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A Cross-species Transcriptional Profile Analysis of Heartwood Formation in Black Walnut

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Abstract The value of black walnut (*Juglans nigra* L.) is determined by the quality and quantity of darkly colored heartwood in its stem. We are exploring the regulation of heartwood production by identifying genes associated with the transition from sapwood to heartwood. We analyzed microarray data from a cross-species hybridization where black walnut cDNA was probed using a 7K gene aspen array. Results showed that only about 17% (1,253 vs 7K) of the probes in the microarray hybridized with genes from black walnut. Genes showing differential abundance in response to time change and developmental stage in the stem were identified and investigated. Eleven genes were identified as upregulated only in the transition zone (TZ) or interior sapwood of a tree harvested in fall; 55 genes were upregulated only in the TZs of trees harvested in summer; 74 genes were upregulated in the TZ of trees harvested in summer and in fall. Most of these genes were classified as "no hits", but some, such as the orthologs of *Arabidopsis* genes At2g14900 and At3g04710, were putatively related to cell rescue and defense. Genes related to other functional classifications such as signal transduction, metabolism, and protein fate and synthesis were also identified in this experiment. Overall, these analyses provide insight into the mechanism regulating heartwood formation in black walnut.

Keywords Microarray · Gene expression · Heartwood formation · Black walnut

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Introduction

Black walnut (*Juglans nigra* L.) is an industrially important tree species because of the excellent qualities of its heartwood. Unlike sapwood, which contains a small number of living parenchyma cells, heartwood is dead. As such, heartwood formation is likely to involve programmed cell death (PCD; Magel et al. 2001; Yang et al. 2004). Despite its economic importance, the mechanism of heartwood formation remains poorly understood. Previous research on heartwood has focused on metabolic, physiological, and ultrastructural changes, and the formation of extractive compounds in a specific area between sapwood and heartwood called the transition zone (TZ) (Frey-Wyssling and Bosshard 1959; Carrodus 1971; Shain and Mackay 1973; Miller et al. 1985; Hillis 1987; Nobuchi et al. 1987a, b; Abeles et al. 1992). Researchers have reported that some of the genes involved in the biosynthesis of polypropanoids and phenolics during heartwood formation include cinnamate 4-hydroxylase, 4-coumarate CoA ligase, chalcone synthase (CHS), flavanone 3-hydroxylase, and dihydroflavonol reductase (Hauch and Magel 1998; Magel 2000; Beritognolo et al. 2002).

The studies cited above involved and found only a handful of plant genes that may play a role in heartwood

formation. To gain a more global perspective on heartwood formation, DNA microarrays and other genomics methods are beginning to be applied. The first microarray study in tree research has determined a unique wood-forming tissue specific transcript profile in *Populus tremula*×*Populus tremuloides*, clone T89 (Hertzberg et al. 2001). Since the release of the poplar genomic sequence, many more microarray analyses of poplar have been performed to detect changes in gene expression, including responses to biotic or abiotic stress (Andersson et al. 2004; Taylor et al. 2005; Harding et al. 2005). Hertzberg et al. (2001) characterized and described how gene expression varied throughout cell division, expansion, secondary wall formation, lignification, and cell death by using a spotted 2,995 unique expressed sequence tags (ESTs) microarray in hybrid aspen. In hardwood research, Yang et al. (2004) investigated gene expression across the trunkwood of black locust (*Robinia pseudoacacia* L.) trees in order to gain insight into the molecular regulation of heartwood and extractives formation through cDNA microarray analysis of 2,278 unigenes.

Cross-species microarray hybridization has become a popular approach for transcriptome analyses in less-annotated genomes, in particular for comparative genomics analyses of gene expression, and for evolutionary and ecological studies of closely related species (Bar-Or et al. 2007). Cross-species microarray studies use a microarray platform designed with sequences from one species on a second species for which a microarray platform is not available. The major concern with cross-species microarray hybridization is the effect of sequence divergence on probe affinity. In other words, a probe that closely matches its target in one species may not match the same region in the second species because of sequence divergence that is described by the phylogenetic distance between the two species.

Heartwood formation is closely related to the process by which parenchyma cells undergo PCD. This complicated biological process includes the breakdown of storage materials, biosynthesis of secondary metabolites, and collapse of the cellular organelles, including mitochondria and vacuoles (Frey-Wyssling and Bosshard 1959). Extractives, the nonstructural wood components, are known to be powerful antimicrobial agents (DeBell et al. 1999), and they have been shown to contribute to the dark color of heartwood (Helm 2000). It is believed that heartwood formation starts in midsummer, continues in the late fall in black locust and sugi (*Cryptomeria japonica* L.; Nobuchi et al. 1987a, b), and then ends with dormancy. Heartwood is formed when the cambium is dormant in pine (*Pinus thunbergii* L.), black walnut, and cherry (*Prunus serrulata* L.; Shain and Mackay 1973; Nelson 1978). Therefore, heartwood formation is associated with seasonal change in some species, but the details of heartwood formation and its genetic regulation remain unclear. To gain insight into changes in gene expression that accompany heartwood formation in black walnut, an aspen 7K array was used in this experiment to hybridize with cDNA from TZ and sapwood of black walnut trees harvested during periods of active growth in summer (July) and dormancy onset in fall (October).

Materials and Methods

Plant Material and Growth Conditions

In order to investigate differences in gene expression between the sapwood and the TZ in black walnut, mature black walnut trees grown at the Martell Research Forest, Tippecanoe County, IN, USA, were cut down on July 1, 2004 and October 14, 2004. These trees were labeled summer tree (Tree 1) and fall tree (Tree 3). Immediately after the trees were felled, stem cross-sections ("cookies"), about 2.5 cm thick and 20 cm in diameter, were cut with a chainsaw from the base of the trees. The cookies were immediately submerged in liquid nitrogen. The time from initial cutting to completely frozen samples was about 15 min. After returning to the lab, the cookies were transferred to an ultra-low freezer (−80°C) for storage. The sapwood was divided into three zones: an interior sapwood zone that was next to TZ, a middle sapwood zone, and an exterior sapwood zone that was close to cambium (Fig. 1). TZs were identified under UV light (Fig. 1). Xylem from each zone (TZ, inner sapwood, middle sapwood, and exterior sapwood) was excised from the cookie using a chisel.

RNA Isolation

RNA was isolated as described previously (Kolosova et al. 2004). Xylem tissue was ground to a fine powder in a 6750 SPEX CertiPrep freezer mill (SPEX CertiPrep, Inc; Metuchen, NJ, USA). The modified extraction buffer consisted of the following: 200 mM Tris, pH8.5, 1.5% lithium dodecyl sulfate, 300 mM lithium chloride, 10 mM ethylenediaminetetraacetic acid, 1% sodium deoxycholate, 1% tergito powder P-40, 5 mM thiourea, 1 mM aurintricarboxylic acid, 10 mM dithiothreitol, 2% polyvinylpolypyrrolidone, and 2% ß-mercaptoethanol. RNA concentration was measured at 260 nM with a NanoDrop 1000 spectrophotometer (Wilmington, DE, USA).

Microarray Design and Hybridization

cDNA was synthesized from total RNA (via random hexamer priming) and labeled with fluorescent dyes (Cy3 and Cy5). To analyze the expression of genes found in the

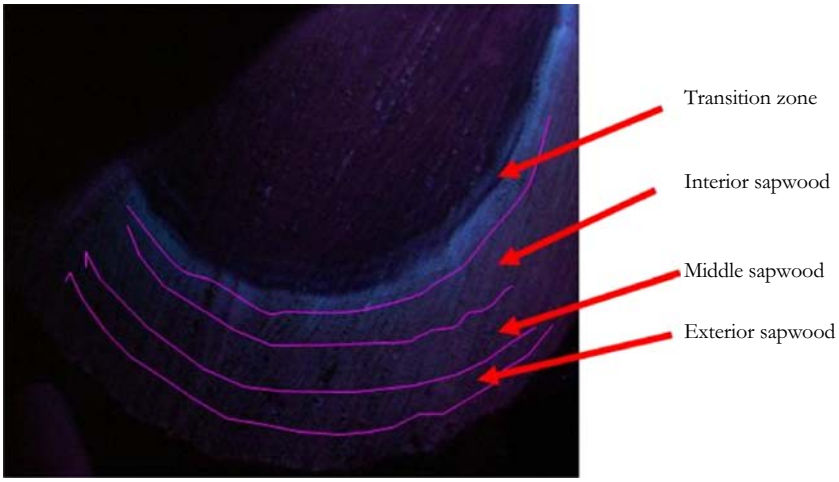


Fig. 1 Cross-section of a stem of black walnut under UV light. Transition zone tissue fluoresces blue and is next to the heartwood; interior sapwood: the layers next to the transition zone; middle sapwood: the layers next to the interior sapwood; exterior sapwood: the layers next to the middle sapwood and inside the vascular cambium

TZ of the walnut, we used an aspen 7K array, representing approximately 5,000 unique aspen sequences associated with wood formation. Cy3 and Cy5 were used to label cDNA from Tree 1 and Tree 3. Reference design (the mixture of all the samples) was used (Churchill 2002). Array hybridization was performed in an HS 400 (Tecan Instruments HS 400), following the protocols of Harding et al. (2005).

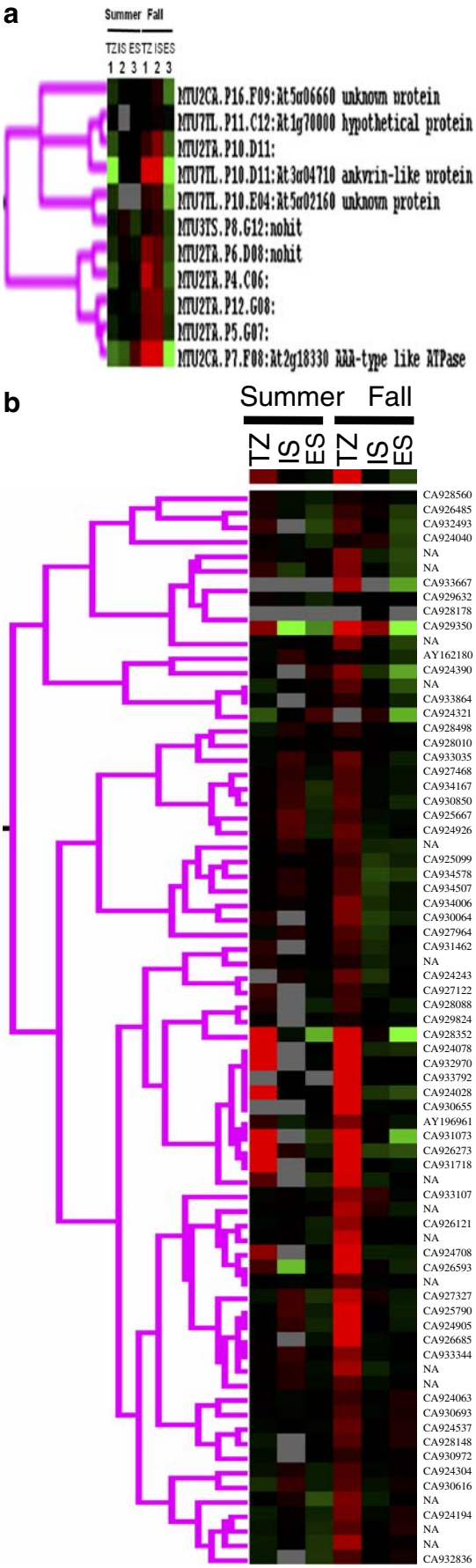
Microarray Data Analysis

Microarray slides were scanned with a GenePix 4000B Scanner (Axon Instruments; Union City, CA, USA), and the fluorescence intensity was acquired and quantified from the GenePix Pro 5.1 image analysis software (Axon Instruments; Union City, CA, USA). Fluorescence intensities were computed by subtracting local median background from median signal in both channels. Hybridization signals satisfying the criteria were used for expression analysis as described by Harding et al. (2005). After filtering, 1,253 features were submitted to Genespring 6.2 software for analysis. For each experiment, differentially expressed genes were considered up- or downregulated when the expression ratios were ≥1.5 or ≤0.67, respectively. Expression profiles of 1,253 genes were also grouped using the average linkage-hierarchical clustering function in Cluster program, and the output was displayed using Treeview (Eisen et al. 1998).

Results and Discussion

We explored the expression of black walnut genes in the TZ and sapwood in response to time change using a poplar 7K microarray, representing approximately 5,410 unique JGI *Populus* gene models. Only about 17% (1,253 vs 7K) of the probes in the microarray hybridized with genes from black walnut, in other words, only around 1/5 of the 7K cDNAs, were investigated for this purpose, perhaps because of sequence difference between the two species and because the aspen 7K array represents a portion of poplar genes (Ranjan et al. 2004; Harding et al. 2005). Hierarchical clustering enabled us to visualize the relationships between sampled tissues. Only 11 genes were significantly upregulated in the TZ or the interior sapwood zone of Tree 3 (fall), and most of them were unclassified (Fig. 2a; Table 2). We found a greater number of genes in clusters, such as genes related to general metabolism, protein synthesis, and cell rescue and defense (Fig. 2b, c; Table 2). We were interested in genes that were differentially expressed between TZ of Tree 1 (summer) and Tree 3 (fall) in response to time change and that had expression profiles that were differentially expressed in the TZ versus sapwood (ratio is as ≥1.5 or ≤0.67; Fig. 2). These differences reflect time change in the transition from sapwood to heartwood. Previous research indicated that heartwood formation is related to seasonal change in some species (Nobuchi et al. 1987a, b; Shain and Mackay 1973; Nelson 1978). Because black walnut is deciduous and becomes dormant in late fall, it is reasonable that the number of upregulated genes in TZ of Tree 3 (fall) decreased dramatically (Fig. 2a).

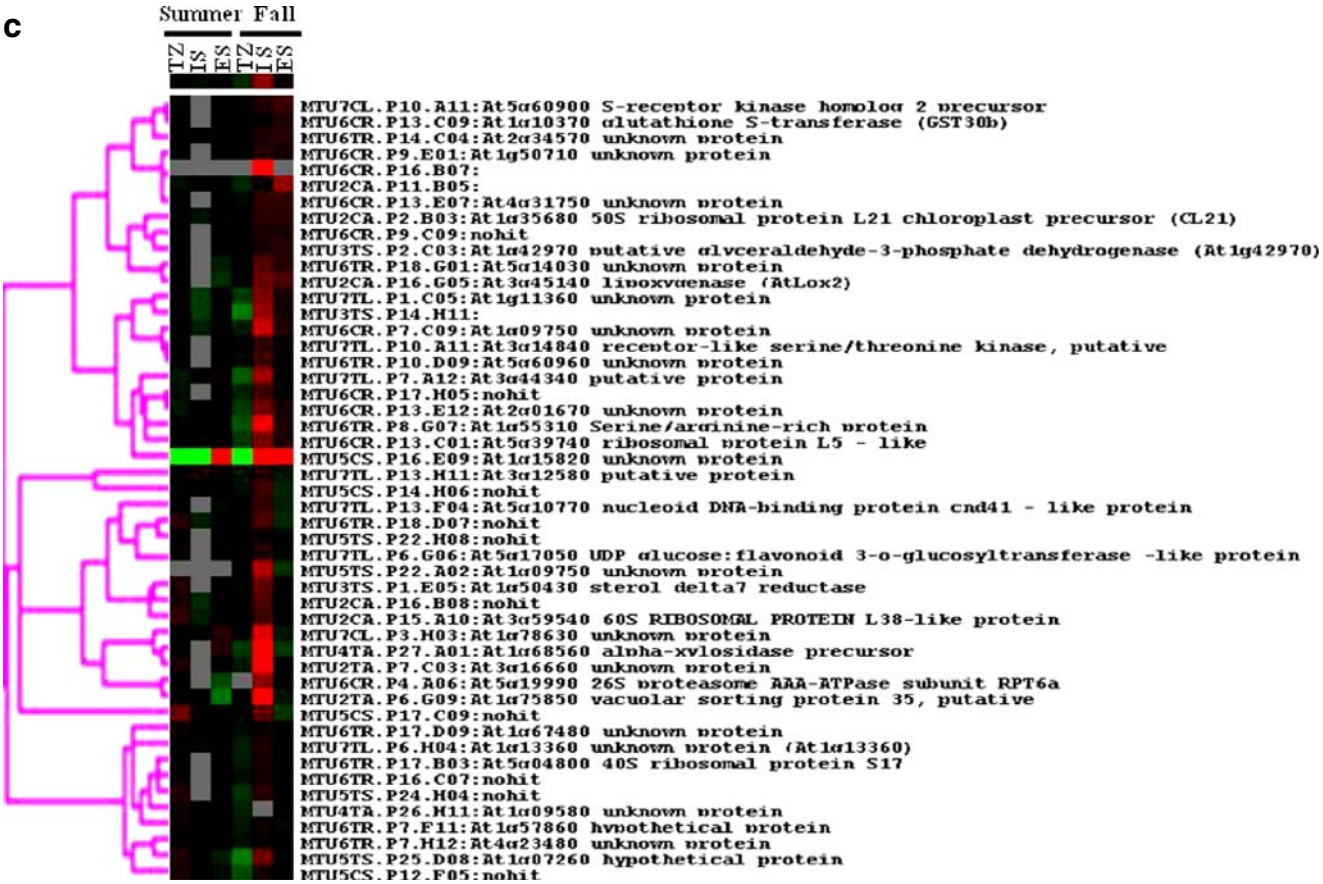
Our results showed that the number of upregulated genes was higher than the number of downregulated genes in every tissue (Table 1), indicating that heartwood formation, as with the development of other xylem tissues, is an active process involving many genes related to wood formation. We speculated that a bias toward upregulated genes was observed because the sequences from a portion of poplar EST databases printed on the chips were from upregulated genes or genes generally upregulated during development.



◀ **Fig. 2** Results of the hierarchical clustering displayed by Treeview. **a** Genes that were induced at TZ of Tree 1. **b** Genes that were induced in the TZ. **c** Genes that were induced in both TZ of Tree 1 and Tree 3 and the interior sapwood of Tree 3. Each *colored box* represents the mean signals of two replicates for a particular sample. These data suggest that expression of each cluster of genes is significantly different in the specific tissue in contrast to other tissues and in response to season. Each *column* represents a different tissue, and a *row* represents a gene. The mean expression level was determined for each gene across the six tissues, and for each tissue, a gene was assigned *red* if expression was above the mean, *green* if expression was below the mean, and *black* if expression was near the mean. The tissues are indicated vertically on the top (TZ (*1*)=transition zone; IS (*2*)=interior sapwood zone; ES (*3*)=exterior sapwood zone; summer=Tree 1 harvested in the summer; Fall=Tree 3 harvested in the fall). Genes are designated by Michigan Technological University gene identity (ID) number of *Populus trichocarpa* (the detailed annotation of these genes is in the Appendix)

As mentioned above, heartwood formation is likely to be closely related to seasonal change. Hierarchical clustering (Fig. 2a) showed that only 11 of 1253 genes were upregulated only in the TZ or the interior sapwood of the tree harvested in the fall (Fig. 2a), including one energy metabolism gene (the ortholog of At2g18330 AAA-type like ATPase), one signal transduction gene (the ortholog of At3g04710 ankyrin-like protein), and nine putative genes that have not been functionally characterized. The ortholog of At2g18330 was a putative AAA-type ATPase similar to 26S proteosome regulatory subunit 8 required for ubiquitin-dependent protein degradation in *Arabidopsis thaliana* (Rhee et al. 2003). The ortholog of At3g04710 was shown to be similar to stress-inducible proteins in *A. thaliana* (Dong 2004). While it is not surprising that heartwood formation might require protein degradation or that a tree entering to dormancy in the fall needs cold-response genes, it is notable that genes regulating these processes were differentially upregulated in the TZ.

Fifty-five genes were downregulated in the TZ of Tree3 (fall), including 23 unclassified or unknown genes (Fig. 2b). One of the downregulated genes, the ortholog of At2g14900, was similar to a gibberellin-related protein (Rhee et al. 2003) that might play a role in cell rescue and defense (Table 2), indicating that heartwood formation may involve downregulation of defense signaling or cell rescue. Interestingly, the ortholog of At5g13930 was downregulated as well. At5g13930 encodes *CHS*, which is a key enzyme in the biosynthesis of flavonoids and required for the accumulation of purple anthocyanins in leaves and stems in *Arabidopsis*. *CHS* is often used as a marker of ethylene biosynthesis (Heller and Forkmann 1988; Rhee et al. 2003; Chen et al. 2004). The chemical composition and distribution of flavonoids and anthocyanins contribute to wood's dark color, which is one of the distinguishing characteristics of heartwood (Mayer et al. 2006). Previous



research showed that *CHS* was most highly expressed in the TZ of black walnut (Beritognolo et al. 2002). These conflicting results may be resolved if there are several members of the *CHS* family in black walnut, each with different patterns of regulation in different tissues. Unlike *A. thaliana*, where only one *CHS* gene exists (Feinbaum and Ausubel 1988), soybean (*Glycine max* L.) has an eight-member *CHS* family, and the members display different functions in different tissues (Dhawale et al. 1989; Dhaubhadel et al. 2003).

We detected 74 genes upregulated in the TZ of both Tree 1 and Tree 3 (Fig. 2c, Table 2). In this group, three of the upregulated genes were related to transport. Perhaps these genes function in movement of extractives or precursors to or from the vacuole or other organelles or cells. Previous research has shown that loss of vacuolar transport resulted in loss of color in tissues. For example, when transcript of *ZmMRP3*, the vacuolar ABC transporter, was reduced, the levels of anthocyanin accumulation were decreased in different plant parts of *Zea mays* as well (Goodman et al. 2004). *Arabidopsis* mutant *transparent testa 12* (*tt12*) is deficient in the vacuolar accumulation of proanthocyanidin precursors in the seed (Debeaujon et al. 2001). The *A. thaliana transparent testa 10* (*tt10*) mutant seed coat exhibits delayed browning compare with that of the wild type, suggesting that this gene encoding a laccase-like enzyme, may be involved in oxidative polymerization of flavonoids (Shirley et al. 1995; Pourcel et al. 2005). Several genes related to protein fate were upregulated in the TZ of the fall trees. As mentioned above, an abrupt change in biological process occurs in the TZ when trees form heartwood. Before this stage, cells near the TZ still need nutrients and minerals to support growth and development, and energy for maintenance until the end of PCD. According to our data, a number of energy metabolism, general metabolism, and secondary metabolism genes were found upregulated in the TZ (Appendix C). Many of these genes could play roles heartwood formation, as described by Higuchi (1997). Higuchi proposed that metabolites or critical genes in living parenchyma cells activated by endogenous or exogenous factors were altered, leading to increased biosynthesis of extractives and finally releasing extractives stored in vacuoles by chemical or enzymatic processes.

Cross-species microarray hybridization has become a popular approach for transcriptome analyses in less-

Table 1 Number of up- and downregulated genes by season and tissue types as detected by hybridization of black walnut onto a 7K poplar array

Tissue	Summer		Fall	
	Upregulated	Downregulated	Upregulated	Downregulated
Transition zone	254	170	235	55
Interior sapwood zone	239	90	184	107
Exterior sapwood zone	212	97	194	91

Up- or downregulated genes were determined as ≥1.5- or ≤0.6-fold change over standard control. See "Materials and Methods" for details

annotated genomes, in particular for comparative genomic analyses of gene expression, evolutionary, and ecological studies of closely related species (Bar-Or et al. 2007). The major concern with cross-species microarray hybridization is the effect of sequence divergence on probe affinity. Low probe affinity may explain why we observed only 17% hybridization between aspen and black walnut sequences. Cross-species hybridization is still a developing and controversial tool, but it can provide a guide for species with limited genomic resources (Bar-Or et al. 2007).

Our study showed that genes related to heartwood formation are a subset of or overlap with those processes necessary for xylem formation, including transcriptional regulation of phytohormones, polyphenol and flavonoid biosynthesis, and PCD-associated events. Unlike the work of Yang et al. (2004) that provides the first comprehensive analysis of the global gene expression changes occurring in the TZ during different seasons in black locus, we did not obtain sufficient information of the mechanism of heartwood formation in black walnut from this cross-species microarray analysis. However, our research should shed light on the mechanism of heartwood and the relationship between heartwood formation and wood formation. The exact mechanism of heartwood formation remains to be further explored.

Table 2 Functional classification of upregulated genes in transition zone tissue harvested in summer and fall

Functional classification	Genes upregulated in TZ of fall tree only	Genes downregulated in TZ of fall tree only	Genes upregulated in TZ of both summer and fall trees
Cell rescue and defense		2	2
Cellular organization		1	3
Development systemic		2	1
Energy metabolism	1	2	2
General metabolism	1	10	7
Protein fate		1	6
Protein synthesis	1	8	10
Regulation interaction with cellular environment			1
Secondary metabolism		1	1
Signal transduction	1	1	2
Subcellular localization		1	1
Systemic response and sensing			1
Transcription	1	1	1
Transport			1
Transposable element		1	
Unclassified	6	21	18
Total number	11	55	74

Numbers are of selected clones for microarray analysis

Appendix

A	
CA929649	MTU2CA.P16.F09:At5g06660 unknown protein
CA924909	MTU7TL.P11.C12:At1g70000 hypothetical protein
NA	MTU2TA.P10.D11:
CA924854	MTU7TL.P10.D11:At3g04710 ankyrin-like protein
CA924857	MTU7TL.P10.E04:At5g02160 unknown protein
CA934520	MTU3TS.P8.G12:no hit
CA931368	MTU2TA.P6.D08:no hit
NA	MTU2TA.P4.C06:
NA	MTU2TA.P12.G08:
NA	MTU2TA.P5.G07:
CA930140	MTU2CA.P7.F08:At2g18330 AAA-type-like ATPase
B	
CA928560	MTU6TR.P4.A12:At5g14470 putative protein
CA926485	MTU6CR.P15.E11:At5g47720 acetoacetyl-CoA-thiolase
CA932493	MTU5CS.P13.F12:At4g26690 unknown protein
CA924040	MTU7CL.P1.A07:At5g20280 sucrose-phosphate synthase-like protein
NA	SP_c8:
NA	PtrSuSy:
CA933667	MTU3TS.P10.G02:no hit
CA929632	MTU2CA.P16.D11:no hit
CA928178	MTU6TR.P17.F05:
CA929350	MTU2CA.P12.E02:At5g48930 anthranilate N-benzoyltransferase
NA	MTU2TA.P5.H06:
AY162180	PtrCesA7:
CA924390	MTU7CL.P5.B04:At3g25690 unknown protein
NA	MTU7TL.P10.D05:
CA933864	MTU3TS.P13.F07:At4g16070 unknown protein
CA924321	MTU7CL.P4.C10:At1g24020 pollen allergen-like protein
CA928498	MTU6TR.P3.D05:At3g49910 60S ribosomal protein-like
CA928010	MTU6TR.P15.F09:At2g45290 putative transketolase precursor
CA933035	MTU5CS.P2.F04:At3g12580 putative protein
CA927468	MTU6CR.P9.D08:At2g36530 enolase (2-phospho-D-glycerate hydroylase)
CA934167	MTU3TS.P4.A05:At2g30570 photosystem II reaction center 6.1KD protein
CA930850	MTU2TA.P11.B04:At5g02500 dnaK-type molecular chaperone hsc70.1
CA925667	MTU7TL.P4.D09:no hit
CA924926	MTU7TL.P11.E10:no hit
NA	MTU5TS.P21.C07:
CA925099	MTU7TL.P13.E10:At4g00430 transmembrane protein
CA934578	MTU3TS.P9.G08:At5g60390 protein synthesis elongation factor eEF-1 alpha chain (gene A4)
CA934507	MTU3TS.P8.F08:At5g60390 protein synthesis elongation factor eEF-1 alpha chain (gene A4)
CA934006	MTU3TS.P1.F03:At5g60390 protein synthesis elongation factor eEF-1 alpha chain (gene A4)
CA930064	MTU2CA.P6.F01:
CA927964	MTU6TR.P15.B03:At5g60390 protein synthesis elongation factor eEF-1 alpha chain (gene A4)
CA931462	MTU2TA.P7.F04:At4g16190 cysteine proteinase like protein
NA	MTU2TA.P6.H09:
CA924243	MTU7CL.P3.D11:At1g11380 unknown protein
CA927122	MTU6CR.P5.C02:At4g02040 hypothetical protein
CA928088	MTU6TR.P16.F03:At1g77750 putative 30S ribosomal protein S13
CA929824	MTU2CA.P3.C02:At1g67430 ribosomal protein L17-like protein
CA928352	MTU6TR.P1.F03:At3g51090 unknown protein
CA924078	MTU7CL.P1.D12:no hit
CA932970	MTU5CS.P1.G10:At2g36870 putative xyloglucan endotransglycosylase
CA933792	MTU3TS.P12.F09:At1g76490 3-hydroxy-3-methylglutaryl CoA reductase (AA 1-592)
CA924028	MTU7CL.P17.H06:At1g12820 transport inhibitor response 1, putative
CA930655	MTU4CA.P25.A10:At5g56710 60S ribosomal protein L31
AY196961	PtrCesA6:
CA931073	MTU2TA.P1.F01:At1g48630 unknown protein
CA926273	MTU6CR.P13.A11:no hit
CA931718	MTU4TA.P22.A09:At3g02790 unknown protein
NA	MTU5CS.P3.C04:
CA933107	MTU5CS.P3.F02:no hit
NA	MTU2TA.P7.C05:
CA926121	MTU6CR.P10.C05:At1g11910 putative aspartic proteinase
NA	MTU2TA.P3.E02:
CA924708	MTU7CL.P9.A10:no hit
CA926593	MTU6CR.P16.H01:At2g46140 putative desiccation related protein
NA	MTU6CR.P11.F07:
CA927327	MTU6CR.P7.F06:At3g12580 putative protein
CA925790	MTU7TL.P5.H09:At1g56410 hypothetical protein
CA924905	MTU7TL.P11.C07:At1g23310 putative alanine aminotransferase
CA926685	MTU6CR.P18.A02:At4g30330 small nuclear ribonucleoprotein homolog
CA933344	MTU5CS.P6.G04:no hit
NA	MTU5TS.P22.C12:
NA	MTU5TS.P21.G11:
CA924063	MTU7CL.P1.C08:At1g09940 putative glutamyl-tRNA reductase 2 precursor
CA930693	MTU4CA.P25.E05:no hit
CA924537	MTU7CL.P6.H08:At1g07560 protein kinase, putative
CA928148	MTU6TR.P17.C09:At3g11210 unknown protein
CA930972	MTU2TA.P13.B06:At5g14040 mitochondrial phosphate translocator

CA924304	MTU7CL.P4.B05:no hit
CA930616	MTU4CA.P24.F03:At2g16660 nodulin-like protein
NA	MTU2TA.P12.E05:
CA924194	MTU7CL.P2.H01:no hit
NA	MTU5TS.P23.C09:
NA	MTU5TS.P25.F03:
CA932836	MTU5CS.P18.B02:At3g49910 60S ribosomal protein-like
C	
CA923405	MTU7CL.P10.A11:At5g60900 S-receptor kinase homolog 2 precursor
CA926291	MTU6CR.P13.C09:At1g10370 glutathione S-transferase (GST30b)
CA927896	MTU6TR.P14.C04:At2g34570 unknown protein
CA927472	MTU6CR.P9.E01:At1g50710 unknown protein
NA	MTU6CR.P16.B07:
NA	MTU2CA.P11.B05:
CA926310	MTU6CR.P13.E07:At4g31750 unknown protein
CA929742	MTU2CA.P2.B03:At1g35680 50S ribosomal protein L21 chloroplast precursor (CL21)
CA927462	MTU6CR.P9.C09:no hit
CA934059	MTU3TS.P2.C03:At1g42970 putative glyceraldehyde-3-phosphate dehydrogenase (At1g42970)
CA928274	MTU6TR.P18.G01:At5g14030 unknown protein
CA929654	MTU2CA.P16.G05:At3g45140 lipoxygenase (AtLox2)
CA925439	MTU7TL.P1.C05:At1g11360 unknown protein
NA	MTU3TS.P14.H11:
CA927297	MTU6CR.P7.C09:At1g09750 unknown protein
CA924821	MTU7TL.P10.A11:At3g14840 receptor-like serine/threonine kinase, putative
CA927569	MTU6TR.P10.D09:At5g60960 unknown protein
CA925881	MTU7TL.P7.A12:At3g44340 putative protein
CA926678	MTU6CR.P17.H05:no hit
CA926315	MTU6CR.P13.E12:At2g01670 unknown protein
CA928953	MTU6TR.P8.G07:At1g55310 Serine/arginine-rich protein
CA926285	MTU6CR.P13.C01:At5g39740 ribosomal protein L5-like
CA932719	MTU5CS.P16.E09:At1g15820 unknown protein
CA925134	MTU7TL.P13.H11:At3g12580 putative protein
CA932582	MTU5CS.P14.H06:no hit
CA925105	MTU7TL.P13.F04:At5g10770 nucleoid DNA-binding protein cnd41-like protein
CA928248	MTU6TR.P18.D07:no hit
CA934722	MTU5TS.P22.H08:no hit
CA925855	MTU7TL.P6.G06:At5g17050 UDP glucose:flavonoid 3-o-glucosyltransferase-like protein
CA934658	MTU5TS.P22.A02:At1g09750 unknown protein
CA933998	MTU3TS.P1.E05:At1g50430 sterol delta7 reductase
CA929612	MTU2CA.P16.B08:no hit
CA929525	MTU2CA.P15.A10:At3g59540 60S ribosomal protein L38-like protein
CA924279	MTU7CL.P3.H03:At1g78630 unknown protein
CA932107	MTU4TA.P27.A01:At1g68560 alpha-xylosidase precursor
CA931431	MTU2TA.P7.C03:At3g16660 unknown protein
CA927024	MTU6CR.P4.A06:At5g19990 26S proteasome AAA-ATPase subunit RPT6a
CA931399	MTU2TA.P6.G09:At1g75850 vacuolar sorting protein 35, putative
CA932778	MTU5CS.P17.C09:no hit
CA928159	MTU6TR.P17.D09:At1g67480 unknown protein
CA925864	MTU7TL.P6.H04:At1g13360 unknown protein (At1g13360)
CA928130	MTU6TR.P17.B03:At5g04800 40S ribosomal protein S17
CA928057	MTU6TR.P16.C07:no hit
CA934859	MTU5TS.P24.H04:no hit
CA932105	MTU4TA.P26.H11:At1g09580 unknown protein
CA928857	MTU6TR.P7.F11:At1g57860 hypothetical protein
CA928879	MTU6TR.P7.H12:At4g23480 unknown protein
CA934893	MTU5TS.P25.D08:At1g07260 hypothetical protein
CA932412	MTU5CS.P12.F05:no hit

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