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ORIGINAL ARTICLE

Alterations in the phenylpropanoid pathway affect poplar ability for ectomycorrhizal colonisation and susceptibility to root-knot nematodes

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Abstract

This study investigates the impact of the alteration of the monolignol biosynthesis pathway on the establishment of the in vitro interaction of poplar roots either with a mutualistic ectomycorrhizal fungus or with a pathogenic root-knot nematode. Overall, the five studied transgenic lines downregulated for *caffeoyl-CoA* O-*methyltransferase* (*CCoAOMT*), *caffeic acid* O-*methyltransferase* (*COMT*), *cinnamoyl-CoA reductase* (CCR), *cinnamyl alcohol dehydrogenase* (*CAD*) or both *COMT* and *CAD* displayed a lower mycorrhizal colonisation percentage, indicating a lower ability for establishing mutualistic interaction than the wild-type. The susceptibility to root-knot nematode infection was variable in the five lines, and the CAD-deficient line was found to be less susceptible than the wild-type. We discuss these phenotypic differences in the light of the large shifts in the metabolic profile and gene expression pattern occurring between roots of the CAD-deficient line and wild-type. A role of genes related to trehalose metabolism, phytohormones, and cell wall construction in the different mycorrhizal symbiosis efficiency and nematode sensitivity between these two lines is suggested. Overall, these results show that the alteration of plant metabolism caused by the repression of a single gene within phenylpropanoid pathway results in significant alterations, at the root level, in the response towards mutualistic and pathogenic associates. These changes may constrain plant fitness and biomass production, which are of economic importance for perennial industrial crops such as poplar.

Keywords Ectomycorrhiza · Monolignol · Root-knot nematode · Secondary metabolites · Transcriptomics · Transgenic poplar

Marc Behr and Fabien Baldacci-Cresp contributed equally to this work.

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Introduction

The phenylpropanoid pathway is responsible for the biosynthesis of a wide range of phenolic compounds and related polymers, including hydroxycinnamic acids, flavonoids, isoflavonoids, stilbenes, tannins, lignans, and lignin (Vogt 2010). Phenylpropanoids are known to be part of the plant response towards (a)biotic stresses and mutualistic interactions (Naoumkina et al. 2010; Ohri and Pannu 2010). The analysis of mutants and/or transgenic plant species with modified lignin content and composition revealed significant alterations in the response towards diverse fungal and bacterial pathogens (reviewed by Zhao and Dixon 2014). However, the majority of these studies did not include plant-soil organism interactions (Bennett et al. 2015).

In trees, numerous investigations on the possibility to alter the lignin content and/or composition have been performed in poplar, and several field trials have been implemented (reviewed in Pilate et al. 2012; Chanoca et al. 2019). Some of these studies reported that while modified lignin transgenic poplar lines showed an apparently normal development in the greenhouse, their biomass was negatively affected in field conditions. This outcome was reported for instance in lines of transgenic poplars downregulated for *cinnamoyl-CoA reductase* (*CCR*; Leplé et al. 2007; Van Acker et al. 2014) and *4-coumarate CoA ligase* (*4CL*; Voelker et al. 2011; Stout et al. 2014). Although the growth of homozygous *cinnamyl alcohol dehydrogenase* (*cad*) pine mutant was normal under greenhouse conditions (Mackay et al. 1997), this mutant line was characterised by a leaning stem and a reduced growth (2.5 m tall and 6–7 cm in diameter at breast height) as compared to the wild-type (8 m tall and 20–22 cm in diameter at breast height) after 12 years of growth in the field (Dimmel et al. 2001).

In field conditions, trees are confronted by environmental conditions, including (a)biotic stresses, that can influence their growth (Chanoca et al. 2019). For instance, the establishment of mutualistic interaction with ectomycorrhizal fungi is essential for tree growth and tolerance to various stress conditions, as this fungus utilises plant-derived carbohydrates to grow and assimilates essential nitrogen and phosphate compounds in the soil transferring them back to the trees (Martin and Selosse 2008; Szuba 2015; Guerrero-Galán et al. 2019). Ectomycorrhizal fungi are the most predominant fungi on poplar roots representing 87% in their abundance (Danielsen et al. 2012). Previously, the colonisation process of poplar roots by the ectomycorrhizal fungus *Laccaria bicolor* was demonstrated to alter the metabolic programme within the symbiotic tissue (Tschaplinski et al. 2014). In addition, when comparing compatible (*Populus trichocarpa*) and incompatible (*Populus deltoides*) poplar host species, these authors showed differences in the diversity and the half-life of defensive secondary metabolites produced by the host (such as salicin, phloroglucinol, and tryptophan), as well as in the general metabolic responsiveness of roots in the primary stages of colonisation.

As well, poplar was described as a model to study tree and root-knot nematodes (RKN) interaction (Baldacci-Cresp et al. 2016). A downregulation of the genes from the phenylpropanoid pathway upon RKN infection was observed in rice (Kyndt et al. 2012) and poplar (Baldacci-Cresp et al. 2016), while overexpressing *ferulate-5-hydroxylase* (*F5H*) in Arabidopsis led to reduced reproduction of nematodes in vitro (Wuyts et al. 2006a). These results suggest an interaction between RKN development and the phenylpropanoid profile of their hosts.

The availability of poplar lines downregulated for genes of the monolignol pathway allowed the study of the potential effects of these metabolic modifications during particular root-microorganism interactions. This might help to explain at least partly the differences in growth observed between controlled greenhouse and field conditions (Pilate et al. 2002; Chanoca et al. 2019). Previously, shifts in the bacterial community have been reported by profiling the bacterial endosphere microbiome of leaf, root, and stem harvested from field-grown *CCR*-downregulated poplars, but no impact on the rhizosphere microbiota was evidenced (Beckers et al. 2016). Particularly, these authors measured an increased abundance of bacterial cell counts in all tested tissues together with a decrease in operational taxonomic unit richness, evenness, and diversity in roots and stems of the *CCR*-downregulated plants compared with that of the wild-type. In a field trial with *CAD* and *caffeic acid 3-O-methyltransferase* (*COMT*)-downregulated poplars (aged between 2 and 4 years), there was no difference in microbial biomass between soils beneath wild-type and transgenic line root system (Pilate et al. 2002). In contrast, poplar downregulated for *COMT*, *CCR*, or *CAD* grown in the field had a different ectomycorrhizal fungal community composition 2 years after plantation (Danielsen et al. 2012, 2013).

Herein, we evaluated the impact of changes of the monolignol pathway on the capacity of soil-borne microorganisms to establish interactions with poplar roots. Two in vitro interacting systems were evaluated with different transgenic lines downregulated for genes of the monolignol pathway: (i) *Populus tremula* x *P. alba* - *Laccaria bicolor* as a mutualistic interaction, and (ii) *P. tremula* x *P. alba* - *Meloidogyne incognita* as a RKN pathosystem. Our studies revealed modifications in colonisation efficiency by ectomycorrhizal fungi as well as in RKN infection and reproduction. These results are further discussed in the light of molecular changes evidenced by a comparative root transcriptomic analysis in wild-type versus a CAD-deficient line, the latter displaying significant differences in both ectomycorrhizal and nematode interactions.

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Material and methods

Plant, root-knot nematodes, and ectomycorrhizal fungus growth conditions

The different transgenic poplar lines and the wild-type (*P. tremula* x *P. alba* clone INRA 717-1B4) were grown aseptically as described in Baldacci-Cresp et al. (2015). The selected transgenic lines (Online resource 3) are sccoaomt-16 (for *CCoAOMT*; Meyermans et al. 2000), 70SOMT-3 (for *COMT*; Jouanin et al. 2000), FS3 (for *CCR*; Leplé et al. 2007; Van Acker et al. 2014), p70-ASCAD34.2 (for *CAD*; Awad et al. 2012), and ASCAD21 x ASOMT7 (for *CAD*/*COMT*; Lapierre et al. 1999).

RKN infection and analysis was performed as described by Baldacci-Cresp et al. (2016). Counting of galls and egg masses was performed on a total of 41 to 56 plants per line spread on four independent biological replicates, and the statistical validation was performed with a Student's t test (P value <0.05).

The protocol described by Felten et al. (2009) was used for ectomycorrhizae development on in vitro 5-week-old poplar root by *L. bicolor* strain S238N (Maire P.D. Orton). Quantification of the number of lateral roots and the mycorrhizal colonisation percentage (number of mycorrhizal lateral roots/total number of lateral roots) was performed 3 weeks post-inoculation on 9 to 51 individual poplar plants per condition spread on two independent biological replicates. The statistical validation was performed with a Student's t test (P value <0.05). Observation and images were taken using a Discovery V.8 stereomicroscope (Zeiss).

Molecular characterisation of the CAD line used in this study

The ASCAD34.2 line was previously described for its xylem properties and hydraulic traits (Awad et al. 2012), but was not characterised at the molecular level. Measures of lignin content and composition as well as molecular characterisation of the used CAD line are reported in Online resource 4. As the *CAD* downregulation results from the expression of the full-length cDNA sequence of *P. deltoides CAD*, an artefact linked to the inappropriate mapping of sequenced transcripts was suspected. Therefore, a comparison of RNA-Seq reads mapping to *CAD* sequences from *P. tremula x P. alba* and *P. deltoides* was made. Following this analysis, 20% residual *CAD* expression was measured in roots of the CAD line.

Metabolomic and RNA-Seq analyses

Metabolomics and RNA-Seq analyses were performed as described by Baldacci-Cresp et al. (2020). For the metabolomics analysis, roots from the CAD (modified) and the wild-type lines ($n=4$) were frozen in liquid N_2 and ground. A total of 200 mg of root samples was extracted by two volumes of methanol. The RNA-Seq results were validated with RT-qPCR on a set of 6 genes, implicated in several different metabolic pathways. Fold changes of both methods were highly correlated ($r^2=0.98$), confirming the robustness of the transcriptomic data. *PT1*, *CYC063*, and *UBQ22* were used as reference genes (Baldacci-Cresp et al. 2016). The complete RNA-Seq data was submitted to GEO (GSE112673).

Results

Ability for ectomycorrhizal colonisation and susceptibility to root-knot nematodes in transgenic lines

Poplar transgenic lines with genes downregulated in the monolignol biosynthetic pathway, *caffeoyl CoA O-methyltransferase* (*CCoAOMT*), *COMT*, *CCR*, *CAD*, or both *CAD* and *COMT*, were assessed for their ability for mycorrhizal symbiosis and their RKN susceptibility (Fig. 1).

At first, we have evaluated the phenotype of roots grown in the presence of the ectomycorrhizal fungus. A significant increase in the number of lateral roots was observed in both COMT and CCR lines compared with those developed in the absence of the fungus (Fig. 1a). In addition, 3 weeks after in vitro inoculation of poplar roots, all tested transgenic lines had a reduction (by 1.3–2-fold) of the percentage of mycorrhizae compared with the wild-type (Fig. 1b).

Subsequently, the susceptibility of the same transgenic lines to the RKN *M. incognita* was assessed. The number of both galls and egg masses per plant was measured on in vitro plants at 21 and 42 days after infection (dai), respectively (Fig. 1c–d). The susceptibility of the CCoAOMT, COMT, and CAD/COMT lines was not significantly affected as compared with that of the wild-type. However, for the CCR line, the infection rate (increased number of galls) and the reproduction efficiency of RKN (more egg masses) increased. Conversely, a reduction in both infection rate and reproduction efficiency was recorded for the CAD line, as compared with those of the wild-type, indicating a lower susceptibility of this line to RKN.

Comparative metabolite and gene expression profiles in the roots of CAD vs. wild-type lines

To highlight potential molecular factors explaining the observed alterations of the interaction between transgenic poplar lines with either ectomycorrhizae or RKN, the consequences of the genetic alteration on the metabolite and gene expression profiles were investigated. The CAD line was selected as a case study because it was both negatively affected in

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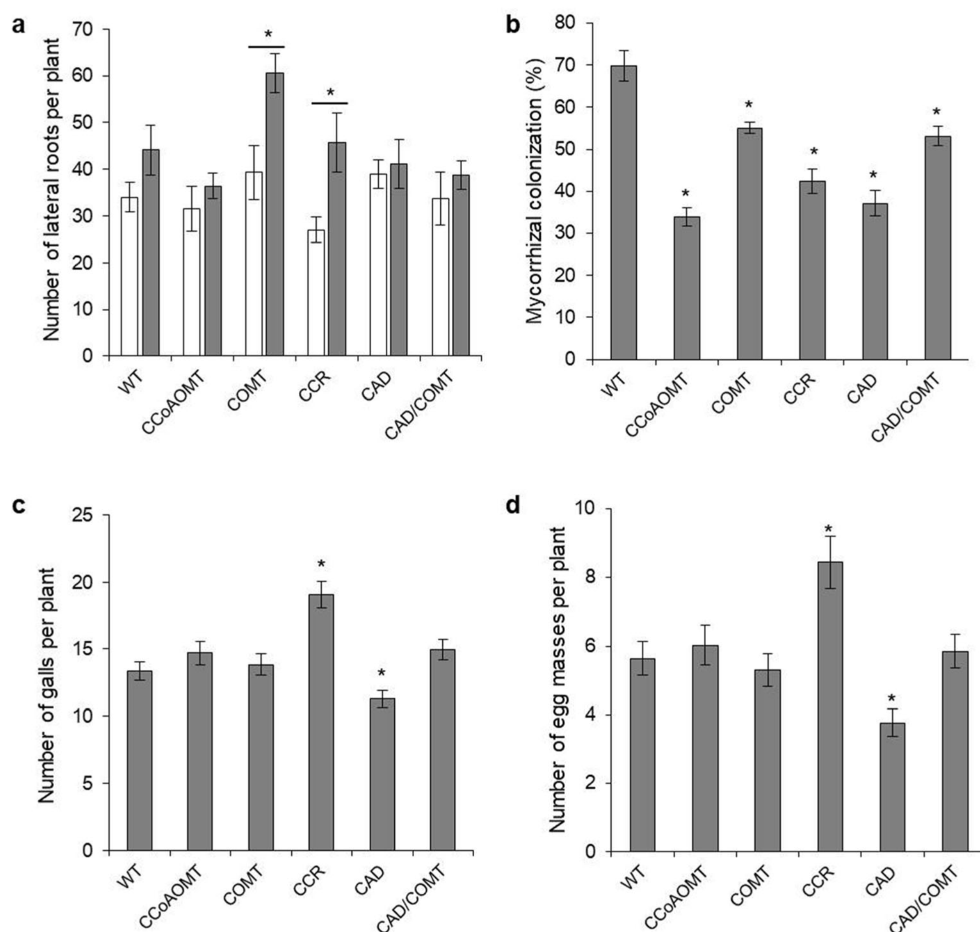

Fig. 1 Impact of the alteration of monolignol biosynthesis pathway gene expression on colonisation by *L. bicolor* and *M. incognita* on in vitro transgenic poplars and wild-type (WT) poplar roots. **a** Number of lateral roots per plant in poplar grown 3 weeks in the absence (white bars) or in the presence (grey bars) of *L. bicolor*. **b** Percentage of ectomycorrhizal lateral roots 3 weeks after inoculation with *L. bicolor*. **c** Number of galls per plant 3 weeks after infection by *M. incognita*. **d** Number of egg masses per plant 6 weeks after inoculation. Data represent means ± SD from 9 to 36 plants from two to three independent biological replicates for *L. bicolor* colonisation test and 41 to 56 plants per line from four independent biological replicates for *M. incognita* resistance test. Pairwise comparisons were made using Student's *t* test ($P < 0.05$)

ectomycorrhizal colonisation efficiency and RKN infection rates. Additionally, several poplar CAD lines have already been investigated towards their association with mycorrhizal fungi, but the molecular network underlying this interaction, as well as interaction with RKN, is still unknown. This line is used as an example of the consequences of an alteration of a metabolite profile on ectomycorrhizal fungus- and RKN-host interactions and is hereafter referred to as the "modified line".

We first investigated the magnitude of the impact of *CAD* downregulation in the modified line by looking at the differential abundance of secondary metabolites. This untargeted metabolomic analysis revealed that 5033 and 6600 mass-to-charge ratio (m/z) features were respectively more and less abundant in roots of the modified line compared with that of the wild-type (false discovery rate (FDR)-adjusted P value < 0.05), with 905 and 2097 of them showing a fold change > 2 (Online resource 1). This result indicates that perturbing the expression of a single gene has deep metabolic consequences that may shape the interaction between the root and its surrounding environment. From the pool of molecules showing a fold change > 2, eight were successfully identified, deriving from hydroxycinnamic and salicylic acids (Online resource 1).

To get a comprehensive overview of roots' differential expression in the two genotypes (wild-type and modified line), a RNA-Seq analysis was performed on roots of 1-month-old in vitro poplar. This comparison revealed 1084 up- and 610 downregulated genes in the modified line vs. wild-type (FDR-adjusted P value < 0.05, fold change > 2) (Online resource 2). Overall, the gene ontologies enrichment (GOE) analysis highlighted modifications in cell wall metabolism, including lignin biosynthesis, trehalose (Tre) metabolism, and phytohormone homeostasis (Fig. 2).

Several cell wall–related gene ontologies were enriched in the modified line, such as regulation of lignin biosynthetic process, cell wall modification, xyloglucan metabolic process, and pectin catabolic process (Fig. 2). More specifically, an upregulation of genes encoding several XTHs, expansins, fasciclin-like arabinogalactan proteins, laccases and pectin lyases/methylesterases was detected (Online resource 2). Genes involved in signal recognition were also differentially expressed, such as a peptidoglycan-binding LysM domain-containing protein and several receptor lectin kinases (Table 1).

GOE analysis highlighted a higher expression of genes involved in Tre metabolism in the modified line (Fig. 2). This includes 5 trehalose phosphatases (*TPP*, yielding Tre

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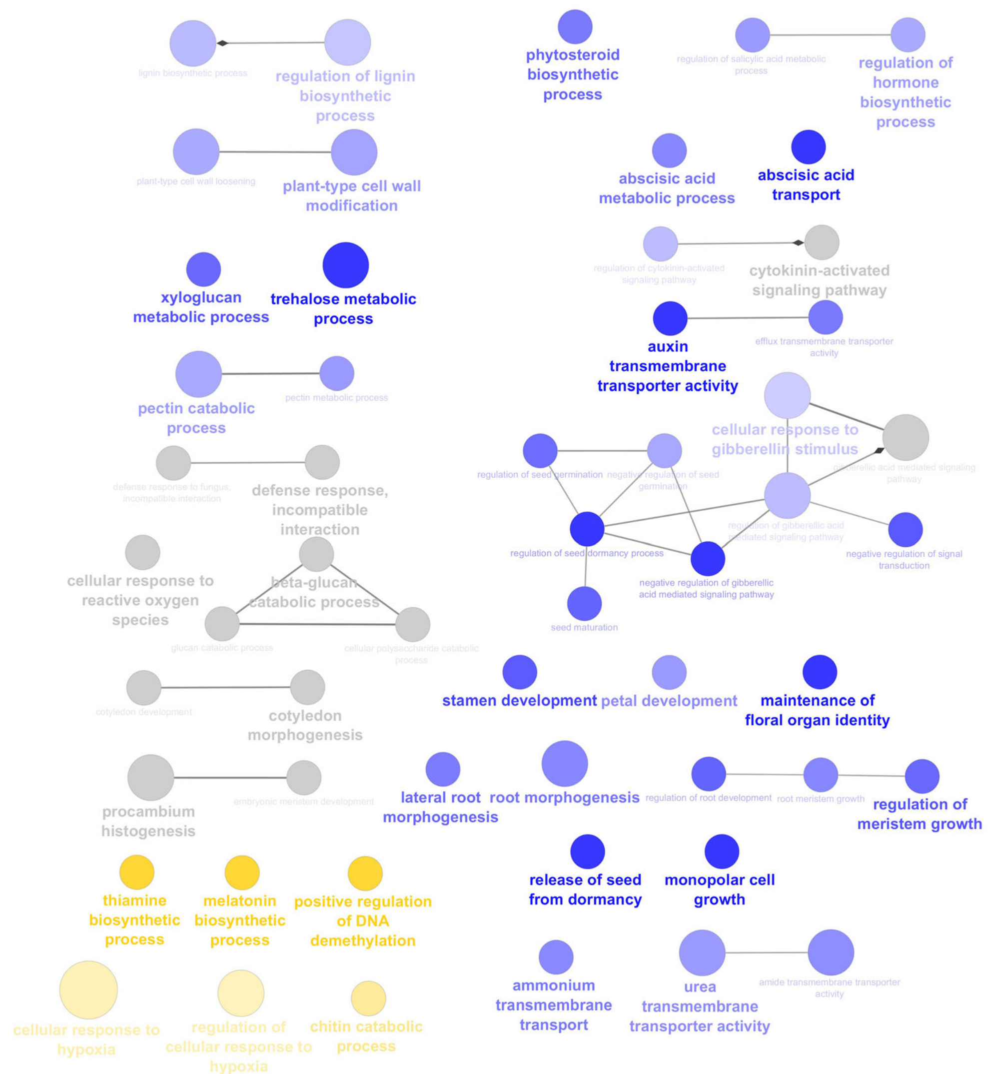

Fig. 2 Gene ontology enrichment performed with the subset of genes showing −2 > fold change > 2 in Cytoscape using ClueGO and CluePedia (Bindea et al. 2009, 2013). Gene ontologies with genes downregulated or upregulated in the CAD line are shown in orange and blue, respectively. Gene ontologies represented in grey are not significantly enriched neither in the wild-type nor in the CAD lines

from Tre-6-phosphate Tre6P) and one trehalase (homologous to Arabidopsis *TRE1*, hydrolyzing Tre into glucose).

Several GOEs related to phytohormone biosynthesis and transport were over-represented in the modified line (Fig. 2 and Table 1), including abscisic acid (ABA) transport and metabolism, cytokinin-activated signalling, auxin transmembrane transporter activity and cellular response to gibberellin (GA) stimulus. For instance, *YUC8*, which participates in auxin biosynthesis (Korasick et al. 2013), was upregulated in the modified line, as were three genes encoding the auxin efflux carrier protein PIN1 and PIN2 and the auxin influx carrier LAX3 (Table 1). This may indicate that auxin biosynthesis and transport are changed in the modified line. Several genes involved in response to GA were more highly expressed in the modified line such as two genes homologous to *GA20OX2*, which is part of the GA biosynthetic pathway (Hedden and Thomas 2012), and two genes coding for GA-regulated family proteins. Finally, several ABA-related genes were also differentially expressed. Genes coding for two ABA transporters, ABCG25 and ABCG40, and ABF2, an activator of ABA-responsive genes (Cutler et al. 2010), as well as NCED3 and ABA1, both involved in ABA biosynthesis (Nambara and Marion-Poll 2005), were more expressed in the modified line as compared with that of the wild-type.

Discussion

Manipulation of the monolignol biosynthesis pathway is widely reported to lead to changes in phenolic profiles (Meyermans et al. 2000; Leplé et al. 2007; Vanholme et al. 2012). It may be anticipated that the accumulation or depletion of phenolic compounds with particular biological activities in the roots could impede the interaction of roots with soil-borne organisms. In this respect, these phenolics could either (i) be toxic resulting in a reduced fitness/virulence of the microorganism (Wuyts et al. 2006b), (ii) be used as a carbon source allowing a better growth and therefore enhanced fitness/

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Table 1 Genes involved in Tre metabolism, phytohormone homeostasis, signal recognition and monolignol metabolism differentially expressed in CAD vs. wild-type (WT) lines

Poplar gene ID	Log_2 ratio CAD vs. WT	Best TAIR10 hit name	Best *Arabidopsis* symbol	Best *Arabidopsis* defline
Trehalose metabolism				
Potri.003G112400	2.81	AT4G12430.1	*TPPF*	Haloacid dehalogenase–like hydrolase (HAD) superfamily protein
Potri.012G126100	1.61	AT5G51460.2	*ATTPPA*	Haloacid dehalogenase–like hydrolase (HAD) superfamily protein
Potri.015G126900	1.25	AT5G51460.2	*ATTPPA*	Haloacid dehalogenase–like hydrolase (HAD) superfamily protein
Potri.001G120500	1.20	AT4G22590.1	*TPPG*	Haloacid dehalogenase–like hydrolase (HAD) superfamily protein
Potri.012G001000	1.04	AT1G35910.1	*TPPD*	Haloacid dehalogenase–like hydrolase (HAD) superfamily protein
Potri.001G087100	1.26	AT4G24040.1	*ATTRE1*	Trehalase 1
Phytohormone biosynthesis, transport and signalling				
Potri.002G254200	2.44	AT4G28720.1	*YUC8*	Flavin-binding monooxygenase family protein
Potri.018G139400	3.04	AT5G57090.1	*ATPIN2*	Auxin efflux carrier family protein
Potri.006G037000	1.20	AT1G73590.1	*ATPIN1*	Auxin efflux carrier family protein
Potri.016G035300	1.01	AT1G73590.1	*ATPIN1*	Auxin efflux carrier family protein
Potri.005G174000	1.13	AT1G77690.1	*LAX3*	Like AUX1 3
Potri.012G132400	1.98	AT5G51810.1	*ATGA20OX2*	Gibberellin 20 oxidase 2
Potri.005G184400	1.32	AT5G51810.1	*ATGA20OX2*	Gibberellin 20 oxidase 2
Potri.001G350600	2.58	AT5G14920.1	*GASA14*	Gibberellin-regulated family protein
Potri.007G051300	1.96	AT2G14900.1		Gibberellin-regulated family protein
Potri.019G083000	1.25	AT1G71960.1	*ATABCG25*	ATP-binding cassette family G25
Potri.018G032900	1.23	AT1G15520.1	*ATABCG40*	Pleiotropic drug resistance 12
Potri.009G101200	1.33	AT1G45249.1	*ABF2*	Abscisic acid responsive element-binding factor 2
Potri.011G112400	1.94	AT3G14440.1	*ATNCED3*	Nine-*cis*-epoxycarotenoid dioxygenase 3
Potri.005G138400	2.66	AT5G67030.1	*ABA1*	Zeaxanthin epoxidase
Potri.007G044300	2.41	AT5G67030.1	*ABA1*	Zeaxanthin epoxidase
Signal recognition				
Potri.013G021600	− 1.22	AT1G55000.1		Peptidoglycan-binding lysm domain-containing protein
Potri.007G111100	− 3.59	AT5G60900.1	*RLK1*	Receptor-like protein kinase 1
Potri.007G120600	3.34	AT3G22060.1		Receptor-like protein kinase-related family protein
Potri.007G120300	2.80	AT3G22060.1		Receptor-like protein kinase-related family protein
Potri.017G039900	− 1.44	AT3G22060.1		Receptor-like protein kinase-related family protein
Monolignol biosynthesis and polymerisation				
Potri.001G036900	1.26	AT3G21240.1	*4CL2*	4-Coumarate:CoA ligase 2
Potri.T071600	1.75	AT1G65060.1	*4CL3*	4-Coumarate:CoA ligase 3
Potri.019G049500	1.66	AT1G65060.1	*4CL3*	4-Coumarate:CoA ligase 3
Potri.018G146100	− 1.94	AT2G30490.1	*ATC4H*	Cinnamate-4-hydroxylase
Potri.006G024300	− 1.48	AT1G72680.1	*ATCAD1*	Cinnamyl-alcohol dehydrogenase 1

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Table 1 (continued)

Poplar gene ID	Log_2 ratio CAD vs. WT	Best TAIR10 hit name	Best *Arabidopsis* symbol	Best *Arabidopsis* defline
Potri.014G100600	2.24	AT2G46570.1	*LAC6*	Laccase 6
Potri.009G102700	2.07	AT5G03260.1	*LAC11*	Laccase 11
Potri.007G023300	1.14	AT5G03260.1	*LAC11*	Laccase 11
Potri.001G054600	1.23	AT5G60020.1	*LAC17*	Laccase 17
Potri.001G133200	1.24	AT1G32100.1	*ATPRR1*	Pinoresinol reductase 1
Potri.001G133300	− 1.17	AT1G32100.1	*ATPRR1*	Pinoresinol reductase 1

virulence of the microorganism (Beckers et al. 2016) and/or (iii) be signalling/recognition molecules that result in an enhanced susceptibility or resistance of the plant (Wuyts et al. 2006a; Mandal et al. 2010).

Perturbation in the monolignol pathway impacts ectomycorrhizal colonisation

The cell wall is a key compartment for a large range of plant biological processes, including physical resistance to attacks, due to cell wall lignification, and perception of external stimuli, such as interaction with ectomycorrhizal fungi. The mutual recognition of host roots and ectomycorrhizal fungi relies on specific plasma membrane receptors, such as G-type lectin receptor-like kinase and LysM-receptor-like kinases, which may be required for ectomycorrhizal or arbuscular mycorrhizal (AM) symbiosis, respectively (Desaki et al. 2018; Labbé et al. 2019). These receptors may be activated by chitin-derived oligosaccharides released by the fungi to initiate the reciprocal interaction (Garcia et al. 2015; Cope et al. 2019). Since the expression of genes coding for several of these receptors was different in the analysed CAD line (Table 1), we hypothesised that the lower mycorrhizal colonisation efficiency is related to the modification of their relative abundances.

The expression pattern of genes involved in cell wall formation and remodelling points to differences in its structure between modified and wild-type lines (Online resource 2). Such chemical modifications may decrease the efficiency of the communication between the root and the fungal cells, either physically by modifying the accessibility of the required receptors, or at the molecular level by altering the expression of genes required for the interaction. For instance, the ultrastructure of cell walls of a double *cad* Arabidopsis mutant appears disorganised (Anderson et al. 2015). Downregulation of *CCR* or *CAD* expression in poplar leads, respectively, to defects in the cohesion of secondary cell walls concomitant with the upregulation of genes related to stress response (Leplé et al. 2007) and decreased elastic modulus and yield stress (Özparpucu et al. 2017). A phenotype similar to the CCR line was also described for downregulation of *cinnamate 4-hydroxylase* (Bjurhager et al. 2010). Observations made in transgenic poplar with reduced cellulose content and secondary cell wall thickness through downregulation of *KORRIGAN*-like genes (involved in cellulose biosynthesis, Vain et al. 2014) also highlight the importance of cell wall structure in the colonisation process. Root metabolites were modified in these RNAi trees (Bali et al. 2016; Veach et al. 2018), in addition to a lower lignin content with a decreased ratio of syringyl/guaiacyl units and an increased mycorrhizal colonisation rate (Kalluri et al. 2016). We may, therefore, propose that decreased ectomycorrhizal colonisation in all transgenic lines analysed is most likely related to modification in cell wall ultrastructure. This variation may

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also ease the penetration of the fungal hyphae into the apoplastic space, therefore resulting in higher fungal biomass within the Hartig net. This assumption requires additional experiments, which will provide a better understanding of the relation between the fungi and its host.

The roles of Tre and Tre6P in plant growth, plant development, and compatible interaction between *L. bicolor* and *P. trichocarpa* have been previously evidenced (Tschaplinski et al. 2014; Lunn et al. 2014). For instance, Tre content was increased almost 40-fold in the symbiotic tissue during the establishment of the *P. trichocarpa*-*Laccaria* compatible relationship. By contrast, Tre was substantially less accumulated (25-fold) during the incompatible *P. deltoides*-*Laccaria* interaction 12 weeks after contact (Tschaplinski et al. 2014). We may thus speculate that the lower mycorrhizal colonisation efficiency measured in the modified line is related to the increased Tre metabolism in the host root. The upregulation of both *TPP* (Tre biosynthesis) and *TRE1* (Tre signalling) in roots of the modified line in the current study may lead to a different Tre content, resulting in changes in its signalling pathway, and ultimately in a different response to ectomycorrhizal establishment. Alternatively, an increase in Tre content is considered a hallmark of functional mycorrhizal root system (Tschaplinski et al. 2014). We may hypothesise that this higher Tre metabolism in the modified line might be perceived as a signal of successful mycorrhizal interaction by the root, ultimately leading to inhibition or decrease of fungal colonisation.

Phytohormones, in addition to regulate most of plant developmental processes, also play a key role in the interaction with mycorrhizal fungi (Pozo et al. 2015; Liao et al. 2018). The differential response of the modified line towards ectomycorrhizal colonisation may, therefore, lie within the altered expression of genes involved in phytohormone biosynthesis, transport and signalling. The presence of the fungus triggered a significant increase in the number of lateral roots in both COMT and CCR lines compared with those developed in the absence of the fungus (Fig. 1a). This outcome suggests either an increased response to auxin in these lines and/or an amplified response to fungal volatiles. The last would induce auxin biosynthesis in plant cells, since *L. bicolor*–released volatiles have been shown to be sufficient to stimulate lateral root formation in *Arabidopsis thaliana* (Felten et al. 2009). The early phase of the interaction between tree roots and ectomycorrhizal fungi, prior to symbiosis establishment, is accompanied by a stimulation of lateral root development through auxin transport and signalling, which is independent on ectomycorrhizal fungi root colonisation (Felten et al. 2009). Auxin favours root colonisation, as evidenced with a transgenic *Hebeloma cylindrosporum* strain over-producing this hormone, but also the formation of a pluriseriate Hartig net, probably through modifications of the cell wall structure (Gay et al. 1994). Later during the colonisation process, auxin is inactivated as a result of conjugation with amino acids (Vayssières et al. 2015). Whether the modification of expression of genes involved in auxin-related process in the modified line has consequences on the last step of the colonisation is worth investigating.

GA is known to inhibit arbuscule formation (Gutjahr 2014; Liao et al. 2018). Since the increase in GA signalling suggested by our transcriptomic dataset is correlated with the deficient mycorrhizal colonisation of the modified line, we may speculate that GA signalling is also negatively associated with ectomycorrhizal colonisation. This is consistent with a recent study showing that exogenous GA treatment of poplar roots impedes the ectomycorrhizal colonisation process (Basso et al. 2020). DELLA proteins suppress GA signalling, as demonstrated in *Medicago truncatula* double *della* mutant (Gutjahr 2014).

Overall, it is expected that crosstalk among these different classes of phytohormones shapes the relation between ectomycorrhizal fungi and roots (Pozo et al. 2015). Whether the reduced mycorrhizal fungi colonisation at the early stages of plant development affects the overall growth and development of their plant hosts in the field deserves further analysis.

Perturbation in the monolignol pathway is associated with differences in root-knot nematode sensitivity

By contrast with the establishment of the root-ectomycorrhizal fungus interaction, the investigated transgenic lines do not display the same sensitivity regarding the infection process with RKN. Indeed, the susceptibility of the CCoAOMT, COMT and CAD/COMT lines was not significantly affected as compared with that of the wild-type, while the CCR line was less resistant and the CAD line showed a lower susceptibility. It is therefore challenging to suggest a common mechanism of resistance/susceptibility to RKN in relation with alteration of the monolignol pathway. Possibly, each specific transcriptomic and metabolomic landscape induced by the downregulation of each of these 5 genes underlies different responses towards RKN, either in a positive or negative way.

The roles of Tre and Tre6P in nematode parasitism have been previously suggested (Hofmann et al. 2010). Since TRE1 localised in the plasmalemma and its active site faces the apoplast, it may act as a sensor for extracellular Tre, which may be a threat signal for the plant during nematode infection (Hofmann et al. 2010). The previously described mechanism involving *TPP* and *TRE1*, which may lead to a different Tre content, may also result in a different response to nematode infection.

The transcriptomic data show the upregulation of *AtSR1*, a gene coding for a transcription factor homologous to a serine/threonine protein kinase, in the modified line (Online resource 2). AtSR1 downregulates the expression of *WRKY53* and negatively regulates the biosynthesis of salicylic acid (SA) (Du et al. 2009), consistent with the lower accumulation of salicortin, salireposide and SA glucoside in the modified line

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(Online resource 1). Furthermore, the expression of *WRKY53* is induced by SA (Yu et al. 2001). It is therefore of special interest to observe in the roots of the modified line a lower abundance of SA-derived molecules and a decreased abundance of the *WRKY53* transcript (Online resources 1 and 2).

Calmodulin (CaM) binding is a critical element of the AtSR1-mediated plant resistance to herbivore attack, as well as to the WRKY53-regulated response to microbial attacks (Poovaiah et al. 2013). AtSR1 and WRKY53 are both CaM-binding transcription factors (Popescu et al. 2007; Laluk et al. 2012). Functional analyses of several of these transcription factors evidenced their roles in plant response to (a)biotic stresses (Reddy et al. 2011). Notably, our transcriptomic data reveals that most of the differentially expressed CaM-related genes, including *CaM-like CML11*, *41* and *42* and *CaM*-binding protein-like, are significantly upregulated in the modified line as compared with the wild-type (Online resource 2). Overexpression of a *CaM* gene in soybean strongly reduced the number of adult female cyst nematodes in roots (Ali et al. 2017). These data points to an important role of CaM and CML in the response of poplar to RKN and deserves further investigation, particularly concerning their interaction with the reported transcription factors.

AtWRKY18 is induced in response to the generalist herbivory *Spodoptera littoralis* (Schweizer et al. 2013), and its poplar homologue was found to be upregulated in the modified line. Mutant defective in *AtWRKY18* favours larval growth and is characterised by the downregulation of genes involved in anthocyanin and flavonol biosynthesis (Schweizer et al. 2013). Two genes from this pathway, a *dihydroflavonol 4-reductase* (*DFR*) and a *leucoanthocyanidin dioxygenase*, were upregulated in the modified line. In addition, *Potri.006G221800* (encoding PtrMYB134, a positive and specific regulator of proanthocyanidin biosynthesis, James et al. 2017), is also upregulated in the modified line (Online resource 2). DFR is a key enzyme of the proanthocyanidin pathway, and its coding gene is strongly induced upon herbivory attack in aspen (Peters and Constabel 2002) and in poplar overexpressing *MYB134* (Mellway et al. 2009). Mellway and colleagues also show that MYB134 upregulates the expression of *4-coumarate:CoA ligase*, as observed in our transcriptomic dataset. All together, these data suggest that phenylpropanoids may be involved in resistance to nematode feeding, as observed for other plant defence mechanisms (Dixon et al. 2002).

Lectins are proteins showing nematicidal activity in several species (Ali et al. 2017). Lectins disrupt the intestinal function of organisms ingesting them, constituting an efficient defence against nematodes. Ten genes related to lectins were upregulated in the modified line as compared to the wild-type, including genes coding for a mannose-binding lectin superfamily protein, several concanavalin A-like lectin protein kinases and S-locus lectin protein kinases (Online resource 2). The overexpression of a *mannose-binding lectin* from snowdrop (*Galanthus nivalis*) in Arabidopsis decreases the quantity of root galls following *M. incognita* infection in several lines, despite a weak correlation between lectin abundance and gall number (Ripoll et al. 2003).

Perturbation in the monolignol pathway may modify plant fitness under field conditions

Genetic manipulation of crops for enhanced agronomic assets has been shown to alter the relationship between hosts and mycorrhizal fungi in annual crops (Wróbel-Kwiatkowska et al. 2012; Londoño et al. 2019) or in perennial species (Danielsen et al. 2013; Veach et al. 2018). For instance, transgenic poplar lines with decreased activities of CAD, COMT and CCR did not show differences in colonisation rate 2 years after out planting, but significant shifts in the ectomycorrhizal community species were observed, although this variation was similar to that found on different commercial poplar genotypes (Danielsen et al. 2012, 2013). Danielsen and colleagues investigated the community pattern of mycorrhizal fungi in three different CAD lines, characterised by contrasting residual expression and, therefore, with different metabolomic signatures. Interestingly, these three lines did not share a common mycorrhizal community, further suggesting that modification in the metabolite profile has a direct impact on the community structure of ectomycorrhizal fungi. Whether the differences observed in both fungal communities and growth and biomass parameters share a causal link may seem speculative, but a significant correlation between biomass and ectomycorrhizal species richness was reported (Danielsen et al. 2013).

Colonisation by mycorrhizal fungi and resistance to nematodes are determinant for resource acquisition and biomass production. Modifications in tree-mycorrhizal fungi interactions may have a broad impact on plant growth and fitness since ectomycorrhizal fungi provide notably nitrogen, phosphate and water to its host (Casieri et al. 2013; Desai et al. 2014). When considering tree improvement for biomass production, the evaluation of the fitness of the value-added trees especially regarding their susceptibility to the biotic environment is worth to be investigated. Several lines of evidence suggest that altered water availability and rising temperatures associated to global warming will have a significant impact on interactions between plants and mycorrhizal fungi (León-Sánchez et al. 2018; Cotton 2018). For instance, mean annual precipitation is negatively associated with an increased dominance of AM trees in USA (Jo et al. 2019). This demonstrates that the tree-fungus system is sensitive to perturbations in its environment that may be attenuated or aggravated by possible consequent metabolic alterations. In addition, global warming will favour the spread of tropical RKN species towards northern regions, potentially threatening various species

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(Wesemael et al. 2011). Modifications in the transcriptomic and metabolic patterns may influence this trend and should be considered both an opportunity to design crops tolerant to RKN or a side effect of plant manipulation which should be carefully investigated before commercial applications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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