Alzabaidi *et al.*
*Fungal Biology and Biotechnology* (2025) 12:2
https://doi.org/10.1186/s40694-025-00193-w

Fungal Biology and Biotechnology

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# Transcriptome response of the white-rot fungus *Trametes versicolor* to hybrid poplar exhibiting unique lignin chemistry

Anbarah R. Alzabaidi[1], Noor Alabbasi[1], Richard Meilan[2], Scott J. Meiners[1] and Thomas Canam[1,3*]

## Abstract

**Background** Production of biofuels and bioproducts from lignocellulosic material is limited due to the complexity of the cell wall structure. This necessitates the use of physical, chemical, and/or physico-chemical pretreatment technologies, which adds significant capital, operational, and environmental costs. Biological pretreatment strategies have the potential to mitigate these expenses by harnessing the innate ability of specialized bacteria and fungi to deconstruct lignocellulose. White-rot fungi (*e.g. Trametes versicolor*) have been shown to be effective at biological pretreatment of lignocellulose, yet it was uncertain if these fungi are feedstock agnostic or are able to sense subtle changes in cell wall chemistry.

**Results** The present study examined the transcriptome response by *Trametes versicolor* to transgenic hybrid poplar (*Populus tremula* × *alba*) lines with altered syringyl (S) and guaiacyl (G) lignin. Specifically, the transcriptional response of the fungus to wild-type wood was compared to that from the wood of six transgenic lines within three lignin phenotypes, LSX (low S with hydroxy-G), LSHG (low S with high G), and HS (high S), with 350 transcripts showing significant differences among the samples. The transcriptome of *T. versicolor* varied according to the lignin phenotype of the wood, with the LSX wood resulting in the most substantial changes in *T. versicolor* transcript abundance. Specifically, the LSX wood led to 50 upregulated and 48 downregulated transcripts from WT at the twofold or greater threshold. For example, transcripts for the lignin peroxidases LiP3 and LiP10 were downregulated (approximately 12X and 31X lower, respectively) by the fungus on LSX wood compared to wild-type wood. LSX wood also resulted in approximately 11X lower transcript numbers of endo-β-1,4-glucanase yet led to an increase in expression of certain hemicellulases, further highlighting the altered deconstruction strategy by the fungus on this wood type.

**Conclusions** Overall, the results of this study demonstrated that *T. versicolor* was able to respond to transgenic poplar wood with the same genetic background, which has important implications for biological pretreatment strategies involving feedstocks that are genetically modified or have considerable natural variations in cell wall chemistry.

**Keywords** White-rot fungi, Pretreatment, Lignocellulose, Transcriptome, Hybrid poplar, Trametes

*Correspondence:
Thomas Canam
tcanam@eiu.edu
[1] Department of Biological Sciences, Eastern Illinois University, 600 Lincoln Avenue, Charleston, IL 61920-3099, USA
[2] Forestry and Natural Resources, Purdue University, 715 West State Street, West Lafayette, IN 47907-2061, USA
[3] Center for Clean Energy Research and Education, Eastern Illinois University, 600 Lincoln Avenue, Charleston, IL 61920-3099, USA

## Background

The challenges of efficiently deconstructing lignocellulose for biofuel and bioproduct applications are well documented, which include the chemically recalcitrant and complex structure of lignin as well as the crystallinity of cellulose [1–4]. A variety of physical, chemical, or physico-chemical pretreatment options exist to mitigate these constraints, although most are either economically

restrictive [5, 6] or environmentally problematic [7, 8]. Biological pretreatment of lignocellulosic material has been explored as a possible substitute or supplement to these processes to help reduce severity parameters [9, 10]. In particular, white-rot fungi (e.g. *Phanerochaete* spp., *Trametes* spp., and *Pleurotus* spp.) have received significant attention as pretreatment agents for lignocellulosic biomass conversion due to their innate ability to deconstruct the material using peroxidases, laccases, and carbohydrate-active enzymes [11–15].

An added challenge to using lignocellulosic material for industrial applications is the variation in composition of the feedstock, whether it is seasonal differences within a single species or intrinsic variation between species [16, 17]. As a result, conversion facilities that are feedstock agnostic is the ultimate goal, where the process can accept a wide range of carbohydrates and lignin moieties as well as types of chemical bonds [18–20]. However, this ideal seems particularly vulnerable when the processing facilities directly or indirectly utilize enzymes, which have inherent specificity limitations [21–23]. Further complicating matters is the potential use of transgenic material that may have unexpected alterations to lignocellulosic structures despite an improvement in one or more trait [24–26].

Progress has been made with respect to the understanding the breadth of lignocellulosic feedstocks utilized by white-rot fungi, including agricultural products, such as straw from rice [27], canola [28], and wheat [29]. When examining the transcriptomes and proteomes of these fungi during solid-state fermentation, the evidence suggests that the transcriptional and translational responses are unique to varying feedstocks [30–33]. Additional studies have examined expression differences by wood-grading fungi in response to variation in lignocellulosic chemistry of a particular substrate. For example, a microarray-based transcriptome and MS-based secretome study from *P. chrysosporium* examined the response of that fungus to transgenic hybrid poplar with high syringyl lignin content (85 and 94% of total lignin; [34]). The milled wood containing the highest syringyl content resulted in the highest number of fungal genes showing significant upregulation, which included those coding for enzymes involved in lignin degradation. Further research on the wood-decay fungi *Phanerochaete chrysosporium* and *Postia placenta* explored transcriptional responses to poplar wood (*P. trichocarpa*) with naturally occurring variation in cell wall chemistry [35]. This microarray-based work uncovered 64 and 84 genes with significantly altered expression from *P. chrysosporium* and *P. placenta*, respectively. Among the traits that were measured, the wood varied in total lignin from approximately 22–28% and 44–54% for glucose.

Despite these efforts, additional research is needed to better understand how wood-degrading fungi respond to variations in lignocellulosic substrates, including the presence of novel or unusual chemistry, which would have important implications for both pretreatment applications and process optimization where feedstock variation within a species or related species may occur. Furthering our understanding of such biological pretreatment agents and their interactions with plant biomass is an important component for state-of-the-art biofuel and bioproduct production systems [36, 37]. The goal of the present study was to explore the transcriptional response of a white-rot fungus, *Trametes versicolor*, to wood from wild-type hybrid poplar as well as transgenic lines with the same genetic background that were genetically modified for altered lignin composition [24]. Specifically, three transgenic lines exhibiting high syringyl lignin (HS), low syringyl with high guaiacyl lignin (LSHG), and low syringyl with hydroxy-G lignin (LSX), were used as solid-state substrates for *T. versicolor*. Transcriptome profiles were compared to assess the response of this white-rot fungus to altered cell wall structure within the same hybrid poplar background.

## Methods

### Wood source

Wood (1 year of growth from coppice) from wild-type and transgenic hybrid poplar trees was provided by Dr. Richard Meilan (Purdue University) in conjunction with the Center for Direct Catalytic Conversion of Biomass to Biofuels (C3Bio). The clone INRA 717-1B4 (*Populus tremula* × *P. alba*; 38) was used as the wild-type control and served as the genetic background for the transgenic lines (Table 1). Six transgenic clones representing

**Table 1** Phenotypes, line numbers, modification histories, and syringyl lignin contents for six transgenic trees as well as WT used in this study that were described previously [24, 38]

| Lignin Phenotype | Label | Modification | Line Number | %S |
|---|---|---|---|---|
| Wild-type | WT | none | INRA 717 | 64 |
| Low S/Hydroxy-G | LSX-1 LSX-2 | C4H::COMTa RNAi | 1036–73 | 28 |
| | | 35S::COMTa RNAi | 0998–45 | 5.2 |
| Low S/High G | LSHG-1 LSHG-2 | 35S::F5H2 RNAi | 1020–24 | 23 |
| | | C4H::F5H2 RNAi | 1035–41 | 34 |
| High S | HS-1 HS-2 | C4H::F5H overexpression | F5H 37 | 77 |
| | | C4H::F5H overexpression | F5H 64 | 82 |

*S* = syringyl lignin, *G* = guaiacyl lignin, *35S* = cauliflower mosaic virus promoter, *COMTa* = caffeic acid-O-methyltransferase from *Populus trichocarpa* × *deltoides*, *C4H* = cinnamate-4-hydroxylase promoter from *Arabidopsis thaliana*, *F5H* = ferulate 5-hydroxylase from *Arabidopsis thaliana*, *F5H2* = ferulate 5-hydroxylase 2 from *Populus trichocarpa*, *RNAi* = RNA interference

three lignin phenotypes were used along with wild-type in this experiment [24, 38]. First, the phenotype of low syringyl lignin with hydroxy-guaiacyl lignin (LSX) included line 1036–73 (C4H::COMTa RNAi; LSX-1) and line 0998–45 (35S::COMTa RNAi; LSX-2). Second, the lignin phenotype of low syringyl and high guaiacyl lignin (LSHG) included line 1020–24 (35S::F5H2 RNAi; LSHG-1) and line 1035–41 (C4H::F5H2 RNAi; LSHG-2). Third, the lignin phenotype of high syringyl lignin (HS) included line F5H37 (C4H::F5H overexpression; HS-1), and line F5H64 (C4H::F5H overexpression; HS-2). Bark was carefully removed from the air-dried poplar stems (Additional Fig. 1) using a belt sander and the wood was cut into discs (average width of 11.6 mm) using a scroll saw. The wood pieces were autoclaved in aluminum foil for 15 min at 121 °C prior to fungal inoculation.

**Wood inoculation**

*Trametes versicolor* (52 J) was purchased from the American Type Culture Collection (ATCC; ID: 96186). The fungus was maintained on malt extract agar at room temperature and was subcultured weekly to fresh media under sterile conditions. Fungal biomass was scraped from the surface of 30 Petri plates containing malt extract agar and blended in 200 mL of malt extract broth using a handheld mixer. This mixture was added to an additional 800 mL of malt extract broth in a 2 L glass bottle. The broth with the fungus was incubated at 100 rpm at room temperature for four days. The fungal biomass was separated from the broth using a 0.22 µm bottle-top filter and rinsed once with 100 mL of sterile water. The rinsed fungal biomass was added to 300 mL of sterile water and mixed gently. Approximately 5 mL of this mixture was added to Petri plates (10 wood discs per plate) for each of the seven hybrid poplar lines. The plates were sealed with

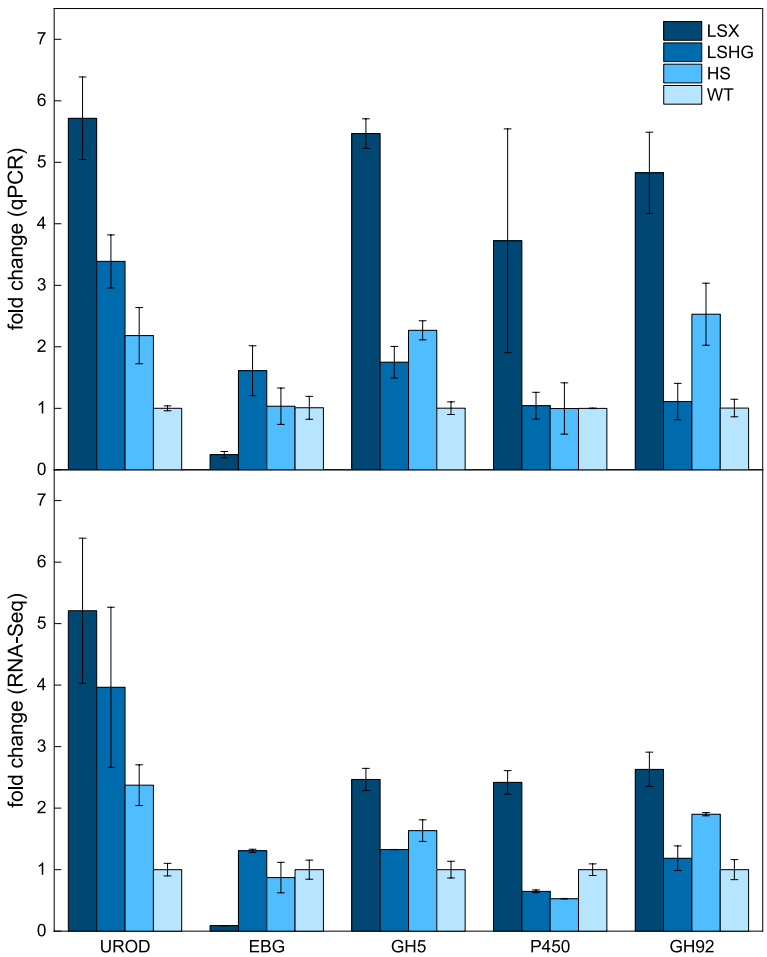


**Fig. 1** Fold-change comparison of RNA-Seq and qPCR data of five genes expressed by *T. versicolor* after three weeks of growth on lignin-modified hybrid poplar (LSX, LSHG, HS) compared to WT. Error bars represent standard deviation (n = 2)

micropore tape and kept in an incubator at room temperature for 3 weeks.

#### RNA extraction and analysis

Five of the ten wood discs with fungal mycelia from each Petri plate (Additional Fig. 2) were ground to a fine powder using a liquid nitrogen-based freezer mill (SPEX 6850; SPEX Certiprep). Total RNA was extracted from the powder using a phenol/chloroform/CTAB-based RNA extraction procedure optimized for wood [39]. RNA was quantified using a NanoDrop Lite (Thermo Scientific) and then diluted to approximately 100 ng/μL using DEPC-treated water. The diluted RNA samples were assessed for quality using an Experion Automated Electrophoresis System with a Standard Sensitivity RNA chip (Bio-Rad). The RQI scores of the fungal RNA ranged from 8.3 to 9.6 (with 7–10 considered to be good quality).

#### Transcriptome sequencing

For transcriptome analysis, a total of 2.5 μg of RNA from each of the eight samples (two samples for each phenotype) was submitted to the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign (Urbana, IL). The RNA-Seq libraries were prepared using TruSeq Stranded mRNAseq Sample Prep kit (Illumina) and sequenced using the HiSeq 4000 system. The RNA-Seq analysis generated a total of 702,087,446 reads (both sense and antisense strands) of 150 nucleotides in length for all eight samples.

#### Transcriptome analysis

The raw sequence files generated by RNA-Seq were processed by ArrayStar software (DNASTAR, Inc.) using a Joint Genome Institute (JGI) transcript file for *Trametes versicolor* (*Trave1_GeneCatalog_transcripts_20101111.nt.fasta*) as a reference [40]. The data were represented as reads per kilobase million (RPKM). The ANOVA function in ArrayStar with false discovery rate (FDR) P value adjustment [41] was used to identify transcripts with significant variation between all sample types (LSX, LSHG, HS, WT) with $P < 0.05$ considered significant. The Gene Ontology (GO) annotation file for *T. versicolor* from JGI (*Trave1_GeneCatalog_proteins_20101111_ GO.tab*) was used for preliminary functional characterization. The data were then transformed to Excel for further processing. Transcripts with total RPKM of less than 8 (*i.e.* less than an average of 1 RPKM per sample) were considered background and removed from further analysis. Heatmaps were created in OriginPro Version 2022 (OriginLab Corporation) with the Heat Map and Dendrogram plugin from OriginLab Technical Support using Euclidian distance and average clustering.

#### Principal coordinates analysis

To visualize variation in gene expression, transcript data were analyzed by a Principal Coordinates Analysis (PCoA). This analysis was conducted using Bray–Curtis dissimilarity of the $\log_{10}(x+1)$ data of transcript abundance (RPKM values). Significance of differences among wood types was assessed with a permutational multivariate analysis of variance using the *adonis* function of the *vegan* package in R based on 1000 permutations. These analyses were conducted on the subset found to have significant changes (described in previous section).

#### qPCR analysis

A subset of each of the eight RNA samples analyzed using RNA-Seq were treated with the TURBO DNA-*free* Kit (Invitrogen) to remove genomic DNA according to the manufacturer's instructions. An aliquot of DNase-treated RNA samples (1 μg) was used to generate cDNA using the SuperScript III First-Strand Synthesis for RT-PCR Kit (Invitrogen). Five representative *T. versicolor* transcripts with twofold expression differences when grown on at least one of the lignin-modified woods compared to WT were examined using qPCR: endo-β-1,4-glucanase (EBG; transcript ID 34218), cytochrome P450 (P450; 130124), glycoside hydrolase family 5 (GH5; 164424), glycoside hydrolase family 92 (GH92; 116603), and UROD/MetE-like superfamily (UROD; 60497). The forward and reverse primers (1 ρmol) specific for each representative gene and the housekeeping gene phosphoglycerate kinase (Additional Table 1) were combined with 1 μL of cDNA template, 10 μL of 2X SYBR Green PCR Master Mix (Applied Biosystems), and nuclease-free water to a total of 20 μL. The reaction parameters for the StepOne Real-Time PCR System (Applied Biosystems) were 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Data were collected during the final step of each cycle. Ct values were generated by the instrument software and transformed using the ΔΔCt method. The fold-change expression patterns of the five representative genes from qPCR data were consistent to those from RNA-Seq analysis (Fig. 1), thereby providing confirmation of the transcriptome data.

## Results and discussion

White-rot fungi have been studied as pretreatment agents for lignocellulosic plant material, with data generally suggesting that these fungi respond uniquely to different types of biomass based on transcriptome and proteome profiles [30–33]. However, it remains unclear how *Trametes versicolor* responds to changes in lignocellulosic chemistry within a species. This has particular importance for biotechnological processes that rely

on feedstocks that show variation between cultivars or transgenic lines of a particular species and/or experience seasonal variation. To investigate the gene expression dynamics of a white-rot fungus to lignocellulosic variation within the same genetic background, *Trametes versicolor* was exposed to wood discs from WT hybrid poplar as well as from two unique transgenic lines with one of three lignin phenotypes: LSHG (low syringyl with high guaiacyl lignin), HS (high syringyl lignin), and LSX (low syringyl with hydroxy-guaiacyl lignin). After three weeks of growth on the wood discs (Additional Fig. 2), RNA-Seq was used to generate transcriptome profiles as a means to assess the response of *T. versicolor* to altered chemical linkages within the lignin.

#### Holistic expression patterns

Broad differences in expression patterns of *T. versicolor* when growing on the four phenotypes of wood (WT, LSHG, HS, and LSX) were examined using a heatmap prepared from transcripts that were significantly different (350 total) across all samples (Fig. 2). The heatmap indicated that the expression patterns of *T. versicolor* on the two representatives of each lignin-modified phenotype showed excellent consistency relative to the other phenotypes, despite being uniquely generated lines. For example, the lines producing the phenotype with the hydroxy version of guaiacyl (G) lignin (LSX-1 and LSX-2) were transformed with different promoters (C4H and 35S, respectively) driving the suppression of caffeic acid-O-methyltransferase (COMT), which led to syringyl (S) lignin contents of 28% and 5.2%, respectively (Table 1). This similarity of gene expression by *T. versicolor* on the two lines with comparable phenotypes is thereby attributed to similarities in the S and G compositions and corresponding altered structural and physical attributes of the wood.

The transcripts with significant differences (350 total) were also analyzed with Principal Coordinates Analysis (PCoA) to further visualize variation in *T. versicolor* expression patterns. The first two axes of the PCoA explained a total of 93.3% of the total variation in the data, clearly separating the wood phenotypes (Fig. 3). The subsequent permutational MANOVA demonstrated significant variation between the LSX, LSHG, HS, and WT wood ($F_{3,4}=38.74$, $P=0.006$, $R^2=0.97$). The PCoA analysis as well as the heatmap visualization highlight the unique transcription patterns by *T. versicolor* on the various hybrid poplar phenotypes, and provides strong evidence for the ability of this fungus to respond to changes to lignocellulose composition from wood derived from the same genetic background (INRA 717-1B4; 33).

#### Differential expression analysis

For a more detailed analysis of transcripts that were significantly upregulated by *T. versicolor* when growing on transgenic lines, the average transcript RPKM values for each transgenic phenotype were compared to the average transcript RPKM value for the WT phenotype. A threshold of > 2X expression of transcripts from *T. versicolor* on transgenic lines compared to the WT was used to define upregulated transcripts. A Venn diagram was then created to show similarities and differences among upregulated transcripts between the transgenic phenotypes (Fig. 4). This analysis revealed 50 unique transcripts with upregulated expression (at the > 2X threshold) when *T. versicolor* was growing on LSX compared to WT wood, while the HS and LSHG phenotypes had 13 and 1 uniquely overexpressed transcripts at this same threshold, respectively (Figs. 4, 5, 6). This suggests that LSX phenotype was perceived much differently by the fungus than the other two transgenic phenotypes, which may be attributed to the significant increase in hydroxy-G lignin in the LSX lines compared to the HS and LSHG lines.

Hydroxy-G compounds have been established as intermediates toward the production of S lignin in hardwoods (Additional Fig. 3) [42]. The suppressed expression of COMT by the LSX transgenic lines resulted in the accumulation of hydroxy-G [24], which *T. versicolor* would not be expected to encounter in wild-type poplar species or hybrids. Therefore, the presence of this unusual lignin moiety in the LSX lines could at least partially explain the substantially higher number of transcripts upregulated by *T. versicolor* on those lines compared to the HS and LSHG lines. However, additional traits of the wood (e.g. density, hemicellulose linkages) could also be responsible for the observed response of the fungus rather than the specific chemical composition of the lignin.

When exploring upregulated transcripts that met the > 2X threshold in two or more phenotypes compared to the WT, the HS and LSHG phenotypes did not share a single transcript (Fig. 4), which could be due to HS having substantially higher percentages of syringyl lignin (77% and 82%) compared to LSHG that had a high rate of guaiacyl lignin with low rates of syringyl lignin (23% and 34%; Table 1). Similarly, HS and LSX phenotypes led to just two shared *T. versicolor* transcripts at the > 2X threshold (Fig. 6), where LSX samples had relatively low percentages of syringyl lignin (5.2% and 28%) compared to HS (Table 1).

In contrast, the low-S lignin phenotypes (LSHG and LSX) shared 7 upregulated transcripts when compared to those from the fungus grown on WT wood (Fig. 6), indicating that there are some similarities to these responses that are likely in part due to the low percentages of syringyl lignin found in both phenotypes. Finally, only a single

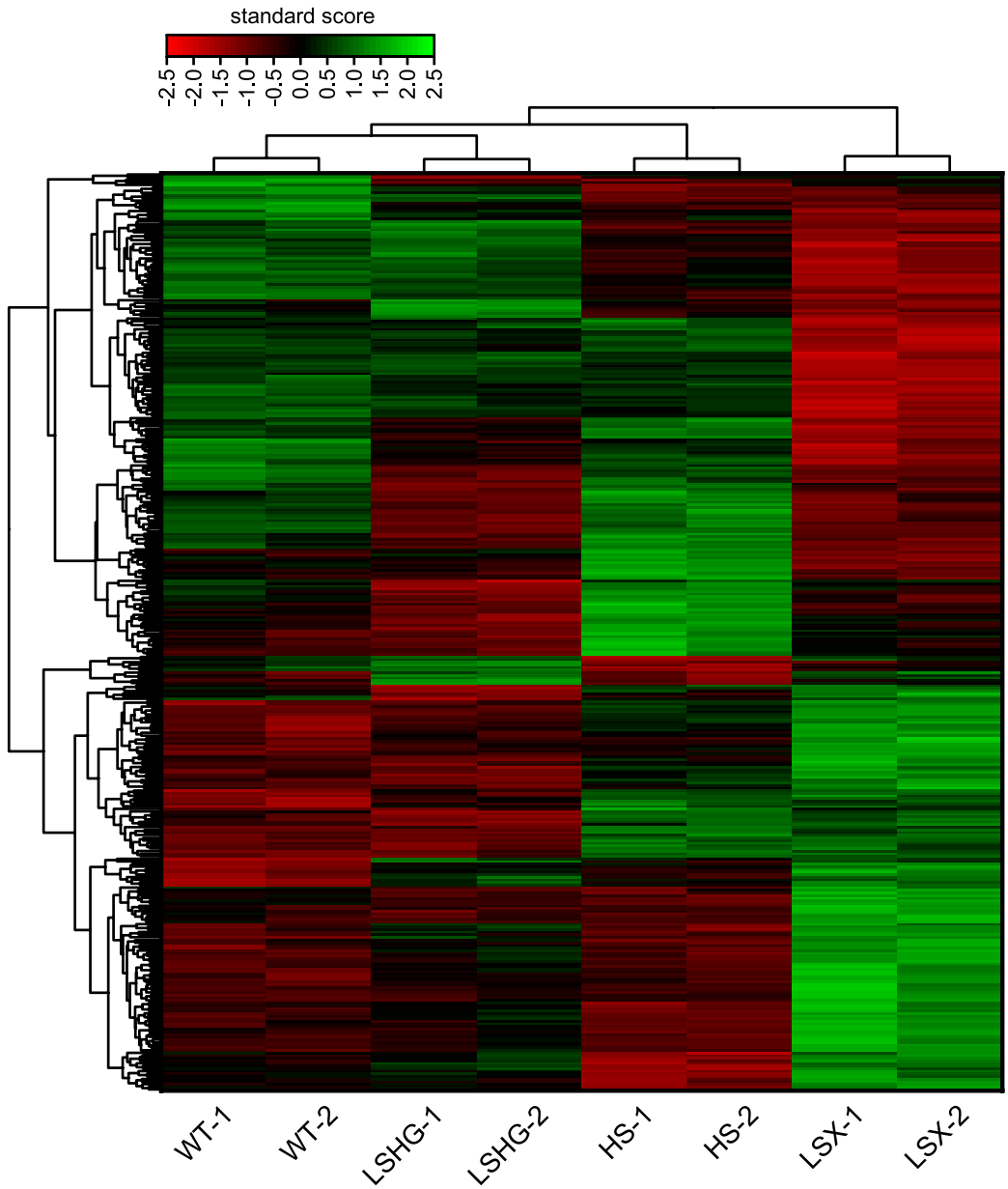


**Fig. 2** Heatmap of the 350 T. *versicolor* transcripts with significant differences in RPKM values (ANOVA F-test, α = 0.05) after three weeks of growth on hybrid poplar wood (LSX, LSHG, HS, or WT). Transcripts were grouped and color coded using Euclidian distance and average clustering using OriginPro Version 2022 (OriginLab Corporation) with the Heat Map and Dendrogram plugin from OriginLab Technical Support

transcript was upregulated at the > 2X threshold on all three transgenic phenotypes compared to WT wood (Fig. 6), which reinforces the conclusion that the white-rot fungus was able to adjust its gene expression levels in response to a variety of lignocellulose modifications rather than use a common suite of enzymes.

Similar to the upregulation comparisons, transcripts with an expression difference of > 2X expression from *T. versicolor* on WT wood compared to transgenic lines was considered to be downregulated. These comparisons resulted in 48 uniquely downregulated transcripts when the fungus was grown on the WT wood compared to LSX (Figs. 4 and 7). As discussed in the upregulation section above, differences in the LSX phenotype appear to exert more of an influence on *T. versicolor* gene expression than the other two transgenic phenotypes (HS and LSHG). For example, HS and LSHG led to just 4 uniquely downregulated transcripts each (Fig. 8).

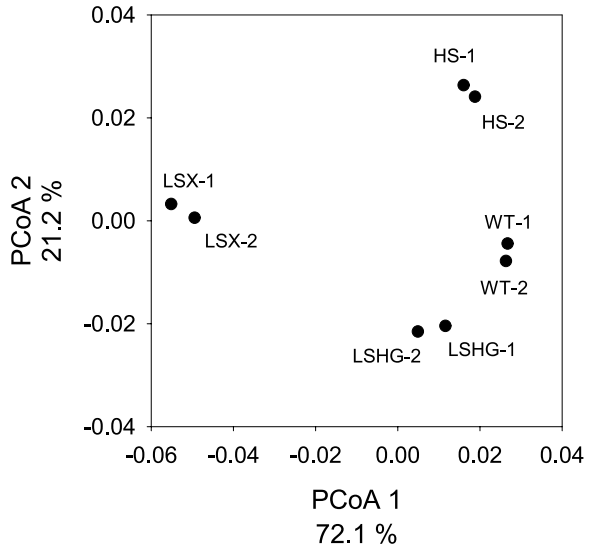


**Fig. 3** Principal coordinates analysis (PCoA) of the 350 transcripts from *T. versicolor* with significant differences in RPKM (ANOVA F-test, α = 0.05) after three weeks of growth on hybrid poplar (LSX, LSHG, HS, and WT)

When examining transcripts that were downregulated on more than one transgenic phenotype, LSX and LSHG shared 7 transcripts (Fig. 8), which was the same number of transcripts that were upregulated on these transgenic lines (Figs. 4 and 6). LSX and HS phenotypes led to five common downregulated transcripts, and two transcripts were downregulated beyond the 2X threshold in LSHG and HS compared to the WT (Fig. 8). Finally, two transcripts were downregulated by the fungus on all three transgenic phenotypes compared to the WT wood (Fig. 8).

When considering both the upregulated and downregulated transcripts by *T. versicolor* at the > 2X thresholds, the LSX wood phenotype led to the most extreme response in both cases. Specifically, there were a total of 60 upregulated transcripts and 62 downregulated transcripts by the fungus on this phenotype compared to WT wood. By comparison, the next highest number of transcripts were with the upregulated transcripts by HS (16 total) and downregulated by LSHG (15 total). Once again, this elevated response by the fungus to LSX suggests that this wood, which has unusually high levels of hydroxy-G lignin [24], presents a unique set of deconstruction challenges to the fungus and/or elicits signals that lead to variations in transcriptional responses. Although, the response by the fungus could be due at least in part to other changes in the lignocellulosic structure or changes to the physical properties of the wood.

### Lignocellulose-degrading enzymes

As expected, several of the identified transcripts (using GO and/or BLAST databases) that were expressed among the > 2X threshold lists (both upregulated and downregulated) are known to be associated with the breakdown of lignocellulose. For example, there were many genes coding for enzymes that efficiently degrade lignocellulose, such as four lignin peroxidases (LiP), which use hydrogen peroxide to oxidatively depolymerize lignin [43]. Specifically, LiP12 (transcript ID 115214) was the most downregulated transcript (13.6 times lower than WT) for the fungus when growing on the LSX wood (Fig. 7). LiP3 and LiP10 were downregulated on both LSX and HS (Fig. 8). However, the downregulation for LSX was much more extreme with 11.6X and 30.7X lower expression than WT for LiP3 and LiP10, respectively,

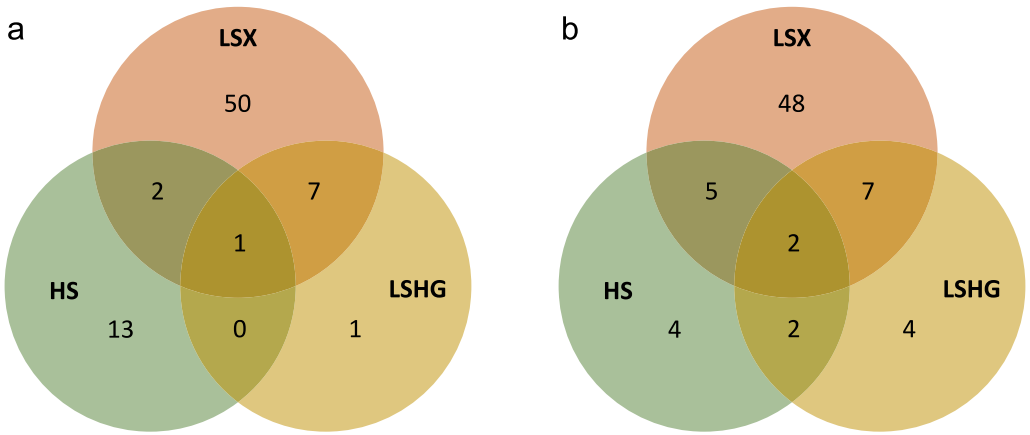


**Fig. 4** Total number of unique and shared transcripts overexpressed by two-fold or greater (**a**) or underexpressed by two-fold or lower (**b**) levels in *T. versicolor* that was grown for 3 weeks on transgenic poplar (LSX, HS, LSHG) compared to wild-type poplar. Excluded were transcripts that were not significantly different among the substrates (ANOVA F-test, α = 0.05)

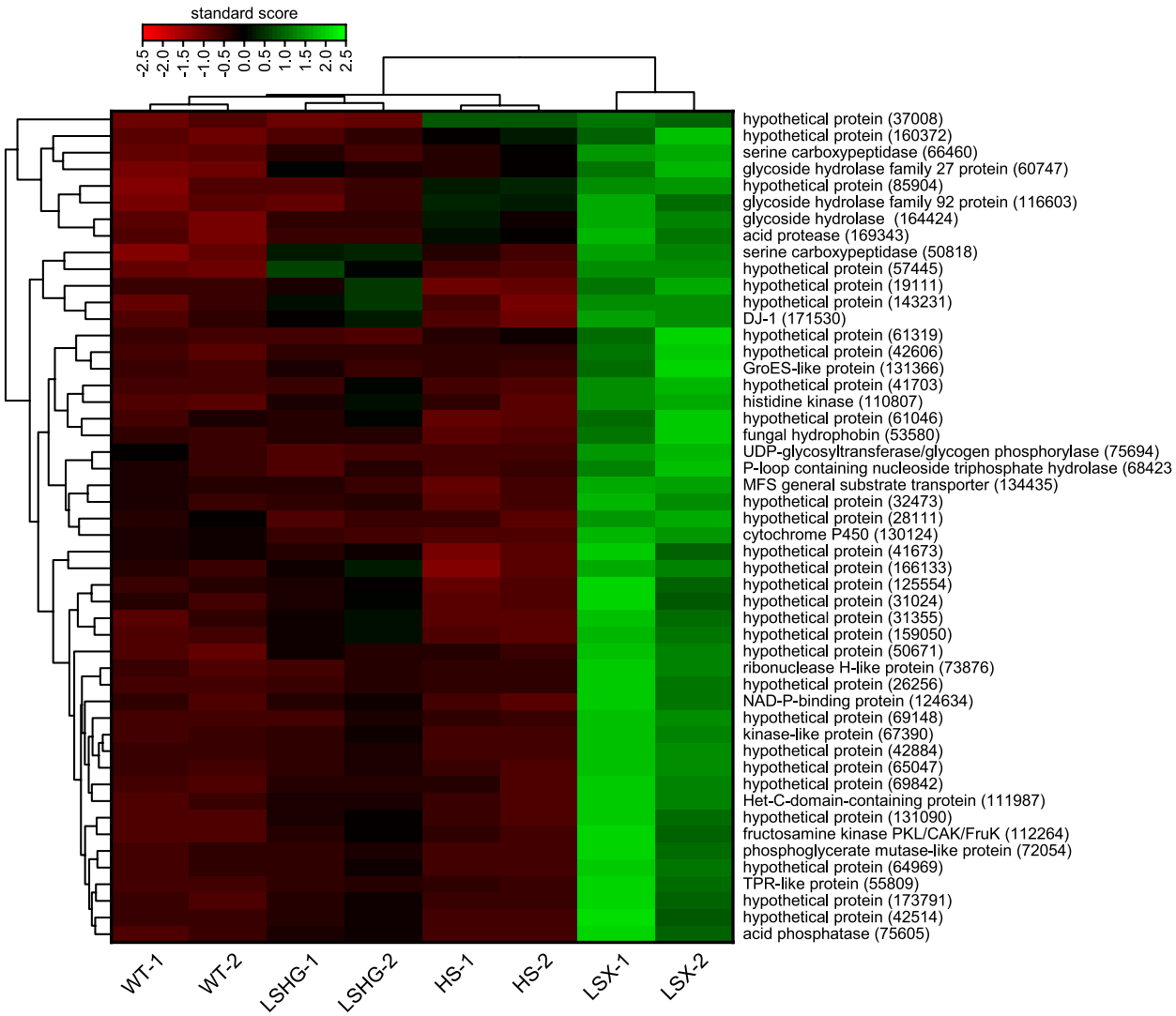


**Fig. 5** Heatmap of the 50 T. *versicolor* transcripts with at least twofold or greater RPKM values after three weeks of growth on LSX wood compared to WT wood. These transcripts were from the 350 significantly different transcripts shown in Fig. 2. Grouping was as described in Fig. 2

while the expression levels of LiP3 and LiP10 in HS were 2.5X and 2.1X lower than WT (Fig. 8). LiP4 (133596) was downregulated in both of the low-S lignin phenotypes (LSX and LSHG; Fig. 8), although the reduction for the LSX phenotype (26.4X) was much more extreme than LSHG (2.5X).

It is unclear why the transcript levels of these important lignin-modifying enzymes were reduced when the fungus was growing on the transgenic phenotypes; however the more extreme downregulation of these four LiP transcripts exhibited by the fungus on LSX wood may be a function of the unusually high levels of hydroxy-G lignin (and other resultant alternations to lignin structure) in the LSX lines. The downregulation of these LiP transcripts was in contrast to a cytochrome P450 transcript (130124) that was upregulated by the fungus when growing on LSX (Fig. 5). Cytochrome P450 enzymes are known to be major players in lignin degradation, especially among white-rot fungi species [44]. As with the LiP examples, it is unclear why the fungus produced more of this cytochrome P450 transcript when growing on the two LSX lines, while the levels of this transcript were approximately 4X less when exposed to the other transgenic phenotypes.

Transcripts encoding enzymes in *T. versicolor* that degrade cellulose, hemicellulose, and other carbohydrate compounds also met the upregulation and downregulation > 2X thresholds in the current study. For example, the transcript levels of endo-β-1,4-glucanase (34218) was second only to LiP12 for highly

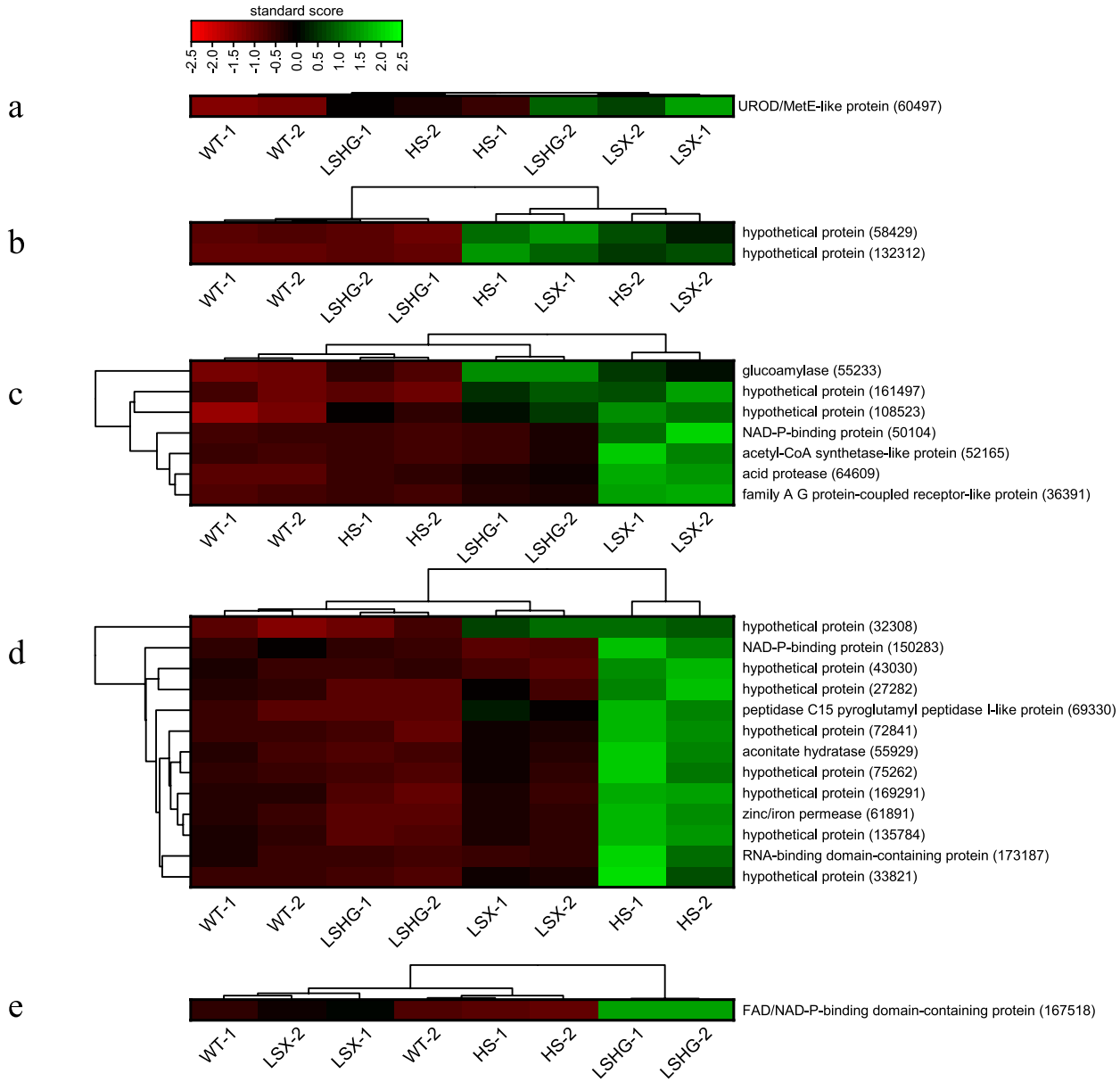


**Fig. 6** Heatmaps of the *T. versicolor* transcripts with twofold or higher RPKM values after three weeks of growth on **a** all three types of wood, **b** LSX and HS, **c** LSX and LSHG, **d** HS, and **e** LSHG. All fold-changes were calculated using transcripts from growth on wild-type (WT) wood as the comparison. These transcripts were from the 350 significantly different transcripts shown in Fig. 2. Grouping was as described in Fig. 2

downregulated transcripts by the fungus on LSX, with 11.3X less transcript compared to the WT (Fig. 7). Endo-β-1,4-glucanases catalyze the cleavage of cellulose bonds internally at amorphous regions, and are central to depolymerization of this polymer [45]. In contrast, the LSX wood led to the upregulation of three glycoside hydrolases (164424, 60747, and 116603; Fig. 5) at approximately 2.5X compared to WT wood. Although the specific substrate for transcript 164424 is unknown, 60747 was identified as a member of the GH family 27 that contains galactosidases [46, 47] while transcript 116603 is a member of the GH family 92 of mannosidases [47], which suggests a shift in activity towards hemicellulose hydrolysis. It could be that the extreme downregulation of the endo-β-1,4-glucanase and upregulation of the three glycoside hydrolases, as well as other transcripts, is a result of altered timing of deconstruction with the LSX wood, whereby the fungus may be lagging with respect to exposure of cellulose with this particular phenotype, although examining the

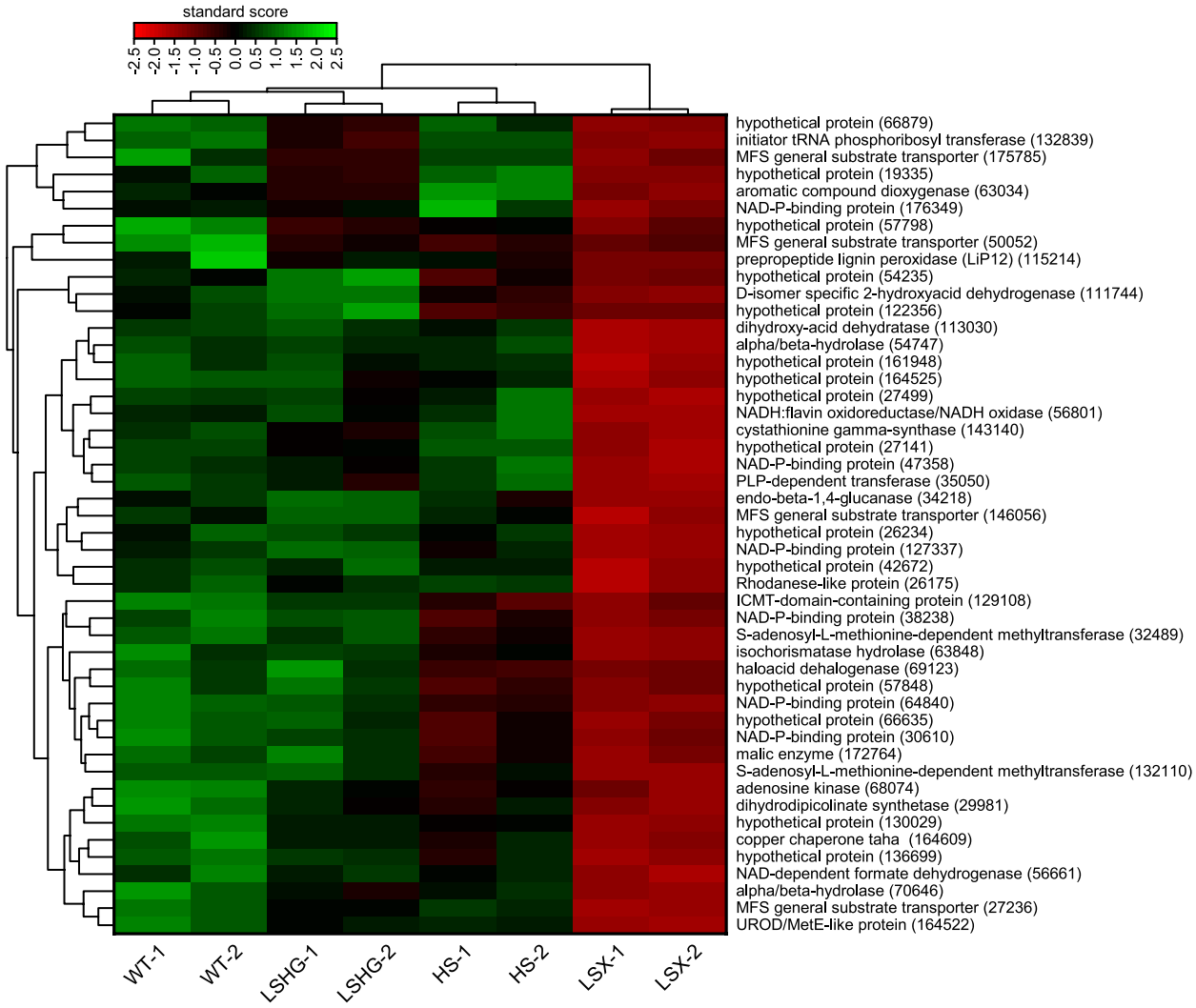


**Fig. 7** Heatmap of the 48 T. *versicolor* transcripts with twofold lower RPKM values after three weeks of growth on LSX wood compared to WT wood. These transcripts were from the 350 significantly different transcripts shown in Fig. 2. Grouping was as described in Fig. 2

transcriptomes at different time points would be necessary to explore this hypothesis further.

### Hypothetical proteins

Almost 46% of the significantly different transcripts in this study (160 of 350) were hypothetical proteins, which are predicted proteins from known transcripts but without a functional identification. Although this high number of unknown proteins prevents further understanding of the biochemical mechanisms used by *T. versicolor* during growth on hybrid poplar, it does highlight the necessity for further functional characterization studies, many of which are expected to contain proteins involved with lignocellulosic breakdown. For example, the unidentified transcripts uniquely upregulated and downregulated by the fungus in response to LSX was 56% (28 of 50) and 31% (15 of 48), respectively. This lack of functional characterization limits the understanding of the biochemical response of the fungus to this wood containing the unusual hydroxy-G lignin chemistry.

## Conclusions

The present study demonstrated that *T. versicolor* has unique transcriptome responses to lignin-modified poplar lines with the same genetic background (*Populus tremula*×*P. alba*). A total of 350 transcripts showed significant expressional differences when growing on the genetically altered wood compared to unmodified wood. Among the three major types of modified wood, the LSX lines elicited the most unique transcriptome response,

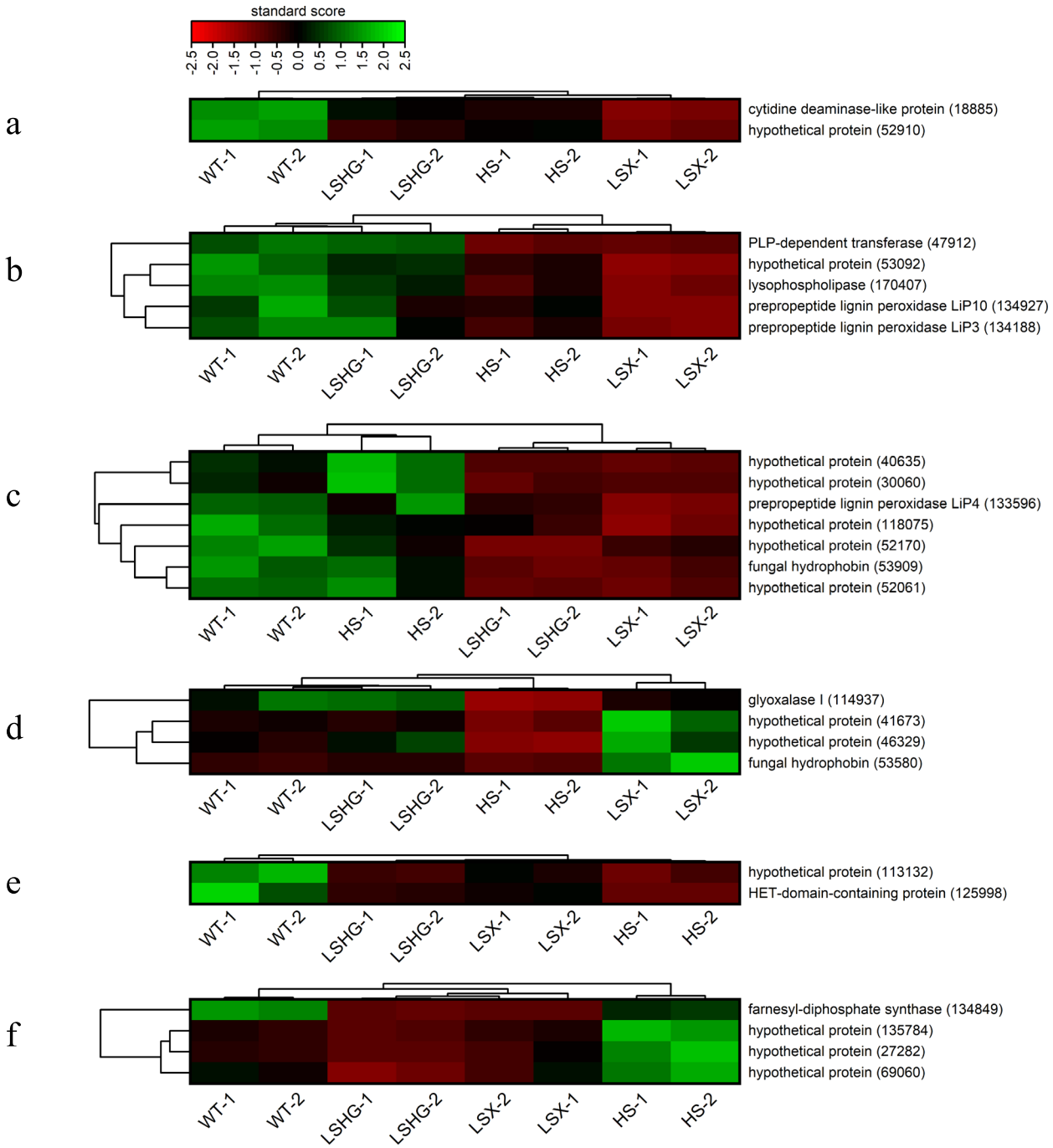


**Fig. 8** Heatmaps of the *T. versicolor* transcripts with twofold or lower RPKM values after three weeks of growth on **a** all three types of wood, **b** LSX and HS, **c** LSX and LSHG, **d** HS, **e** HS and LSHG, and **f** LSHG. All fold-changes were calculated using transcripts from growth on wild-type (WT) wood as the comparison. These transcripts were from the 350 significantly different transcripts shown in Fig. 2. Grouping was as described in Fig. 2

which is largely attributed to the abundance of hydroxyguaiacyl moieties and potential changes to the structural and physical attributes of the wood as a result of the unusual lignin chemistry. Due to the single time point nature of these studies, it is unclear if the transcriptome variations observed were due to the chemical composition of the lignocellulose and/or a function of the stage of deconstruction. Future time course experiments of the transcriptome of *T. versicolor* on these substrates should help discern the effects of time on transcription profiles

during the deconstruction process. Nevertheless, the results of the present study have important implications for industrial processes that use white-rot fungi (directly or as a source of enzymes) for processing lignocellulose, especially those that use direct fungal pretreatment of transgenic or chemically-inconsistent feedstocks.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40694-025-00193-w.

Additional file 1.

**Acknowledgements**
Not applicable.

**Author contributions**
ARA and NA were involved with RNA extraction and processing, transcriptome analysis, and manuscript preparation. RM provided the wood samples and provided guidance with manuscript preparation, and SJM provided statistical analyses and data interpretation assistance. TC designed the experiments, interpreted the data, and prepared the manuscript.

**Funding**
Funding for this work was provided by the Saudi Arabian Cultural Mission (SACM) as well as internal funding provided by Eastern Illinois University.

**Availability of data and materials**
Raw sequencing files and summary file generated from this study are available from GEO Series accession number GSE286220 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE286220).

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

Received: 4 December 2024 Accepted: 19 February 2025
Published online: 05 March 2025

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