in poplar roots, and on its role in ectomycorrhizal development, remains circumstantial.

To unravel the role of phytohormones implied in defense and development during the establishment of ECM, we looked for differences in hormonal levels between colonized and uncolonized poplar lateral roots. We found that *L. bicolor* alters poplar GA and SA

metabolism. Furthermore, we defined phytohormone-responsive and crosstalk-regulated genes and investigated their transcriptional patterns in developing ECM. We found that *L. bicolor* inoculation impairs poplar root sensitivity to JA, while enhancing its sensitivity to SA, ET and GA. However, a branch of JA/ET signaling may be activated during ectomycorrhizal development to ensure the correct balance between cell wall integrity and elasticity for efficient hyphal penetration in the root apoplast. Our analysis reveals multifaceted roles of JA signaling, and of its crosstalk with other phytohormones implied in defense and growth, during the establishment of symbiotic structures.

2. Article published in Plant, Cell & Environment (December 2019)

Title: An ectomycorrhizal fungus alters sensitivity to jasmonate, salicylate, gibberellin, and ethylene in host roots

Authors: Veronica Basso[1], Annegret Kohler[1], Shingo Miyauchi[1], Vasanth Singan[2], Frédéric Guinet[1], Jan Šimura[3], Ondřej Novák[3], Kerrie W. Barry[2], Mojgan Amirebrahimi[2], Jonathan Block[1], Yohann Daguerre[1,4], Hyunsoo Na[2], Igor V. Grigoriev[2,5], Francis Martin[1,6 †], Claire Veneault-Fourrey[1 †‡]

Contact information: [1]Université de Lorraine, INRA, UMR Interactions Arbres/Microorganismes (IAM), Laboratoire d'excellence Recherches Avancés sur la Biologie de l'Arbre et les Ecosystèmes Forestiers (LabEx ARBRE), Centre INRA Grand-Est, 54280 Champenoux, France.

[2]US Department of Energy Joint Genome Institute (JGI), Walnut Creek, California, USA.

[3]Laboratory of Growth, Palacký University, Faculty of Science & The Czech Academy of Sciences, Institute of Experimental Botany, Šlechtitelů 27, 783 71 Olomouc, The Czech Republic.

[4]Forest Genetics and Plant Physiology, Umeå Plant Science Centre, 90736 UMEÅ, Sweden.

[5]Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA 94720 USA.

[6]Beijing Forestry University, 35 Qinghua E Rd, Haidian Qu, Beijing Shi, China, 100083.

† Joint senior authors.

‡ Corresponding author.

2.1. Abstract

The phytohormones jasmonate, gibberellin, salicylate, and ethylene regulate an interconnected reprogramming network integrating root development with plant responses against microbes. The establishment of mutualistic ectomycorrhizal symbiosis requires the suppression of plant defense responses against fungi as well as the modification of root architecture and cortical cell wall properties. Here, we investigated the contribution of phytohormones and their crosstalk to the ontogenesis of ectomycorrhizae (ECM) between grey poplar (*Populus tremula x alba*) roots and the fungus *Laccaria bicolor*. To obtain the

hormonal blueprint of developing ECM, we quantified the concentrations of jasmonates, gibberellins, and salicylate via liquid chromatography-tandem mass spectrometry. Subsequently, we assessed root architecture, mycorrhizal morphology, and gene expression levels (RNA-sequencing) in phytohormone-treated poplar lateral roots in the presence or absence of *L. bicolor*. Salicylic acid accumulated in mid-stage ECM. Exogenous phytohormone treatment affected the fungal colonization rate and/or frequency of Hartig net formation. Colonized lateral roots displayed diminished responsiveness to jasmonate but regulated some genes, implicated in defense and cell wall remodeling, that were specifically differentially expressed after jasmonate treatment. Responses to salicylate, gibberellin, and ethylene were enhanced in ECM. The dynamics of phytohormone accumulation and response suggest that jasmonate, gibberellin, salicylate, and ethylene signaling play multifaceted roles in poplar-*L. bicolor* ectomycorrhizal development.

Keywords: ectomycorrhizae, symbiosis, phytohormones, jasmonate, MiSSP, crosstalk, defense, cell wall remodeling, *Laccaria bicolor*, *Populus tremula x alba*.

2.2. Introduction

Most trees of temperate boreal forests interact with soil-borne beneficial fungi to form mutualistic interactions termed ectomycorrhizae (ECM; van der Heijden et al., 2015; Martin et al., 2016). Such ectomycorrhizal symbioses are of fundamental importance for forest ecosystems (Perez-Moreno and Read, 2000; Tibbett and Sanders, 2002; Clemmensen et al., 2015), but the molecular mechanisms of their establishment have not yet been fully unraveled. Most ECM show a distinct phenotype composed of three main features: (i) an extramatrical mycelium gathering nutrients from the soil, (ii) a mantle of aggregated hyphae ensheathing the tree's lateral roots (LRs) (Horan et al., 1988), and (iii) an internal hyphal network between the epidermis and root cortex, the Hartig net, where mineral nutrients and carbohydrates are exchanged (Blasius et al., 1986; Massicotte et al., 1987). The availability of sequenced genomes for ectomycorrhizal fungi such as *Laccaria bicolor* (Martin et al., 2008) and black truffle (*Tuber melanosporum*) (Martin et al., 2010), as well as host trees such as black cottonwood (*Populus trichocarpa*) (Tuskan et al., 2006) and English oak (*Quercus robur*) (Plomion et al., 2018), has facilitated the genetic dissection of ectomycorrhizal development. Distinct lineages of ECM fungi have acquired the ectomycorrhizal lifestyle independently upon the convergent loss of plant cell wall-degrading enzymes and expanded repertoires of effector-like secreted proteins with respect to the ancestral wood decayers. However, given the polyphyletic evolution of ECM symbiosis, the

identity of the fungal and tree gene repertoires required for its establishment depend on the fungus-host tree pair (Kohler et al., 2015). For example, the establishment of *P. trichocarpa*-*L. bicolor* ECM partly relies on the Common Symbiosis Signaling Pathway (CSSP; Cope et al., 2019), a highly conserved pathway in land plants necessary for the development of the arbuscular mycorrhizal symbiosis and the root nodule symbiosis (Oldroyd, 2008). However the genomes of Pinaceae trees lost key genes belonging to the CSSP, and are thus unlikely to exploit it to regulate mutualistic associations with ectomycorrhizal fungi (Garcia et al., 2015).

The formation of ECM involves complex developmental reprogramming of host tree morphology, including enhanced LR initiation (Tranvan et al., 2000; Rincón et al., 2003), root hair decay (Horan et al., 1988; Béguiristain and Lapeyrie, 1997), and elongation of epidermal and cortical cells (Kottke and Oberwinkler, 1987; Horan et al., 1988). Apoplastic hyphal penetration is accompanied by changes in plant and fungal cell wall composition (Mello and Balestrini, 2018 and references therein). These changes result in the aggregation of fungal hyphae and their adhesion to the plant cell wall (Tagu and Martin, 1996; Laurent et al., 1999; Tagu et al., 2001), hemicellulose and pectin degradation (Veneault-Fourrey et al., 2014; Sillo et al., 2016), and plant cell wall expansion and *de novo* biogenesis (Luo et al., 2009; Veneault-Fourrey et al., 2014; Sebastiana et al., 2014).

The reprogramming of root development during ECM formation partially depends on altered plant metabolism or sensitivity to phytohormones, the master regulators of plant responses to developmental and environmental cues (Garcia et al., 2015). Several ectomycorrhizal basidiomycetes and ascomycetes can produce auxins, facilitating root colonization (Gea et al., 1994; Splivallo et al., 2009; Vayssières et al., 2015). Moreover, ectomycorrhizal fungi can manipulate plant auxin and ethylene (ET) signaling to stimulate LR initiation and counteract root hair elongation (Ditengou et al., 2000; Reboutier et al., 2002; Splivallo et al., 2009; Felten et al., 2009, Vayssières et al., 2015). In addition, exogenous jasmonate (JA) is detrimental for Hartig net development (Plett et al., 2014a). *L. bicolor* hijacks JA signaling through the secretion of the Mycorrhiza-induced Small Secreted Protein 7 (MiSSP7), which enters the nuclei of *Populus* roots and stabilizes PtJAZ6, a corepressor of JA signaling (Plett et al., 2011; 2014b). Since JA signaling mediates plant defense responses against pests and necrotrophic fungi (Howe and Jander, 2008; Antico et al., 2012), *L. bicolor* might manipulate JA signaling in poplar to escape plant immunity. Consistent with the synergistic effect of ET and JA signaling on plant defense responses (Pieterse et al., 2009; 2012), fungal hyphae failed to form Hartig nets also in the roots of transgenic (*$35S_{pro}$:ACO1*) poplar plants overproducing ET (Plett et al., 2014a). Salicylic acid (SA) signaling also functions in plant defense, playing an antagonistic role with JA/ET signaling (Glazebrook, 2005; Spoel and Dong, 2008; Pieterse et al., 2009; 2012). However, exogenous SA

treatment does not affect fungal colonization (Plett et al., 2014a). Finally, the crosstalk between JA and gibberellin (GA) signaling regulates plant responses thought to function in the defense versus development trade-off (Hou et al., 2010; Wild et al., 2012; Yang et al., 2012; Song et al., 2014; Guo et al., 2018). Early reports suggest that exogenous GA inhibits the hyphal growth of several ectomycorrhizal species (Santoro and Casida, 1962; Gogala, 1971; Župančić and Gogala, 1980). GA signaling plays a role in symbiotic reprogramming during the establishment of arbuscular mycorrhizal symbiosis (Foo et al., 2013; Takeda et al., 2015). However, the contribution of GA signaling to the process of ectomycorrhizal colonization is currently unknown.

The differences in defense phytohormone levels and responsiveness between colonized and uncolonized poplar LRs have not yet been investigated. Therefore, in this study, we surveyed the hormonal landscape of ectomycorrhizal development by quantifying several classes of phytohormones in poplar-*L. bicolor* ECM, uncolonized poplar LRs, as well as *L. bicolor* free-living mycelia (FLM). Moreover, the role of the crosstalk between JA signaling and other hormone signaling pathways during ECM formation is currently unclear. Therefore, in second instance, we exogenously treated fungus-colonized and uncolonized poplar plants, as well as *L. bicolor* FLM, with JA, GA, SA, ET, and their combinations. Our aims were to (i) identify specifically differentially expressed genes after phytohormone treatment (phytohormone-sDEGs) in poplar LRs, to be used as a proxy for active phytohormone signaling; (ii) analyze the phenotypes of hormone-treated poplars in terms of root architecture and ectomycorrhizal colonization; and (iii) dissect the transcriptomic responses of poplar and *L. bicolor* to phytohormones under symbiotic and nonsymbiotic conditions via RNA-sequencing (RNA-seq) at two time points.

Dosage of phytohormone content revealed that SA content was enhanced in ECM, while assessment of transcriptomic and physiological responses of poplar roots to exogenous hormonal treatment showed that fungus-colonized LRs are less sensitive than uncolonized LRs to JA. In particular, their diminished responses to JA involve genes putatively associated with plant defense responses and cell wall modification. However, the overlap between JA-sDEGs, ET-sDEGs, and ECM-responsive genes suggests that residual JA/ET signaling modulates transient stress responses and plant cell wall modification. On the contrary, colonized LRs were more sensitive to SA, GA, and ET. All exogenous phytohormone treatments except SA affected the fungal colonization rate and/or frequency of Hartig net formation. Together, these results highlight the pivotal role of phytohormonal balance in the regulation of ECM symbiosis. With this study we also provide for the first time a list of phytohormone-sDEGs in poplar lateral roots and highlight within this belowground organ the antagonism and synergy between the main phytohormones implied in the trade-off between defense and development.

2.3. Materials and methods

Plant and fungal materials, hormonal treatments, and growth conditions

Plant and fungal materials were cultured as described by Felten et al. (2009). Briefly, grey poplar (*Populus tremula* x *Populus alba* line INRA 717-1-B4) clones were micropropagated *in vitro* and grown in half-strength Murashige and Skoog (MS/2) medium in glass culture tubes in a growth chamber at 24°C and 150 µmol/(m^2 x s) light intensity under a 16-h photoperiod. Light came from OSRAM Fluorescent tubes (50/50 Fluora / Cool white) placed 15 cm from poplar plants. The dikaryotic vegetative mycelia of strain S238N of the ectomycorrhizal fungus *Laccaria bicolor* were maintained on modified Pachlewski agar medium P5 at 25°C in the dark (Deveau et al., 2007). For *in vitro* coculture of poplar with *L. bicolor*, we used the sandwich system described by Felten et al. (2009). Briefly, ~10-mm-long rooted stem cuttings from *in vitro*-grown poplar plants were transferred to Petri dishes containing Pachlewski agar medium with reduced sugar (P20) covered with a cellophane membrane and a second, mycelium-covered cellophane membrane was placed on the roots. For single cultures, poplar plants and *L. bicolor* FLM were grown separately. The Pachlewski agar medium was supplemented with 2-morpholinoethanesulfonic acid sodium salt (MES Na) to maintain the pH at 5.8, along with the following phytohormones: JA treatment: 50 µM methyl-jasmonic acid (MeJA, Sigma, in 100% EtOH); GA treatment: 1 µM GA_3 (Sigma, in 100% EtOH); SA treatment: 500 µM SA (Sigma, in 100% EtOH); ET treatment: 250 µM 1-aminocyclopropane-1-carboxylic acid (ACC, Sigma, in H_2O). Combined GA-JA, SA-JA, and ET-JA treatments were performed by mixing MeJA with each of the other phytohormones in turn. Untreated plants were grown in non-hormone-supplemented medium. The Petri dishes were positioned vertically and incubated for 1 or 2 weeks in a growth chamber at 20°C under a 16-h photoperiod. Uncolonized poplar lateral roots (ULR) and *L. bicolor* FLM were collected separately for transcriptomic analysis; while colonized lateral roots (CLR) formed a mixed tissue of vegetal and fungal origin, and was subjected to metatranscriptomic analysis. Samples were collected at 1 or 2 weeks post-treatment (wpt). The chosen time points recapitulate two fundamental stages of ectomycorrhizal development: mantle formation (1 week post-contact (wpc), early stage ECM) and Hartig net development (2 wpc, mid-stage ECM). The samples were snap-frozen and stored at −80°C for subsequent RNA extraction or LC-MS/MS. For JA-treated plants with poorly developing LRs, the central parts of the adventitious roots were sampled. A summary of our experimental approach can be found in Figure 1.

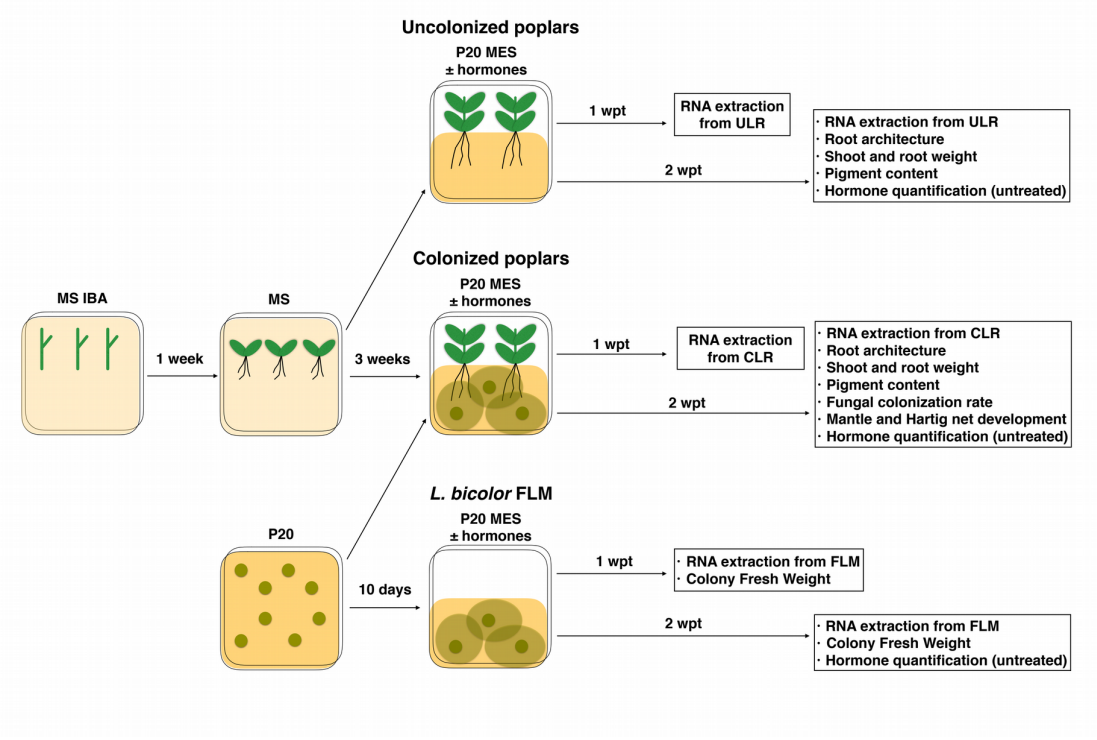


Figure 1. Experimental approach.

P. tremula x alba *cuttings were propagated in vitro in modified Murashige and Skoog basal agar medium (MS) supplemented with indole-3-butyric acid (IBA) for 1 week and transferred to modified MS without IBA for 3 weeks to stimulate rooting. Plugs of* L. bicolor *mycelia were propagated on sugar-reduced Pachlewski agar medium (P20) covered with a cellophane membrane for 10 days. Poplar-*L. bicolor *cocultures were set up on P20 plates containing the pH stabilizer 2-morpholinoethanesulfonic acid sodium salt (MES Na). The medium was supplemented with 50 µM MeJA, 1 µM* GA_3*, 500 µM SA, 250 µM ACC, combinations of MeJA and other hormones, or no phytohormones (untreated). Plants deposited on such plates between two layers of cellophane without fungal mycelia are referred to as uncolonized plants. Poplar cuttings in contact with fungal mycelia constituted colonized plants. L. bicolor mycelia grown on P20 MES in the absence of poplar were termed free-living mycelia (FLM). Uncolonized LRs (ULR), colonized LRs (CLR), and FLM were sampled for RNA extraction was performed at two time points: 1 and 2 weeks post-treatment (wpt). CLR-derived RNA was a mixture of poplar and* L. bicolor *RNA, due to the composite nature of root-fungal symbiotic tissues. Root architecture parameters, shoot weight, root weight, and pigment content were also assessed in poplar cuttings at 2 wpt. In addition, fungal colonization rate, mantle development, and Hartig net development were assessed for colonized LRs at 2 wpt. LRs of untreated uncolonized cuttings, plugs of untreated FLM, and untreated ECM at 2 wpt were collected for phytohormone quantification.*

Quantification of phytohormones

Endogenous levels of plant hormones (JAs, GAs, and SA) were measured using 20 mg (fresh weight) of uncolonized poplar LRs and ECM, as well as *L. bicolor* FLM, as described by Šimura et al. (2018). Five biological replicates per condition were produced. Briefly, the phytohormones were extracted using an aqueous solution of acetonitrile (50% ACN/H_2O, v/v). A cocktail of stable isotope-labeled standards was added (all from Olchemim Ltd., Czech Republic) per sample to validate the LC-MS method. The extracts were purified using Oasis HLB columns (30 mg/1 ml, Waters), and the analytes were eluted using 30% ACN/ H_2O (v/v). The eluent (containing hormones and their metabolites) was gently evaporated to dryness under a stream of nitrogen. Separation was performed on an Acquity I-Class System (Waters, Milford, MA, USA) equipped with an Acquity UPLC® CSH C18 RP column (150×2.1 mm, 1.7 µm; Waters), and the effluent was introduced into the electrospray ion source of a triple quadrupole mass spectrometer (Xevo™ TQ-S, Waters). To highlight statistically significant differences in hormone levels between LR, ECM, and FLM samples, we performed a Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction ($n = 5$, $p < 0.05$, R package agricolae, https://CRAN.R-project.org/package=agricolae).

Analysis of ectomycorrhizal colonization and development

The quantification of ectomycorrhizal colonization and the observation of ectomycorrhizal structures were performed as described by Felten et al. (2009). Briefly, colonized plants were observed under a Discovery V.8 stereomicroscope (Zeiss), and short, rounded LRs ensheathed by fungal mantle were considered to be colonized. The rate of ectomycorrhizal colonization was defined as the ratio of colonized LRs to the total number of LRs (expressed as a percentage). Between 25 and 53 plants per treatment were observed. Untreated colonized LRs displayed a ~40% colonization rate, which is in line with previous reports (Plett et al., 2015). To confirm the development of intraradical Hartig nets, three to five ECM per treatment were fixed with 4% paraformaldehyde in 1X phosphate-buffered saline and embedded in 4% (w/v) agarose. Transverse 30-µm-thick sections of ECM obtained 200 to 400 µm from the LR tip were produced with a Leica VT1200 S vibratome and stained with propidium iodide (1:100 dilution; Sigma-Aldrich) and WGA-488 (1:100 dilution; Wheat Germ Agglutinin, Alexa Fluor™ 488 Conjugate, Thermo Fischer Scientific) to highlight plant and fungal structures, respectively. The sections were observed under a Zeiss LSM 700 laser scanning microscope and the images analyzed with Fiji software (RRID:SCR_002285, Schindelin et al., 2012). Root diameter, fungal mantle thickness, and Hartig net depth were measured. Hartig net frequency (expressed as the percentage of root

apoplastic spaces occupied by fungal hyphae) was also calculated. At least three sections per sample were analyzed.

Analysis of root and shoot phenotypes

The root architectures of 32 to 37 plants per treatment were assessed by analyzing scanned (Epson Perfection V700 PHOTO) plates with Fiji software (RRID:SCR_002285, Schindelin et al., 2012). LR density was defined as the number of LRs over the length (mm) of the respective adventitious root. To measure shoot and root system weight, 32 plants were pooled in eight biological replicates immediately after sampling (FW) or after lyophilization for 48 h in a Univapo 100H evaporator centrifuge (dry weight, DW). Lyophilized shoots were subsequently used for total chlorophyll (Hall and Rao, 1999) or anthocyanin measurements (Ticconi et al., 2001), with four biological replicates per assay. Anthocyanin content was measured for seven to eight biological replicates of lyophilized roots. In brief, shoots and roots were ground into a fine powder with a tissue lyser (MM 400, Retsch). Chlorophyll was extracted in 80% acetone, while anthocyanin was extracted by boiling the sample for 3 min in 15% (v/v) isopropanol 9.96 N HCl buffer. The supernatants were diluted and their absorbance (A) measured with a Tecan Infinite M200 PRO plate reader. The quantity of chlorophyll was determined using the following formula:

$$Total\,chlorophyll\left(mg/g\,DW\right)=\frac{A_{652}*dilution\,factor*1000}{DW\left(mg\right)*34.5}$$

Anthocyanin was quantified using the following formula:

$$Anthocyanin\left(A_{535}/g\,DW\right)=\frac{\left(A_{535}-A_{650}\right)*1000}{DW\left(mg\right)}$$

Differences in root and shoot architectural and physiological parameters among samples were tested through Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction (R package agricolae, https://CRAN.R-project.org/package=agricolae). The results were manually converted into a numeric matrix preserving statistically significant differences. This matrix was then used to build models representing the phenotypes of poplar cuttings under each treatment. Average values for each parameter were used to generate a heat map and to group phenotypically similar treatments through hierarchical clustering (R package pheatmap, RRID:SCR_016418, https://CRAN.R-project.org/package=pheatmap).

Analysis of *L. bicolor* FLM growth phenotypes

Diametral growth of *L. bicolor* FLM colonies grown on P20 medium supplemented with phytohormones, or with EtOH 0.061% v/v, or non supplemented was monitored daily for 14 days. Linear regression models were built for every growth curve on a different medium, and χ^2 test was used to test differences between linear models of EtOH- or phytohormone-treated colonies with respect to untreated colonies ($p < 0.05$). At the same time Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction was used to highlight significant differences among treatments at every time point ($p < 0.05$). Six to 16 biological replicates per conditions were used. Three to four FLM colonies were also harvested at 1 and 2 wpt for fresh weight (FW) measurement. Significant differences in FW among samples were tested via Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction ($p < 0.05$, R package agricolae, https://CRAN.R-project.org/package=agricolae).

RNA-seq and gene expression data analysis

RNA was extracted with an RNeasy Mini Kit (Qiagen) from three types of samples: (i) uncolonized poplar LRs, (ii) *L. bicolor* FLM, and (iii) colonized poplar LRs composed of mixed poplar and fungal tissue (Figure 1). LRs of ~12 plants were pooled to form a biological replicate, and three replicates per treatment were produced. Similarly, three biological replicates of FLM were sampled from independent plates. Preparation of stranded libraries and 2 × 150 bp Illumina HiSeq2000/2500 mRNA sequencing (RNA-seq) were performed by the Department of Energy Joint Genome Institute (JGI) facilities. Raw reads were filtered and trimmed using the JGI QC pipeline. Briefly, using BBDuk (RRID:SCR_016969, https://sourceforge.net/projects/bbmap/) raw reads were evaluated for artifact sequence by k-mer matching (k-mer=25), allowing 1 mismatch, and detected artifact was trimmed from the 3'-end of the reads. RNA spike-in reads, PhiX reads and reads containing any Ns were removed. Quality trimming was performed using the phred trimming method set at Q6. Finally, reads under the length threshold of 25 bases or 1/3 of the original read length were removed.

Filtered reads from each library were aligned to the reference genomes (https://genome.jgi.doe.gov/Lacbi2/Lacbi2.home.html or Ptrichocarpa_444_v3.1- available at https://phytozome.jgi.doe.gov/pz/portal.html) using HISAT2 version 2.1.0 (RRID:SCR_015530). A summary of the alignments is given in Supplemental Data Set 1. FeatureCounts was used to generate raw gene counts. Only primary hits assigned to the reverse strand were included in the raw gene counts (-s 2 -p --primary options). FPKM- and TPM-normalized gene counts are also provided. The $\log_2$ fold change ($\log_2$ FC) in gene

expression level between conditions was calculated with the R package DESeq2 (RRID:SCR_015687, Love et al., 2014). Genes with statistically significant differences in expression were selected based on Bonferroni adjusted p-value < 0.05. Normalized read counts of the genes were also produced with DESeq2 and were subsequently log_2 transformed. The consistency of normalized transcript levels from biological replicates was confirmed by visualizing the distribution of read counts (Supplemental Figure 1A). Spearman's rank correlation was calculated with normalized read counts from the biological replicates from all conditions. The estimated correlation coefficients were visualized and examined (Supplemental Figure 1B).

Identification of phytohormone-sDEGs in poplar LRs

To identify genes specifically expressed after phytohormone treatment (phytohormone-sDEGs) in poplar LRs we analyzed log_2 FC of gene expression in uncolonized LRs treated with JA, GA, SA, and ET at 1 wpt versus untreated uncolonized LRs. Normalization was performed on the entire set of uncolonized LR counts. JA-, SA-, and ET-sDEGs were defined as DEGs whose expression varied significantly in response to treatment with only JA, SA, or ET, respectively (> 4-fold, $p < 0.05$). To highlight groups of synergistically or antagonistically regulated genes, we performed Weighted Gene Co-expression Network Analysis (WGCNA, RRID:SCR_003302, Langfelder et al., 2008) using DESeq2-normalized counts of untreated or JA-, SA-, ET-, SA-JA-, and ET-JA-treated uncolonized LRs at 1 wpt. Normalization was performed on the entire set of uncolonized LR counts. WGCNA was previously applied to subsets of RNA-seq data to balance an acceptable computational time with biological meaningfulness (Zhan et al., 2015; Baumgart et al., 2016; Drag et al., 2017). We implemented WGCNA for semisupervised analysis, including only DEGs (> 4-fold, $p < 0.05$) of uncolonized LRs treated with one or multiple hormones. The resulting 5134 genes were binned into 13 color-coded modules (Supplemental Figure 2C). Modules were correlated to one or more treatment (Pearson's correlation test, $p < 0.05$). The results of the correlation test and the expression profile of each module were taken into account to regroup interesting modules into four clusters: the red, green, black, and purple clusters, representing synergistic SA-JA and ET-JA signaling, synergistic ET-JA signaling, antagonistic SA-JA and ET-JA signaling, and antagonistic SA-JA signaling, respectively (Supplemental Data Set 2 and Supplemental Figure 2D). We defined poplar ECM-responsive genes as DEGs (> 4-fold, $p < 0.05$) in untreated colonized LRs versus untreated uncolonized LRs (Supplemental Data Set 3). Gene Ontology (GO) enrichment analysis for groups of phytohormone-sDEGs or groups of crosstalk-responsive genes was carried out via the online software AgriGO (Tian et al., 2017), by Singular Enrichment Analysis (SEA) against the reference background of the *Populus trichocarpa*

genome v3.0. GO terms in query sets were tested for significant enrichment via Fisher's exact test with Benjamini-Yekutieli correction for multiple testing (FDR < 0.05).

Unsupervised analysis of the transcriptomic data

To obtain a comprehensive view of the impact of hormone treatments on the poplar and *L. bicolor* transcriptomes, we constructed transcriptomic models using SHIN+GO (Miyauchi et al., 2016; 2017; 2018). A self-organizing map (SOM) was trained with the normalized read count of the selected replicates using Rsomoclu (Wittek et al., 2013). A 37 x 31 (1147) matrix with rectangular connections (i.e. formed by four neighboring nodes) was used for analysis. A resolution of 25 genes per node was used for clustering, which was empirically optimized (Miyauchi et al., 2016; 2017). An epoch of 1000 times more than the map size was applied (i.e. 1,147,000: 1147 map size x 1000). The initial radius for SOM calculation was determined using the neighbor distance function in the R kohonen package (Wehrens and Buydens 2007). The following graphic outputs (i.e., Tatami maps) were examined: (i) genome-wide transcriptomic patterns of all biological replicates and (ii) genome-wide condition-specific transcriptomic patterns (Supplemental Figure 3). Mean transcription values were calculated based on the values of grouped genes per condition in each node (i.e., node-wise transcription; Supplemental Data Sets 4 and 5). Nodes whose mean transcription value showed > 4-fold regulation ($p < 0.05$) under a specific treatment compared to the respective untreated tissue were considered to be differentially regulated and were highlighted in the summary Tatami maps. Functional annotation sets were integrated into the constructed model using the following databases: Carbohydrate Active Enzyme (CAZy, RRID:SCR_012909, Levasseur et al., 2013; Lombard et al., 2014), Gene Ontology (GO, RRID:SCR_002811, The Gene Ontology Consortium, 2015), Kyoto Encyclopedia of Genes and Genomes (KEGG, RRID:SCR_012773, Ogata et al., 1999), EuKaryotic Orthologous Groups (KOG, RRID:SCR_008223, Tatusov et al., 2003), PFAM (RRID:SCR_004726, Finn et al., 2016), Panther (RRID:SCR_004869, Thomas et al., 2003), and Proteases (MEROPS, RRID:SCR_007777, Rawlings et al., 2018). KOG, GO, KEGG, PFAM, and Panther best-hit Arabidopsis TAIR10 homologs were obtained from Phytozome, JGI (RRID:SCR_006507, https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Ptrichocarpa_er). CAZymes and MEROP were obtained based on PFAM and KEGG IDs using R packages KEGG.db and PFAM.db (Carlson 2016; Carlson et al., 2018). R was used to operate the pipeline (R Core Team, 2013). All procedures were performed with the SHIN module of SHIN+GO. A set of custom scripts for SHIN is available upon request.

Finally, to highlight genes specifically regulated by hormonal treatment in colonized or uncolonized poplar LRs, we analyzed $\log_2$ FC of gene expression in hormone-treated

colonized or uncolonized LRs versus untreated colonized or uncolonized LRs, respectively. DEGs were defined as > 4-fold ($p < 0.05$) regulated genes in treated versus untreated LRs. Normalization was performed on the entire set of colonized and uncolonized poplar LR counts.

Accession numbers

Sequencing data from this article can be found in the NCBI BioProject data libraries under accession numbers PRJNA443942 to PRJNA444766.

2.4. Results

SA accumulates in mid-stage ECM *in vitro*

To investigate the dynamics of phytohormone metabolism during ectomycorrhizal development, we quantified the concentration of some precursor and bioactive JA and GA species, as well as SA, using liquid chromatography-tandem mass spectrometry (LC-MS/MS; Šimura et al., 2018) in (i) ECM of *in vitro*-grown colonized poplar cuttings at 2 weeks post-contact (wpc), (ii) uncolonized poplar LRs, and (iii) *L. bicolor* FLM. The results are summarized in Table 1.

We detected JA, jasmonoyl-L-isoleucine (JA-Ile), and the JA precursor *cis*-(+)-12-oxo-phytodienoic acid (*cis*-OPDA) in concentrations of 0.14 ± 0.06 pmol/g FW to 5.36 ± 1.70 nmol/g FW. These compounds were 4, 5.8, and 3.9 times less concentrated in ECM than in LRs. However, their concentrations in FLM were very low or below the detection limit, suggesting that these compounds were mostly synthetized by poplar roots. Since the proportion of poplar tissue in ECM was ~36% (Supplemental Data Set 1) and metabolite content was normalized by sample FW, the reduced content of JAs in ECM could be explained by the dilution of poplar tissue in ECM due to the presence of fungal hyphae (data not shown). We also detected SA in all samples ranging from 67.08 ± 22.68 to 863.82 ± 251.32 pmol/g FW. SA levels increased 11.9-fold in ECM compared to LRs and 4.8-fold compared to FLM. Finally, we detected low levels of two bioactive gibberellins (GA_4 and GA_6) and the GA_6 precursor GA_{19}, ranging from 0.25 ± 0.07 to 7.33 ± 1.77 pmol/g FW. GA_4 levels were low in LRs and were below the detection limit in ECM. By contrast, GA_6 and GA_{19} concentrations were higher in ECM compared to LRs and FLM. Such variations in hormonal contents suggest that altered phytohormone turnover occurs during ectomycorrhizal development, which may affect the expression of hormone-related gene networks.

Class	Hormone Type	Metabolism	LRs	ECM	FLM
Gibberellins	GA_4	bioactive hormone	0.25 ± 0.07	<LOD †	<LOD
	GA_6	bioactive hormone	<LOD	1.32 ± 0.18 †	0.82 ± 0.25
	GA_{19}	precursor	4.51 ± 0.87	7.33 ± 1.77 †§	<LOD
Jasmonates	*cis*-OPDA	precursor	5357.8 ± 1701.2	1364.8 ± 267.3 †§	<LOD
	JA	bioactive hormone	48.65 ± 14.36	11.98 ± 2.74 †§	0.14 ± 0.06
	JA-Ile	bioactive hormone	32.86 ± 11.19	5.7 ± 1.35 †§	0.05 ± 0.02
Salicylates	SA	bioactive hormone	67.08 ± 22.68	863.82 ± 251.32 †§	148.1 ± 47.19

Table 1. Ectomycorrhizae accumulate salicylic acid.

Average and standard error of the concentrations of gibberellins, jasmonate and salicylate in uncolonized poplar lateral roots (LRs), ectomycorrhizae (ECM) at 2 weeks post-contact (wpc), and L. bicolor free-living mycelium (FLM), as measured by LC-MS/MS and reported in pmol/g fresh weight (FW). <LOD represents concentrations below the detection limit. † or § indicate significant differences between ECM and LRs, or between ECM and FLM, respectively, according to Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction (n = 5, p < 0.05).

Phytohormonal treatments affect ectomycorrhizal development and physiology of poplar cuttings

To investigate the contributions of JA, GA, SA, and ET signaling pathways and their crosstalk to ectomycorrhizal development, we treated colonized and uncolonized poplar cuttings, as well as *L. bicolor* FLM, with exogenous JA, GA, SA, and ET, as well as GA-JA, SA-JA, and ET-JA combinations. We then sampled RNA for transcriptomic analysis and examined the phenotype of hormone-treated fungi and plants at 1 or 2 weeks post-treatment (wpt) (Figure 1). First we assessed whether phytohormone treatment impaired the growth of *L. bicolor* FLM. SA treatment resulted in a transient tendency towards higher radial growth of *L. bicolor* colonies, while ET treatment significantly reduced colony fresh weight at 1 wpt. None of the other hormonal treatments, or the highest concentration of EtOH used as solvent (0.061% v/v), altered FLM phenotypes (Supplemental Figure 4). However, all phytohormone treatments except SA treatment impaired the fungal colonization rate of poplar plants (Figure 2A and Supplemental Data Set 6). To assess the development of symbiotic structures *in*

planta, we harvested colonized root tips for microscopy observation. ECM from JA, GA-JA-, and ET-JA-treated plants displayed 3-, 2.5-, and 4.3-fold thinner mantles, respectively, than untreated ECM. Moreover, although Hartig net depth did not vary significantly among samples, the frequency of fungal penetration events (Hartig net frequency) was significantly reduced in ECM of JA- (0.9%) and ET-JA- (1.4%) treated colonized plants compared to untreated ECM (51%; Figure 2A and Supplemental Data Set 6). ET-JA-treated ECM, being very short, showed strong accumulation of red fluorescent compounds in root cells, a phenotype typical of poplar root meristem.

All exogenous phytohormone treatments except GA treatment also affected the phenotypes of colonized and uncolonized poplar plants, including weight, root architecture (number and length of adventitious roots, density and length of LRs, secondary LR density, and total root system length), and both chlorophyll and anthocyanin contents. A picture of representative hormone-treated and/or fungus-inoculated poplar cuttings can be found as Supplemental Figure 5. Hierarchical clustering of measures relative to these parameters, as well as to the fungal colonization rate, revealed four groups of plants with similar phenotypes (Figure 2B). GA- and ET-treated colonized plants were the most similar to untreated colonized plants (Group 1). This group displayed the highest root and shoot weights, LR density, colonization rate, and Hartig net frequency. Within Group 2, ET-treated uncolonized plants displayed higher LR density than untreated uncolonized plants. Group 4 was formed by all uncolonized plants treated with JA, alone or in combination with SA or ET, as well as ET-JA-treated colonized plants. This group displayed the lowest chlorophyll content, the highest anthocyanin content, and the lowest LR density. Anthocyanin content can be used as a proxy for active plant defenses against biotic or abiotic stress, while chlorophyll content is related to the rate of photosynthetic activity and plant nitrogen nutrition (Lev-Yadun and Gould, 2008; Wang et al., 2014). Therefore, plants belonging to Group 4 likely displayed the strongest activation of defense mechanisms at the expense of carbon fixation. Mycorrhizal colonization of JA-, GA-JA-, and SA-JA-treated plants (Group 3) partially rescued these phenotypes (Supplemental Figure 6 and Supplemental Data Set 6). These findings suggest that JA-triggered plant defense responses counteract fungal colonization.

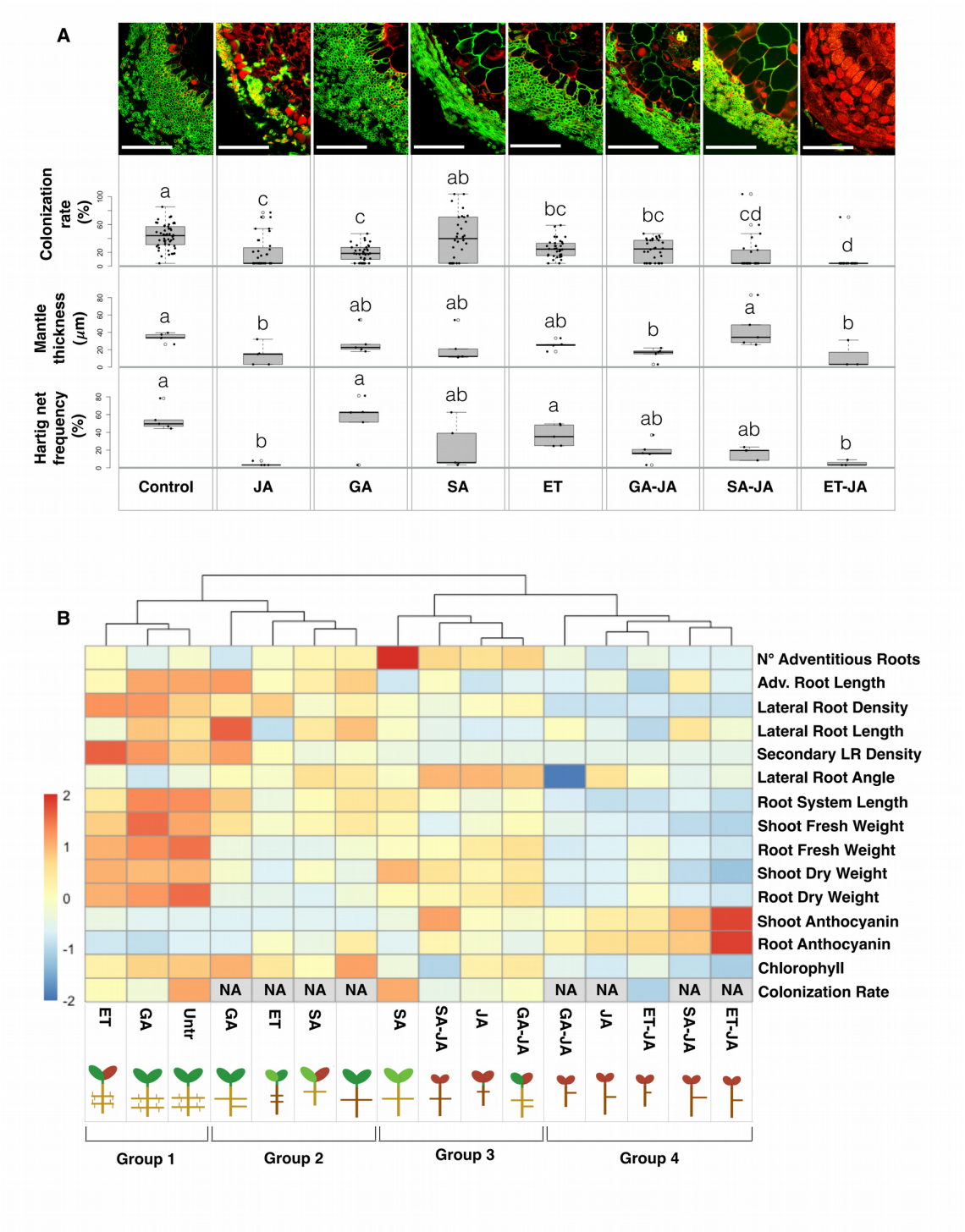


Figure 2. Exogenous phytohormone treatments affect ectomycorrhizal development and physiology of poplar cuttings.

A. Ectomycorrhizal phenotypes of phytohormone-treated colonized poplar plants. Upper panels: confocal microscopy images of representative sections of untreated or JA-, GA-, SA-, ET-, GA-JA-, SA-JA-, and ET-JA-treated ectomycorrhizae (ECM). Propidium Iodide (red) stains the root cell wall,

while AlexaFluor® WGA-288 (green) stains the fungal cell wall. Scale bar: 50 µm. Lower panels: boxplots representing fungal colonization rate, mantle thickness, and Hartig net frequency in untreated or phytohormone-treated ECM. Whiskers represent the limits of the 1.5 interquartile range. Letters indicate significant groups based on the results of Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction ($p < 0.05$). B. Heat map showing the clustering of untreated and hormone-treated poplar cuttings according to the average values of physiological parameters. Row-scaled Z-score values were used to build the heat map. Values range from −2 to 2 standard deviations from the center. NA indicates the lack of colonization in the uncolonized poplar cuttings. Four groups were established according to the hierarchical clustering results. Phenotypic models were drawn taking into account statistically significant differences resulting from Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction for each parameter (Supplemental Data Set 6). Adv.: Adventitious. LR: Lateral root. Untr: Untreated.

Phytohormone treatment has minor effects on the *L. bicolor* transcriptome

To untangle the complex responses of poplar and *L. bicolor* to phytohormones, we performed an unsupervised analysis using the entire gene expression sets derived from RNA sequencing of hormone-treated colonized and uncolonized poplar LRs, as well as *L. bicolor* FLM. To this end, we exploited the SHIN+GO (Self-organizing map Harboring Informative Nodes with Gene Ontology) pipeline (Miyauchi et al., 2016; 2017; 2018). Based on the normalized levels of the 28,502 transcripts detected in poplar LRs and the 15,129 transcripts detected in *L. bicolor* mycelia, we built transcriptomic models using the SHIN+GO pipeline on the R platform (Methods, Supplemental Data Sets 4 and 5). In the resulting summary Tatami maps, the nodes (i.e. groups of genes with similar expression profiles) that were regulated by one or more treatments are highlighted with different colors (Figure 3). This analysis revealed few or no nodes regulated in *L. bicolor* mycelium upon phytohormone treatment (Figure 3A-B). However, root-colonizing *L. bicolor* hyphae were more sensitive to exogenous hormonal treatments than FLM, with modified expression of between 25 (GA treatment at 2 wpt) and 703 (GA-JA treatment at 2 wpt) genes. Both FLM and root-colonizing hyphae responded to SA and SA-JA treatments, triggering the regulation of 123 to 665 genes (Supplemental Data Set 7).

To gain insight into the response of *L. bicolor* to phytohormone-derived signals, we examined the expression of genes uniquely regulated by JA, GA, SA, or ET treatment in FLM and root-colonizing hyphae at 1 wpt (Supplemental Data Set 8). SA treatment of FLM and root-colonizing hyphae triggered the upregulation of 15 and 22 genes, respectively, with a predicted role in lipid transport and metabolism. In addition, SA treatment of root-colonizing hyphae upregulated 10 genes putatively involved in glycolysis. On the other hand, GA treatment of root-colonizing hyphae downregulated 20 genes putatively involved in signal transduction and eight genes encoding predicted von Willebrand factor-related coagulation

proteins (Fischer et al., 1988). Finally, ET treatment of root-colonizing hyphae downregulated 10 genes putatively involved in replication, transcription, and RNA processing.

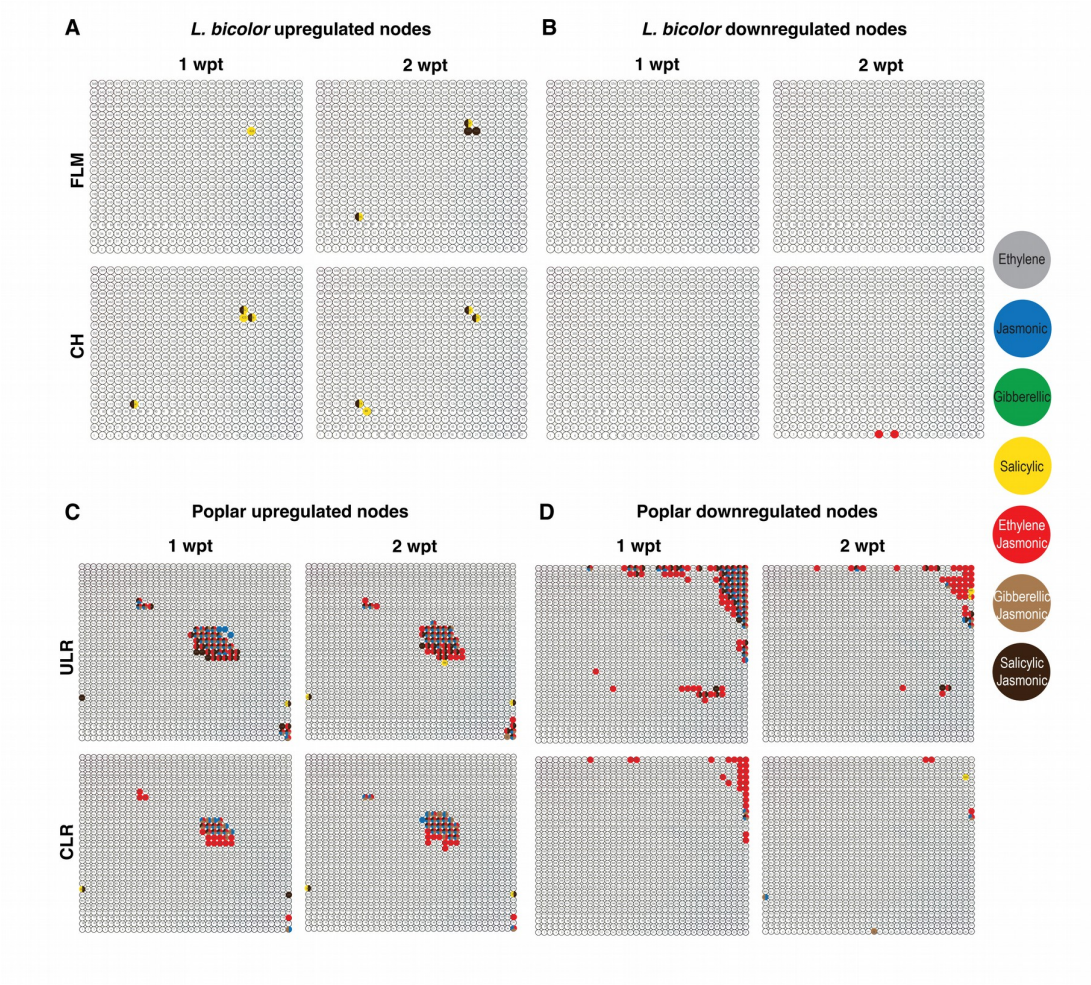


Figure 3. Transcriptomic analysis reveals decreased sensitivity to JA in colonized versus uncolonized poplar lateral roots.

A-B. Summary Tatami maps representing significantly upregulated (A) and downregulated (B) nodes in the transcriptomes of hormone-treated L. bicolor *FLM versus untreated FLM (upper panels), as well as of hormone-treated root-colonizing hyphae versus untreated root-colonizing hyphae (lower panels) at 1 (left panels) or 2 wpt (right panels). C-D. Summary Tatami maps representing significantly upregulated (C) and downregulated (D) nodes in the transcriptomes of hormone-treated uncolonized lateral roots (LRs) versus untreated uncolonized LRs (upper panels), as well as hormone-treated colonized LRs versus untreated colonized LRs (lower panels) at 1 (left panels) or 2 weeks post-treatment (wpt) (right panels). Colors represent nodes differentially regulated by one or more hormonal treatment. ULR: uncolonized LRs. CLR: colonized LRs. CH: root-colonizing hyphae.*

L. bicolor colonization affects the sensitivity of poplar LRs to JA treatment

Unsupervised analysis revealed that the transcriptomes of uncolonized and colonized poplar LRs were strongly affected by exogenous phytohormones. ET-JA treatment had the greatest effect, leading to the differential regulation of 130 nodes in uncolonized LRs and 61 nodes in colonized LRs (Figure 3C-D; Supplemental Data Set 4). Higher numbers of differentially regulated nodes in uncolonized LRs versus colonized LRs were also detected after JA, GA-JA, and SA-JA treatment, especially at 1 wpt (Figure 3C-D). These findings point to the diminished sensitivity of poplar LRs to JA treatment upon inoculation with *L. bicolor*, especially at the 1-week time point.

To gain deeper insight into the functional consequences of diminished JA sensitivity in early colonized LRs, we explored the nodes that were exclusively regulated by JA treatment in uncolonized LRs (Supplemental Data Set 9). The presence of the mycorrhizal fungus mitigated JA-induced expression of 16 genes predicted to encode serine protease inhibitors, including eight putative Kunitz trypsin inhibitors. Also, the contact with *L. bicolor* counteracted the JA-triggered downregulation of genes likely involved in cell shape modification, cell wall remodeling, and root hair elongation, such as homologs of Arabidopsis genes encoding six expansins, seven extensins, six xyloglucan endotransglycosylases, and four root hair-specific pectate lyases. Albeit reduced, the activation of plant defense was still detectable in the transcriptome of JA-treated colonized LRs, as revealed by the induction of genes putatively encoding ten protease inhibitors, six basic chitinases, four genes predicted to encode white-brown complex homolog protein 11 (WBC11), and nine genes likely involved in terpene biosynthesis (Supplemental Data Set 9).

L. bicolor colonization increases the sensitivity of poplar LRs to GA, SA and ET treatment

The SHIN+GO pipeline revealed few or no nodes specifically regulated by GA, SA, or ET treatment in colonized or uncolonized poplar LRs (Figure 3C-D, Supplemental Data Set 4), possibly due to the lower number of specifically regulated genes in response to these phytohormones compared to JA (Supplemental Data Set 7). Therefore, to explain the phenotypes of GA-, SA-, and ET-treated plants, we compared DEGs in GA-, SA-, and ET-treated colonized and uncolonized LRs to their respective untreated LRs. The response to GA treatment was more pronounced in colonized LRs (465 genes at 1 wpt and 103 genes at 2 wpt) than in uncolonized LRs (5 genes at 1 wpt and 5 genes at 2 wpt). GA-downregulated genes in colonized LRs included four genes likely involved in ATP synthesis, six genes putatively encoding ribosome subunits, and nine genes predicted to encode RNA-binding proteins. The sensitivity to SA was slightly higher in colonized LRs (494 genes at 1 wpt and 1325 genes at 2 wpt) than in uncolonized LRs (334 genes at 1 wpt and 624 genes at 2 wpt).

The specific responses of colonized LRs to exogenous SA included the upregulation of seven genes encoding predicted Ca^{2+}-binding proteins, seven putative chaperones, and 18 predicted cell wall- or membrane-associated protein kinases. Interestingly, among these were two predicted calmodulin binding protein-like genes (Potri.015G045300 and Potri.012G054900). These genes encode homologs of Arabidopsis SAR DEFICIENT1 (SARD1), which is required for SA biosynthesis during pattern-triggered immunity (PTI; Zhang et al., 2010; Wang et al., 2011; Truman and Glazebrook, 2012). Finally, ET treatment triggered the regulation of more genes in colonized LRs (502 genes at 1 wpt and 491 genes at 2 wpt) than in uncolonized LRs (275 genes at 1 wpt and 402 genes at 2 wpt). ET-treated colonized LRs specifically upregulated genes encoding eight phospholipase A 2A, Ca^{2+}-dependent lipid binding proteins putatively involved in PTI (La Camera et al., 2005; 2009; Supplemental Data Set 7 and Supplemental Data Set 9).

The crosstalk between JA, SA, and ET signaling regulates transient defense responses and cell wall remodeling during ectomycorrhizal development

After assessing differential responsivity to exogenous phytohormones in poplar colonized and uncolonized LRs, we aimed at investigating if the JA, GA, SA, and ET signaling pathways were specifically triggered during ectomycorrhizal development. We first searched for specifically differentially expressed genes after phytohormone treatment (phytohormone-sDEGs) in uncolonized poplar LRs at 1 wpt and used them as proxies of activated phytohormone signaling pathways. We detected 2452 JA-sDEGs, 232 SA-sDEGs, and 97 ET-sDEGs. By contrast, we only detected six genes that were specifically regulated upon GA treatment of LRs (Supplemental Figure 2A, Supplemental Data Set 7, and Supplemental Data Set 10). Gene Ontology enrichment analysis (Supplemental Data Set 11) revealed that JA treatment enhanced response to wounding (FDR = 9.4 e^{-6}) and lipid metabolism (FDR = 0.00026), while repressing genes involved in cell wall modifications (FDR = 2.4 e^{-8}) and possessing pectinesterase activity (FDR = 1.8 e^{-7}), xyloglucan:xyloglucosyl transferase activity (FDR = 5.6 e^{-5}) and polygalacturonase activity (FDR = 0.0034). In contrast, SA treatment triggered cell wall modifications (FDR = 0.00012) based on pectinesterase activity (FDR= 0.0013) but inhibited the expression of photosynthesis-related genes (FDR = 3.5 e-^{20}). ET-upregulated sDEGs were enriched in oxidoreductases (FDR = 0.0043) and hydrolases (FDR = 0.0079), but no GO category was overrepresented in ET-downregulated sDEGs. In addition to phytohormone-sDEGs, we searched for crosstalk-regulated genes in uncolonized poplar LRs. We could not explore the potential GA-JA crosstalk because of the low number of detected GA-sDEGs, but we detected 5134 DEGs in LRs under at least one among JA, SA, and ET treatment or combined SA-JA or ET-JA treatment at 1 wpt (Supplemental Figure 2B). To highlight clusters of genes regulated by SA-

JA or ET-JA crosstalk, we binned these 5134 DEGs into 13 coexpression modules via Weighted Gene Co-expression Network Analysis (WGCNA (Methods, Supplemental Figure 2C). Subsequently, we analyzed the expression profiles of these modules and merged 10 of the modules into four clusters, representing genes synergistically or antagonistically regulated by SA-JA or ET-JA signaling. Gene Ontology enrichment analysis revealed the main biological processes these four clusters modulate (Supplemental Data Set 11). The red cluster includes genes regulated by synergy between SA-JA and ET-JA signaling and responsive to wounding (FDR = 1.2 e^{-8}) and to oxidative stress (FDR = 7.6 e^{-7}). The green cluster contains genes regulated by synergy between ET and JA signaling, part of which involved in carbohydrate metabolism (FDR = 5.9 e^{-8}) and photosynthesis (FDR = 0.00011). Genes in the black cluster are antagonistically regulated by SA-JA and ET-JA and enriched in terpene synthases (FDR = 1.1 e^{-7}). Finally, genes in the purple cluster are antagonistically regulated by SA and JA signaling and part of these genes act in cell wall modification (FDR = 2.6 e^{-11}) and protein phosphorylation (FDR= 0.00042) (Supplemental Figure 2D, Supplemental Data Set 2, and Supplemental Data Set 11).

We found substantial consistency in the expression patterns of JA-, SA-, and ET-sDEGs, as well as crosstalk-regulated genes, in hormone-treated colonized and uncolonized poplar LRs at 1 week post-treatment (1 wpt) and 2 wpt (Supplemental Figure 7). The only exception was downregulated JA-sDEGs, most of which were not regulated by JA treatment in colonized LRs. Thus, these genes represent *bona fide* LR responses to the respective hormones. Therefore, we utilized these gene sets to investigate whether JA, SA, and ET signaling and their crosstalk are active along two stages of ectomycorrhizal development: before (1 week post-contact (wpc)) and after (2 wpc) Hartig net initiation. Indeed, 167 out of 2452 JA-sDEGs, 28 out of 232 SA-sDEGs, and 17 out of 97 ET-sDEGs were also differentially expressed in untreated colonized LRs at 1 or 2 wpc (Figure 4A and Supplemental Data Set 3). Moreover, 136 out of the 1055 red cluster genes, 178 out of the 3178 green cluster genes, 25 out of the 234 black cluster genes, and 19 out of the 207 purple cluster genes were differentially regulated in untreated colonized LRs (Figure 4C and Supplemental Data Set 3). These results suggest that (at least a branch of) JA, SA, and ET signaling, as well as their crosstalk, are activated during ectomycorrhizal development.

Within this gene set 16 JA-upregulated sDEGs and 22 genes in the red cluster with predicted roles in defense were upregulated in untreated colonized LRs at 1 wpc. Among these were genes encoding three putative Kunitz trypsin inhibitors and four putative terpene synthase-like proteins. Other ECM-responsive genes belonging to the red cluster encode predicted oxidative stress-related proteins; six of these genes were also ET-sDEGs. By contrast, 24 JA-downregulated sDEGs and 32 genes in the green cluster likely involved in cytoskeleton/cell wall remodeling and root hair elongation were downregulated in untreated

colonized LRs at 2 wpc. Among these were genes for three predicted root hair-specific pectin lyases, two putative xyloglucan endotransglucosylases/hydrolases, and one cellulose synthase-like protein D4 (Figure 4B,D, Table 2 and Supplemental Data Set 12).

In addition, we found that one putative ACC synthase gene and two putative ACC oxidase genes, possibly mediating ET biosynthesis, were upregulated 4.6- to 11.3-fold in colonized LRs versus uncolonized LRs. Also, four genes predicted to encode 2-oxoglutarate-dependent dioxygenases, which function in GA biosynthesis and inactivation, were upregulated 4.3- to 4.9-fold in colonized LRs (Supplemental Data Set 3). These results suggest that both phytohormone signaling and metabolism are altered during ECM development.

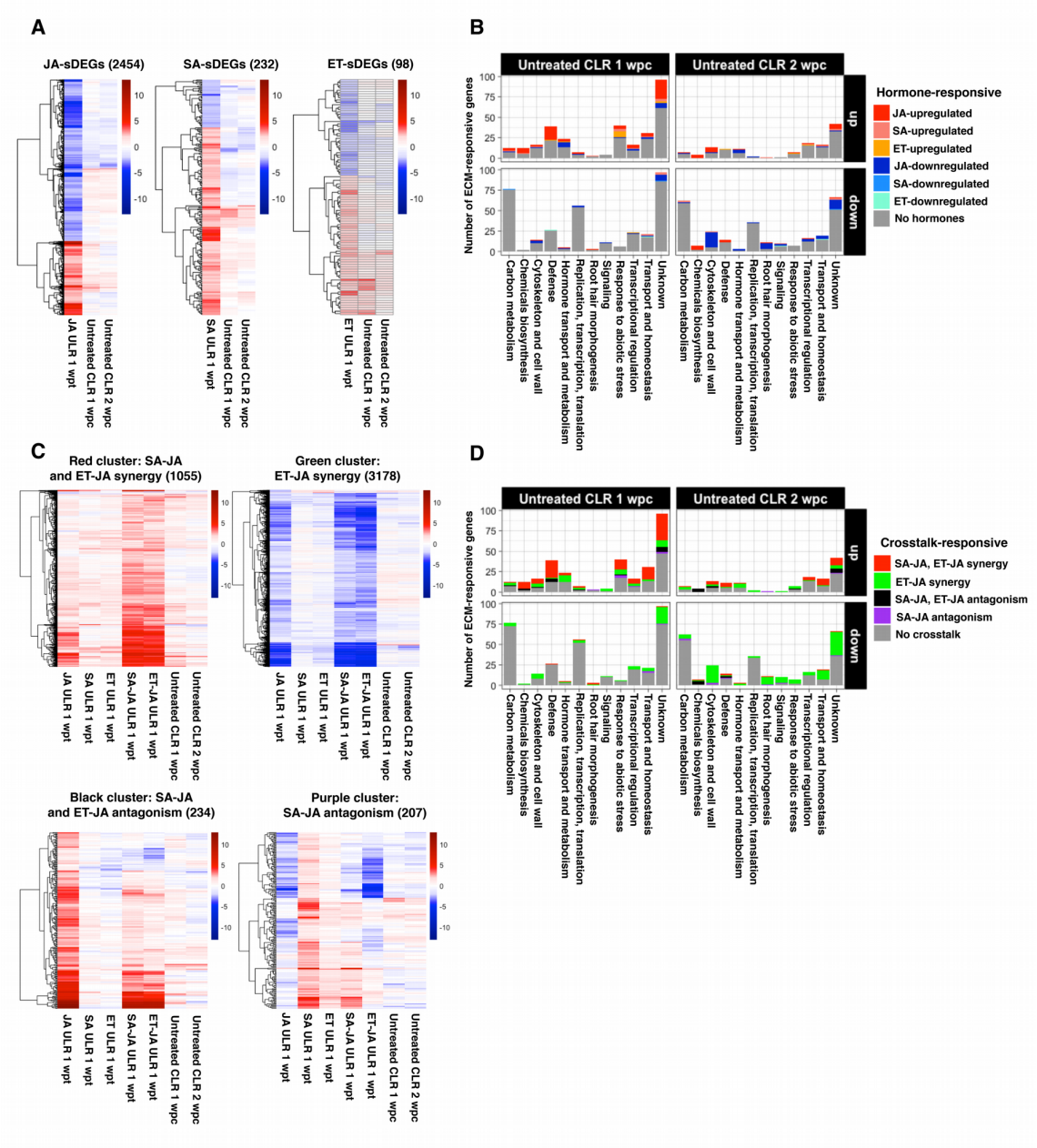


Figure 4. Ectomycorrhizal development induces the activation of subsets of phytohormone-specifically differentially expressed genes.

A, C. Heat maps of the log_2 FC in expression of genes specifically differentially expressed upon JA, SA and ET treatment (JA-, SA-, and ET-sDEGs) (A) or crosstalk-regulated genes (C) in hormone-treated uncolonized poplar lateral roots (LRs) versus untreated uncolonized LRs at 1 week post-treatment (wpt), as well as untreated colonized LRs versus untreated uncolonized LRs at 1 and 2 weeks post-contact (wpc). B, D. Bar charts representing the number of upregulated (upper panels) and downregulated (lower panels) genes in colonized LRs versus uncolonized LRs at 1 wpc (left panels) and 2 wpc (right panels) sorted by functional annotation. Colors indicate ECM-responsive genes that are also regulated by hormone treatment (B) or hormone crosstalk (D). ULR: uncolonized LRs. CLR: colonized LRs.

JA-sDEGs

Defense

Gene ID	JA 1wpt log2FC	p-value JA 1wpt	CLR vs ULR 1wpc log2FC	CLR vs ULR 1wpc p-value	CLR vs ULR 2wpc log2FC	CLR vs ULR 2wpc p-value	Arabidopsis homolog	Arabidopsis symbol	Protein name
Potri.006G188400	4	1.6E-09	3.2	6.2e-05	-0.61	0.5	AT4G19810.1		Glycosyl hydrolase family protein with chitinase insertion domain
Potri.004G000400	4.4	4.4E-12	2.2	0.0025	-0.19	0.85	AT1G73260.1	ATKTI1,KTI1	Kunitz trypsin inhibitor 1
Potri.007G111600	5.3	4.8E-17	2.7	0.00026	-0.67	0.4	AT1G73260.1	ATKTI1,KTI1	Kunitz trypsin inhibitor 1
Potri.007G111700	4.5	1.8E-12	2.2	0.0041	-0.39	0.67	AT1G73260.1	ATKTI1,KTI1	Kunitz trypsin inhibitor 1
Potri.008G213100	2.9	1.5E-05	2.9	0.00011	0.73	0.42	AT1G24020.1	MLP423	MLP-like protein 423
Potri.T111200	2.9	1.3E-05	2.4	0.0032	1.5	0.094	AT1G24020.1	MLP423	MLP-like protein 423
Potri.013G041700	2.7	6.5E-05	2.2	0.0095	-1.3	0.17	AT3G04720.1	HEL,PR-4,PR4	pathogenesis-related 4
Potri.013G041900	2.2	0.00087	2.2	0.0095	-1.8	0.062	AT3G04720.1	HEL,PR-4,PR4	pathogenesis-related 4
Potri.017G134100	3.5	1.7E-08	2.1	0.007	0.58	0.51	AT2G26560.1	PLA IIA,PLA2A,PLP2	phospholipase A 2A
Potri.006G212200	2.6	5.3E-06	2.6	0.00057	0.029	0.96	AT2G38870.1		Serine protease inhibitor, potato inhibitor I-type family protein
Potri.010G075800	4.9	1.6E-14	2.9	0.0001	0.52	0.58	AT2G38870.1		Serine protease inhibitor, potato inhibitor I-type family protein
Potri.016G079100	4.1	8.2E-09	2.1	0.0075	-0.7	0.33	AT2G38870.1		Serine protease inhibitor, potato inhibitor I-type family protein
Potri.001G308200	11	7.3E-193	2.1	0.0015	-2.4	0.0011	AT3G25830.1	ATTPS-CIN,TPS-CIN	terpene synthase-like sequence-1,8-cineole
Potri.001G308300	12	1.6E-40	2.8	0.00058	-1.1	0.16	AT3G25820.1	ATTPS-CIN,TPS-CIN	terpene synthase-like sequence-1,8-cineole
Potri.T072900	11	2.8E-249	2.5	0.00018	-2.3	0.0016	AT3G25830.1	ATTPS-CIN,TPS-CIN	terpene synthase-like sequence-1,8-cineole
Potri.T073000	11	3.2E-63	2.5	0.00082	-1.4	0.051	AT3G25830.1	ATTPS-CIN,TPS-CIN	terpene synthase-like sequence-1,8-cineole

Cytoskeleton and cell wall remodeling

Gene ID	JA 1wpt log2FC	p-value JA 1wpt	CLR vs ULR 1wpc log2FC	CLR vs ULR 1wpc p-value	CLR vs ULR 2wpc log2FC	CLR vs ULR 2wpc p-value	Arabidopsis homolog	Arabidopsis symbol	Protein name
Potri.012G141600	-3.3	9.3E-08	-0.56	0.66	-2.6	0.025	AT4G25590.1	ADF7	actin depolymerizing factor 7
Potri.016G045500	-3.6	6.3E-15	-0.97	0.12	-2.6	1.9e-05	AT3G12110.1	ACT11	actin-11
Potri.006G192700	-2.8	1.6E-15	-0.72	0.24	-2.3	0.00025	AT3G12110.1	ACT11	actin-11
Potri.010G227000	-3.4	1.1E-17	-2.3	0.00058	-2.1	0.0047	AT1G01950.1	ARK2	armadillo repeat kinesin 2
Potri.001G112866	-3.7	1E-09	-1.7	0.13	-2.6	0.021	AT1G62980.1	ATEXP18, ATEXPA18, ATHEXP ALPHA 1.25,EXP18,EXPA18	expansin A18
Potri.007G071200	-4.3	1.2E-11	-0.63	0.58	-2.8	0.0084	AT1G48930.1	AtGH9C1,GH9C1	glycosyl hydrolase 9C1
Potri.016G006900	-3	0.00011	-2.5	0.029	-3	0.022	AT4G17220.1	ATMAP70-5, MAP70-5	microtubule-associated proteins 70-5
Potri.T040400	-3.6	3.8E-09	-0.37	0.79	-2.4	0.041	AT1G11920.1		Pectin lyase-like superfamily protein
Potri.010G175100	-3.3	5E-11	-0.45	0.74	-2.3	0.047	AT1G30870.1		Peroxidase superfamily protein
Potri.010G247600	-3.3	5.2E-13	-0.25	0.87	-2.3	0.047	AT3G10710.1	RHS12	root hair specific 12
Potri.011G008100	-3.8	0.000001	-1.3	0.25	-3.5	0.0019	AT4G22080.1	RHS14	root hair specific 14
Potri.T040300	-4.1	2.4E-07	-2	0.047	-3.2	0.0025	AT4G22080.1	RHS14	root hair specific 14
Potri.011G008000	-4.1	2.6E-09	-0.39	0.77	-2.9	0.008	AT4G22080.1	RHS14	root hair specific 14

Potri.015G139000	-4.2	5E-11	-1.3	0.24	-2.9	0.0094	AT2G45750.1		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Potri.011G106200	-3.2	9.6E-05	-1.5	0.19	-2.4	0.038	AT1G29050.1	TBL38	TRICHOME BIREFRINGENCE-LIKE 38
Potri.016G079100	4.1	8.2E-09	2.1	0.0075	-0.7	0.33	AT2G38870.1		Serine protease inhibitor, potato inhibitor I-type family protein
Potri.001G308200	11	7.3E-193	2.1	0.0015	-2.4	0.0011	AT3G25830.1	ATTPS-CIN,TPS-CIN	terpene synthase-like sequence-1,8-cineole
Potri.001G308300	12	1.6E-40	2.8	0.00058	-1.1	0.16	AT3G25820.1	ATTPS-CIN,TPS-CIN	terpene synthase-like sequence-1,8-cineole
Potri.T072900	11	2.8E-249	2.5	0.00018	-2.3	0.0016	AT3G25830.1	ATTPS-CIN,TPS-CIN	terpene synthase-like sequence-1,8-cineole
Potri.T073000	11	3.2E-63	2.5	0.00082	-1.4	0.051	AT3G25830.1	ATTPS-CIN,TPS-CIN	terpene synthase-like sequence-1,8-cineole

Cytoskeleton and cell wall remodeling

Gene ID	*JA 1wpt log2FC*	*p-value JA 1wpt*	*CLR vs ULR 1wpc log2FC*	*CLR vs ULR 1wpc p-value*	*CLR vs ULR 2wpc log2FC*	*CLR vs ULR 2wpc p-value*	*Arabidopsis homolog*	*Arabidopsis symbol*	*Protein name*
Potri.012G141600	-3.3	9.3E-08	-0.56	0.66	-2.6	0.025	AT4G25590.1	ADF7	actin depolymerizing factor 7
Potri.016G045500	-3.6	6.3E-15	-0.97	0.12	-2.6	1.9e-05	AT3G12110.1	ACT11	actin-11
Potri.006G192700	-2.8	1.6E-15	-0.72	0.24	-2.3	0.00025	AT3G12110.1	ACT11	actin-11
Potri.010G227000	-3.4	1.1E-17	-2.3	0.00058	-2.1	0.0047	AT1G01950.1	ARK2	armadillo repeat kinesin 2
Potri.001G112866	-3.7	1E-09	-1.7	0.13	-2.6	0.021	AT1G62980.1	ATEXP18, ATEXPA18, ATHEXP ALPHA 1.25,EXP18,EXPA18	expansin A18
Potri.007G071200	-4.3	1.2E-11	-0.63	0.58	-2.8	0.0084	AT1G48930.1	AtGH9C1,GH9C1	glycosyl hydrolase 9C1
Potri.016G006900	-3	0.00011	-2.5	0.029	-3	0.022	AT4G17220.1	ATMAP70-5, MAP70-5	microtubule-associated proteins 70-5
Potri.T040400	-3.6	3.8E-09	-0.37	0.79	-2.4	0.041	AT1G11920.1		Pectin lyase-like superfamily protein
Potri.010G175100	-3.3	5E-11	-0.45	0.74	-2.3	0.047	AT1G30870.1		Peroxidase superfamily protein
Potri.010G247600	-3.3	5.2E-13	-0.25	0.87	-2.3	0.047	AT3G10710.1	RHS12	root hair specific 12
Potri.011G008100	-3.8	0.000001	-1.3	0.25	-3.5	0.0019	AT4G22080.1	RHS14	root hair specific 14
Potri.T040300	-4.1	2.4E-07	-2	0.047	-3.2	0.0025	AT4G22080.1	RHS14	root hair specific 14
Potri.011G008000	-4.1	2.6E-09	-0.39	0.77	-2.9	0.008	AT4G22080.1	RHS14	root hair specific 14
Potri.015G139000	-4.2	5E-11	-1.3	0.24	-2.9	0.0094	AT2G45750.1		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Potri.011G106200	-3.2	9.6E-05	-1.5	0.19	-2.4	0.038	AT1G29050.1	TBL38	TRICHOME BIREFRINGENCE-LIKE 38
Potri.018G084300	-3	0.00000002	-0.93	0.47	-3	0.0062	AT4G28850.1	ATXTH26,XTH26	xyloglucan endotransglucosylase/hydrolase 26
Potri.006G160700	-3.5	6.3E-08	-1.3	0.26	-2.6	0.035	AT4G28850.1	ATXTH26,XTH26	xyloglucan endotransglucosylase/hydrolase 26

Signaling and root hair development

Gene ID	*JA 1wpt log2FC*	*p-value JA 1wpt*	*CLR vs ULR 1wpc log2FC*	*CLR vs ULR 1wpc p-value*	*CLR vs ULR 2wpc log2FC*	*CLR vs ULR 2wpc p-value*	*Arabidopsis homolog*	*Arabidopsis symbol*	*Protein name*
Potri.013G057500	-2.8	1.6E-08	-1.3	0.079	-2.5	0.0011	AT5G56540.1	AGP14,ATAGP14	arabinogalactan protein 14
Potri.002G072200	-2.2	0.0019	0.04	0.95	-2.9	0.0054	AT1G77110.1	PIN6	Auxin efflux carrier family protein
Potri.006G081200	-4.3	1E-14	-1.9	0.067	-2.5	0.018	AT1G62440.1	LRX2	leucine-rich repeat/extensin 2
Potri.015G061700	-4.8	6.8E-10	-1.5	0.19	-2.8	0.011	AT5G61350.1		Protein kinase superfamily protein
Potri.016G059600	-3.3	1.4E-05	-1.1	0.24	-2.9	0.0042	AT2G41970.1		Protein kinase superfamily protein
Potri.002G201800	-3.5	3E-12	-0.49	0.71	-2.4	0.04	AT4G02270.1	RHS13	root hair specific 13
Potri.007G091200	-4	1.3E-07	-1.8	0.044	-3.1	0.0022	AT5G65160.1		tetratricopeptide repeat (TPR)-containing protein

SA-sDEGs

Gene ID	*JA 1wpt log2FC*	*p-value JA 1wpt*	*CLR vs ULR 1wpc log2FC*	*CLR vs ULR 1wpc p-value*	*CLR vs ULR 2wpc log2FC*	*CLR vs ULR 2wpc p-value*	*Arabidopsis homolog*	*Arabidopsis symbol*	*Protein name*
Potri.013G125100	3.1	0.00044	3.7	3.6e-07	2.3	0.013	AT3G54420.1	ATCHITIV,ATEP3, CHIV,EP3	homolog of carrot EP3-3 chitinase
Potri.017G005400	2.6	1.2E-10	2.8	2.2e-09	1.8	0.0016	AT5G37980.1		Zinc-binding dehydrogenase family protein
Potri.017G006000	2.8	4.6E-14	2.8	5.6e-10	1.6	0.004	AT5G37980.1		Zinc-binding dehydrogenase family protein
Potri.017G005700	2.9	8.9E-17	2.8	6.4e-11	1.5	0.0051	AT1G26320.1		Zinc-binding dehydrogenase family protein

Potri.007G036501	2.8	1.00E-05	2.3	0.0025	1.4	0.099	AT4G39230.1		NmrA-like negative transcriptional regulator family protein
Potri.018G094900	2.2	0.011	2.1	0.014	1.6	0.1	AT4G25810.1	XTH23,XTR6	xyloglucan
Potri.005G138800	2.1	0.0029	0.2	0.84	-2.4	0.02	AT5G67050.1		alpha/beta-Hydrolases superfamily protein
Potri.001G012700	3.1	0.000014	0.89	0.36	-2.3	0.015	AT3G12120.2	FAD2	fatty acid desaturase 2
Potri.006G256600	2.3	0.0012	-0.95	0.33	-2.3	0.02	AT3G14310.1	ATPME3,PME3	pectin methylesterase 3

ET-sDEGs

Gene ID	*JA 1wpt log2FC*	*p-value JA 1wpt*	*CLR vs ULR 1wpc log2FC*	*CLR vs ULR 1wpc p-value*	*CLR vs ULR 2wpc log2FC*	*CLR vs ULR 2wpc p-value*	*Arabidopsis homolog*	*Arabidopsis symbol*	*Protein name*
Potri.016G039050	2.2	1.2E-07	3.3	4.3e-10	2.1	0.00049	AT2G41380.1		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Potri.T163900	2.1	0.000004	3.3	1.1e-11	1.9	0.00069	AT2G41380.1		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Potri.012G006800	3.7	6.9E-11	4.1	1.6e-13	1.2	0.1	AT1G05260.1	RCI3,RCI3A	Peroxidase superfamily protein
Potri.011G113000	2.3	0.00015	2.8	7.2e-05	1.8	0.02	AT1G78380.1	ATGSTU19,GST8, GSTU19	glutathione S-transferase TAU 19
Potri.011G162800	2.1	3.9E-06	2.3	3.2e-07	0.91	0.11	AT5G44440.1		FAD-binding Berberine family protein
Potri.009G109300	2.6	0.00015	3.3	2.1e-06	1	0.22	AT2G15960.1		
Potri.012G110300	2.1	0.00028	2.2	9.5e-05	0.81	0.21	AT5G61820.1		

Table 2. Notable overlaps between poplar specifically differentially expressed genes after phytohormone treatment and ectomycorrhiza-responsive genes suggest that JA and ET signaling function in ectomycorrhizal development.

Gene expression levels and annotation of ectomycorrhiza (ECM)-responsive genes that are also regulated upon hormonal treatment. Log_2 fold change (FC) and Bonferroni adjusted p-value of gene expression are reported for hormone-treated uncolonized poplar lateral roots (ULR) versus untreated ULR at 1 week post-treatment (wpt), as well as untreated colonized lateral roots (CLR) versus untreated ULR at 1 and 2 weeks post-contact (wpc). When available, the name of the closest Arabidopsis homologous gene, its symbol and its protein name are also reported.

2.5. Discussion

The multifaceted roles of JA signaling in poplar LRs during symbiosis development

Transcriptomic network analysis highlighted a generalized suppression of JA-triggered gene expression in colonized poplar LRs compared to uncolonized LRs (Figure 3C-D and Supplemental Data Set 7), impairing the activation of genes likely involved in defense and cell wall remodeling (Supplemental Data Set 9). Therefore it is likely that *L. bicolor* impairs the activation of JA-triggered responses by altering poplar sensitivity to JA, rather than affecting JA accumulation. However, some JA-sDEGs were regulated in untreated ECM, suggesting that one or more branches of JA signaling play a functional role in ECM development. Indeed, 17 to 21% of ECM-responsive genes were also JA-sDEGs (Figure 4A-B and Table 2). This finding suggests that a subset of JA-regulated genes is co-opted for ectomycorrhizal development. Many ECM-responsive genes regulated by JA treatment, or by the synergistic effects of SA-JA and ET-JA signaling, are likely involved in defense

responses against pathogens and oxidative stress resistance. Among these are several genes coding for putative protease inhibitors. The synthesis of such enzymes upon cell wall damage often determines the outcome of plant-pathogen interactions. Plant protease inhibitors can possess antimicrobial activity (Kim et al., 2009), while pathogen-derived protease inhibitors can suppress plant defenses and promote infection (Jashni et al., 2015). The role of plant protease inhibitors in the development of mutualistic interactions remains to be investigated. In addition, four TPS-b monoterpene synthase genes (Irmsch et al., 2014) were regulated in ECM. Two of these genes, *PtTPS21* (Potri.001G308300) and *PtTPS16* (Potri.001G308200), are expressed during root herbivory in *P. trichocarpa*. Their respective enzymes synthesize a wide range of monoterpenes, including camphene, α-pinene, β-pinene, limonene, and γ-terpinene (Lackus et al., 2018). Plants can emit monoterpenes to deter chewing insects and pathogenic fungi (Stamopoulos et al., 2007; López et al., 2008; Marei et al., 2012; Tak et al., 2016; Chiu et al., 2017; Quintana-Rodriguez et al., 2018) or to communicate with other plants, insects, and microbes (Seybold et al., 2006; Junker and Tholl, 2013; Schmidt et al., 2016). Therefore, we cannot exclude the possibility that monoterpenes serve as chemical cues rather than defense compounds in poplar roots. In addition, mid-stage ECM might co-opt JA/ET signaling to dampen excessive cell wall loosening through the repression of genes encoding putative pectin lyases, pectinesterases, xyloglucan endotransglucosylases, expansins, and glycosyl hydrolases (Table 2 and Supplemental Data Set 12). This may confer optimal rigidity to the cell wall in order to restrict excessive fungal apoplastic penetration. JA signaling might also be co-opted for the inhibition of root hair growth in ECM, via the downregulation of 10 putative root-hair-specific genes involved in cell wall loosening (Won et al., 2009), and two genes encoding homologs of the Arabidopsis bHLH transcription factor ROOT HAIR DEFECTIVE SIX-LIKE2 (RSL2) (Table 2; Yi et al., 2010; Vijayakumar et al., 2016).

The suppression of responses to JA may be required to deter plant immunity during ectomycorrhizal colonization. Indeed, JA treatment of colonized LRs enhanced the expression of genes predicted to function in plant defense, as well as four genes putatively encoding WBC11, an ATP-binding cassette protein belonging to a family of transporters required for cutin and suberin secretion (Panikashwili et al., 2007; Yadav et al., 2014; Supplemental Data Set 9). Suberins are biopolyesters that act as barriers between the plant cell and the environment, limiting water and gas exchange as well as pathogen invasion (Franke and Schreiber, 2007). In addition, the phenotype of JA-treated poplar cuttings, with reduced root systems and enhanced anthocyanin accumulation, suggests that systemic defense responses were activated in these plants, limiting fungal colonization (Figure 2). Alternatively, modified root architecture or physiology upon JA treatment may have affected colonization by *L. bicolor*. The manipulation of poplar JA signaling by *L. bicolor* is essential

for Hartig net formation (Plett et al., 2014a,b). Indeed, *L. bicolor* secretes the effector MiSSP7 to stabilize PtJAZ6, a corepressor of JA signaling in poplar. Consistently, our data confirm that Hartig net frequency is reduced in JA-treated colonized poplar plants (Figure 2A).

In conclusion, colonized poplar LRs displayed reduced sensitivity to JA. However, a subset of JA-sDEGs were expressed in untreated colonized LRs, suggesting that JA signaling, in synergy with SA and ET signaling, is co-opted for transient defense and stress responses in early ECM as well as the inhibition of cell wall loosening in mid-stage ECM. Therefore, consistent with the role of JA signaling in other beneficial plant-microbe interactions (Basso and Veneault-Fourrey, 2020), we propose that moderate JA signaling contributes to ectomycorrhizal development, whereas strong JA signaling, which triggers excessive plant immunity and cell wall stiffening, is detrimental for this symbiosis.

ET signaling promotes LR initiation but affects fungal colonization

Exogenous ET treatment of colonized poplar LRs impaired the fungal colonization rate (Figure 2A) and upregulated eight genes putatively encoding phospholipase A 2As; these Ca^{2+}-dependent lipid binding proteins are involved in PTI (La Camera et al., 2005; 2009; Supplemental Data Set 9). ET treatment also reduced the expression of genes involved in replication, transcription, and RNA processing in colonizing *L. bicolor* hyphae (Supplemental Data Set 8) and transiently decreased the fresh weight of *L. bicolor* colonies (Supplemental Figure 4). Together, these results suggest that the induction of PTI in ET-treated colonized poplar LRs delays the completion of the fungal cell cycle and affects *L. bicolor* colonization. Alternatively, reduced LR length of ET-treated plants may have counteracted the contact of fungal hyphae with the root surface. However, ET treatment did not affect Hartig net depth or frequency (Figure 2A), in contrast to a previous report (Plett et al., 2014a). Such discrepancy may be explained by the different pH of the culture medium, which in our experimental setup was stabilized at 5.8. Indeed, exogenous ACC inhibits H^+-ATPase activity in Arabidopsis and rice, resulting in alkalinization of the culture medium and inhibited longitudinal root cell elongation (Staal et al., 2011; Chen et al., 2017). Therefore, ACC treatment may affect Hartig net development via pH-dependent inhibition of cortical root cell elongation. Alternatively, pH might affect the growth of fungal hyphae. Indeed, the optimal pH range for growth of ectomycorrhizal fungi is pH 5 to 7 (Yamanaka, 2003; Sundari and Adholeya, 2003), and soil alkalinization reduces the frequency of ectomycorrhizal colonization (Marx, 1990; Aggangan et al., 1996).

In contrast to exogenous ET, endogenous ET signaling might promote ECM establishment. ET signaling functions in the morphogenetic responses of *Cistus incanus* and Arabidopsis roots to indirect contact with *Tuber* spp. mycelium (black truffle). Indeed,

although free-living truffle mycelia do not produce ET above the detection limit, Arabidopsis ET-insensitive mutants fail to exhibit enhanced LR density in response to fungal volatiles (Splivallo et al., 2009). In the current study, we detected the enhanced expression of three predicted ET-biosynthetic genes in early colonized LRs, pointing to colonization-promoted ET biosynthesis in poplar LRs (Supplemental Data Set 3). In addition, ET-treated uncolonized poplar cuttings displayed very dense, short LRs, a phenotype reminiscent of mycorrhizal root systems (Supplemental Data Set 6). Our results, together with those of Splivallo et al. (2009), suggest that ectomycorrhizal fungi stimulate ET biosynthesis in host roots to regulate LR patterning.

In conclusion, we propose that moderate ET signaling promotes the establishment of ECM symbiosis by enhancing the density of LR primordia. However, intense ET signaling might affect the fungal colonization rate by activating PTI and affecting fungal replication, transcription, and translation. In addition, excessive ET signaling might affect Hartig net development through a yet-to-be-elucidated pH-dependent mechanism.

SA accumulates in ECM but does not promote symbiosis

Through LC-MS/MS, we showed that SA levels were higher in ECM compared to uncolonized poplar LRs and that FLM could synthesize this hormone. Thus, the SA detected in ECM may have been derived from the plant, the fungus, or both. However, such accumulation was not mirrored by the activation of many SA-sDEGs in colonized poplar LRs (Figure 4A-B); also, exogenous SA did not promote ectomycorrhizal development (Figure 2A). We propose two main hypotheses to explain this phenomenon: (i) fungal signals influence plant responses to SA or (ii) SA accumulation in ECM occurs in fungal hyphae and SA is not perceived by poplar cells. SA treatment of colonized LRs triggers the regulation of genes putatively involved in systemic defense (Supplemental Data Set 9; Zhang et al., 2010; Wang et al., 2018; Guerra et al., 2019), suggesting that *L. bicolor* cannot suppress poplar responses to exogenous SA. However, we cannot exclude the possibility that fungal MiSSPs target endogenous SA signaling in poplar, similar to JA signaling (Plett et al., 2014b). Our second hypothesis is that SA accumulation in ECM is localized to fungal hyphae. Since *L. bicolor* FLM metabolizes the β-glucoside salicin to SA (Tchaplinski et al., 2014), the increase in SA concentration in ECM might be due to an enhanced metabolic rate of hyphal feeding on plant-derived glycosides. In our experiment, *L. bicolor* FLM and root-colonizing hyphae responded to exogenous SA via the activation of carbohydrate and lipid transport and metabolism (Supplemental Data Set 8), suggesting that SA triggers energy production or storage in *L. bicolor*. In conclusion, the increased SA concentration in ECM may be derived from fungal metabolism and may not be functional for the establishment of ECM symbiosis.

Altered GA metabolism in ECM may regulate hyphal adhesion to host roots

Our assessment of phytohormone concentration and the expression of genes related to hormone metabolism showed that GA biosynthesis and inactivation were enhanced in ECM. On the other hand, GA treatment did not affect Hartig net frequency, but it had a negative impact on the fungal colonization rate (Figure 2A). Since the LR density of GA-treated plants did not differ from that of untreated plants (Supplemental Data Set 6), the reduced colonization rate may depend on GA-driven inhibition of hyphal growth or adhesion to host roots. Interestingly, colonizing *L. bicolor* hyphae responded to GA treatment via the downregulation of genes encoding eight glycoproteins homologous to an animal blood coagulation factor (Supplemental Data Set 8). Cytological observations of early mycorrhizal development have shown that the fungal secretion of oriented fibrillar materials containing polysaccharides and glycoproteins is important for adhesion to the host root (Lei et al., 1991; Tagu and Martin, 1996). Therefore, we propose that GA treatment affects glycoprotein biosynthesis in *L. bicolor*, thereby reducing hyphal adhesion to poplar roots.

Uncolonized poplar LRs were insensitive to GA treatment (Supplemental Data Set 7), possibly due to the low dosage (Busov et al., 2006). We predict that higher doses of exogenous GA would affect poplar root architecture and Hartig net development. Indeed, GA-deficient and -insensitive poplar mutants display LR proliferation and elongation (Gou et al., 2010), while GA-overproducing transgenic poplar lines exhibit enhanced xylose and glucose deposition in their cell walls (Park et al., 2015). In conclusion, GA signaling might affect fungal adhesion to the host root, but the other roles of GA signaling in ECM formation remain to be investigated.

2.6. Conclusion

Here we investigated the role of the plant hormones JA, GA, SA, and ET in the development of ECM between roots of *P. tremula x alba* and hyphae of *L. bicolor*. We demonstrated that fungal colonization alters the endogenous hormonal levels and the sensitivity to exogenous phytohormones of poplar LRs. In particular, diminished sensitivity to JA may be required for Hartig net formation, although a branch of JA/ET signaling is activated during ectomycorrhizal development. Altogether, this work illustrates that accumulation, perception, and responses to phytohormones implied in plant defense and development must be tightly regulated to ensure ectomycorrhizal development.

2.7. Acknowledgements

The authors acknowledge Maíra de Freitas Pereira and Amit Verma for their help with the analysis of RNA-seq data. This work was supported by the Genomic Science Program, US Department of Energy, Office of Science, Biological and Environmental Research as part of the Plant-Microbe Interfaces Scientific Focus Area (http://pmi.ornl.gov), the Laboratory of Excellence ARBRE (grant no. ANR-11-LABX-0002_ARBRE), Institut National de la Recherche Agronomique, University of Lorraine, Région Lorraine Research Council and the European Fund for Regional Development. The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. The work conducted in Czech Republic is supported by the Ministry of Education, Youth and Sports of the Czech Republic (European Regional Development Fund-Project 'Plants as a tool for sustainable global development' no. CZ.02.1.01/0.0/0.0/16_019/0000827) and the Czech Science Foundation (project no. 17-06613S).

2.8. Author contributions

V.B. performed the phenotyping of poplar cuttings, interpreted the RNA-seq data, and wrote the manuscript. A.K., S.M. and V.S. analyzed the RNA-seq data. K.W.B. and I.G. supervised the RNA sequencing. H.N and M.A. prepared and sequenced RNA libraries. F.G., Y.D. and J.B. performed the *in vitro* experiments and the phenotyping of ectomycorrhizae. F.G. and C.V-F. performed total RNA extractions. O.N. and J.S. performed LC/MS-MS and analyzed the output. C.V-F. and F.M. designed research and significantly contributed to the writing of the manuscript. A.K. agrees to serve as responsible distribution of materials integral to the findings presented in this article. C.V.F. agrees to serve as the author responsible for contact and ensures communication.

2.9. References

Aggangan, N.S., Dell, B., and Malajczuk, N. (1996). Effects of soil pH on the ectomycorrhizal response of *Eucalyptus urophylla* seedlings. New Phytol. 134: 539–546.

Antico, C.J., Colon, C., Banks, T., and Ramonell, K.M. (2012). Insights into the role of jasmonic acid-mediated defenses against necrotrophic and biotrophic fungal pathogens. Front. Biol. 7: 48–56.

Basso, V. and Veneault-Fourrey, C. (2020). The role of jasmonates in beneficial microbe-root interactions. In Jasmonate in Plant Biology, in press. Jasmonate in Plant Biology: Methods and Protocols, Methods in Molecular Biology; 2085:43-67. Antony Champion and Laurent Laplaze

(eds). Springer. doi: 10.1007/978-1-0716-0142-6_4.

Baumgart, M. et al. (2016). Longitudinal RNA-seq analysis of vertebrate aging identifies mitochondrial complex I as a small-molecule-sensitive modifier of lifespan. Cell Syst. 2: 122–132.

Blasius, D., Feil, W., Kottke, I., and Oberwinkler, F. (1986). Hartig net structure and formation in fully ensheathed ectomycorrhizas. Nord. J. Bot. 6(6): 837–842.

Busov, V., Meilan, R., Pearce, D.W., Rood, S.B., Ma, C., Tschaplinski, T.J., and Strauss, S.H. (2006). Transgenic modification of *gai* or *rgl1* causes dwarfing and alters gibberellins, root growth, and metabolite profiles in *Populus*. Planta 224: 288–299.

La Camera, S., Balagué, C., Göbel, C., Geoffroy, P., Legrand, M., Feussner, I., Roby, D., and Heitz, T. (2009). The Arabidopsis Patatin-Like Protein 2 (PLP2) plays an essential role in cell death execution and differentially affects biosynthesis of oxylipins and resistance to pathogens. Mol. Plant Microbe Interact. 22: 469–481.

La Camera, S., Geoffroy, P., Samaha, H., Ndiaye, A., Rahim, G., Legrand, M., and Heitz, T. (2005). A pathogen-inducible patatin-like lipid acyl hydrolase facilitates fungal and bacterial host colonization in Arabidopsis. Plant J. 44: 810–825.

Carlson, M. (2016). KEGG.db: A set of annotation maps for KEGG. R package version 3.2.3

Carlson, M., Liu, T., Lin, C., Falcon, S., Zhang, J. and MacDonald, J.W. (2018). PFAM.db: A set of protein ID mappings for PFAM. R package version 3.6.0.

Chen, H., Zhang, Q., Cai, H., and Xu, F. (2017). Ethylene mediates alkaline-induced rice growth inhibition by negatively regulating plasma membrane H^+-ATPase activity in roots. Front. Plant Sci. 8: 1839.

Chiu, C.C., Keeling, C.I., and Bohlmann, J. (2017). Toxicity of pine monoterpenes to mountain pine beetle. Sci. Rep. 7: 8858.

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., and Wardle, D.A. (2015). Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytol. 205: 1525–1536.

Cope, K.R. et al. (2019). The Ectomycorrhizal Fungus *Laccaria bicolor* Produces Lipochitooligosaccharides and Uses the Common Symbiosis Pathway to Colonize *Populus* Roots. Plant Cell 31(10): 2387-2410.

Deveau, A., Palin, B., Delaruelle, C., Peter, M., Kohler, A., Pierrat, J.C., Sarniguet, A., Garbaye, J., Martin, F., and Frey-Klett, P. (2007). The mycorrhiza helper *Pseudomonas fluorescens* BBc6R8 has a specific priming effect on the growth, morphology and gene expression of the ectomycorrhizal fungus *Laccaria bicolor* S238N. New Phytol. 175: 743–755.

Ditengou, F.A., Béguiristain, T., and Lapeyrie, F. (2000). Root hair elongation is inhibited by hypaphorine, the indole alkaloid from the ectomycorrhizal fungus *Pisolithus tinctorius*, and restored by indole-3-acetic acid. Planta 211: 722–728.

Ditengou, F.A., Müller, A., Rosenkranz, M., Felten, J., Lasok, H., Van Doorn, M.M., Legué, V., Palme, K., Schnitzler, J.-P., and Polle, A. (2015). Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. Nat. Commun. 6: 6279.

Drag, M., Skinkyté-Juskiené, R., Do, D.N., Kogelman, L.N., and Kadarmideen, H.N. (2017). Differential

expression and co-expression gene networks reveal candidate biomarkers of boar taint in non-castrated pigs. Sci. Rep. 7: 12205.

Felten, J., Kohler, A., Morin, E., Bhalerao, R.P., Palme, K., Martin, F., and Ditengou, F.A. (2009). The ectomycorrhizal fungus *Laccaria bicolor* stimulates lateral root formation in poplar and Arabidopsis through auxin transport and signaling. Plant Physiol. 151: 1991–2005.

Finn, R.D. et al. (2015). The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res. 44: 279–285.

Fischer, R.R., Giddingst, J.C., and Roisenberg, I. (1988). Hereditary combined deficiency of clotting factors V and VIII with involvement of von Willebrand factor. Int. J. Lab. Hematol. 10: 53–62.

Foo, E., Ross, J.J., Jones, W.T., and Reid, J.B. (2013). Plant hormones in arbuscular mycorrhizal symbioses: an emerging role for gibberellins. Ann. Bot. 111: 769–779.

Franke, R. and Schreiber, L. (2007). Suberin- a biopolyester forming apoplastic plant interfaces. Curr. Opin. Plant Biol. 10: 252–259.

Garcia, K., Delaux, P.-M., Cope, K.R., and Ané, J.-M. (2015). Molecular signals required for the establishment and maintenance of ectomycorrhizal symbioses. New Phytol. 208: 79–87.

Gea, L., Normand, L., Vian, B., and Gay, G. (1994). Structural aspects of ectomycorrhiza of *Pinus pinaster* (Ait.) Sol. formed by an IAA-overproducer mutant of *Hebeloma cylindrosporum* Romagnési. New Phytol. 128: 659–670.

The Gene Ontology Consortium (2014). Gene Ontology Consortium: going forward. Nucleic Acids Res. 43: D1049–D1056.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and nectrotrophic pathogens. Annu. Rev. Phytopathol. 43: 205–227.

Gogala, N. (1970). Einfluß der natürlichen Cytokinine von *Pinus sylvestris* L. und anderer Wuchsstoffe auf das Myzelwachstum von *Boletus edulis* var. pinicolus Vitt. Österreichische Bot. Zeitschrift 118: 321–333.

Gou, J., Strauss, S.H., Tsai, C.J., Fang, K., Chen, Y., Jiang, X., and Busov, V.B. (2010). Gibberellins regulate lateral root formation in *Populus* through interactions with auxin and other hormones. Plant Cell 22: 623–639.

Guerra, T., Schilling, S., Sylvester, F.-P., Conrads, B., and Romeis, T. (2019). Calcium-dependent protein kinase 5 links calcium-signaling with SARD1-dependent immune memory in systemic acquired resistance. bioRxiv doi: https://doi.org/10.1101/553750.

Guo, Q., Major, I.T., and Howe, G.A. (2018). Resolution of growth-defense conflict: mechanistic insights from jasmonate signaling. Curr. Opin. Plant Biol. 44: 72–81.

Hall, D.O. and Rao, K. (1999). Photosythesis, 6th edition. (Cambridge University Press: Cambridge, UK).

van der Heijden, M.G.A., Martin, F.M., Selosse, M.A., and Sanders, I.R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol. 205: 1406–1423.

Horan, D.P., Chilvers, G.A., and Lapeyrie, F.F. (1988). Time sequence of the infection process in eucalypt ectomycorrhizas. New Phytol. 109: 451–458.

Hou, X., Lee, L.Y.C., Xia, K., Yan, Y., and Yu, H. (2010). DELLAs modulate jasmonate signaling via

competitive binding to JAZs. Dev. Cell 19: 884–894.

Howe, G.A. and Jander, G. (2008). Plant immunity to insect herbivores. Annu. Rev. Plant Biol. 59: 41–66.

Irmisch, S., Jiang, Y., Chen, F., Gershenzon, J., and Köllner, T.G. (2014). Terpene synthases and their contribution to herbivore-induced volatile emission in western balsam poplar (*Populus trichocarpa*). BMC Plant Biol. 14: 270.

Jashni, M.K., Mehrabi, R., Collemare, J., Mesarich, C.H., and de Wit, P.J.G.M. (2015). The battle in the apoplast: further insights into the roles of proteases and their inhibitors in plant–pathogen interactions. Front. Plant Sci. 6: 1–7.

Junker, R.R. and Tholl, D. (2013). Volatile organic compound mediated interactions at the plant-microbe interface. J. Chem. Ecol. 39: 810–825.

Kim, J.Y., Park, S.C., Hwang, I., Cheong, H., Nah, J.W., Hahm, K.S., and Park, Y. (2009). Protease inhibitors from plants with antimicrobial activity. Int. J. Mol. Sci. 10: 2860–2872.

Kohler, A. et al. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat. Genet. 47: 410–415.

Kottke, I. and Oberwinkler, F. (1987). The cellular structure of the Hartig net: coenocytic and transfer cell-like organization. Nord. J. Bot. 7: 85–95.

Lackus, N.D., Lackner, S., Gershenzon, J., Unsicker, S.B., and Köllner, T.G. (2018). The occurrence and formation of monoterpenes in herbivore-damaged poplar roots. Sci. Rep. 8: 17936.

Langfelder, P. and Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9: 559.

Laurent, P., Voiblet, C., Tagu, D., de Carvalho, D., Nehls, U., De Bellis, R., Balestrini, R., Bauw, G., Bonfante, P., and Martin, F. (1999). A novel class of ectomycorrhiza-regulated cell wall polypeptides in *Pisolithus tinctorius*. Mol. Plant Microbe Interact. 12: 862–871.

Lei, J., Wong, K.K.Y., and Piché, Y. (1991). Extracellular Concanavalin A-binding sites during early interactions between *Pinus banksiana* and two closely related genotypes of the ectomycorrhizal basidiomycete *Laccaria bicolor*. Mycol. Res. 95: 357–363.

Lev-Yadun, S. and Gould, K.S. (2009). Role of anthocyanins in plant defence. In Anthocyanins: Biosynthesis, Functions, and Applications, C. Winefield, K. Davies, and K. Gould, eds (Springer New York: New York, NY), pp. 22–28.

Levasseur, A., Drula, E., Lombard, V., Coutinho, P.M., and Henrissat, B. (2013). Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. Biotechnol. Biofuels 6: 41.

Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., and Henrissat, B. (2014). The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 42: 490–495.

López, M.D., Jordán, M.J., and Pascual-Villalobos, M.J. (2008). Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests. J. Stored Prod. Res. 44: 273–278.

Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biol. 15:550.

Luo, Z., Janz, D., Jiang, X., Göbel, C., Wildhagen, H., Tan, Y., Rennenberg, H., Feussner, I., and

Polle, A. (2009). Upgrading root physiology for stress tolerance by ectomycorrhizas: insights from metabolite and transcriptional profiling into reprogramming for stress anticipation. Plant Physiol. 151: 1902–1917.

Marei, G.I.K., Abdel Rasoul, M.A., and Abdelgaleil, S.A.M. (2012). Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. Pestic. Biochem. Physiol. 103: 56–61.

Martin, F. et al. (2010). Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464: 1033–1038.

Martin, F. et al. (2008). The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature 452: 88–92.

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., and Hibbett, D.S. (2016). Unearthing the roots of ectomycorrhizal symbioses. Nat. Rev. Microbiol. 14: 760–773.

Marx, D.H. (1990). Soil pH and nitrogen influence *Pisolithus* ectomycorrhizal development and growth of loblolly pine seedlings. For. Sci. 36: 224–245.

Massicotte, H.B., Peterson, R.L., Ackerley, C.A., and Ashford, A.E. (1987). Ontogeny of *Eucalyptus pilularis-Pisolithus tinctorius* ectomycorrhizae. II. Transmission electron microscopy. Can. J. Bot. 65: 1940–1947.

Mello, A. and Balestrini, R. (2018). Recent insights on biological and ecological aspects of ectomycorrhizal fungi and their interactions. Front. Microbiol. 9: 216.

Miyauchi, S. et al. (2018). Integrative visual omics of the white-rot fungus *Polyporus brumalis* exposes the biotechnological potential of its oxidative enzymes for delignifying raw plant biomass. Biotechnol. Biofuels 11: 201.

Miyauchi, S., Navarro, D., Grigoriev, I. V., Lipzen, A., Riley, R., Chevret, D., Grisel, S., Berrin, J.G., Henrissat, B., and Rosso, M.N. (2016). Visual comparative omics of fungi for plant biomass deconstruction. Front. Microbiol. 7: 1335.

Miyauchi, S., Navarro, D., Grisel, S., Chevret, D., Berrin, J.G., and Rosso, M.N. (2017). The integrative omics of white-rot fungus *Pycnoporus coccineus* reveals co-regulated CAZymes for orchestrated lignocellulose breakdown. PLoS One 12: e0175528.

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., and Kanehisa, M. (1999). KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 27: 29–34.

Oldroyd, G.E.D. (2013). Speak, friend, and enter: Signalling systems that promote beneficial symbiotic associations in plants. Nat. Rev. Microbiol. 11: 252–263.

Panikashvili, D., Savaldi-Goldstein, S., Mandel, T., Yifhar, T., Franke, R.B., Schreiber, L., Chory, J., and Aharoni, A. (2007). The Arabidopsis DESPERADO/AtWBC11 transporter is required for cutin and wax secretion. Plant Physiol. 145: 1345–1360.

Park, E.-J., Kim, H.-T., Choi, Y.-I., Lee, C., Nguyen, V.P., Jeon, H.-W., Cho, J.-S., Funada, R., Pharis, R.P., Kurepin, L. V, and Ko, J.-H. (2015). Overexpression of gibberellin 20-oxidase1 from *Pinus densiflora* results in enhanced wood formation with gelatinous fiber development in a transgenic hybrid poplar. Tree Physiol. 35: 1264–1277.

Perez-Moreno, J., & Read, D.J. (2000). Mobilization and transfer of nutrients from litter to tree

seedlings via the vegetative mycelium of ectomycorrhizal plants. New Phytol. 145: 301–309.

Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S., and Van Wees, S.C.M. (2009). Networking by small-molecule hormones in plant immunity. Nat. Chem. Biol. 5: 308–316.

Pieterse, M.J., Van Der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C.M. (2012). Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. 28: 489–521.

Plett, J.M., Daguerre, Y., Wittulsky, S., Vayssieres, A., Deveau, A., Melton, S.J., Kohler, A., Morrell-Falvey, J.L., Brun, A., Veneault-Fourrey, C., and Martin, F. (2014b). Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. Proc. Natl. Acad. Sci. 111: 8299–8304.

Plett, J.M., Kemppainen, M., Kale, S.D., Kohler, A., Legué, V., Brun, A., Tyler, B.M., Pardo, A.G., and Martin, F. (2011). A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. Curr. Biol. 21: 1197–1203.

Plett, J.M., Khachane, A., Ouassou, M., Kohler, A., and Martin, F. (2014a). Ethylene and jasmonic acid act as negative modulators during mutualistic symbiosis between *Laccaria bicolor* and *Populus* roots. New Phytol. 202: 270–286.

Plett, J.M., Tisserant, E., Brun, A., Morin, E., Grigoriev, I. V, Kuo, A., Martin, F., and Kohler, A. (2015). The mutualist *Laccaria bicolor* expresses a core gene regulon during the colonization of diverse host plants and a variable regulon to counteract host-specific defenses. Mol. Plant Microbe Interact. 28: 261–273.

Plomion, C. et al. (2018). Oak genome reveals facets of long lifespan. Nat. Plants 4: 440–452.

Quintana-Rodriguez, E., Rivera-Macias, L.E., Adame-Alvarez, R.M., Molina Torres, J., and Heil, M. (2018). Shared weapons in fungus-fungus and fungus-plant interactions? Volatile organic compounds of plant or fungal origin exert direct antifungal activity in vitro. Fungal Ecol. 33: 115–121.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rawlings, N., Barrett, A., Thomas, P., Huang, X., Bateman, A. and Finn, R. (2018). The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. Nucleic Acids Res. 46:D624-D632.

Reboutier, D., Bianchi, M., Brault, M., Roux, C., Dauphin, A., Legué, V., Lapeyrie, F., and Bouteau, F. (2002). The indolic compound hypaphorine produced by ectomycorrhizal fungus interferes with auxin action and evokes early responses in nonhost *Arabidopsis thaliana*. Mol. Plant Microbe Interact. 15: 932–938.

Rincón, A.N.A., Priha, O., Sotta, B., Bonnet, M., and Le, F. (2003). Comparative effects of auxin transport inhibitors on rhizogenesis and mycorrhizal establishment of spruce seedlings inoculated with *Laccaria bicolor*. Tree Physiol. 23: 785–791.

Santoro, T. and Casida, L.E. (1962). Growth inhibition of mycorrhizal fungi by gibberellin. Mycologia 54: 70–71.

Schindelin, J. et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods 9: 676–682.

Schmidt, R., Etalo, D.W., de Jager, V., Gerards, S., Zweers, H., de Boer, W., and Garbeva, P. (2016). Microbial small talk: volatiles in fungal-bacterial interactions. Front. Microbiol. 6: 1495.

Sebastiana, M., Vieira, B., Lino-Neto, T., Monteiro, F., Figueiredo, A., Sousa, L., Salomé Pais, M., Tavares, R., and Paulo, O.S. (2014). Oak root response to ectomycorrhizal symbiosis establishment: RNA-seq derived transcript identification and expression profiling. PLoS One 9: e98376.

Seybold, S.J., Huber, D.P.W., Lee, J.C., Graves, A.D., and Bohlmann, J. (2006). Pine monoterpenes and pine bark beetles: a marriage of convenience for defense and chemical communication. Phytochem. Rev. 5: 143–178.

Sillo, F., Fangel, J.U., Henrissat, B., Faccio, A., Bonfante, P., Martin, F., Willats, W.G.T., and Balestrini, R. (2016). Understanding plant cell-wall remodelling during the symbiotic interaction between *Tuber melanosporum* and *Corylus avellana* using a carbohydrate microarray. Planta 244: 347–359.

Šimura, J., Antoniadi, I., Široká, J., Tarkowska, D., Strnad, M., Ljung, K., and Novak, O. (2018). Plant hormonomics: multiple phytohormone profiling by targeted metabolomics. Plant Physiol. 177: 476–489.

Song, S., Qi, T., Wasternack, C., and Xie, D. (2014). Jasmonate signaling and crosstalk with gibberellin and ethylene. Curr. Opin. Plant Biol. 21: 112–119.

Splivallo, R., Fischer, U., Göbel, C., Feussner, I., and Karlovsky, P. (2009). Truffles regulate plant root morphogenesis via the production of auxin and ethylene. Plant Physiol. 150: 2018–2029.

Spoel, S.H. and Dong, X. (2008). Making sense of hormone crosstalk during plant immune responses. Cell Host Microbe 3: 348–351.

Staal, M., De Cnodder, T., Simon, D., Vandenbussche, F., Van Der Straeten, D., Verbelen, J.-P., Elzenga, T., and Vissenberg, K. (2011). Apoplastic alkalinization is instrumental for the inhibition of cell elongation in the Arabidopsis root by the ethylene precursor 1-Aminocyclopropane-1-carboxylic acid. Plant Physiol. 155: 2049–2055.

Stamopoulos, D.C., Damos, P., and Karagianidou, G. (2007). Bioactivity of five monoterpenoid vapours to *Tribolium confusum* (du Val) (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 43: 571–577.

Sundari, S.K. and Adholeya, A. (2003). Growth profile of ectomycorrhizal fungal mycelium: emphasis on substrate pH influence. Antoine van Leeuwenhoek, J. Microbiol. 83: 209–214.

Tagu, B.Y.D. and Martin, F. (1996). Molecular analysis of cell wall proteins expressed during the early steps of ectomycorrhiza development. New Phytol. 133: 73–85.

Tagu, D., Bellis, R. De, Balestrini, R., Vries, O.M.H. De, Piccoli, G., Stocchi, V., Bonfante, P., and Martin, F. (2001). Immunolocalization of hydrophobin HYDPt-1 from the ectomycorrhizal basidiomycete *Pisolithus tinctorius* during colonization of *Eucalyptus globulus* roots. New Phytol. 149: 127–135.

Tak, J.-H., Jovel, E., and Isman, M.B. (2016). Contact, fumigant, and cytotoxic activities of thyme and lemongrass essential oils against larvae and an ovarian cell line of the cabbage looper, *Trichoplusia ni*. J. Pest Sci. (2004). 89: 183–193.

Takeda, N., Handa, Y., Tsuzuki, S., Kojima, M., Sakakibara, H., and Kawaguchi, M. (2015). Gibberellins interfere with symbiosis signaling and gene expression and alter colonization by arbuscular mycorrhizal fungi in *Lotus japonicus*. Plant Physiol. 167: 545–557.

Tatusov, R.L. et al. (2003). The COG database: an updated version includes eukaryotes. BMC Bioinformatics 4: 41.

Thomas, P.D., Campell, M.J., Kejariwal, A., Mi, H., Karlak, B., Daverman, R., Diemer, K., Muruganujan, A., and Narechania, A. (2003). PANTHER: a library of protein families and subfamilies indexed by function. Genome Res. 13: 2129–2141.

Tian, T., Liu, Y., Yan, H., You, Q., Yi X., Du, Z., Xu, W., and Su, Z. (2017). agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. Nucl. Ac. Res. 45(W1): W122-W129.

Tibbett, M. and Sanders, F.E. (2002). Ectomycorrhizal symbiosis can enhance plant nutrition through improved access to discrete organic nutrient patches of high resource quality. Ann. Bot. 89: 783–789.

Ticconi, C.A., Delatorre, C.A., and Abel, S. (2001). Attenuation of phosphate starvation responses by phosphite in Arabidopsis. Plant Physiol. 127: 963–972.

Tranvan, H., Habricot, Y., Jeannette, E., Gay, G., and Sotta, B. (2000). Dynamics of symbiotic establishment between an IAA-overproducing mutant of the ectomycorrhizal fungus *Hebeloma cylindrosporum* and *Pinus pinaster*. Tree Physiol. 20: 123–129.

Truman, W. and Glazebrook, J. (2012). Co-expression analysis identifies putative targets for CBP60g and SARD1 regulation. BMC Plant Biol. 12: 216.

Tschaplinski, T.J., Plett, J.M., Engle, N.L., Deveau, A., Cushman, K.C., Martin, M.Z., Doktycz, M.J., Tuskan, G.A., Brun, A., Kohler, A., and Martin, F. (2014). *Populus trichocarpa* and *Populus deltoides* exhibit different metabolomic responses to colonization by the symbiotic fungus *Laccaria bicolor*. Mol. Plant Microbe Interact. 27: 546–556.

Tuskan, G.A. et al. (2006). The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313: 1596–1605.

Vayssières, A., Pěnčík, A., Felten, J., Kohler, A., Ljung, K., Martin, F., and Legué, V. (2015). Development of the poplar-*Laccaria bicolor* ectomycorrhiza modifies root auxin metabolism, signaling and response. Plant Physiol. 169: 890–902.

Veneault-Fourrey, C., Commun, C., Kohler, A., Morin, E., Balestrini, R., Plett, J., Danchin, E., Coutinho, P., Wiebenga, A., de Vries, R.P., Henrissat, B., and Martin, F. (2014). Genomic and transcriptomic analysis of *Laccaria bicolor* CAZome reveals insights into polysaccharides remodelling during symbiosis establishment. Fungal Genet. Biol. 72: 168–181.

Vijayakumar, P., Datta, S., and Dolan, L. (2016). ROOT HAIR DEFECTIVE SIX-LIKE4 (RSL4) promotes root hair elongation by transcriptionally regulating the expression of genes required for cell growth. New Phytol. 4: 944–953.

Wang, L., Tsuda, K., Truman, W., Sato, M., Nguyen, L. V, Katagiri, F., and Glazebrook, J. (2011). CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling. Plant J. 67: 1029–1041.

Wang, Y., Schuck, S., Wu, J., Yang, P., Döring, A., Zeier, J., and Tsuda, K. (2018). A MPK3/6-

WRKY33-ALD1-pipecolic acid regulatory loop contributes to systemic acquired resistance. Plant Cell 30: 2480–2494.

Wang, Y., Wang, D., Shi, P., and Omasa, K. (2014). Estimating rice chlorophyll content and leaf nitrogen concentration with a digital still color camera under natural light. Plant Methods 10: 1–11.

Wehrens, R. and Budyens, L.M.C. (2007). Self- and super-organizing maps in R: the kohonen package. J. Stat. Softw. 21(5): 1–19.

Wild, M., Davière, J., Cheminant, S., Regnault, T., Baumberger, N., Heintz, D., Baltz, R., Genschik, P., and Achard, P. (2012). The Arabidopsis DELLA RGA-LIKE3 is a direct target of MYC2 and modulates jasmonate signaling responses. Plant Cell 24: 3307–3319.

Wittek, P., Gao, S.C., Lim, I.S., and Zhao, L. (2017). Somoclu: an efficient parallel library for self-organizing maps. J. Stat. Softw. 78(9): 1–21.

Won, S.-K., Lee, Y.-J., Lee, H.-Y., Heo, Y.-K., Cho, M., and Cho, H.-T. (2009). Cis-element and transcriptome-based screening of root hair-specific genes and their functional characterization in Arabidopsis. Plant Physiol. 150: 1459–1473.

Yadav, V., Molina, I., Ranathunge, K., Queralta Castillo, I., Rothstein, S.J., and Reed, J.W. (2014). ABCG transporters are required for suberin and pollen wall extracellular barriers in Arabidopsis. Plant Cell 26: 3569–3588.

Yamanaka, T. (2003). The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi in vitro. Mycologia 95: 584–589.

Yang, D., Yao, J., Mei, C., Tong, X., Zeng, L., Li, Q., and Xiao, L. (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc. Natl. Acad. Sci. U. S. A. 109: E1192–E1200.

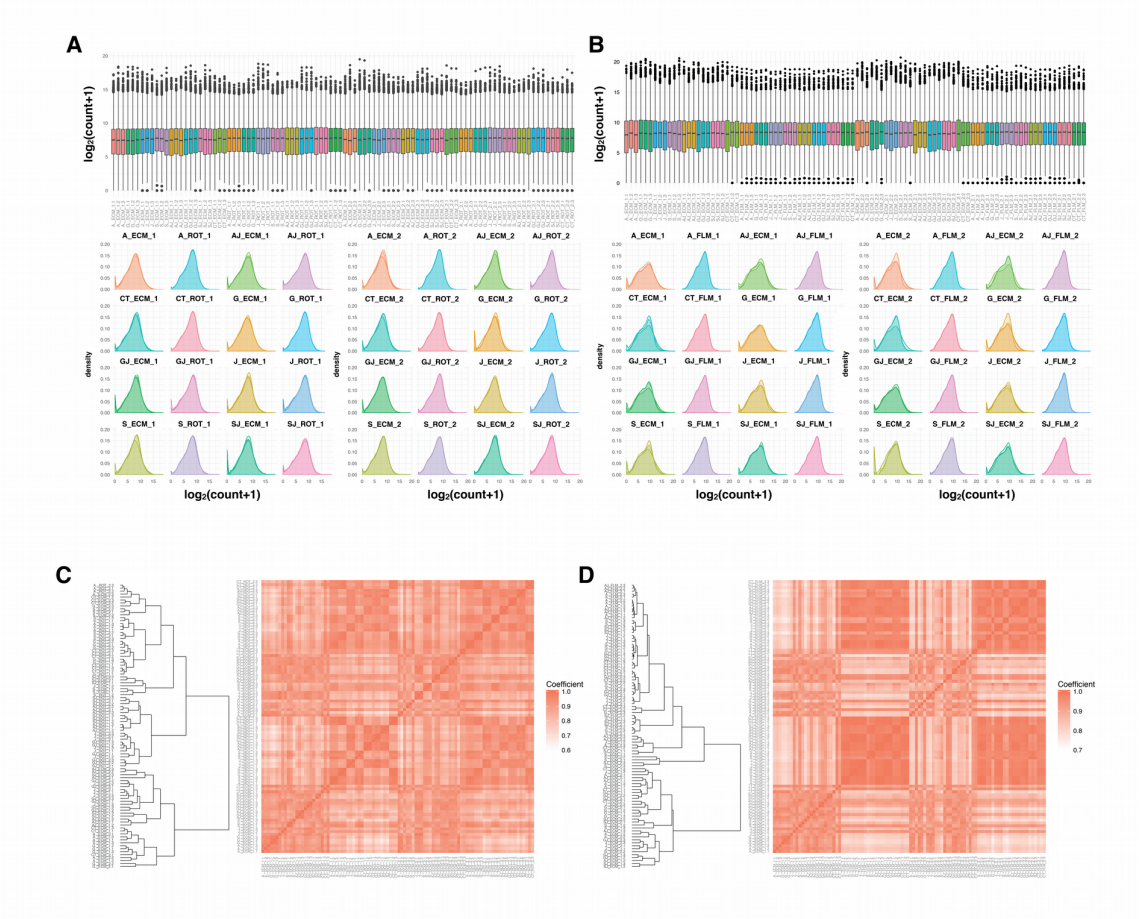
Yi, K., Menand, B., Bell, E., and Dolan, L. (2010). A basic helix-loop-helix transcription factor controls cell growth and size in root hairs. Nat. Genet. 42: 264–267.

Zhan, J., Thakare, D., Ma, C., Lloyd, A., Nixon, N.M., Arakaki, A.M., Burnett, W.J., Logan, K.O., Wang, D., Wang, X., Drews, G.N., and Yadegari, R. (2015). RNA sequencing of laser-capture microdissected compartments of the maize kernel identifies regulatory modules associated with endosperm cell differentiation. Plant Cell 27: 513–531.

Zhang, Y., Xu, S., Ding, P., Wang, D., Ti, Y., He, J., Gao, M., Xu, F., Li, Y., Zhu, Z., Li, X., and Zhang, Y. (2010). Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proc. Natl. Acad. Sci. 107: 18220–18225.

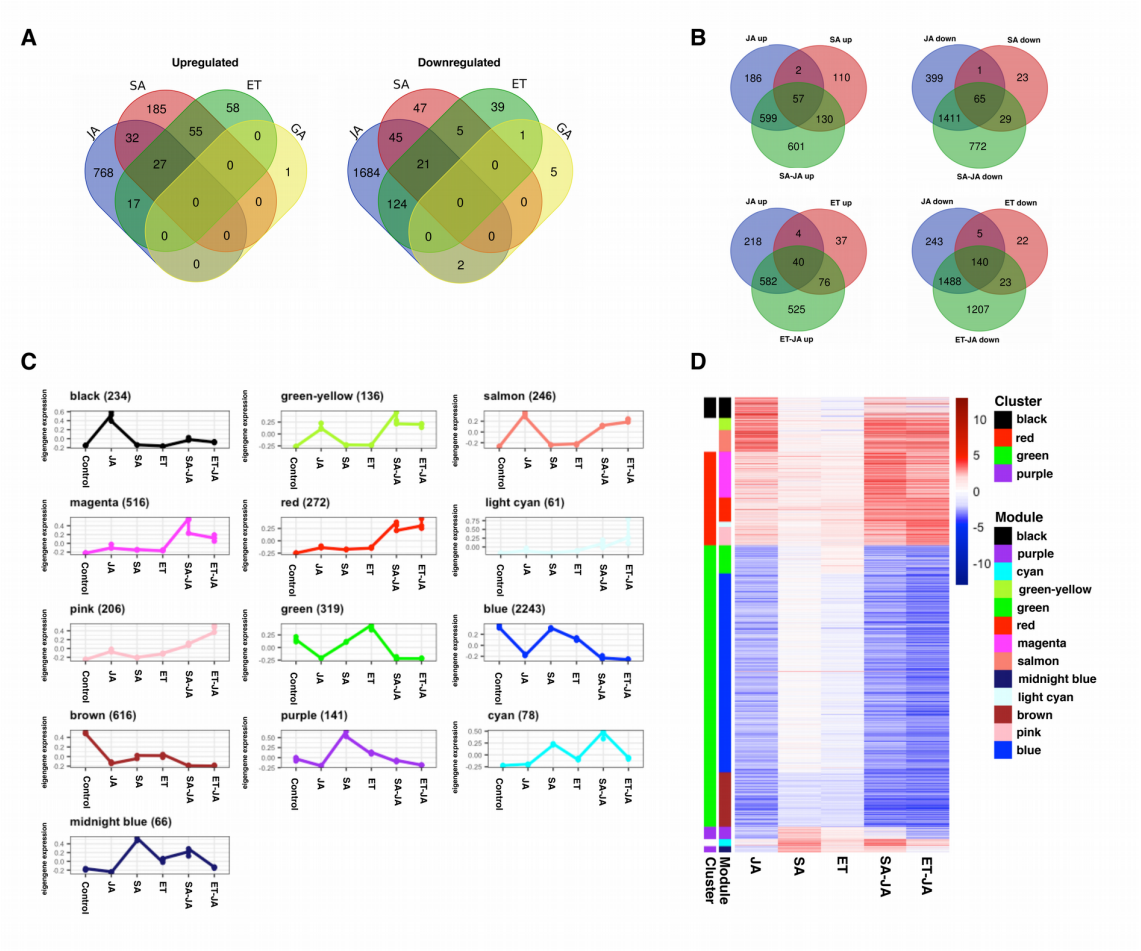
Župančić, A. and Gogala, N. (1980). The influence of root exudate auxins and gibberellins on the growth of *Suillus variegatus* mycelium. Acta Bot. Croat. 39: 85–93.

2.10. Supplemental Material



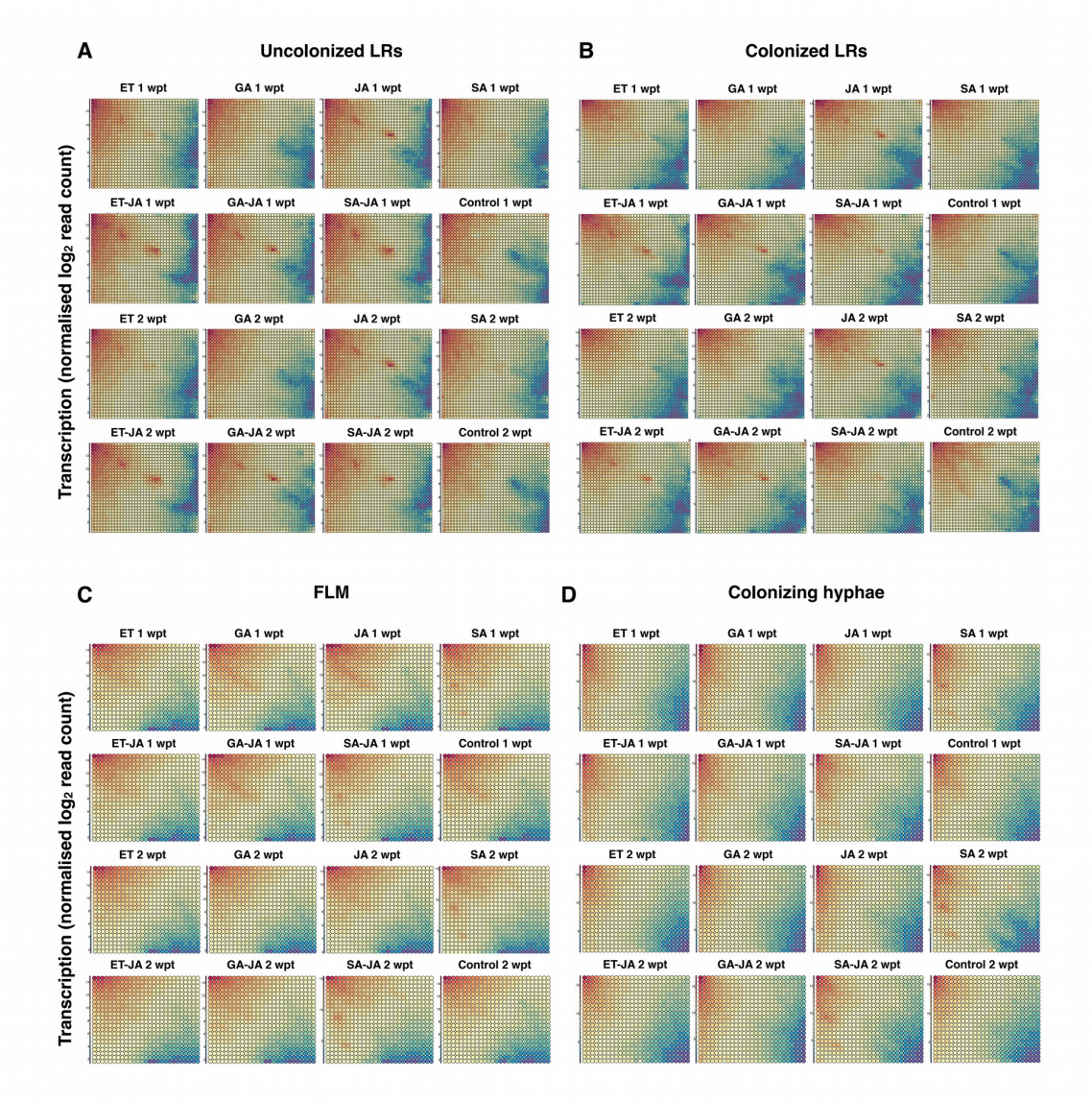
Supplemental Figure 1. Replicate analysis reveals consistency of normalized poplar lateral root and* L. bicolor *mycelium transcriptomes.

(Supports Figure 3.) A. Distribution and density of normalized, log$_2$-transformed read counts of 28,502 genes from 96 biological replicates of colonized and uncolonized poplar lateral roots (LRs) treated or not with exogenous phytohormones. B. Distribution and density of normalized, log$_2$-transformed read counts of 15,129 genes from 96 biological replicates of L. bicolor *free-living mycelia (FLM) and root-colonizing hyphae treated or not with exogenous phytohormones. C-D. Correlation of transcriptomes among 96 biological replicates of colonized and uncolonized poplar LRs (C) or* L. bicolor *FLM and root-colonizing hyphae (D) treated or not with exogenous phytohormones. Left: Hierarchical clusters of biological replicates based on the distances of transcriptomic similarities. Right: Adjacent matrix of the correlation coefficients (p < 0.0001). ECM: Ectomycorrhizae, i.e., colonized poplar LRs. ROT: Roots only, i.e., uncolonized poplar LRs. A/J/G/S/CT: Ethylene, jasmonic acid, gibberellic acid, salicylic acid, and control (no treatment). 1/2: 7/14 days after treatment.*



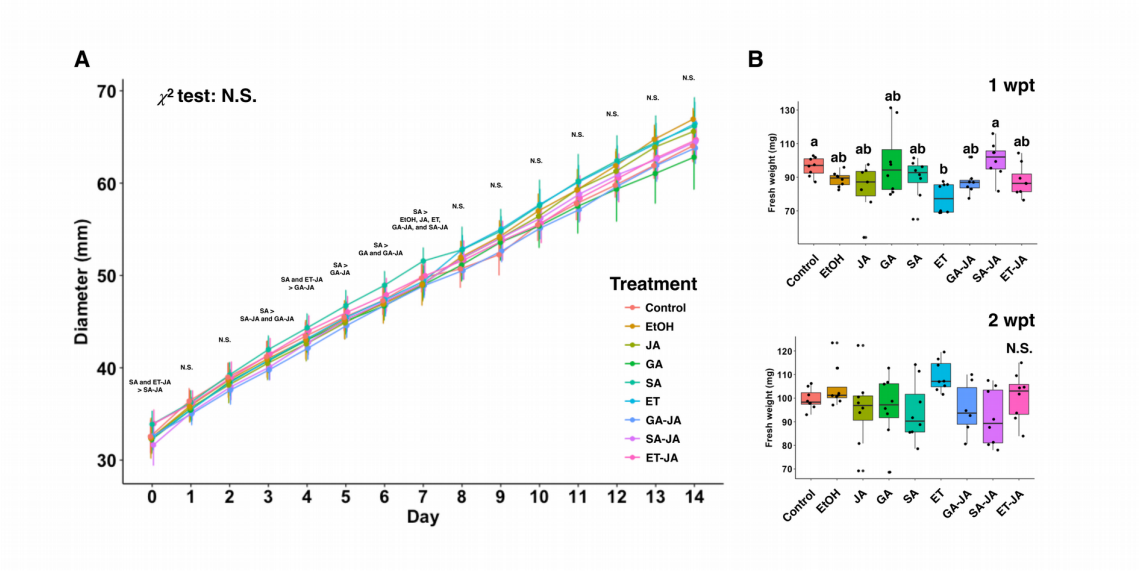
Supplemental Figure 2. Weighted Gene Co-expression Network Analysis reveals four gene clusters regulated by SA-JA and ET-JA crosstalk.

(Supports Figure 4C-D.) A. Venn diagrams showing the number of upregulated (left diagram) and downregulated (right diagram) genes in uncolonized poplar lateral roots (LRs) treated with exogenous JA, GA, SA or ET, as compared to untreated LRs, at 1 week post-treatment (wpt). B. Venn diagrams showing the overlaps in the number of upregulated (left diagrams) and downregulated (right diagrams) genes between uncolonized poplar lateral roots (LRs) treated with exogenous JA, SA, or a combination of JA and SA (upper panels) or JA, ET, or a combination of JA and ET (lower panels), as compared to untreated LRs, at 1 week post-treatment (wpt). C. Eigengene profiles of the 13 modules obtained through Weighted Gene Co-expression Network Analysis (WGCNA). D. Heat map of the $\log_2$ FC in expression of genes belonging to the WGCNA modules in hormone-treated uncolonized LRs versus untreated uncolonized LRs at 1 wpt. Colors denote WGCNA modules or indicate crosstalk-regulated gene clusters. Red cluster: SA-JA and ET-JA synergy. Green cluster: ET-JA synergy. Black cluster: SA-JA and ET-JA antagonism. Purple cluster: SA-JA antagonism.



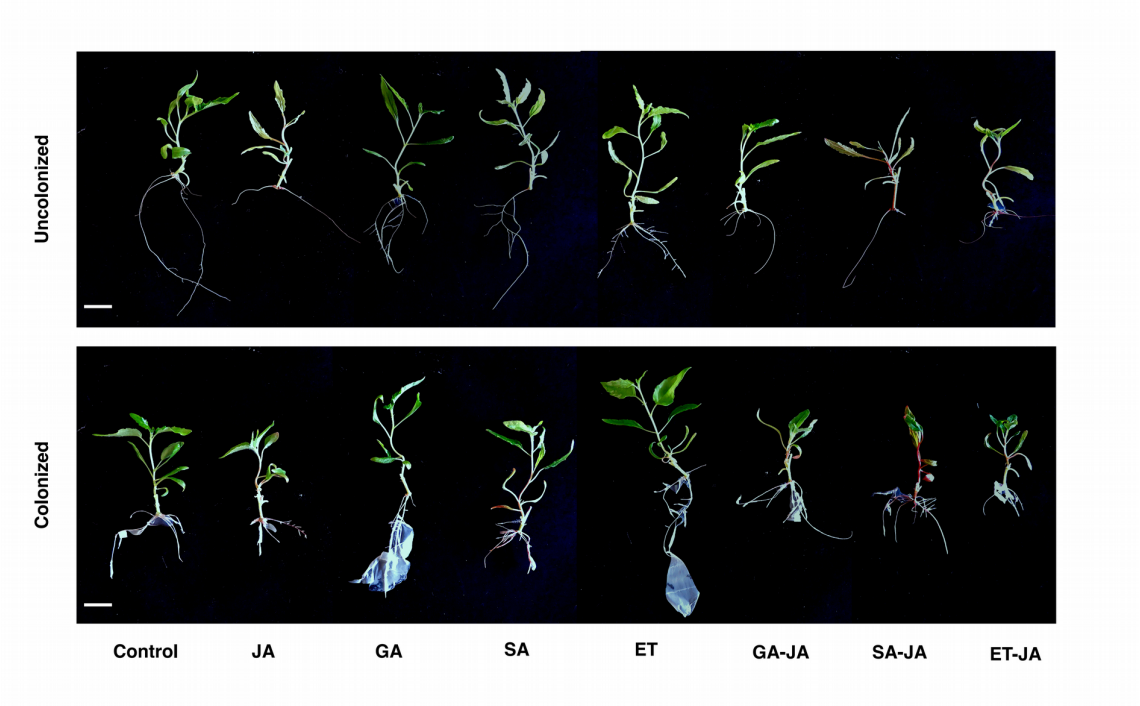
Supplemental Figure 3. Condition-wise Tatami maps.

(Supports Figure 3.) Condition-wise Tatami maps showing the averaged transcriptomic patterns from uncolonized (A) and colonized (B) poplar lateral roots (LRs), as well as L. bicolor *free-living mycelium (FLM) (C) and root-colonizing hyphae (D) at 1 and 2 weeks post-treatment (wpt). Nodes represent groups of genes with similar expression pattern. Red color indicates high node-wise transcription, while blue color indicates low node-wise transcription.*



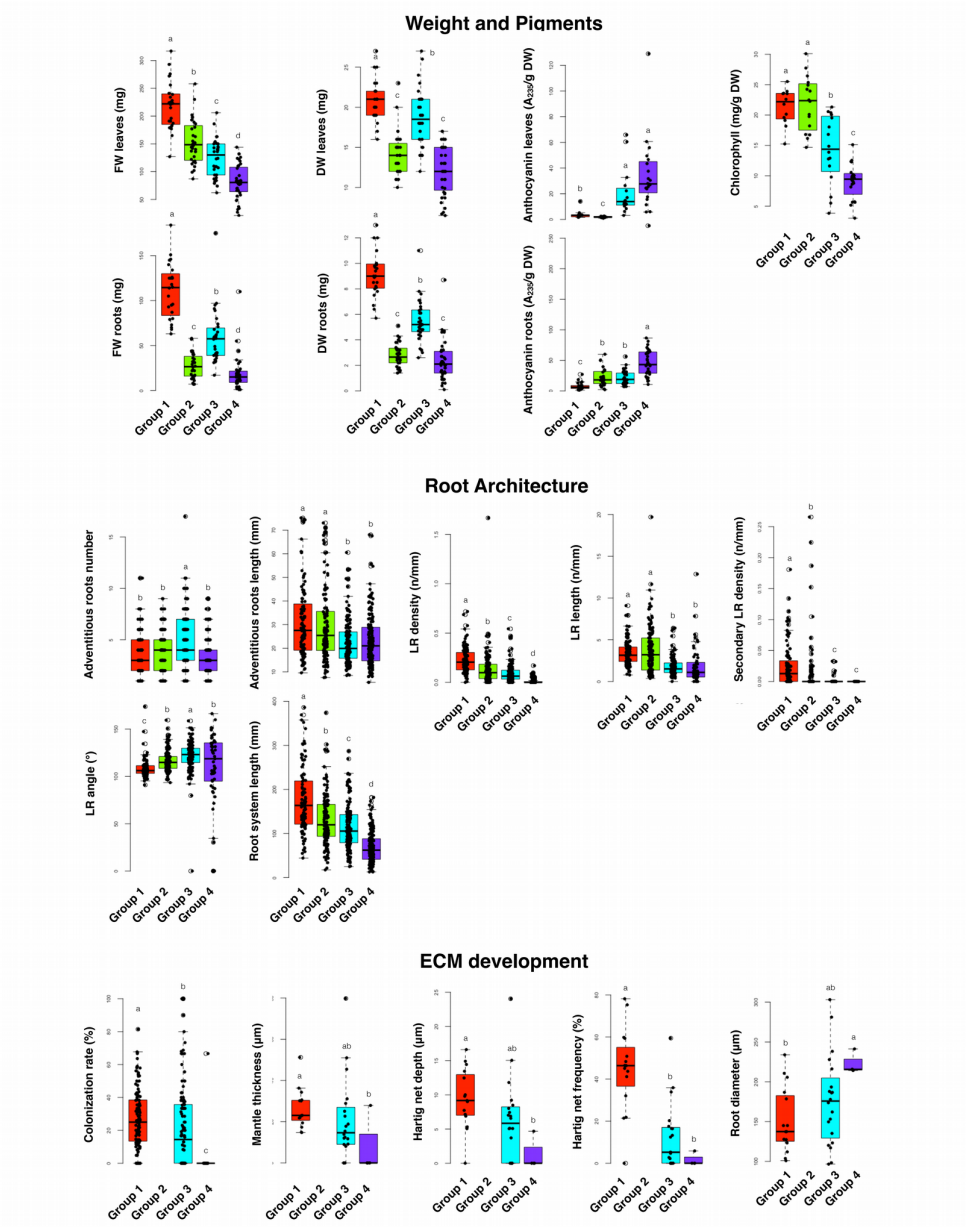
Supplemental Figure 4. ET treatment transiently affects the growth of* L. bicolor *colonies.

(Supports Figure 2.) A. Diameter growth curves of L. bicolor *colonies grown on Pachlewski agar medium supplemented with 2-morpholinoethanesulfonic acid sodium salt (P20 MES medium) with and without the addition of phytohormones or 0.061% v/v EtOH over a 14-day time-course. Error bars indicate standard deviation of six to sixteen replicates per condition. Linear models of growth curves from phytohormone- or EtOH-treated* L. bicolor *colonies do not differ significantly from untreated colonies according to χ^2 test. Equivalences reported on top of growth curves at single time points indicate significant differences between conditions as revealed by Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction ($p < 0.05$), performed at each time point. B. Boxplot charts representing differences in fresh weight among* L. bicolor *colonies treated or not with phytohormones or 0.061% v/v EtOH at 1 week post treatment (wpt, upper panel) or 2 wpt (lower panel). Whiskers represent the limits of the 1.5 interquartile range. Letters indicate the result of Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction ($p < 0.05$). N.S.: non significant.*



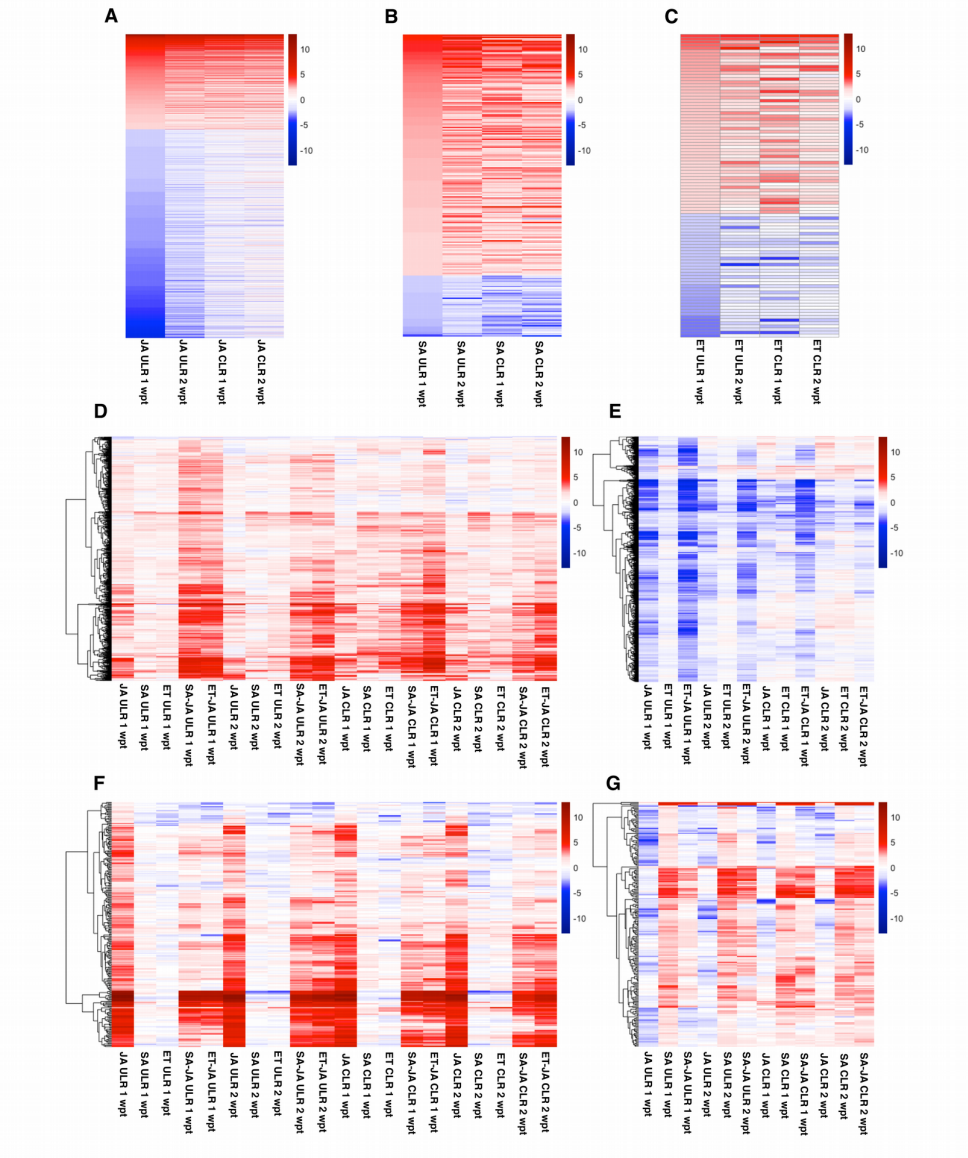
Supplemental Figure 5. Pictures of representative poplar cuttings.

(Supports Figure 2B.) Representative untreated and hormone-treated uncolonized (upper panel) and colonized (lower panel) poplar cuttings at 2 weeks post-treatment (wpt). Scale bar: 1 cm.



Supplemental Figure 6. Groups of phytohormone-treated colonized and uncolonized poplar cuttings showing specific phenotypes.

(Supports Figure 2B.) Boxplot charts representing differences in weight, pigment content, root architecture, and ectomycorrhizal development for groups of poplar plants resulting from hierarchical clustering (Figure 2B). Whiskers represent the limits of the 1.5 interquartile range. Letters indicate the result of Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction ($p < 0.05$). Group 1: Untreated, GA-treated, and ET-treated colonized plants. Group 2: Untreated, GA-treated, SA-treated, and ET-treated uncolonized plants. Group 3: JA-, SA-, GA-JA-, and SA-JA-treated colonized plants. Group 4: JA-, GA-JA-, SA-JA-, ET-JA-treated uncolonized plants, and ET-JA-treated colonized plants.



Supplemental Figure 7. Expression levels of phytohormone-specifically differentially expressed genes (phytohormone-sDEGs) and crosstalk-regulated genes are constant in hormone-treated colonized and uncolonized poplar lateral roots at different time points.

(Supports Figure 4.) Heat maps representing the $\log_2$ FC in expression of specifically differentially expressed genes (sDEGs) after JA (A), SA (B), or ET (C) treatment and in crosstalk- (D-G) responsive genes in hormone-treated uncolonized lateral roots (LRs) versus untreated uncolonized LRs, as well as hormone-treated colonized LRs versus untreated colonized LRs, at 1 or 2 wpt. Crosstalk-responsive genes are regulated by: SA-JA and ET-JA synergy (D, red cluster); ET-JA synergy (E, green cluster); SA-JA and ET-JA antagonism (F, black cluster); and SA-JA antagonism (G, purple cluster). ULR: uncolonized LRs. CLR: colonized LRs.

Supplemental Data Set 1. Summary of RNA-seq statistics.

RNA was extracted from three biological replicates per each of untreated or hormone-treated uncolonized poplar lateral roots (LRs), colonized poplar LRs, and L. bicolor *free-living mycelium (FLM) samples. Sampling was performed at two time points: 1 and 2 weeks post-treatment (wpt). The resulting 143 RNA samples were utilized to construct stranded sequencing libraries. After 2 × 150 bp Illumina HiSeq2000/2500 sequencing, raw reads were obtained. Reads were quality-filtered and trimmed to give the total number of sequenced fragments. Reads were mapped on the* L. bicolor *genome (v1.1) and/or on the* Populus trichocarpa *genome (v3.1). The percentages of fragments mapped on each genome and of total mapped fragments are provided. ULR: uncolonized LRs. CLR: colonized LRs.*

Supplemental Data Set 2. Weighted Gene Co-expression Network Analysis (WGCNA) reveals four gene clusters regulated by SA-JA and ET-JA crosstalk.

A. Ten WGCNA-derived modules are grouped into four clusters of crosstalk-regulated genes. *Correlation coefficients and p-values of Pearson's correlation tests between each of the 13 color-coded modules obtained via WGCNA (Supplemental Figure 2C) and each hormonal treatment of uncolonized plants at 1 week post-treatment (wpt). Significant correlations ($p < 0.05$) and inspection of expression profiles of each module (Supplemental Figure 2D) allowed regrouping of 10 modules into four clusters, representing genes synergistically or antagonistically regulated by SA-JA and ET-JA. It is reported whether genes belonging to each cluster are up- or downregulated upon phytohormone crosstalk.* ***B-E. Crosstalk-regulated gene clusters.*** *Gene expression levels and annotation of crosstalk-regulated genes. Log_2 FC and Bonferroni adjusted p-values are reported for hormone-treated uncolonized poplar lateral roots (LRs) versus untreated uncolonized LRs at 1 wpt. When available, the name of the closest Arabidopsis homologous gene, its symbol and its protein name are also reported. ULR: uncolonized LRs.* ***B. Red cluster (SA-JA and ET-JA synergy). C. Green cluster (ET-JA synergy). D. Black cluster (SA-JA and ET-JA antagonism). E. Purple cluster (SA-JA antagonism).***

Supplemental Data Set 3. Several ectomycorrhiza-responsive poplar genes are also specifically differentially expressed genes after phytohormone treatment or respond to hormonal crosstalk.

Gene expression levels and annotation of ectomycorrhiza (ECM)-responsive differentially expressed poplar genes (DEGs). ECM-responsive DEGs were defined as > 4-fold ($p < 0.05$) regulated genes in untreated colonized poplar lateral roots (LRs) compared to untreated uncolonized LRs at 1 or 2 weeks post-contact (wpc). Log_2 FC and Bonferroni adjusted p-values are reported for ECM-responsive genes. Annotations describe whether these genes are also specifically differentially expressed genes after JA, SA, or ET treatment (JA-, SA- or ET-sDEGs) (Supplemental Data Set 10), whether they are regulated by more than one of these treatments and whether they respond to hormonal crosstalk (Supplemental Data Set 2). When available, the name of the closest Arabidopsis homologous gene, its symbol and its protein name are also reported. The manual annotation of gene function, based on

the Arabidopsis homolog, is reported. Finally, genes with similar functions were grouped onto 12 categories of biological functions, as reported in Figure 4B,D. CLR: colonized LRs. ***A. ECM-responsive genes at 1 wpc. B. ECM-responsive genes at 2 wpc.***

Supplemental Data Set 4. Genome-wide co-regulated gene clusters for colonized and uncolonized poplar lateral root transcriptomes using Self-organizing map Harbouring Informative Nodes with Gene Ontology (SHIN+GO).

The annotations per protein IDs in 1147 nodes (gene clusters). The nodes with high/differential transcriptions are labelled. The table also includes JGI protein IDs with following information. Log_2 transformed normalized read counts of the genes averaged from the triplicates under all conditions at two time points; the log_2 fold difference of the transcriptions (i.e. hormone-treated against non-treated) at two time points with statistical significance (FDR adjusted p-value < 0.05); functional annotation information on Carbohydrate Active Enzyme (CAZyme), Proteases (MEROPS), the Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), EuKaryotic Orthologous Groups (KOG), and Protein families (Pfam). ECM: Ectomycorrhizal root. Rot: Root. S: Salicylic acid. J: Jasmonic acid. G: Gibberellic acid. A: ethylene. CT: Control (no-treatment). 1/2: 1 or 2 week-old.

Supplemental Data Set 5. Genome-wide co-regulated gene clusters for* L. bicolor *free-living mycelium and root-colonizing hyphae transcriptomes using Self-organizing map Harbouring Informative Nodes with Gene Ontology (SHIN+GO).

The annotations per protein IDs in 596 nodes (gene clusters). The nodes with high/differential transcriptions are labelled. The table also includes JGI protein IDs with following information. Log_2 transformed normalized read counts of the genes averaged from the triplicates under all conditions at two growth points; the log_2 fold difference of the transcriptions (i.e. hormone treated against non-treated) at two time points with statistical significance (FDR adjusted p-value < 0.05); functional annotation information on theoretically secreted Carbohydrate Active Enzyme database (CAZy), lipases, proteases, small secreted proteins (< 300 aa), InterPro (IPR), the Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and EuKaryotic Orthologous Groups (KOG), and SignalP for prediction of signal peptides. ECM: Ectomycorrhizae. FLM: Free-living mycelia. S: Salicylic acid. J: Jasmonic acid. G: Gibberellic acid. A: ethylene. CT: Control (no-treatment). 1/2: 1 or 2 week-old.

Supplemental Data Set 6. Phytohormone treatments alter root architecture, weight, pigment content, and ectomycorrhizal colonization of poplar cuttings.

Average and standard error of hormone-treated and untreated uncolonized and colonized poplar cuttings at 2 weeks post-treatment (wpt) in terms of weight, pigment content, root architecture, and ectomycorrhizal development. Letters represent the results of Kruskal-Wallis one-way analysis of variance and post-hoc Fischer's LSD test with Bonferroni correction (p < 0.05). Unc.: uncolonized plants; Col.: colonized plants. ***A. Hormone-treated poplar cuttings.*** *The number of biological replicates per treatment for measurement of weight and pigments ranges from four to eight. The*

number of biological replicates per treatment for assessment of root architecture ranges from 32 to 37. The number of biological replicates per treatment for estimation of the ectomycorrhizal colonization rate ranges from 25 to 53. The number of biological replicates per treatment for assessment of other parameters of ectomycorrhizal development ranges from three to five. ***B. Groups of treatments established according to the hierarchical clustering results (Figure 2B).*** *The number of biological replicates per group of treatments for measurement of weight and pigments ranges from 12 to 40. The number of biological replicates per group of treatments for assessment of root architecture ranges from 109 to 176. The number of biological replicates per group of treatments for estimation of the ectomycorrhizal colonization rate ranges from zero to 123. The number of biological replicates per group of treatments for assessment of other parameters of ectomycorrhizal development ranges from zero to 19.*

Supplemental Data Set 7. Exogenous phytohormones have wider effects on the transcriptomes of poplar lateral roots than of L. bicolor *mycelia.*

A. Hormonal treatments affect poplar lateral root transcriptomes. *Number of up- and downregulated genes, and total differentially expressed genes (DEGs) in hormone-treated colonized and uncolonized poplar lateral roots (LRs) compared to untreated colonized and uncolonized LRs, respectively. Transcriptomic analysis was performed at two time points: 1 and 2 weeks post-treatment (wpt). DEGs were defined as > 4-fold ($p < 0.05$) regulated genes in hormonal treatments as compared to untreated LRs. Prior to DEG calling, reads were normalized with two different methods.* ***1. Normalization on the total reads from uncolonized LR libraries.*** *These DEGs were exploited to define specific DEGs after phytohormone treatment (phytohormone-sDEGs) (Figure 4A) and as input for Weighted Gene Co-expression Network Analysis (WGCNA) (Supplemental Figure 2).* ***2. Normalization on the total reads from colonized and uncolonized LR libraries.*** *These DEGs were used to define specific responses of colonized or uncolonized LRs to GA, SA, and ET treatment (Supplemental Data Set 9). ULR: uncolonized LRs. CLR: colonized LRs.* ***B. Hormonal treatments have minor effects on*** **L. bicolor** ***transcriptomes.*** *Number of up- and downregulated genes, and total DEGs in hormone-treated* L. bicolor *free-living mycelium (FLM) and root-colonizing hyphae compared to untreated FLM and root-colonizing hyphae. Transcriptomic analysis was performed at two time points: 1 and 2 wpt. DEGs were defined as > 4-fold ($p < 0.05$) regulated genes in hormonal treatments as compared to untreated hyphae. Prior to DEG calling, reads were normalized by the total mapped reads from* L. bicolor *FLM and root-colonizing hyphae libraries. CH: root-colonizing hyphae.*

Supplemental Data Set 8. The transcriptome of L. bicolor *responds to exogenous SA, ET and GA.*

Gene expression levels and annotation of genes regulated by hormonal treatment in L. bicolor *transcriptomes. Log_2 FC and Bonferroni adjusted p-values are reported for hormone-treated free-living mycelium (FLM) versus untreated FLM at 1 week post-treatment (wpt), or hormone-treated root-colonizing hyphae versus untreated root-colonizing hyphae at 2 wpt. Functional KOG annotations are also reported. CH: root-colonizing hyphae.* ***A. SA-regulated genes in FLM. B. GA-regulated genes***

in root-colonizing hyphae. C. SA-regulated genes in root-colonizing hyphae. D. ET-regulated genes in root-colonizing hyphae.

Supplemental Data Set 9. Altered sensitivity to phytohormones upon fungal colonization determines selective regulation of genes functioning in defense and root architecture.

Gene expression levels and annotation of notable genes regulated by hormonal treatment in hormone-treated uncolonized lateral roots (LRs) versus untreated uncolonized LRs at 1 week post-treatment (wpt), or in hormone-treated colonized LRs versus untreated colonized LRs at 1 wpt. Log_2 FC and Bonferroni adjusted p-values are reported. When available, the name of the closest Arabidopsis homologous gene, its symbol and its protein name are also reported. ULR: uncolonized LRs. CLR: colonized LRs. ***1. Genes upregulated upon JA treatment in uncolonized, but not in colonized LRs. 2. Genes downregulated upon JA treatment in uncolonized, but not in colonized LRs. 3. Genes upregulated upon JA treatment in uncolonized and colonized LRs. 4. Genes downregulated upon GA treatment in colonized, but not in uncolonized LRs. 5. Genes upregulated upon SA treatment in colonized, but not in uncolonized LRs. 6. Genes upregulated upon ET treatment in colonized, but not in uncolonized LRs.***

Supplemental Data Set 10. Specifically differentially expressed genes after phytohormone treatment.

Gene expression levels and annotation of specifically differentially expressed genes after phytohormone treatment (phytohormone-sDEGs). Phytohormone-sDEGs were defined as > 4-fold ($p < 0.05$) regulated genes in only one of the uncolonized lateral root (LR) transcriptomes upon JA, SA, and ET treatment compared to untreated uncolonized LR transcriptome. Prior to DEG calling, reads were normalized by the total mapped reads from uncolonized LR libraries. Log_2 FC and Bonferroni adjusted p-values are reported. When available, the name of the closest Arabidopsis homologous gene, its symbol and its protein name are also reported. ULR: uncolonized LRs. ***A. JA-sDEGs. B. SA-sDEGs. C. ET-sDEGs. D. GA-sDEGs.***

Supplemental Data Set 11. Gene Ontology enrichment analysis of groups of genes regulated upon phytohormone treatment or responding to hormonal crosstalk.

Results of Gene Ontology (GO) enrichment analysis for groups of genes specifically differentially regulated upon phytohormone treatment (phytohormone-sDEGs) and groups of genes regulated by hormonal crosstalk (see Supplemental Figure 2 and Supplemental Data Set 2), as obtained via AgriGO (see Methods). The table reports significantly enriched GO terms, along with their accession number, type, number of genes belonging to such GO term in the query and in the reference background, total number of genes belonging to the query or the reference background, p-value resulting from Fisher's exact test, false discovery rate (FDR)-adjusted p-value (Benjamini-Yekutieli correction for multiple testing), and the GeneID of the query genes assigned to such GO term

(entries). Only terms scoring FDR<0.01 are shown. Term type: P. Biological process. C. Cellular component. F. Molecular function. Bg: background.

Supplemental Data Set 12. Phytohormone crosstalk regulates genes involved in cell wall modifications and responses to biotic and abiotic stress during fungal colonization.

Gene expression levels and annotation of notable crosstalk-regulated and ectomycorrhiza (ECM)-responsive genes. The entire sets of crosstalk-regulated genes are reported in Supplemental Data Set 2, while the entire sets of ECM-responsive genes are reported in Supplemental Data Set 3. Log_2 FC and Bonferroni adjusted p-values are reported for hormone-treated uncolonized poplar lateral roots (LRs) versus untreated uncolonized LRs at 1 week post-treatment (wpt), as well as untreated colonized LRs versus untreated uncolonized LRs at 1 and 2 weeks post-contact (wpc). When available, the name of the closest Arabidopsis homologous gene, its symbol and its protein name are also reported. ULR: uncolonized LRs. CLR: colonized LRs. 1. Red cluster (SA-JA and ET-JA synergy). 2. Green cluster (ET-JA synergy). 3. Black cluster (SA-JA and ET-JA antagonism). 4. Purple cluster (SA-JA antagonism).

3. Appendix: additional data

In this appendix we report some additional data that has not been included in the submitted manuscript. Indeed, to investigate the dynamics of phytohormone metabolism during ectomycorrhizal development, we screened 101 phytohormones using liquid chromatography-tandem mass spectrometry (LC-MS/MS; Šimura et al., 2018). In addition to JAs, SA and GAs (reported in the article), we also detected bioactive auxins, cytokinins (CKs), abscisates (ABA), as well as their precursors, conjugates and catabolites in (i) ectomycorrhizae (ECM) of *in vitro*-grown colonized poplar cuttings at 2 weeks post-contact (wpc), (ii) uncolonized poplar lateral roots (LRs), and (iii) *L. bicolor* free-living mycelium (FLM). Moreover, we identified genes involved in phytohormone metabolism to be regulated during ectomycorrhizal development. We briefly discuss the perspectives of these preliminary findings.

3.1. Additional results

Auxins, cytokinin ribosides, and phaseic acid accumulate in mid-stage ECM *in vitro*

To obtain the hormonal blueprint of ECM development, we screened 101 phytohormones using liquid chromatography-tandem mass spectrometry (LC-MS/MS; Šimura et al., 2018). Among the auxin-related compounds examined, with concentrations ranging from 2.81 ± 0.53 pmol/g fresh weight (FW) to 129.6 ± 26.8 nmol/g FW, the concentration of bioactive indole-3-acetic acid (IAA) was 1.7 times higher in ECM than in LRs but was comparable to that in FLM. Similar patterns were observed for the IAA precursor tryptophan, its degradation product oxIAA, and its conjugated form, IAA-Glu (Additional Table 1). These results are consistent with previous findings (Vayssières et al., 2015).

We measured the concentrations of total isoprenoid cytokinins (CKs) belonging to four groups, including isopentenyladenine (iP), *trans*-zeatin (*t*Z), *cis*-zeatin (*c*Z), and dihidrozeatin (DHZ), which ranged from 0.0036 ± 0.0011 to 64.40 ± 21.90 pmol/g FW. All of these compounds were present at low levels in FLM, suggesting that detected CKs were mainly synthetized by poplar roots. Therefore, the slight reduction in total CK content in ECM could be explained by the dilution of poplar tissue with fungal hyphae (data not shown). However, some groups of CK metabolites showed specific accumulation patterns. For instance, CK ribosides (transported forms of CK) were 2 times more concentrated in ECM than in LRs. Specifically, the concentration of iP riboside (iPR) increased 2.1-fold in ECM versus LRs. By contrast, CK *O*-glucosides (reversible CK conjugates) were 9.1 times less concentrated in ECM than in LRs. Finally, even though the concentration of total bioactive

CK bases did not differ between samples, *cis*-zeatin was 1.5 times more concentrated in ECM than in LRs.

We also detected the stress hormone abscisic acid (ABA) and its degradation products phaseic acid (PA) and diphaseic acid (DPA), which ranged from 6.22 ± 0.76 to 108.94 ± 17.7 pmol/g FW. The levels of these metabolites were below the detection limit in FLM. The differences in ABA levels between LRs and ECM disappeared if the ECM content was corrected by a dilution factor (data not shown). However, the PA concentration increased 2.7-fold in ECM compared to LRs, while the pool of DPA was completely depleted in ECM.

Such variations in hormonal contents suggest that altered phytohormone turnover occurs during ectomycorrhizal development, which may affect the expression of hormone-related gene networks.

Cytokinin and auxin metabolism is promoted during ectomycorrhizal development

To corroborate the finding that hormone levels are altered in mid-stage ECM, we investigated the expression of ECM-responsive genes with a role in phytohormone turnover by quantifying transcript levels in colonized and uncolonized poplar LRs at 1 and 2 wpc via paired-end Illumina RNA-seq. Several genes involved in phytohormone biosynthesis and degradation were upregulated during ectomycorrhizal development, especially at 1 wpc (>4-fold, $p < 0.05$; Appendix Table 2). For example, the putative cytokinin oxidase 3/dehydrogenase gene *PtCKX3* (Potri.007G066100), which might be involved in bioactive CK degradation, was upregulated 26-fold in colonized versus uncolonized LRs.

In addition, one 2-oxoglutarate and Fe(II)-dependent oxygenase, putatively involved in auxin catabolism, the homolog of the Arabidopsis gene *PLEIOTROPIC DRUG RESISTANCE 9*, a negative regulator of auxin accumulation in the root apex, and a gene putatively coding for a GH3 auxin conjugating enzyme were significantly upregulated at both time points, suggesting active auxin catabolism in ECM (Appendix Table 2). The activation of auxin catabolism in poplar roots during ectomycorrhizal colonization has been previously reported (Vayssières et al., 2015).

3.2. Additional discussion

Auxin signaling in ECM

Our data confirmed that auxin, its precursor, and catabolites accumulate during poplar-*L. bicolor* ECM development. Moreover, fungal colonization of poplar roots triggers the expression of genes implied in auxin catabolism. These data confirm a previous report (Vayssières et al., 2015). Auxin signaling seems to play a dual role during ECM development. In the pre-contact phase between host roots and ECM fungi, induced auxin signaling promotes LR development (Felten et al., 2009), possibly in crosstalk with ET signaling (Splivallo et al., 2009). On the other hand, during ECM maturation (2 wpc *in vitro*), *L. bicolor* appears to inhibit LR elongation via manipulation of root auxin signaling (Vayssières et al., 2015). GA and ET signaling, which alter auxin transport in roots (Splivallo et al., 2009; Gou et al., 2010), might also function in this process. In many tree species an important morphological modification of colonized LRs is the loss of root hairs (Horan et al., 1988; Béguristan and Lapeyrie, 1997). Auxin signaling promotes root hair elongation through induction of the bHLH transcription factor ROOT HAIR DEFECTIVE 6-LIKE4 (RSL4, Yi et al., 2010), while the ectomycorrhizal basidiomycete *Paxillus involutus* hijacks this mechanism through the secretion of the indolic compound hypaphorine (Béguristan and Lapeyrie, 1997; Ditengou et al., 2000). However, our findings suggest that JA/ET signaling contributes to the suppression of root hair formation in poplar-*L. bicolor* ECM (Basso et al., submitted). The role of the crosstalk between auxin and JA/ET signaling pathways in regulating root hair development should be addressed in the future.

CK ribosides and *cis*-zeatin accumulate in ECM

ECM preferentially accumulated CK ribosides, mainly iPR, and exhibited reduced contents of CK *O*-glucosides (Additional Table 1). The latter are inactive conjugated forms of CK that are easily converted into bioactive CK and thus represent a pool of readily available CKs. CK ribosides are transported forms of CK (Sakakibara, 2006). Therefore, colonized poplar LRs might convert CK *O*-glucosides into CK ribosides to mobilize CK or to maintain the concentration of bioactive CK forms at an optimal range. On the other hand, the slight increase in bioactive *cis*-zeatin concentration might be relevant for ECM symbiosis.

Fungal inoculation induced the expression of *PtCKX3*, putatively encoding a CK-degrading enzyme (Additional Table 2). Since *AtCKX3* is CK responsive (Ariel et al., 2012; van Zeijl et al., 2015; Reid et al., 2016), the enhanced expression of *PtCKX3* might result from active CK signaling in colonized LRs. In legumes, CK signaling reduces polar auxin transport and induces ET biosynthesis in rhizobium-infected roots, thereby promoting nodule

development (Plet et al., 2011; van Zeijl et al., 2015). Interestingly, we found that a gene encoding a predicted inhibitor of polar auxin transport and three genes that putatively function in ET biosynthesis were upregulated in 1-week-old colonized LRs (Basso et al., submitted; Additional Table 2). Therefore, it is tempting to speculate that CK signaling is responsible for the reduced polar auxin transport detected in 2-week-old ECM (Vayssières et al., 2015). The possible cross-regulation between CK signaling, auxin transport, and ET biosynthesis in ECM deserves further attention.

ABA/PA signaling may contribute to stress resistance in poplar ECM

Quantification of ABA and its catabolites, PA and DPA, suggested that ABA degradation is perturbed upon ECM formation. Indeed, the PA content of ECM was significantly higher than that of uncolonized poplar LRs, while the pool of PA-derived DPA was depleted in ECM (Additional Table 1). Interestingly, PA activates a subset of ABA-related genes in Arabidopsis, as well as a PA-specific gene set encoding metabolic enzymes such as laccases, acylhydrolases, xyloglucan endotransglucosylases, aspartyl proteases, and flavin-monooxygenases (Weng et al., 2016). ABA plays major roles in mediating plant resistance to abiotic stress (Vishwakarma et al., 2017). Consistently, our transcriptomic analysis revealed the upregulation of 29 genes putatively involved in responses to oxidative stress, salinity, drought, heat, and heavy metals in colonized LRs (Basso et al., submitted, Supplemental Data Set 5). Therefore, ABA/PA signaling might mediate a transient abiotic stress response in developing ECM.

3.3. Additional tables

Class	Hormone Type	Metabolism	LRs			ECM			FLM		
Abscisic acid	**ABA**	bioactive hormone	17.62	±	2.39	6.22	±	0.76 *§	<LOD		
	PA	degradation product	48.49	±	13.23	133.25	±	38.68 *§	<LOD		
	DPA	degradation product	108.94	±	17.7	<LOD *			<LOD		
Auxins	**Tryptophan**	precursor	37560.6	±	8336.6	129619.4	±	26832.1 *§	71817	±	9145.3
	IAA	bioactive hormone	68.43	±	3.92	115.47	±	17.38 *	105	±	16.6
	oxIAA	degradation product	<LOD			80.09	±	8.95 *	33.32	±	7.53
	IAA-Glu	conjugated form	2.81	±	0.53	0.95	±	0.24 *	<LOD		
Cytokinins	**Total Cytokinins**		73.63	±	23.09	22.74	±	4.94 *§	0.66	±	0.22
	Total CK Bases	bioactive hormones	1.49	±	0.09	1.55	±	0.31 §	0.01	±	0.01
	trans-zeatin (*tZ*)	bioactive hormone	0.83	±	0.11	0.8	±	0.21 §	<LOD		
	cis-zeatin (*cZ*)	bioactive hormone	0.5	±	0.06	0.75	±	0.2 *§	<LOD		
	Isopentenyladenine (iP)	bioactive hormone	0.17	±	0.04	<LOD *			<LOD		
	2-methyltio-iP (2MeSiP)	bioactive hormone	<LOD			<LOD §			0.016	±	0.005
	Total CK Ribosides	transport forms	7.02	±	1.69	14.13	±	3.6 *§	0.65	±	0.22
	tZ riboside (*tZR*)	transport form	0.22	±	0.03	0.1	±	0 ***§**	<LOD		
	cZ riboside (cZR)	transport form	2.59	±	0.69	0.75	±	0.2 *§	0.27	±	0.09
	iP riboside (iPR)	transport form	4.1	±	0.99	8.78	±	1.99 *§	0.37	±	0.13
	2-methylthio-*cis*-zeatin riboside (2MeScZR)	transport form	0.1	±	0.02	0.21	±	0.07 *§	<LOD		
	2MeSiP riboside (2MeSiPR)	transport form	0.0093	±	0.0013	*<LOD *§*			0.0036	±	0.0011
	Total CK O-glucosides	conjugated forms	64.38	±	22.44	7.06	±	3.53 *§	<LOD		
	tZ O-glucoside (*tZOG*)	conjugated form	2.8	±	1.03	0.97	±	0.33 *§	<LOD		
	tZR O-glucoside (*tZROG*)	conjugated form	0.1	±	0.03	<LOD *			<LOD		
	cZ O-glucoside (cZOG)	conjugated form	59.34	±	21.44	6.68	±	2.37 *§	<LOD		
	cZR O-glucoside (cZROG)	conjugated form	1.97	±	0.29	0.94	±	0.18 *§	<LOD		
	dihidrozeatin riboside O-glucoside (DHZROG)	conjugated form	0.17	±	0.04	<LOD *			<LOD		
	Total CK N-glucosides	conjugated forms	1.21	±	0.13	<LOD *			<LOD		
	tZ 7-N-glucoside (*tZ7G*)	conjugated form	1.21	±	0.13	<LOD *			<LOD		
	Total tZ-types	various	4.67	±	1.16	1.65	±	0.52 *§	<LOD		
	Total cZ-types	various	64.4	±	21.9	12.09	±	4.08 *§	0.27	±	0.09
	Total DHZ-types	various	0.17	±	0.04	<LOD *			<LOD		
	Total iP-types	various	4.27	±	1.02	8.78	±	1.99 *§	0.37	±	0.13
	Total MeSCKs	various	0.11	±	0.02	0.21	±	0.07 *§	0.013	±	0.009

Additional Table 1. ECM accumulate auxins, CK ribosides, and phaseic acid.

*Average and standard error of the concentrations of selected phytohormones, their precursors, and their degradation/inactivation products in uncolonized poplar LRs, ECM at 2 wpc, and L. bicolor FLM, as measured by LC-MS/MS and reported in pmol/g FW. <LOD represents concentrations below the detection limit. * or § indicate significant differences between ECM and LRs, or between ECM and FLM, respectively, according to Kruskal-Wallis one-way analysis of variance and post-hoc Fisher's LSD test with Bonferroni correction (n = 5, p < 0.05).*

Gene ID	*CLR vs ULR 1wpc log2FC*	*CLR vs ULR 1wpc p-value*	*CLR vs ULR 2wpc log2FC*	*CLR vs ULR 2wpc p-value*	*Arabidopsis homolog*	*Arabidopsis symbol*	*Protein name*	*Biological process*
Potri.007G050300	2.8	1.1e-10	2.7	1.9e-09	AT2G14960.1	GH3.1	Auxin-responsive GH3 family protein	IAA conjugation
Potri.011G146600	2.2	3.3e-06	1.6	0.0032	AT1G14130.1		2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	auxin catabolism
Potri.010G153700	2.3	0.00097	4.3	3e-11	AT3G53480.1	ABCG37,ATPDR9,PDR9,PIS1	pleiotropic drug resistance 9	negative regulation of auxin accumulation in the root apex
Potri.007G066100	4.7	7.4e-16	3.9	1.6e-10	AT5G56970.1	ATCKX3,CKX3	cytokinin oxidase 3	CK catabolism

Additional Table 2. Expression of genes that function in auxin and CK metabolism in early colonized poplar LRs.

Gene expression level and annotation of genes that function in phytohormone biosynthesis, conjugation, and degradation in control colonized poplar LRs versus control uncolonized LRs at 1 and 2 wpc. Log_2 FC and adjusted Bonferroni p-value of gene expression are reported. When available, the name of the closest Arabidopsis homologous gene, its symbol and its protein name are also reported. The last column indicates the biological process in which each gene is involved.

3.4. Additional references

Ariel, F. et al. (2012). Two Direct Targets of Cytokinin Signaling Regulate Symbiotic Nodulation in Medicago truncatula. Plant Cell **24**: 3838–3852.

Lorenzo, O., Piqueras, R., Sánchez-Serrano, J.J., and Solano, R. (2003). ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell **15**: 165–178.

Plet, J., Wasson, A., Ariel, F., Le Signor, C., Baker, D., Mathesius, U., Crespi, M., and Frugier, F. (2011). MtCRE1-dependent cytokinin signaling integrates bacterial and plant cues to coordinate symbiotic nodule organogenesis in Medicago truncatula. Plant J. **65**: 622–633.

Reid, D.E., Heckmann, A.B., Kelly, S., and Stougaard, J. (2016). CYTOKININ OXIDASE/DEHYDROGENASE3 Maintains Cytokinin Homeostasis during Root and Nodule Development in Lotus japonicus. Plant Physiol. **170**: 1060–1074.

Rojo, E., León, J., and Sánchez-Serrano, J.J. (1999). Cross-talk between wound signalling pathways determines local versus systemic gene expression in Arabidopsis thaliana. Plant J. **20**: 135–142.

Sakakibara, H. (2006). CYTOKININS: Activity, Biosynthesis, and Translocation. Annu. Rev. Plant Biol. **57**: 431–449.

Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R.K., Kumar, V., Verma, R., Upadhayay, R.G., Pandey, M., and Sharma, S. (2017). Abscisic Acid Signaling and

Abiotic Stress Tolerance in Plants : A Review on Current Knowledge and Future Prospects. Front. Plant Sci. **8**: 161.

Weng, J., Ye, M., Li, B., and Noel, J.P. (2016). Co-evolution of Hormone Metabolism and Signaling Networks Expands Plant Adaptive Plasticity. Cell **166**: 881–893.

van Zeijl, A., Op Den Camp, R.H.M., Deinum, E.E., Charnikhova, T., Franssen, H., Op Den Camp, H.J.M., Bouwmeester, H., Kohlen, W., Bisseling, T., and Geurts, R. (2015). Rhizobium Lipo-chitooligosaccharide Signaling Triggers Accumulation of Cytokinins in Medicago truncatula Roots. Mol. Plant **8**: 1213–1226.

Chapter 5: Discussion and perspectives

Chapter 5: Discussion and perspectives

1. Distinct branches of JA signaling might differentially regulate poplar-*L. bicolor* ECM development

In the course of this PhD project we provided new knowledge on the role of plant hormones in the transcriptomic reprogramming associated to the development of ECM between *Populus* spp. and the basidiomycete *L. bicolor*. We focused our study on the phytohormone JA, since it had been previously demonstrated that the effector protein MiSSP7 of *L. bicolor* stabilizes the poplar JA co-receptor PtJAZ6, represses JA signaling, and allows Hartig net development by dampening plant defense reactions (Plett et al., 2014a). Since JA signaling regulates many aspects of plant physiology and development, our overarching goal was to determine whether a specific subset of JA-mediated molecular responses is implied in the regulation of ECM development. The aim of such investigation is to unravel whether fungal manipulation of plant immunity and/or development is required for ECM symbiosis. Within this line of research, our main findings are:

- The transcriptional regulator PtJAZ6 interacts with poplar homologs of the *Arabidopsis* bHLH transcription factors MYC2 and JAM1, and the fungal effector MiSSP7 strengthens the binding of PtJAZ6 to PtMYC2.1, while weakening the PtJAZ6-PtMYC2.2 interaction;
- The overexpression of *PtaMYC2.1* in poplar roots inhibits the Hartig net development, while the overexpression of *PtaJAM1.1* promotes hyphal colonization of the root apoplast. Fungal colonization slightly reduces the expression of these genes in transgenic poplar roots;
- A subset of JA-responsive genes is regulated during ECM development, suggesting that moderate JA signaling may be co-opted during fungal colonization and mediates a transient stress response, the suppression of root hair elongation, and optimal rigidity of the plant cell wall;
- Fungal inoculation suppresses the sensitivity of poplar roots to exogenous JA, thus hampering the expression of many JA-responsive genes implied in plant defense against biotic and abiotic stress.

Altogether, our results suggest that JA signaling is a crucial pathway determining ECM development and that the different branches of JA signaling may differentially regulate the extent of fungal colonization *in planta* (Figure 1). We speculate that PtMYC2.1

preferentially controls the expression of defense-related genes, and that the suppression of its activity is required for intercellular fungal penetration. On the other hand, PtMYC2.2 may regulate the expression of JA-dependent genes involved in root hair suppression and modification of plant cell wall properties, required for optimal ECM development. Therefore, preferential binding of PtJAZ6 to PtMYC2.1 in presence of MiSSP7 may inhibit the activation of defense-related genes while PtMYC2.2, free from inhibition, may mediate a moderate response to JA and promote optimal fungal colonization. Further experimental evidence is required to validate this model.

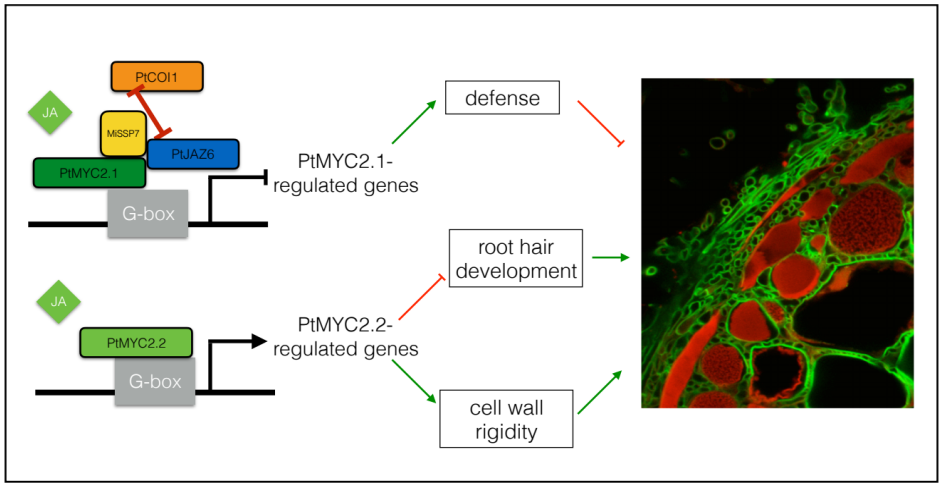


Figure 1. Working model: distinct branches of JA signaling differentially regulate ECM development.

Hypothetic model describing the role of JA signaling and JA-related TFs in the development of ECM between poplar and L. bicolor*. The fungal effector MiSSP7 stabilizes the poplar JA co-receptor PtJAZ6 by inhibiting its interaction with the F-box protein PtCOI1. MiSSP7 also strengthens the binding of PtJAZ6 to the TF PtMYC2.1, possibly limiting its activity. PtMYC2.1-regulated genes may be involved in defense, and the repression of such genes may be essential for the apoplastic penetration of fungal hyphae. On the other hand, MiSSP7 negatively affects the interaction between PtJAZ6 and PtMYC2.2, a paralog of PtMYC2.1. It is thus possible that moderate JA signaling in colonized poplar roots activates the expression of PtMYC2.2-regulated genes, which may contribute to the suppression of root hair development and to attaining the optimal level of cell wall rigidity, two processes that might promote ECM development. Further experimental evidence is necessary to validate this model.*

Assuming our working hypothesis proved correct, moderate or transient activation of JA signaling may be beneficial for ECM development, while sustained activation of responses to JA may lead to the rejection of the symbiotic fungus. In this respect, the role of JA signaling in ECM symbiosis would resemble the function of this hormonal signaling pathway in the

establishment of the arbuscular mycorrhizal (AM) symbiosis (Basso & Veneault-Fourrey, in press). In fact, moderate activation of JA signaling promotes root colonization of several host species by AM fungi (Regval et al., 1996; Kiers et al., 2010; Landgraf et al., 2012), while massive or repeated exogenous application of JA results in lower frequency of fungal colonization (Herrera-Medina et al., 2008; Riedel et al., 2008; Fernandez et al., 2014). Fine-tuning of JA-dependent responses, therefore, is emerging as a common mechanism to regulate the extent of several types of mycorrhizal symbioses, in accordance to environmental stimuli (Kiers et al., 2010) and to the level of compatibility between host and microbe (Fernandez et al., 2014). Since poplar species can also interact with AM fungi, it would be interesting to assess if JA signaling plays similar roles in regulating the establishment of the AM and the ECM symbiosis in poplar roots. Also, to verify if JA signaling is implied in the definition of symbiotic compatibility between trees and ectomycorrhizal fungi, it would be worth investigating whether the activation of JA-dependent defense mechanisms underlies the diminished colonization of recalcitrant host species *Populus deltoides* by *L. bicolor* (Tschaplinski et al., 2014).

2. The hormonal landscape of ECM development

To generate an overview of the role of phytohormones in ECM establishment, we described the hormonal landscape of developing ECM and investigated possible interactions between JA signaling and other phytohormone signaling pathways in poplar roots. In brief, our main findings are:

- Phytohormone metabolism is altered during ECM development, with increased accumulation of several auxin species, SA, CK ribosides, and phaseic acid in mid-stage ECM;
- *L. bicolor* inoculation modifies the sensitivity of poplar roots to phytohormones, inhibiting poplar responses to exogenous JA, while rendering roots more sensitive to exogenous SA, GA, and ET;
- Exogenous supply of JA, GA and ET, alone or in combination, negatively affects the frequency of fungal colonization and/or ECM development, indicating that an optimal hormonal balance is required for the establishment of mutualism;
- Positive crosstalk with SA and ET signaling may contribute to the role of moderate JA signaling in ECM development.

Within the framework of published research and our current data, we propose that several hormonal pathways contribute to ectomycorrhizal development and functional output (Figure 2).

In the pre-contact phase of the ectomycorrhizal symbiosis (3 days post indirect contact *in vitro*), fungal volatiles and/or diffusible molecules determine enhanced lateral root initiation in host and non-host plants (Felten et al., 2009; Splivallo et al., 2009). Auxin signaling, in combination with volatiles, like sesquiterpenes and ethylene, participates to such induction (Figure 2a; Felten et al., 2009; Splivallo et al., 2009; Ditengou et al., 2015). Several mycorrhizal fungi produce bioactive auxins (Frankenberger and Poth, 1987; Niemi et al., 2002; Splivallo et al., 2009; Vayssières et al., 2015). For the poplar-*L. bicolor* interaction, it was suggested that fungal auxin is uptaken by the host root apex and transiently stimulates the expression of the basipetal auxin transporter PtaPIN9 and the auxin-responsive protein PtaIAA19.3. Consequently, apical/lateral transport of auxin in the elongation zone likely primes pericycle cells for lateral root initiation (Felten et al., 2009; Laskowski et al., 2008). On the other hand, both auxin transport and ethylene signaling were necessary to reproduce the full range of root phenotypes stimulated by truffle volatiles on the non-host Arabidopsis (Splivallo et al., 2009). Moreover, in our experiment, ET treatment enhanced poplar lateral

root density. Auxin promotes ET biosynthesis by increasing the expression and the stability of ACC synthases (Lee et al., 2017), while ET signaling modulates the expression of polar auxin transporters (Lewis et al., 2011). Therefore, ET signaling may prompt the establishment of optimal auxin gradients for lateral root emergence upon fungal inoculation. Surprisingly, in Arabidopsis ET signaling negatively regulates auxin-mediated lateral root emergence (Ivachenko et al., 2008; Negi et al., 2008; Lewis et al., 2011). Thus, fungi likely stimulate additional plant signaling pathways to induce positive ethylene-auxin crosstalk in lateral root initiation.

In the early contact phase between the symbiotic partners (1 wpc *in vitro*), a transitory response to biotic and abiotic stress takes place in the plant. This has been reported also for other kinds of mutualistic interactions (Liu et al., 2003; Duplessis et al., 2005; Siciliano et al., 2007; Jacobs et al., 2011; Vahabi et al., 2015) and it is possibly determined by a premature plant defense response prior to recognition of the symbiont. Our data suggest that poplar JA signaling and its crosstalk with SA and ET signaling are implied in this early reaction to the fungus (Figure 2b). During this stage, fungal hyphae need to anchor to the host's root surface through the secretion of polysaccharides and glycoprotein microfibrils (Lei et al., 1991; Tagu and Martin, 1996). Our results suggest that root response to GA leads to an inhibition of the expression of putative glycoprotein-coding genes of *L. bicolor*, possibly impacting hyphal adhesion to the roots (Figure 2b).

During ECM maturation (2 wpc *in vitro*), poplar lateral roots become shortened and surrounded by a thick fungal mantle. Vayssières et al. (2015) proposed that fungal auxins are synthesized in hyphae in the vicinity of roots, secreted and transferred to root cells, where they modify host auxin metabolism and trigger the expression of a set of auxin-responsive genes, leading into root growth arrest and increased lateral root development. GA and ET signaling, altering auxin transport in plant roots (Splivallo et al., 2009; Gou et al., 2010), may also be important players in this process. Another important morphological modification of mycorrhizal lateral roots is the root hairs decay (Horan et al., 1988; Ditengou et al., 2000). Auxin signaling promotes root hair elongation through induction of the bHLH TF ROOT HAIR DEFECTIVE 6-LIKE 4 (RSL4, Yi et al., 2010). The ectomycorrhizal basidiomycete *Paxillus involutus* can hijack this mechanism through the secretion of the indolic compound hyphaporine (Ditengou et al., 2000). On the other hand, our findings suggest that JA/ET signaling contributes to root hair suppression in poplar-*L. bicolor* ECM. The crosstalk between auxin and JA/ET signaling pathways in the regulation of root hair development needs to be addressed in the future (Figure 2c).

Finally, during ECM maturation fungal hyphae grow in the root cortex apoplast to form the Hartig net (Blasius et al., 1986; Massicotte et al., 1987; Balestrini & Kottke, 2016). Our findings suggest that JA/ET signaling induces the downregulation of genes coding for pectin

lyases, xyloglucan endotransglucosylases/hydrolases and extensins in *Populus* roots in interaction with *L. bicolor*. On the other hand, mycorrhizal *L. bicolor* secretes fungal Carbohydrate Active enZymes (CAZymes) that likely contribute to cellulose and pectin degradation, allowing apoplastic penetration (Martin et al., 2008; Veneault-Fourrey et al., 2014; Zhang et al., 2018; Zhang et al., unpublished results). Therefore, our data support the hypothesis that mycorrhizal poplar roots activate a branch of JA signaling to counteract excessive cell wall loosening and hyphal invasion (Plett et al., 2014b). However, excessive JA can hamper fungal apoplastic growth (Plett et al., 2014a,b). Therefore, *L. bicolor* hijacks the JA signaling pathway through secretion of the effector protein MiSSP7 (Plett et al., 2014a). We speculate that MiSSP7 diminishes JA sensitivity of poplar roots, mitigating JA-mediated defense responses and cell wall rigidity (Figure 2c). In this phase, the concentration of several hormonal species is modified. For instance, we found that SA accumulation is enhanced in poplar mycorrhizal roots. However, since exogenous SA did not promote symbiotic development, nor many poplar SA-responsive genes responded to fungal inoculation, the role of SA accumulation in ECM remains to be elucidated. Since SA treatment increased the expression of carbohydrate and lipid metabolic enzymes in *L. bicolor* free-living mycelium, we propose that SA perception may trigger fungal energetic metabolism rather than plant responses. Alternatively, if the SA and JA signaling pathways were proved to be mutually antagonistic in poplar roots, as they are in Arabidopsis, increased SA accumulation might hamper tree responses to JA, and mediate the observed decrease of sensitivity to JA in *L. bicolor*-colonized poplar roots.

Phytohormone signaling likely mediates also the functional outputs of the ectomycorrhizal symbiosis, like nutrient exchange and growth promotion (Figure 2d). For instance, SA and ABA signaling have been implied in enhanced salt stress tolerance of poplar upon *P. involutus* inoculation (Luo et al., 2009). Also, ECM-induced resistance to aboveground herbivory in poplar relies on systemic activation of JA-dependent defense mechanisms (Kaling et al., 2018). Thus, the rearrangement of hormone content and perception in colonized plants might not only be significant for the regulation of symbiosis development, but also required to ensure enhanced host fitness.

In conclusion, phytohormone biosynthesis, transport and perception must undergo tight regulation to ensure ECM development. Ectomycorrhizal fungi may achieve this through: (i) emission of volatile/diffusible plant hormones or molecules stimulating plant hormone biosynthesis (Felten et al., 2009; Splivallo et al., 2009; Ditengou et al., 2015); (ii) secretion of effector proteins hijacking phytohormone signaling pathways (Plett et al., 2014a); (iii) epigenetic regulation of gene expression through differential DNA methylation, similarly to arbuscular mycorrhizal fungi (Cicatelli et al., 2014). Further studies are necessary to unravel

the molecular mechanisms through which the ectomycorrhizal symbionts modulate phytohormone accumulation, perception and responses.

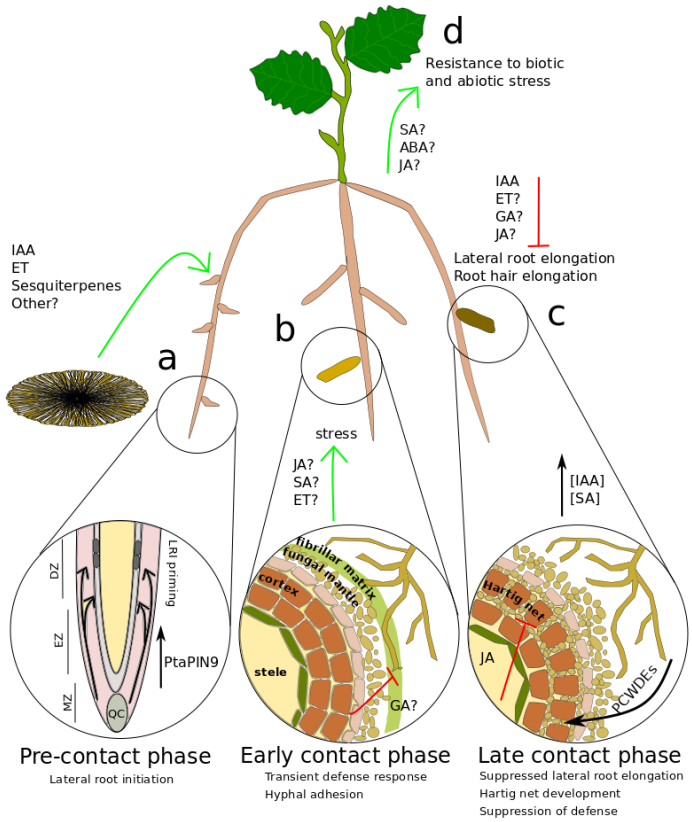


Figure 2. The roles of phytohormones in the development of the ECM symbiosis.

a. During the pre-contact phase of the symbiosis, L. bicolor *mycelium emits volatile signals, such as ET and sesquiterpenes, and secretes phytohormones, such as auxins, which promote lateral root development in the host plant. The effect of fungal auxin can be attributed to the increased expression of the poplar auxin transporter PtaPIN9 with subsequent increased basipetal auxin flux and enhanced lateral root priming. b.The fungal mantle forms at 1 wpc* in vitro*. During this phase, a transient response of poplar roots to biotic and abiotic stress takes place, possibly mediated by the crosstalk between JA, SA, and ET signaling. GA signaling may affect hyphal adhesion to host roots by affecting fungal secretion of adhesive proteins. c. During ECM maturation, LR elongation and root hair development are suppressed. GA and JA/ET signaling may perturb auxin transport and/or responses to influence root morphology. In mid-stage ECM, fungal hyphae penetrate the apoplast to generate the Hartig net. Apoplastic growth requires the expression of* L. bicolor *plant cell wall degrading enzymes (PCWDEs), possibly counteracted by JA-induced inhibition of pectin and cellulose degradation. During this phase, auxin and SA contents increase, but the reason for this is currently unknown. d. Phytohormones like JA, SA, and ABA are also implied in ECM-induced plant systemic resistance to biotic and abiotic stress. QC: quiescent centre. MZ: meristematic zone. EZ: elongation zone. DZ: differentiation zone. IAA: Indole-3-acetic acid.*

3. Microbial manipulation of phytohormone signaling through effectors across different trophic strategies

This research project provided novel evidence on the role of phytohormones in the transcriptional reprogramming occurring during the establishment of poplar-*L. bicolor* ECM. Altered phytohormone signaling is a common outcome of plant-microbe interactions. Microorganisms of different kingdoms and lifestyles, ranging from pathogens to mutualists, can influence phytohormone accumulation and responses to ensure colonization and/or infection of their hosts (Jacobs et al., 2011; Gutjahr, 2014; Kazan & Lyons, 2014; Ferguson & Mathesius, 2014). The negative crosstalk between JA and SA signaling, determining the specific panel of defense mechanisms activated by the plant, is a major target of such microbe-driven regulation (Gimenez-Ibanez et al., 2016; Zhang et al., 2017). Microbes and insects perturb JA and SA signaling either through the production of phytohormones and phytohormone-mimics or upon secretion of specialized small secreted proteins, termed effectors, targeting plant signaling components (Gimenez-Ibanez et al., 2016; Zhang et al., 2017). For instance, the SA-sensitive pathogen *Pseudomonas syringae* synthetizes coronatin, a JA-mimic that suppresses SA signaling, thus ensuring infection (Zheng et al., 2012). *P. syringae* can activate JA signaling also through the secretion of specialized effectors. For example, XopX1 and HopZ1a promote JA signaling by interacting with JAZ repressors and inducing their degradation (Gimenez-Ibanez et al., 2014; Jiang et al., 2013), while the effector HopBB1 activates a subset of JA-dependent responses by dissociating the JAZ3-MYC2 complex and leading the transcriptional repressor TCP14 to proteasomal degradation (Yang et al., 2017). Our findings suggest that the mutualistic effector MiSSP7 of *L. bicolor* might also induce specific JA-related gene expression by influencing the activity of PtJAZ6-interacting TFs in poplar roots (Figure 1). Since the MiSSP7 effector specifically strengthens the binding of PtJAZ6 to PtMYC2.1, but not to its paralog PtMYC2.2, it seems that the function of this fungal effector is very precise and targeted, pointing at a close co-evolution between the genomes of poplar and *L. bicolor*. Indeed, even though *L. bicolor* is a generalist ectomycorrhizal fungus, the expression of a wide repertoire of fast-evolving, species-specific small secreted proteins may confer selective advantage during the colonization of different host species. However, MiSSP7 is part of the core regulon expressed by *L. bicolor* upon root colonization of both *Populus trichocarpa* and *Pseudotsuga menziesii* (Plett et al., 2015), indicating that this mutualistic effector may play a similar role in the roots of different host tree species than poplars. To understand the evolutionary dynamics of the *L. bicolor* genome, it will be worth investigating whether MiSSP7 affects JA

signaling also in the roots of different hosts of *L. bicolor*, or performs different functions in the roots of distinct tree species.

The discovery that also mutualistic microbes can manipulate plant immune signaling in order to ensure infection challenged the idea of a clear-cut distinction between beneficial and detrimental microbes in evolutionary and functional terms (Plett & Martin, 2015; Martin et al., 2016). As such, differences in colonization strategies among microbial species would depend more on their evolutionary history rather than on their trophic mode and on their impact on plant fitness. The initial conception that symbionts and pathogens exploited separate strategies for plant colonization came from the study of the mechanisms of arbuscular mycorrhizal (AM) symbiosis and root nodule symbiosis (RNS) development, which utilize partially overlapping signaling pathways whose common components form the ancient Common Symbiosis Pathway (CSP; Oldroyd, 2013; Martin et al., 2017). The CSP seemed a more refined and concerted pathway than effector-dependent evasion of plant immunity, promoting the idea that plants were “willingly” colonized by beneficial microbes. However, the CSP seems only marginally involved in the establishment of ECM symbiosis between poplar and *L. bicolor* (Cope et al., 2019), and the absence of many CSP genes in Pinaceae suggests that this signaling pathway is not required for a wide range of mutualistic fungus-tree interactions (Garcia et al., 2015). Therefore, some ECM fungi may have evolved the ability to co-opt the CSP for host colonization, while others employ different molecular mechanisms to communicate with their hosts, such as the emission of volatile signals, the biosynthesis of phytohormones, and the secretion of effector proteins (Garcia et al., 2015). This variety reflects the phylogenetic history of ECM fungi, which rose several times independently from saprotrophic ancestors and do not form a compact phylogenetic clade like AM fungi (Kohler et al., 2015). Thus, by means of convergent evolution, different species of ectomycorrhizal fungi acquired the ability to develop morphologically and functionally similar structures in association to tree roots, albeit using different molecular mechanisms and expressing species-specific sets of symbiosis-related genes (Martin et al., 2016).

The observation that ectomycorrhizal basidiomycetes display an enlarged array of candidate effectors in their genomes suggests that convergent evolution of MiSSPs is relevant for the appearance of the ECM lifestyle (Kohler et al., 2015). As stated earlier, effector proteins are fundamental for successful infection of many pathogenic bacteria and fungi (Kazan & Lyons, 2014). Moreover, also the AM fungus *Glomus intraradices* and the beneficial endophyte *Piriformospora indica* secrete effector proteins promoting colonization (Kloppholtz et al., 2011; Akum et al., 2015). These observations suggest that the evolution of effectors is a common mechanism for microbes of different lifestyles to avoid rejection from the target host plant. Thus, the study of effector biology also hints that the classification of microbes into saprotrophs, pathogens, and mutualists is rather artificial and human-biased,

and does not necessarily reflect the evolutionary history or the colonization strategy of such microorganisms (Plett & Martin, 2015).

4. Future research and perspectives

4.1. Further experiments needed to complete this work

Despite the key highlights provided by our research project, additional experimental evidence is required to validate our working model predicting the involvement of different branches of JA signaling in ECM development. For instance, high-throughput RNA sequencing will be useful to determine the transcriptomic responses to overexpression of PtaMYC2 and PtaJAM1 TFs in colonized poplar roots. We expect that these TFs regulate the expression of at least part of the JA-responsive genes identified in this research project. If specific transcriptional patterns were found for each PtJAZ6-associated TF, it would suggest that MiSSP7-driven alterations in the strength of PtJAZ6-TF interactions bear defined functional consequences. In particular, we are interested in unraveling whether PtaMYC2.1 and PtaMYC2.2 regulate the expression of different groups of target genes. The identification of genes belonging to either the PtaMYC2.1- or the PtaMYC2.2-branch of JA signaling will indicate which JA-dependent responses need to be specifically repressed during ectomycorrhizal colonization of poplar roots, and will confirm whether a subset of JA-responsive genes needs to be activated for optimal apoplastic penetration of fungal hyphae.

Since Arabidopsis MYC2 hierarchically regulates a transcriptional cascade involving the activation of additional TFs (Kazan & Manners, 2013), it could be interesting to assess if such regulatory cascade is conserved in poplar. Performing Chromatine ImmunoPrecipitation coupled to sequencing (ChIP-seq) on HA-tagged PtaMYC2s in transgenic poplar lines would allow to identify the promoters directly bound by these TFs, and thus determine the direct targets of PtaMYC2.1 and PtaMYC2.2. Secondly, the comparison between this data and the results of RNA-seq may provide information on the extent of signal amplification via molecular relay to lower-rank TFs. Finally, a short-list of PtaMYC2.1 and PtaMYC2.2 direct targets might unravel previously unknown functions of JA signaling, not related to defense or cell wall remodeling, in the regulation of ECM symbiosis development.

Since the amino acid sequences of PtMYC2.1 and PtMYC2.2 display 86.5% identity, and they are almost identical in their DNA-binding domains, it is possible that these TFs perform similar biochemical functions, binding to the same regulatory proteins and to the same promoter sequences of target genes. If this is the case, it would be interesting to analyze whether tissue- or cell type-specific expression patterns exist for these two paralogous proteins, via the expression of promoter-reporter fusions or antisense RNA-based mRNA localization. Similar techniques could be applied to investigate the extent and the localization of PtaJAM1.1-mediated repression of gene expression. Discoveries in this context might

disclose whether the indirect influence of MiSSP7 on poplar transcription needs to occur locally or systemically for the promotion of fungal colonization.

4.2. Further lines of research

4.2.1. Manipulation of phytohormone signaling by sRNAs produced by trees or mutualistic fungi

We serendipitously discovered that the overexpression of *PtaMYC2.1* is suppressed in mycorrhizal roots, hinting at the regulation of *PtaMYC2.1* expression at the mRNA level. Similarly, an ECM-dependent downregulation of *PtaMYC2.2* and *PtaJAM1.1* expression was detected in lateral roots of transgenic poplar lines, but not in wild-type lateral roots. Also, in transgenic roots, especially when only lateral roots were sampled, the basal expression of *PtaMYC2.1* and *PtaMYC2.2* tended to be higher than in wild-type lateral roots. Altogether, these results suggest that genetic transformation slightly stimulated JA signaling and/or rendered roots more sensitive to wounding, resulting from sampling of lateral roots. However, it is also possible that *PtaMYC2.1*, *PtaMYC2.2*, and *PtaJAM1.1* transcripts accumulate locally in transgenic lateral roots, and that *L. bicolor* inoculation diminishes the pool of *PtaMYC2.1, PtaMYC2.2*, and *PtaJAM1.1* mRNAs locally rather than systemically. This phenomenon and its possible implications in ECM development deserve further attention in future research.

Also, this discovery suggests that the expression of (at least) *PtaMYC2.1* is controlled by RNA interference (RNAi) by non-coding small RNAs (sRNAs). sRNAs and the RNAi machinery are commonly used to regulate gene expression in both plants and microbes (Song et al., 2019 Katiyar-Agarwal & Jin, 2010; Mallory & Vaucheret, 2006). sRNAs are mainly distinguished between microRNAs (miRNAs) and short-interfering RNAs (siRNAs). miRNAs are 20-24 nt-long RNA fragments derived from the cleavage of single-stranded RNA precursors with stem-loop structures, whereas siRNAs are 21-40 nt-long RNA fragments emerging from the maturation of long double-stranded RNA precursors. Both miRNA and siRNA are derived from the enzymatic activity of ribonuclease III-like enzymes Dicer or Dicer-like (DCL) on a precursor RNA molecule, and loaded onto ARGONAUTE (AGO) proteins in order to form the RNA-induced silencing complex (RISC). By targeting complementary mRNA sequences, miRNA and siRNA can affect the expression of their target gene through steric hindrance of the ribosomal machinery, induced mRNA degradation, or the induction of heterochromatic DNA methylation (Song et al., 2019). In recent years it was discovered that pathogenic fungi or symbiotic rhizobia can transfer sRNAs into host cells and exploit the host RNAi machinery to affect the expression of selected host genes and promote infection (Weiberg et al., 2013; Wang et al., 2017; Ren et al., 2019; Huang et al., 2019). Conversely,

plants can transport sRNAs into cells of pathogenic fungi and oomycetes to silence genes involved in pathogen virulence (Zhang et al., 2016; Jiao & Peng, 2018; Cai et al., 2018; Hou et al., 2019). To our knowledge, symbiotic RNA transfer between plants and mycorrhizal fungi has not yet been demonstrated, although *L. bicolor*-colonized *P. trichocarpa* roots display differential expression of 357 putative miRNAs, six of which are complementary to the sequences of four fungal genes (Mewalal et al., 2019). Since *L. bicolor* utilizes effector proteins to alter host responses, similarly to pathogenic fungi, we could expect that this mutualistic microbe has also evolved RNAi-based strategies to control the host transcriptome. Therefore, it would be interesting to assess if suppressed accumulation of *PtaMYC2.1* transcripts in colonized poplar roots depends on sRNAs, and whether such molecules derived from the plant or from the fungus. In a wider perspective, it will be worth investigating if cross-kingdom RNA transfer occurs also in the establishment the ectomycorrhizal symbiosis. sRNA-seq and the annotation of putative *L. bicolor* miRNA and siRNA precursors may disclose a new chapter in the dissection of the molecular dialogue between trees and fungi.

4.2.2. Investigation of additional mutualistic effectors targeting other phytohormone signaling pathways

In addition to MiSSP7, over 50 small-secreted proteins are produced by *L. bicolor* mycelium when in contact with host roots (Martin et al., 2008). One of them has been identified as a β-1,4 endoglucanase possibly involved in host cell wall remodeling (Zhang et al., 2018), while another seems implied in hyphal aggregation for efficient adhesion to the root surface (Pellegrin et al., 2019). In addition, MiSSP7.6 may also influence the transcription of poplar genes related to defense by targeting nuclear trihelix transcription factors (Kang & Martin, unpublished results). However, the function of many other MiSSPs has not yet been investigated. Since the balance of hormonal signaling is fundamental for ECM development, it would be interesting to assess whether additional components of plant hormone signaling pathways are targeted by *L. bicolor* secreted effectors. Such studies would deepen current knowledge on the complexity of the regulatory pathways underpinning the establishment of mutualism between trees and fungi.

4.2.3. Altered phytohormone signaling by mutualistic fungi: does it impact the poplar root microbiome?

In order to set the current model of ECM development between poplar and *L. bicolor* in a wider ecological context, it would be interesting to assess whether fungus-induced changes

in phytohormone homeostasis and perception of host roots affected the interactions between poplar roots and other soil microbes. Indeed, tree-associated microbial communities play key roles in the promotion of plant growth, nutrition, and health (Hacquard & Schadt, 2015). Several plant species can modulate the composition, structure, and function of their root-associated microbiome according to developmental and environmental stimuli, via the secretion of specialized root exudates (Berendsen et al., 2012; Chaparro et al., 2013; Castrillo et al., 2017; Sasse et al., 2018). Phytohormones can either be a component of root exudates or alter their composition by affecting root metabolism. For instance, root-secreted strigolactones stimulate branching of beneficial AM fungi, increasing the likelihood of their contact with host roots (Akiyama et al., 2005); on the other hand, systemic JA signaling can determine the production of specific root exudates in order to select microbial communities that might help plants cope with biotic stress (Carvalhais et al., 2013, 2015), while impaired SA signaling affects the abundance of specific bacterial phyla in Arabidopsis roots (Lebeis et al., 2015).

In this context, it would be interesting to investigate if altered phytohormone metabolism and perception upon *L. bicolor* colonization of poplar roots affected the poplar rhizospheric and endophytic microbial communities, and whether these alterations bore functional consequences for tree physiology, nutrition, and health. For example, the transient response against biotic stress taking place in the early stage of *L. bicolor* colonization might affect the composition and/or the functions of rhizospheric/endophytic bacteria, archea and fungi, with consequences on tree physiology or symbiotic development. Alternatively, the repression of JA signaling by MiSSP7 may hamper tree immunity and allow root colonization by additional microbes that would be otherwise rejected. The potential consequences for the root microbiome of increased SA accumulation during ECM development also deserve attention. Since several bacterial species can promote the development of ECM symbiosis (Frey-Klett et al., 2007), it would be particularly interesting if fungal modulation of plant hormonal signaling were contributing to the recruitment of such mycorrhiza-helper bacteria. Pursuing this line of research may allow to disentangle the complexity of multipartite interactions between trees and microbes in a natural context.

5. References

Akiyama, K., Matsuzaki, K.I., and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature **435**: 824–827.

Akum, F.N., Steinbrenner, J., Biedenkopf, D., Imani, J., and Kogel, K.H. (2015). The *Piriformospora indica* effector PIIN_08944 promotes the mutualistic Sebacinalean symbiosis. Front. Plant Sci. **6**: 906.

Balestrini, R. and Kottke, I. (2016). Structure and development of ectomycorrhizal roots. In Martin F. ed, Molecular Mycorrhizal Symbiosis. Hoboken, NJ, USA: John Wiley & Sons, 47–62.

Basso, V. and Veneault-Fourrey, C. The role of jasmonates in beneficial microbe-root interactions. In Jasmonate in Plant Biology.

Berendsen, R.L., Pieterse, C.M.J., and Bakker, P.A.H.M. (2012). The rhizosphere microbiome and plant health. Trends Plant Sci. **17**: 478–486.

Blasius, D., Feil, W., Kottke, I., and Oberwinkler, F. (1986). Hartig net structure and formation in fully ensheathed ectomycorrhizas. Nord. J. Bot. **6**: 837–842.

Cai, Q., Qiao, L., Wang, M., He, B., Lin, F., Palmquist, J., and Jin, H. (2018). Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science. **360**: 1126–1129.

Carvalhais, L.C., Dennis, P.G., Badri, D. V., Kidd, B.N., Vivanco, J.M., and Schenk, P.M. (2015). Linking Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. Mol. Plant-Microbe Interact. **28**: 1049–1058.

Carvalhais, L.C., Dennis, P.G., Badri, D. V., Tyson, G.W., Vivanco, J.M., and Schenk, P.M. (2013). Activation of the Jasmonic Acid Plant Defence Pathway Alters the Composition of Rhizosphere Bacterial Communities. PLoS One **8**(2): e56457.

Castrillo, G., Teixeira, P.J.P.L., Paredes, S.H., Law, T.F., De Lorenzo, L., Feltcher, M.E., Finkel, O.M., Breakfield, N.W., Mieczkowski, P., Jones, C.D., Paz-Ares, J., and Dangl, J.L. (2017). Root microbiota drive direct integration of phosphate stress and immunity. Nature **543**: 513–518.

Chaparro, J.M., Badri, D. V., Bakker, M.G., Sugiyama, A., Manter, D.K., and Vivanco, J.M. (2013). Root Exudation of Phytochemicals in Arabidopsis Follows Specific Patterns That Are Developmentally Programmed and Correlate with Soil Microbial Functions. PLoS One **8**: 1–10.

Cicatelli, A., Todeschini, V., Lingua, G., Biondi, S., Torrigiani, P., and Castiglione, S. (2014). Epigenetic control of heavy metal stress response in mycorrhizal versus non-mycorrhizal poplar plants. Environ. Sci. Pollut. Res. **21**: 1723–1737.

Cope, K.R. et al. (2019). The Ectomycorrhizal Fungus *Laccaria bicolor* Produces Lipochitooligosaccharides and Uses the Common Symbiosis Pathway to Colonize *Populus* Roots. Plant Cell: tpc.00676.2018.

Ditengou, F.A., Béguiristain, T., and Lapeyrie, F. (2000). Root hair elongation is inhibited by hypaphorine, the indole alkaloid from the ectomycorrhizal fungus *Pisolithus tinctorius*, and restored by indole-3-acetic acid. Planta **211**: 722–728.

Ditengou, F.A., Müller, A., Rosenkranz, M., Felten, J., Lasok, H., Van Doorn, M.M., Legué, V., Palme, K., Schnitzler, J.P., and Polle, A. (2015). Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. Nat. Commun. **6**: 6279.

Duplessis, S., Courty, P.-E., Tagu, D., and Martin, F. (2005). Transcript patterns associated with ectomycorrhiza development in *Eucalyptus globulus* and *Pisolithus microcarpus*. New Phytol. **165**: 599–611.

Felten, J., Kohler, A., Morin, E., Bhalerao, R.P., Palme, K., Martin, F., Ditengou, F.A., and Legué, V. (2009). The Ectomycorrhizal Fungus *Laccaria bicolor* Stimulates Lateral Root Formation in Poplar and Arabidopsis through Auxin Transport and Signaling . Plant Physiol. **151**: 1991-2005.

Ferguson, B.J. and Mathesius, U. (2014). Phytohormone Regulation of Legume-Rhizobia Interactions. J. Chem. Ecol. **40**: 770–790.

Fernández, I., Merlos, M., López-Ráez, J.A., Martínez-Medina, A., Ferrol, N., Azcón, C., Bonfante, P., Flors, V., and Pozo, M.J. (2014). Defense Related Phytohormones Regulation in Arbuscular Mycorrhizal Symbioses Depends on the Partner Genotypes. J. Chem. Ecol. **40**: 791–803.

Frankenberger, W.T. and Poth, M. (1987). Biosynthesis of Indole-3-Acetic Acid by the Pine Ectomycorrhizal Fungus Pisolithus tinctorius. Appl. Environ. Microbiol. **53**: 2908–2913.

Frey-Klett, P., Garbaye, J., and Tarkka, M. (2007). The mycorrhiza helper bacteria revisited. New Phytol. **176**: 22–36.

Garcia, K., Delaux, P.-M., Cope, K.R., and Ané, J.-M. (2015). Molecular signals required for the establishment and maintenance of ectomycorrhizal symbioses. New Phytol. **208**: 79–87.

Gimenez-Ibanez, S., Boter, M., Fernández-Barbero, G., Chini, A., Rathjen, J.P., and Solano, R. (2014). The Bacterial Effector HopX1 Targets JAZ Transcriptional Repressors to Activate Jasmonate Signaling and Promote Infection in Arabidopsis. PLoS Biol. **12**: e1001792.

Gimenez-Ibanez, S., Chini, A., and Solano, R. (2016). How microbes twist jasmonate signaling around their little fingers. Plants **5**: 323–329.

Gou, J., Strauss, S.H., Tsai, C.J., Fang, K., Chen, Y., Jiang, X., and Busov, V.B. (2010). Gibberellins Regulate Lateral Root Formation in *Populus* through Interactions with Auxin and Other Hormones. Plant Cell **22**: 623–639.

Gutjahr, C. (2014). Phytohormone signaling in arbuscular mycorhiza development. Curr. Opin. Plant Biol. **20**: 26–34.

Hacquard, S. and Schadt, C.W. (2015). Towards a holistic understanding of the beneficial interactions across the *Populus* microbiome. New Phytol. **205**: 1424–1430.

Herrera-Medina, M.J., Tamayo, M.I., Vierheilig, H., Ocampo, J.A., and García-Garrido, J.M. (2008). The jasmonic acid signalling pathway restricts the development of the arbuscular mycorrhizal association in tomato. J. Plant Growth Regul. **27**: 221–230.

Horan, D.P., Chilvers, G.A., and Lapeyrie, F.F. (1988). Time sequence of the infection process in eucalypt ectomycorrhizas. New Phytol. **109**: 451–458.

Hou, Y. et al. (2019). A *Phytophthora* Effector Suppresses Trans-Kingdom RNAi to Promote Disease Susceptibility. Cell Host Microbe **25**: 153-165.e5.

Huang, C.-Y., Wang, H., Hu, P., Hamby, R., and Jin, H. (2019). Small RNAs – Big Players in Plant-Microbe Interactions. Cell Host Microbe **26**: 173–182.

Ivanchenko, M.G., Muday, G.K., and Dubrovsky, J.G. (2008). Ethylene-auxin interactions regulate lateral root initiation and emergence in *Arabidopsis thaliana*. Plant J. **55**: 335–347.

Jacobs, S., Zechmann, B., Molitor, A., Trujillo, M., Petutschnig, E., Lipka, V., Kogel, K.-H., and Schafer, P. (2011). Broad-Spectrum Suppression of Innate Immunity Is Required for Colonization of Arabidopsis Roots by the Fungus *Piriformospora indica*. Plant Physiol. **156**: 726–740.

Jiang, S., Yao, J., Ma, K.W., Zhou, H., Song, J., He, S.Y., and Ma, W. (2013). Bacterial Effector Activates Jasmonate Signaling by Directly Targeting JAZ Transcriptional Repressors. PLoS Pathog. **9**(10): e1003715.

Kaling, M., Schmidt, A., Moritz, F., Rosenkranz, M., Witting, M., Kasper, K., Janz, D., Schmitt-Kopplin, P., Schnitzler, J.-P., and Polle, A. (2018). Mycorrhiza-Triggered Transcriptomic and Metabolomic Networks Impinge on Herbivore Fitness. Plant Physiol. **176**: 2639-2656.

Kang, H. and Martin, F. The small secreted effector protein MiSSP7.6 of *Laccaria bicolor* is required for the establishment of ectomycorrhizal symbiosis. Unpublished results.

Katiyar-Agarwal, S. and Jin, H. (2010). Role of Small RNAs in Host-Microbe Interactions. Annu. Rev. Phytopathol. **48**: 225–246.

Kazan, K. and Lyons, R. (2014). Intervention of Phytohormone Pathways by Pathogen Effectors. Plant Cell **26**: 2285–2309.

Kazan, K. and Manners, J.M. (2013). MYC2: The master in action. Mol. Plant **6**: 686–703.

Kloppholz, S., Kuhn, H., and Requena, N. (2011). A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. Curr. Biol. **21**: 1204–1209.

Kohler, A. et al. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat. Genet. **47**: 410.

Landgraf, R., Schaarschmidt, S., and Hause, B. (2012). Repeated leaf wounding alters the colonization of *Medicago truncatula* roots by beneficial and pathogenic microorganisms. Plant, Cell Environ. **35**: 1344–1357.

Laskowski, M., Grieneisen, V.A., Hofhuis, H., Ten Hove, C.A., Hogeweg, P., Marée, A.F.M., and Scheres, B. (2008). Root system architecture from coupling cell shape to auxin transport. PLoS Biol. **6**: e307.

Lebeis, S.L., Paredes, S.H., Lundberg, D.S., Breakfield, N., Gehring, J., McDonald, M., Malfatti, S., Glavina del Rio, T., Jones, C.D., Tringe, S.G., and Dangl, J.L. (2015). Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science. **349**: 860–864.

Lee, H.Y., Chen, Y.C., Kieber, J.J., and Yoon, G.M. (2017). Regulation of the turnover of ACC synthases by phytohormones and heterodimerization in Arabidopsis. Plant J. **91**: 491–504.

Lei, J., Wong, K.K.Y., and Piché, Y. (1991). Extracellular Concanavalin A-binding sites during early interactions between *Pinus banksiana* and two closely related genotypes of the ectomycorrhizal basidiomycete *Laccaria bicolor*. Mycol. Res. **95**(3): 357-363.

Lewis, D.R., Negi, S., Sukumar, P., and Muday, G.K. (2011). Ethylene inhibits lateral root development, increases IAA transport and expression of PIN3 and PIN7 auxin efflux carriers. Development **138**: 3485–3495.

Liu, J., Blaylock, L.A., Endre, G., Cho, J., Town, C.D., Vandenbosch, K.A., and Harrison, M.J. (2003). Transcript Profiling Coupled with Spatial Expression Analyses Reveals Genes Involved in Distinct Developmental Stages of an Arbuscular Mycorrhizal Symbiosis. Plant Cell **15**: 2106–2123.

Luo, Z.-B., Janz, D., Jiang, X., Gobel, C., Wildhagen, H., Tan, Y., Rennenberg, H., Feussner, I., and Polle, A. (2009). Upgrading Root Physiology for Stress Tolerance by Ectomycorrhizas: Insights from Metabolite and Transcriptional Profiling into Reprogramming for Stress Anticipation. Plant Physiol. **151**: 1902–1917.

Mallory, A.C. and Vaucheret, H. (2006). Functions of microRNAs and related small RNAs in plants. Nat. Genet. **38**: S31–S36.

Martin, F. et al. (2008). The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature **452**: 88–92.

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., and Hibbett, D.S. (2016). Unearthing the roots of ectomycorrhizal symbioses. Nat. Rev. Microbiol. **14**: 760–773.

Martin, F.M., Uroz, S., and Barker, D.G. (2017). Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria. Science. **356**: eead4501.

Massicotte, H.B., Peterson, R.L., Ackerley, C.A., and Ashford, A.E. (1987). Ontogeny of *Eucalyptus pilularis – Pisolithus tinctorius* ectomycorrhizae. II. Transmission electron microscopy. Can. J. Bot. **65**: 1940–1947.

Mewalal, R., Yin, H., Hu, R., Jawdy, S., Vion, P., Tuskan, G.A., Tacon, F. Le, Labbé, J.L., and Yang, X. (2019). Identification of *Populus* small RNAs responsive to mutualistic interactions with mycorrhizal fungi, *Laccaria bicolor* and *Rhizophagus irregularis*. Front. Microbiol. **10**: 515.

Negi, S., Ivanchenko, M.G., and Muday, G.K. (2008). Ethylene regulates lateral root formation and auxin transport in *Arabidopsis thaliana*. Plant J. **55**: 175–187.

Niemi, K., Vuorinen, T., Ernstsen, A., and Häggman, H. (2002). Ectomycorrhizal fungi and exogenous auxins influence root and mycorrhiza formation of Scots pine hypocotyl cuttings *in vitro*. Tree Physiol. **22**: 1231–1239.

Oldroyd, G.E.D. (2013). Speak, friend, and enter: Signalling systems that promote beneficial symbiotic associations in plants. Nat. Rev. Microbiol. **11**: 252–263.

Pellegrin, C., Martin, F., and Veneault-Fourrey, C. (2019). Molecular Signalling During the Ectomycorrhizal Symbiosis. Biol. Fungal Cell: 95–109.

Plett, J.M., Daguerre, Y., Wittulsky, S., Vayssieres, A., Deveau, A., Melton, S.J., Kohler, A., Morrell-Falvey, J.L., Brun, A., Veneault-Fourrey, C., and Martin, F. (2014a). Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. Proc. Natl. Acad. Sci. **111**: 8299–8304.

Plett, J.M., Khachane, A., Ouassou, M., Kohler, A., and Martin, F. (2014b). Ethylene and jasmonic acid act as negative modulators during mutualistic symbiosis between *Laccaria bicolor* and *Populus* roots. New Phytol. **202**: 270–286.

Plett, J.M. and Martin, F. (2015). Reconsidering mutualistic plant-fungal interactions through the lens of effector biology. Curr. Opin. Plant Biol. **26**: 45–50.

Plett, J.M., Tisserant, E., Brun, A., Morin, E., Grigoriev, I. V, Kuo, A., Martin, F., and Kohler, A. (2015). The Mutualist *Laccaria bicolor* Expresses a Core Gene Regulon During the Colonization of Diverse Host Plants and a Variable Regulon to Counteract Host-Specific Defenses. Mol. Plant Microbe Interact. **28**: 261–273.

Regvar, M., Gogala, N., and Zalar, P. (1996). Effect of jasmonic acid on mycorrhizal *Allium sativum*. New Phytol **134**: 703–707.

Ren, B., Wang, X., Duan, J., and Ma, J. (2019). Rhizobial tRNA-derived small RNAs are signal molecules regulating plant nodulation. Science. **365**: 919–922.

Riedel, T., Groten, K., and Baldwin, I.T. (2008). Symbiosis between Nicotiana attenuata and Glomus intraradices: Ethylene plays a role, jasmonic acid does not. Plant, Cell Environ. **31**: 1203–1213.

Sasse, J., Martinoia, E., and Northen, T. (2018). Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? Trends Plant Sci. **23**: 25–41.

Siciliano, V., Genre, A., Balestrini, R., Cappellazzo, G., deWit, P.J.G.M., and Bonfante, P. (2007). Transcriptome Analysis of Arbuscular Mycorrhizal Roots during Development of the Prepenetration Apparatus. Plant Physiol. **144**: 1455–1466.

Song, X., Li, Y., Cao, X., and Qi, Y. (2019). MicroRNAs and Their Regulatory Roles in Plant–Environment Interactions. Annu. Rev. Plant Biol. **70**: 489–525.

Splivallo, R., Fischer, U., Göbel, C., Feussner, I., and Karlovsky, P. (2009). Truffles Regulate Plant Root Morphogenesis via the Production of Auxin and Ethylene. Plant Physiol. **150**: 2018–2029.

Tagu, D. and Martin, F. (1996). Molecular analysis of cell wall proteins expressed during the early steps of ectomycorrhiza development. New Phytol.

Toby Kiers, E., Adler, L.S., Grman, E.L., and van der Heijden, M.G.A. (2010). Manipulating the jasmonate response: How do methyl jasmonate additions mediate characteristics of aboveground and belowground mutualisms? Funct. Ecol. **24**: 434–443.

Tschaplinski, T.J., Plett, J.M., Engle, N.L., Deveau, A., Cushman, K.C., Martin, M.Z., Doktycz, M.J., Tuskan, G.A., Brun, A., Kohler, A., and Martin, F. (2014). *Populus trichocarpa* and *Populus deltoides* exhibit different metabolomic responses to colonization by the symbiotic fungus *Laccaria bicolor*. Mol. Plant-Microbe Interact. **27**: 546–556.

Vahabi, K., Sherameti, I., Bakshi, M., Mrozinska, A., Ludwig, A., Reichelt, M., and Oelmüller, R. (2015). The interaction of Arabidopsis with *Piriformospora indica* shifts from initial transient stress induced by fungus-released chemical mediators to a mutualistic interaction after physical contact of the two symbionts. BMC Plant Biol. **15**: 58: 1–15.

Vayssières, A., Pěnčík, A., Felten, J., Kohler, A., Ljung, K., Martin, F., and Legué, V. (2015). Development of the poplar-*Laccaria bicolor* ectomycorrhiza modifies root auxin metabolism, signaling, and response. Plant Physiol. **169**: 890–902.

Veneault-fourrey, C., Commun, C., Kohler, A., Morin, E., Balestrini, R., Plett, J., Danchin, E., Coutinho, P., Wiebenga, A., Vries, R.P. De, Henrissat, B., and Martin, F. (2014). Genomic and transcriptomic analysis of *Laccaria bicolor* CAZome reveals insights into polysaccharides remodelling during symbiosis establishment. Fungal Genet. Biol. **72**: 168–181.

Wang, M., Weiberg, A., Dellota, E., Yamane, D., and Jin, H. (2017). *Botrytis* small RNA *Bc-siR37* suppresses plant defense genes by cross-kingdom RNAi. RNA Biol. **14**: 421–428.

Weiberg, A., Wang, M., Lin, F.-M., Zhao, H., Zhang, Z., Kaloshian, I., Huang, H.-D., Jin, H., Khaloshian, I., Huang, H.-D., and Jin, H. (2014). Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways. Science. **342** (6154): 118–123.

Yang, L., Teixeira, P.J.P.L., Biswas, S., Finkel, O.M., He, Y., Salas-Gonzalez, I., English, M.E., Epple, P., Mieczkowski, P., and Dangl, J.L. (2017). *Pseudomonas syringae* Type III Effector HopBB1 Promotes Host Transcriptional Repressor Degradation to Regulate Phytohormone Responses and Virulence. Cell Host Microbe **21**: 156–168.

Yi, K., Menand, B., Bell, E., and Dolan, L. (2010). A basic helix-loop-helix transcription factor controls cell growth and size in root hairs. Nat. Genet. **42**: 264–267.

Zhang, F. et al. (2018). The ectomycorrhizal basidiomycete *Laccaria bicolor* releases a secreted β-1,4 endoglucanase that plays a key role in symbiosis development. New Phytol. **220**: 1309–1321.

Zhang, F. et al. The ectomycorrhizal basidiomycete *Laccaria bicolor* releases a secreted pectinase that plays a key role in symbiosis establishment. Unpublished results.

Zhang, L., Zhang, F., Melotto, M., Yao, J., and He, S.Y. (2017). Jasmonate signaling and manipulation by pathogens and insects. J. Exp. Bot. **68**: 1371–1385.

Zhang, T., Zhao, Y.L., Zhao, J.H., Wang, S., Jin, Y., Chen, Z.Q., Fang, Y.Y., Hua, C.L., Ding, S.W., and Guo, H.S. (2016). Cotton plants export microRNAs to inhibit virulence gene expression in a fungal pathogen. Nat. Plants **2**: 16153.

Zheng, X.Y., Weaver Spivey, N., Zeng, W., Liu, P.-P., Fu, Z.Q., Klessig, D.F., He, S.Y., and Dong, X. (2014). Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. Cell Host Microbe **11**: 587–596.

Résumé

Les ectomycorhizes (ECM) sont des interactions mutualistes essentielles dans les écosystèmes forestiers, car elles promeuvent la croissance des arbres et participent aux cycles des éléments nutritifs. Cependant, les signaux et les mécanismes moléculaires qui sous-tendent leur développement sont encore mal connus. En utilisant le modèle d'étude entre peuplier et le champignon *Laccaria bicolor*, il a été montré que la protéine fongique MiSSP7 (Mycorrhiza-induced Small Secreted Protein 7 kDa) est sécrétée, pénètre les noyaux des cellules racinaires corticales de son arbre hôte où elle stabilise PtJAZ6, le répresseur de la voie de signalisation de l'acide jasmonique (AJ). Cette stabilisation inhibe les réponses médiées par l'AJ et permettent l'établissement de la symbiose. La signalisation de l'AJ est impliquée dans la défense et le développement des plantes, mais on ignore actuellement quelles sont les réponses dépendantes de l'AJ qui doivent être supprimées pour autoriser le développement de la symbiose ECM. C'est pourquoi, dans cette thèse, nous tentons de répondre aux questions suivantes: (i) Comment la protéine effectrice MiSSP7 influence-t-elle la structure du complexe de perception de l'AJ dans les racines de peuplier? (ii) Des branches distinctes de la signalisation de l'AJ jouent-elles des rôles spécifiques lors de l'établissement de l'ECM? (iii) L'interaction entre la voie de signalisation de l'AJ et d'autres voies de signalisation hormonale influence-t-elle le développement symbiotique?

Nous avons identifié le complexe protéique associé à PtJAZ6 dans les racines de peuplier et avons montré que MiSSP7 module la force des interactions entre PtJAZ6 et ses facteurs de transcription associés (FT). La modification de l'expression des gènes codant pour ces FT dans les racines de peuplier, par transformation génétique, influence la maturation des ECM, indiquant que les FT associés à PtJAZ6 régulent cette interaction mutualiste. De plus, en couplant une analyse hormonomique à une analyse physiologique et transcriptomique de racines de peuplier et de mycélium de *L. bicolor* traités aux hormones, nous avons démontré que des modifications de la biosynthèse et de la perception des phytohormones ont lieu au cours du développement de l'ECM. Pris ensemble, nos résultats suggèrent un double rôle de la signalisation de l'AJ dans le développement de l'ECM avec une branche fonctionnant pour réguler la pénétration apoplastique des hyphes fongiques et une autre étant responsable de l'exclusion du champignon dans des conditions de stress. Nous proposons que la régulation fine de la signalisation des phytohormones, et en particulier de l'AJ, est cruciale pour l'intégration des signaux fongiques et végétaux, afin de reprogrammer à la fois la physiologie des cellules racinaire et des hyphes pour le développement de l'ECM.

Mots-clefs: symbiose, ectomycorhize, phythohormones, acide jasmonique

Abstract

Promoting nutrient cycling and tree fitness, ectomycorrhizae (ECM) are mutualistic interactions pivotal in forest ecosystems. However not much is known about the signals and molecular mechanisms underpinning their establishment. Using *Populus* and *Laccaria bicolor* as a model system, it was shown that the fungal Mycorrhiza-Induced Small Secreted Protein of 7 kDa (MiSSP7) is secreted upon ECM establishment, penetrates the nuclei of cortical root cells of its host and stabilizes the repressor of jasmonic acid (JA) signaling PtJAZ6, dampening plant responses to JA and promoting mutualism. JA signaling is implied in plant defense and development, but it is currently unknown which part of JA-dependent responses in host roots need to be suppressed for ECM formation. Therefore, the thesis aims to answer the following questions: (i) how does the effector protein MiSSP7 impact the structure of the JA perception complex in poplar roots? (ii) do distinct branches of JA signaling play specific roles during ECM establishment? (iii) does the interplay between JA signaling and other hormone signaling pathways influence symbiotic development?
Through protein-protein interaction studies, we deciphered the composition of the PtJAZ6-associated protein complex in poplar roots and showed that MiSSP7 modulates the strength of interactions between PtJAZ6 and its associated transcription factors (TFs). Altered expression of the genes coding for such TFs in poplar roots, through genetic engineering, influences ECM maturation, indicating that PtJAZ6-binding TFs regulate the extent of this mutualistic interaction. In addition, by means of hormonomics coupled with physiologic and transcriptomic analysis of hormone-treated poplar roots and *L. bicolor* mycelium, we demonstrate that a complex rearrangement of phytohormone biosynthesis and perception takes place in host roots during symbiotic development. In particular, we found that fungus-colonized roots are less sensitive to JA. Altogether, our results suggest a dual role of JA signaling in developing ECM, with one branch functioning to regulate fungal apoplastic penetration to an optimal extent, and another being responsible of fungal exclusion under stress conditions. We propose that finely tuned phytohormone signaling, and in particular JA signaling, is crucial for the integration of fungal and plant-derived signals, in order to reprogram root and mycelial physiology for a successful mutualistic interaction.

Keywords: symbiosis, ectomycorrhiza, phytohormones, jasmonate