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Abstract:	Despite the crucial roles of phytohormones in plant development, comparison of the exact distribution profiles of different hormones within plant meristems has thus far remained scarce. Vascular cambium, a wide lateral meristem with an extensive developmental zonation, provides an optimal system for hormonal and genetic profiling. By taking advantage of this spatial resolution, we show here that two major phytohormones, cytokinin and auxin, display different, yet partially overlapping, distribution profiles across the cambium. In contrast to auxin, which has its highest concentration in the actively dividing cambial cells, cytokinins peak in the developing phloem tissue of a Populus trichocarpa stem. Gene expression patterns of cytokinin biosynthetic and signaling genes coincided with this hormonal gradient. To explore the functional significance of cytokinin signaling for cambial development, we engineered transgenic Populus tremula × tremuloides trees with an elevated cytokinin biosynthesis level. Confirming that cytokinins function as major regulators of cambial activity, these trees displayed stimulated cambial cell division activity resulting in dramatically increased (up to 80% in dry weight) production of the lignocellulosic trunk biomass. To connect the increased growth to hormonal status, we analyzed the hormone distribution and genome-wide gene expression profiles in unprecedentedly high resolution across the cambial zone. Interestingly, in addition to showing an elevated cambial cytokinin content and signaling level, also the cambial auxin concentration and auxin responsive gene expression were increased in the transgenic trees. Our results indicate that cytokinin signaling specifies meristematic activity through a graded distribution that influences the amplitude of the cambial auxin gradient.

Cytokinin and auxin display distinct but interconnected distribution and signaling profiles to stimulate cambial activity

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Summary

Despite the crucial roles of phytohormones in plant development, comparison of the exact distribution profiles of different hormones within plant meristems has thus far remained scarce. Vascular cambium, a wide lateral meristem with an extensive developmental zonation, provides an optimal system for hormonal and genetic profiling. By taking advantage of this spatial resolution, we show here that two major phytohormones, cytokinin and auxin, display different, yet partially overlapping, distribution profiles across the cambium. In contrast to auxin, which has its highest concentration in the actively dividing cambial cells, cytokinins peak in the developing phloem tissue of a *Populus trichocarpa* stem. Gene expression patterns of cytokinin biosynthetic and signaling genes coincided with this hormonal gradient. To explore the functional significance of cytokinin signaling for cambial development, we engineered transgenic *Populus tremula* × *tremuloides* trees with an elevated cytokinin biosynthesis level. Confirming that cytokinins function as major regulators of cambial activity, these trees displayed stimulated cambial cell division activity resulting in dramatically increased (up to 80% in dry weight) production of the lignocellulosic trunk biomass. To connect the increased growth to hormonal status, we analyzed the hormone distribution and genome-wide gene expression profiles in unprecedentedly high resolution across the cambial zone. Interestingly, in addition to showing an elevated cambial cytokinin content and signaling level, also the cambial auxin concentration and auxin responsive gene expression were increased in the transgenic

trees. Our results indicate that cytokinin signaling specifies meristematic activity through a graded distribution that influences the amplitude of the cambial auxin gradient.

Results and Discussion

Cytokinin and auxin display distinct distribution profiles across the vascular cambium

The bulk of global lignocellulosic biomass is present in the form of wood, the secondary xylem of a plant stem. Secondary vascular tissues are produced through the activity of the vascular cambium, the cylindrical secondary meristem of a tree trunk. Detailed knowledge about the regulatory mechanisms controlling cambial development provides us powerful tools to boost the lignocellulosic biomass production in forest trees (1-2): the better we understand the molecular mechanisms controlling wood formation, the easier it is to implement this knowledge into forest biotechnology and tree breeding.

Cytokinins and auxin are two major plant hormone classes well-known to regulate a multitude of plant developmental processes. Differential cytokinin and auxin response domains have been shown to contribute to the patterning of both shoot (3-5) and root apical meristems (6-9). Whereas the distribution of auxin in various meristems is well established (10-12), the occurrence of cytokinin has been less studied. To address this question, we have now taken advantage of the high spatial resolution provided by the extensive developmental zonation of the vascular cambium. We report here direct measurements of distribution and signaling profiles of cytokinin and auxin hormones across the cambial zone of both *Populus trichocarpa* (Figures 1, S1) and *Populus tremula* × *tremuloides* stems (see below).

In *P. trichocarpa*, hormonal concentrations were studied in six stem cryofractions encompassing the developmental zonation of the cambial zone: old phloem, conducting phloem, developing phloem, cambium, developing xylem and xylem tissues. We observed that concentration of the major bioactive auxin (IAA) peaked in the middle of the cambial zone, in the domain of dividing cambial cells. This result confirms the pattern of cambial auxin gradient and response domain previously reported in tree species (13-16). The importance of auxin for cambial development has been verified through functional studies: fewer cell divisions took place in the vascular cambium of transgenic *Populus* trees with reduced auxin responsiveness, resulting in compromised radial stem growth (17). In contrast to auxin, the two major bioactive cytokinin species, isopentenyladenine (iP) and trans-zeatin (tZ), have their maximum levels coinciding with each other in the developing phloem cells (Figure 1B). Also iP riboside (iPR), a precursor of bioactive iP, has a similar distribution profile. A third plant hormone, bioactive gibberellin (GA4), peaked in the developing xylem tissue. Similar gibberellin distribution profile has been previously reported by Israelsson et al. (18): in their study bioactive GA4 and GA1 had their highest levels in the expanding xylem cells. The observed distribution coincides with activity of gibberellin signaling in the regulation of xylem development: the rate of secondary xylem production has been shown to be increased in transgenic plants with either enhanced gibberellin signaling (19) or biosynthesis levels (20-23). Taken together, our results show that the two major phytohormones, cytokinin and auxin, form distinct, yet partially overlapping, distribution domains across the vascular cambium.

To verify the observed cytokinin domain, we studied the expression of cytokinin signaling and homeostasis genes (24, 25) across the cambial zone of *P. trichocarpa* stem through RT-qPCR analyses (Figure S1). Expression of the signaling and biosynthesis genes coincided with the

phloem peaking hormonal distribution; the gene expression profiles were further defined through high resolution RNA-sequencing analyses in *P. tremula* × *tremuloides* trees (see below).

Increased cytokinin biosynthesis stimulates cambial cell division rate and increases the production of trunk biomass in transgenic *Populus* trees

Previous work has highlighted the potential of cytokinin signaling as a major positive regulator of cambial activity in a tree trunk: we have shown that a reduced cytokinin concentration leads to compromised radial growth in transgenic *Populus* trees (26). Similarly, cambial activity was abolished in the root of quadruple Arabidopsis *ipt1,3,5,7* mutant, which lacks the four major cytokinin biosynthesis enzymes (27).

Based on our cytokinin profiling data and previous results, we next studied the effect of elevated cambial cytokinin signaling on tree trunk growth. With the aim to stimulate cytokinin biosynthesis in transgenic *P. tremula* × *tremuloides* trees, we over-expressed the *AtIPT7* gene from Arabidopsis. This gene encodes one of the key enzymes in the biosynthesis of major bioactive cytokinins (28-30). The *AtIPT7* transgene was cloned under the *PttLMX5* promoter (31), which drives a high expression in the cambial zone and developing xylem cells. We were able to obtain twelve transgenic lines, from which two, *pLMX5:AtIPT7* 1 and 3, both with a high transgene expression level and highly similar phenotypes (Figure 2), were selected for further analyses.

To evaluate the effect of AtIPT7 activity on tree development, we followed growth of the transgenic trees under greenhouse conditions (Figure 2). The apical growth rate of *pLMX5:AtIPT7* lines was similar to WT plants; transgenic plants had the same height as the controls (Figure 2A). After ten weeks of growth the average height of four individual trees from WT, and lines 1 and 3, was 152±4 cm SE, 150±2 cm and 148±3 cm, respectively. In contrast, stem diameter was increased in the transgenic trees as compared to the WT trees (Figure S2A); the stimulatory effect of cytokinin on the radial growth was therefore independent of the apical growth rate. Accordingly, the stem volume, which was counted as the additive volume of internodes, was larger in the *pLMX5:AtIPT7* trees (Figure 2B). The stem biomass (trunk after the branches were removed) was measured at the age of 13 weeks: the average dry weight of three individual trees from WT, and lines 1 and 3, was 18.5±0.3 g SE, 33.0±0.7 g and 34.4±0.8 g, respectively (*p*-values from Student's t-test: line 1 vs WT 6.56E-04***, 3 vs WT 9.19E-04***). The increase in biomass was up to 80% in dry weight.

To explore in detail the effect of enhanced cytokinin signaling on vascular architecture, we analyzed the anatomy of the transgenic trees. No differences were observed in the dimensions of xylem cells between the WT and *pLMX5:AtIPT7* trees. In contrast, a difference was observed in the cambial anatomy. The vascular cambium of the *pLMX5:AtIPT7* line 1 and line 3 trees contained more meristematic cells in the cambial cell files than the WT trees: respectively in average 23±0.4 (SE), 24±1.2 and 15±0.8 (Figures 3A, S2B). The increased cell number indicates that the cambial cell files were undergoing additional cell divisions as compared to the WT. Our results confirm that cytokinins act as major positive regulators of cambial activity in trees.

Genetic engineering of enhanced cytokinin biosynthesis leads to an increase in cambial cytokinin and auxin concentrations

Next, hormonal responsiveness of the two *pLMX5:AtIPT7* lines was tested in an *in vitro* assay (Figure 2C), where a lower cytokinin to auxin ratio in the growth medium induces root regeneration and a higher cytokinin to auxin ratio promotes shoot regeneration (32). We observed enhanced cytokinin responsiveness in the transgenic trees: several internodes produced shoots, and unexpectedly also roots, already on the medium with no added cytokinin, whereas no WT internodes produced either. As high auxin concentration promotes root formation, these results indicate that the transgenic lines may have had higher concentrations of both cytokinin and auxin than the WT trees.

To validate our transgenic approach to stimulate cytokinin biosynthesis, we compared the hormonal profiles across the cambial zone of WT and *pLMX5:AtIPT7* trees (Figure 3B). The hormonal distribution in WT *P. tremula* × *tremuloides* was similar in *P. trichocarpa*: cytokinins were highest in the developing phloem tissue, and auxin was peaking in the middle of the cambium. When the transgenic trees were compared to the WT, several differences in hormonal levels were observed: concentrations of bioactive iP and tZ were elevated and dramatic increases were seen in the concentrations of iP precursor iPR and IAA contents (Figure 3B). These results confirm an increase in cambial cytokinin content in the *pLMX5:AtIPT7* trees; they further show that this increase also leads to an elevation in the auxin concentration. In Arabidopsis, homeostasis of these two hormones is known to be connected: cytokinin has been shown to contribute to the regulation of both auxin homeostasis (33) and transport (11, 34).

Genome-wide gene expression profiling across the cambial zone confirms the distinct cytokinin and auxin signaling domains

To connect the hormonal distribution to the status of hormonal signaling and homeostasis, we conducted a high-resolution gene expression profiling across the cambial zone of WT and *pLMX5:AtIPT7 P. tremula* × *tremuloides* trees (Figures 4, S4). The data was collected in a genome-wide manner through RNA-sequencing; our focus in the profiling was on cytokinin and auxin signaling, homeostasis and transport genes.

Considering cytokinin signaling, we profiled the components of cytokinin signal transduction pathway: receptors, HPts, type-B RRs and type-A RRs. Cytokinin signaling pathway represents a multistep two-component phosphorelay system: upon binding hormone ligand, cytoplasmic CYTOKININ RESPONSE 1 (CRE1) -like receptors initiate the phosphorelay, and phosphorylate histidine phosphotransfer (HPt) proteins. The HPts cycle between the cytosol and nucleus; in the nucleus, they transfers the phosphoryl onto type-B phospho-accepting response regulators (RRs). The type-B RR are transcription factors that activate transcription of cytokinin primary response genes; among them are the type-A RRs, which provide a negative feedback mechanism on the phosphorelay by repressing the activity of type-B RRs. For cytokinin homeostasis we characterized expression patterns for the biosynthetic *IPT*s, together with the *LONELY GUYS* (*LOG*s), which convert inactive cytokinins to bioactive forms, and the catabolic *CYTOKININ OXIDASES* (*CKX*s). To profile cytokinin transporters, we analyzed the expression patterns of *Populus* orthologs of Arabidopsis *AtENT6* (35) and *AtABCG14* (36, 37). To define the status of auxin response, expression profiles of *PttIAA*s, representing the putative auxin response genes, were analyzed. For auxin homeostasis we characterized expression of the biosynthetic *YUCCA* genes and the auxin transporting *PIN* influx and *AUX/LAX* efflux carriers.

Prior to the profiling, to test cytokinin responsiveness of the selected genes, we conducted an RNA-sequencing analysis of cytokinin treated WT *P. tremula* × *tremuloides* stem discs (Figure S3). As expected, almost all type-A *RR*s were upregulated by the 1 h cytokinin treatment, whereas most other genes were unaffected. Since expression of some *IAA*s is known to be directly upregulated by the B-type RRs (7, 38), our focus was to identify the *PttIAA* genes that are not cytokinin induced. Expression of most *IAA*s was unaffected, indicating that they represent true auxin response marker genes, but two out of 33 were potentially upregulated (Figure S3), and respectively omitted from further profiling.

In the profiling data, we were able to define three distinct patterns for robustly expressed genes in the WT cambial zone; identity of the tissues was verified through marker gene analyses (Figure 4). Pattern 1 (P1) was defined by genes that peaked in the developing phloem tissues (Figures 4, S4A). The pattern was specified by the marker gene *PttCLE41a*, an ortholog of an early phloem abundant Arabidopsis peptide, and ligand of the PXY/WOX regulatory pathway of cambial activity (39, 40). Pattern 2 (P2) was defined by genes with expression peaking at the middle of cambial zone, in the zone of dividing cells, coinciding with the profile of the cambial *PttWOX4a* marker gene (Figures 4, S4). Pattern 3 (P3) was specified by genes with expression maxima in the developing xylem tissue. This pattern coincides with the profile of *PttCOMT2*, a marker gene expressed in differentiating xylem cells (Figures 4, S4).

Members from all analyzed cytokinin signaling and homeostasis gene families were present in the *Populus* cambium. Almost all cytokinin signaling pathway genes (receptors, HPts, type-B and type-A RRs) were defined by the phloem peaking Pattern 1 (Figures 4, S4), but one gene displayed middle peaking Pattern 2 (Figure S4). In contrast, profiles of the putative auxin primary response *PttIAA* genes were more diverse: most of them coincided with the auxin gradient, peaking in the middle of the cambium (Pattern 2) (Figures 4, S4), but several were defined by the two other patterns (Figure S4). Almost all of the *YUCCA*s and auxin transporters followed Pattern 2 (Figure S4), but a few were peaking in the phloem (Pattern 1) (Figure S4).

Considering cytokinin homeostasis, most biosynthetic *IPT* genes were defined by the Pattern 1 (Figures 4, S4), whereas both cytokinin activating *LOG*s and catabolic *CKX*s were peaking more in the middle (Figures 4, S4), implying that the homeostasis genes play a role in shaping of the cambial cytokinin gradient.

Increased cytokinin biosynthesis enhances cytokinin and auxin responses in their distinct cambial domains

To further explore the hormonal status of the *pLMX5:AtIPT7* trees, we compared their cytokinin and auxin response profiles to the WT. As expected, the *AtIPT7* transgene had a robust expression level in the transgenic trees (Figure 4).

To define the status of cytokinin signaling, we analyzed the expression profiles of the *type-A RR*s: three out of four were upregulated in the transgenic trees (Figures 4, S4). Their profiles were similar to the WT with the phloem abundant expression peaks (Pattern 1) confirming that cytokinin response was enhanced in its endogenous domain. Additionally, expression of *PttAINT* was elevated in the cambium of the transgenic trees (Figure 4). This gene is known to be upregulated by a prolonged cytokinin treatment and to act as a positive regulator of cambial activity (41), indicating that it may be involved in the stimulation of cell division rate in the *pLMX5:AtIPT7* trees.

To define the status of auxin response, expression profiles of the non-cytokinin induced *PttIAA*s were analyzed. Among those whose expression was coinciding with the auxin gradient (Pattern 2), we were able to identify one gene, *PttIAA7.1*, which was upregulated in both transgenic tree lines (Figure 4).

Considering cytokinin homeostasis, we observed that the expression level of several *IPT*s and *LOG*s was downregulated in the transgenic trees (Figures 4, S4), indicating that a process of feedback is active in the regulation of cytokinin biosynthesis. In addition, we studied the effect of elevated cytokinin on the expression of auxin biosynthesis and transport genes. No systematic upregulation was observed in the expression level of cambial auxin biosynthesis or transport genes, therefore the manner by which elevated cytokinin increases the amplitude of auxin gradient in the cambial zone remains to be addressed by future studies.

Conclusions

We report here that the two major plant hormones, cytokinin and auxin, have distinct, yet partially overlapping, distribution profiles across the vascular cambium meristem. In contrast to auxin, which peaks in the actively dividing cambial cells (as has been reported before, 13-15), we show for the first time that the cytokinins peak in the developing phloem tissue. Additionally, we confirm the previous report (18) that a third hormone, bioactive gibberellin, has its maximum in the developing xylem cells. Potentially the cambial hormone gradients determine different developmental responses from the respective tissue zones.

To connect the hormone distributions to the status of hormonal signaling and homeostasis, we performed a genome-wide gene expression profiling at a high resolution across the cambial zone. The identified cytokinin and auxin response domains coincided well with the hormonal gradients. Almost all cytokinin signaling and biosynthesis genes peaked in the developing phloem cells with the maximum cytokinin content. In contrast, most of the auxin response genes had maximal expression in the middle of the cambial zone, coinciding with the peak auxin content. The high expression of cytokinin catabolic genes at the same domain may help to define the shape of cambial cytokinin distribution.

We confirmed the importance of cytokinin signaling for cambial activity by showing that secondary development in a tree stem can be by dramatically increased through an elevated cytokinin biosynthesis. Our transgenic *Populus* trees displayed increased cytokinin concentration leading to an elevated level of cytokinin signaling. Furthermore, elevation of the cytokinin content led to an increase in cambial auxin concentration, highlighting the interconnected nature of these two hormonal gradients. Potentially the stimulation of cambial activity occurs through the elevation of both hormonal signaling responses. It remains to be determined by future studies what is the contribution of vertical and lateral transport, as compared to the biosynthesis, to the cambial hormone distributions.

By connecting the hormonal domains to the developmental zonation inside the vascular cambium and addressing the interconnected nature between the hormone distributions, our work complements the recent studies of cell specific auxin and cytokinin profiles in the Arabidopsis root apical meristem (7, 12, 42). Interestingly, observed cytokinin and auxin signaling profiles across the cambial zone differed from those identified during Arabidopsis primary root development: there auxin response status was high in the developing xylem cells, whereas cytokinin response was highest in the dividing procambial cells between the xylem and

phloem tissues (8, 9). These differences reflect the adaptability of hormonal regulation during plant development: the outcome of hormonal signaling is highly dependent by its context; same hormones play versatile role during different developmental processes.

Our observation that lignocellulosic biomass production in woody plants can be boosted through enhanced cytokinin signaling confirms that advanced understanding of the regulatory mechanisms controlling tree development has immense applied value for the forest industry: identified molecular regulators represent optimal target genes for tree breeding and biotechnological implementation.

Experimental Procedures

Hormonal profiling in *Populus trichocarpa*

Samples for hormonal analysis were collected from two 8-month-old greenhouse grown *Populus trichocarpa* "Nisqually-1" trees. A tangential cryosectioning protocol (43) was used to divide the cambial zone of the stem into six cryo-fractions representing old phloem (125 μm), active phloem (125 μm), developing phloem (100 μm), dividing cambial cells (100 μm), developing xylem (125 μm) and lignified xylem (300 μm).

Quantification of hormones

Extraction and determination of hormones in *Populus* tissues were performed as described previously using an ultra-performance liquid chromatography (UPLC)-tandem mass spectrometry (AQUITY UPLC™ System/XEVO-TQS; Waters, Milford, MA, USA) with an ODS column (AQUITY UPLC BEH C_{18}, 1.7 μm, 2.1 x 100 mm, Waters)(44).

RNA isolation and RT-qPCR gene expression analysis

Expression of cytokinin homeostasis and signaling genes was analyzed across the cambial zone of two eight-month-old greenhouse grown *Populus trichocarpa* "Nisqually-1" trees. Tangential cryosectioning protocol (43) was used to section the stem into eight 50 μm fractions. Total RNA was extracted from cryosections using the RNeasy plant mini kit (Qiagen) with ''RLT'' lysis buffer and an RNase-free DNase set (Qiagen) to remove any remaining genomic DNA. cDNA synthesis was performed using the Transcriptor First Strand cDNA Synthesis Kit (Roche) with random hexamer primers and 150 ng of total RNA. A 1:8 dilution of the cDNA was used as reaction template. All qPCR reactions were performed using a LightCycler 480 (Roche) with LightCycler 480 Sybrgreen I master mix (Roche) and the manufacturer's qPCR program. From each sample, four technical repeats were amplified by each primer pair. The analyzed genes and sequences for primers used in RT-qPCR are listed in Supplemental Information.

Cytokinin induction

Two three-months-old *Populus tremula* × *tremuloides* clone T89 stems (30 cm from tip; diameter 4-5mm) were sectioned into 50-100 μm thick cross sections. Sections were incubated 1 h in 20nM NaPi buffer with or without 100nM 2ip.

Transgenic *Populus tremula* × *tremuloides* trees

P. tremula × *tremuloides* clone T89 was transformed with the *pLMX5:AtIPT7* construct. *ARABIDOPSIS THALIANA ISOPENTENYLTRANSFERASE 7* gene (*At3g23630*) (990 bp) was cloned

into a Gateway vector with 1807 bp *LMX5* promoter from *P. tremula* × *tremuloides* (31). Primers used for the cloning of *AtIPT7*: Fwd *ATGAAGTTCTCAATCTCA*; Rev *TCATATCATATTGTGGG*. Agrobacterium-mediated transformation, shoot regeneration and *in vitro* culture of *Populus* lines were conducted as described in (45).

Phenotypic analyses of transgenic tree lines

Growth dynamics of transgenic trees were studied under greenhouse conditions. Height and diameter of trees was measured once per week at the age of 6-11 weeks (Supplemental Information). Cytokinin response assay and maceration study of stem segments were conducted as described in (26). Plastic embedding and sectioning of the 20th internode was done according to (26). The number of meristematic, dividing cambial cells was calculated from 30 cell files of WT (T89) and *pLMX5:AtIPT7* trees. The meristematic cells were defined in cross-sections as flat, thin-walled cells localized in the cambial cell files between differentiating xylem and phloem cells.

Hormonal and gene expression profiling in *Populus tremula* × *tremuloides*

Four (A-D) 100 µm cryo-fractions were collected across the cambial zone for hormonal analysis from three three-month old WT (clone T89) and *pLMX5:AtIPT7* line 1 and 3 trees. Gene expression profiles were analyzed in twelve 35 µm cryosections collected across the cambial zone of three WT and three *pLMX5:AtIPT7* line 1 and 3 trees.

RNA sequencing

For gene expression profiling of cryosections, an Ovation Universal RNA-Seq System kit was used for Illumina library preparations (NuGEN Technologies Inc., CA, USA). Purified total RNA (10-50 ng) was used and primers for ribosomal removal were designed and used as outlined in the kit manual. Libraries were purified with AMPure XP beads (Beckman Coulter Inc., MA, USA), quantified and run on a NextSeq 500 sequencer using 75b single read kits (Illumina, CA, USA). For cytokinin induction, samples were sent for sequencing to SciLife Lab, Sweden. Adapter sequences and low quality reads were removed from the data using cutadapt (46). The data was mapped to the *P. trichocarpa* genome v3.0 using STAR (47). Count data was processed using custom scripts (Supplemental Information), GenomicFeatures (48), GenomicAlignments (48) and DESeq2 (49) in R. The data is publicly available through ArrayExpress (http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-4631 and E-MTAB-4635).

Author contributions

J.I., K.N., L.P., P.A., H.S., and Y.H. designed the study. J.I., K.N., and R.P.B. contributed to the tree transformations. J.I., K.N., O.P.S., M.K., J.A.S., P.K., J.Z., A.E., and N.S. carried out the experimental work. J.I., K.N., O.P.S., J.A.S., and H.S. analyzed the data. J.I., K.N., O.P.S., J.A.S., A.P.M., P.A., H.S., and Y.H. wrote the manuscript.

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Advanced Plant Science Network. R.P.B. was funded by grants from Berzili, TC4F and FUTURE trees. Related to the results of this research, Stora Enso oyj has applied for a patent titled “A method for improving stem volume growth and biomass production in trees.”, application No.: PCT/FI2014/051057. The authors declare that they have no conflicting financial interests.

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Figure legends

Figure 1 A) Hormonal profiles of *Populus trichocarpa* cambial zone were analyzed in six (A-F) stem cryo-fractions representing old phloem (125 µm) (A), active phloem (125 µm) (B), developing phloem (100 µm) (C), dividing cambial cells (100 µm) (D), developing xylem (125 µm) (E) and lignified xylem (300 µm) (F); see also Figure S1. Scale bar 200 µm. **B)** Bioactive auxin (IAA) concentration peaks in the dividing cambial cells (D-fraction). In contrast, bioactive cytokinins (tZ and iP), together with the iP precursor iRP, have their maximum in the developing phloem tissue (C-fraction). Bioactive gibberellin (GA4) peaks in the developing xylem tissue (E-fraction). Values are averages (±SE) from two individual trees. Auxin concentration is given on the right side y-axis.

Figure 2 A) WT and *pLMX5:AtIPT7* line 1 and 3 *Populus tremula* × *tremuloides* trees at the age of ten weeks, with similar height. Scale bar 20 cm. **B)** Total stem volumes of transgenic *pLMX5:AtIPT7 Populus* lines 1 and 3 were increased as compared to the WT; see also Figure S2. Values are averages (±SE) from five individual trees per each line. *p*-values from Student's t-test are given in the tables (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0{,}001$). **C)** Cytokinin responsiveness assay of WT and *pLMX5:AtIPT7* lines 1 and 3. Stem segments were grown on medium supplemented with 0.5 mg/L auxin (IAA) and 0, 0.5 or 1.5 mg/L cytokinin t-zeatin (tZ). At the age of four weeks, transgenic lines regenerated roots and shoots on a medium with 0 mg/L tZ, and shoots already in low cytokinin concentrations (0.5 mg/L), whereas WT required a higher (1.5 mg/L) tZ concentration for shoot regeneration. Scale bar 1 cm.

Figure 3 A) In *pLMX5:AtIPT7* line 1 and 3 trees the vascular cambium contains more dividing, meristematic cells (marked by asterisks), than in WT trees; see also Figure S2. Four 100 µm cryo-fractions (A-D, representing tissues from developing phloem to the developing xylem) were collected across the cambial zone for hormonal analysis. Scale bar 100 µm. **B)** Hormonal profiles of auxin (IAA) and bioactive cytokinins (iP and tZ) together with an iP precursor form (iPR) across the cambial zone of WT and transgenic *Populus pLMX5:AtIPT7* line 1 and 3 stem. Values are averages (±SE) from three individual trees. *p*-values from Student's t-test are given in the tables (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0{,}001$).

Figure 4 Expression profiles for selected cytokinin and auxin signaling and homeostasis and genes across the cambial zone of *P. tremula* × *tremuloides* WT and *pLMX5:AtIPT7* line 1 and 3 trees; see also Figures S3 and S4. Gene expression patterns were analyzed in twelve 35 µm cryofractions collected across the cambial zone (from developing phloem to the developing xylem). Scale bar 100 µm. Gene expression level is given as normalized RNA-sequencing reads per million reads per kb (rpkm).

Three distinct gene expression patterns were identified: developing phloem peaking Pattern 1 (specified by phloem marker gene *PttCLE41a*), middle cambium peaking Pattern 2 (cambium marker gene *PttWOX4a*) and developing xylem peaking Pattern 3 (xylem marker gene *PttCOMT2*). *AtIPT7* was expressed in the cambial zone of the transgenic trees. Cytokinin signaling pathway genes (type-B and type-A *RR*s) were defined by the phloem peaking Pattern 1, whereas the auxin response gene *PttIAA7.1* was peaking in the middle of the cambium (Pattern 2); a similar pattern was observed for the cambial regulator *PttAINT*. Cytokinin biosynthetic *PttIPT5a* gene was defined by the Pattern 1, whereas the catabolic *PttCKX*5*a* was

peaking more in the middle (Pattern 2). *p*-values from Student's t-test are given in the inserted tables and in Table S1 (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0{,}001$).

Figure 1

Click here to download Figure Fig_1.tif

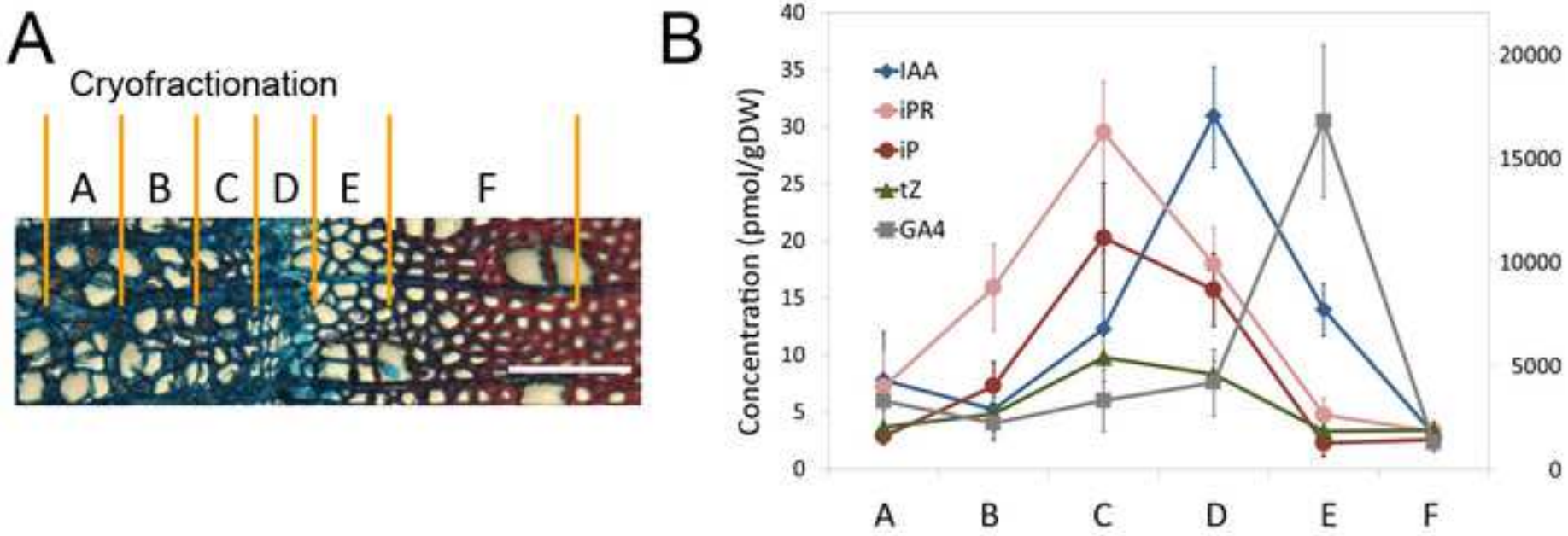

Figure 2

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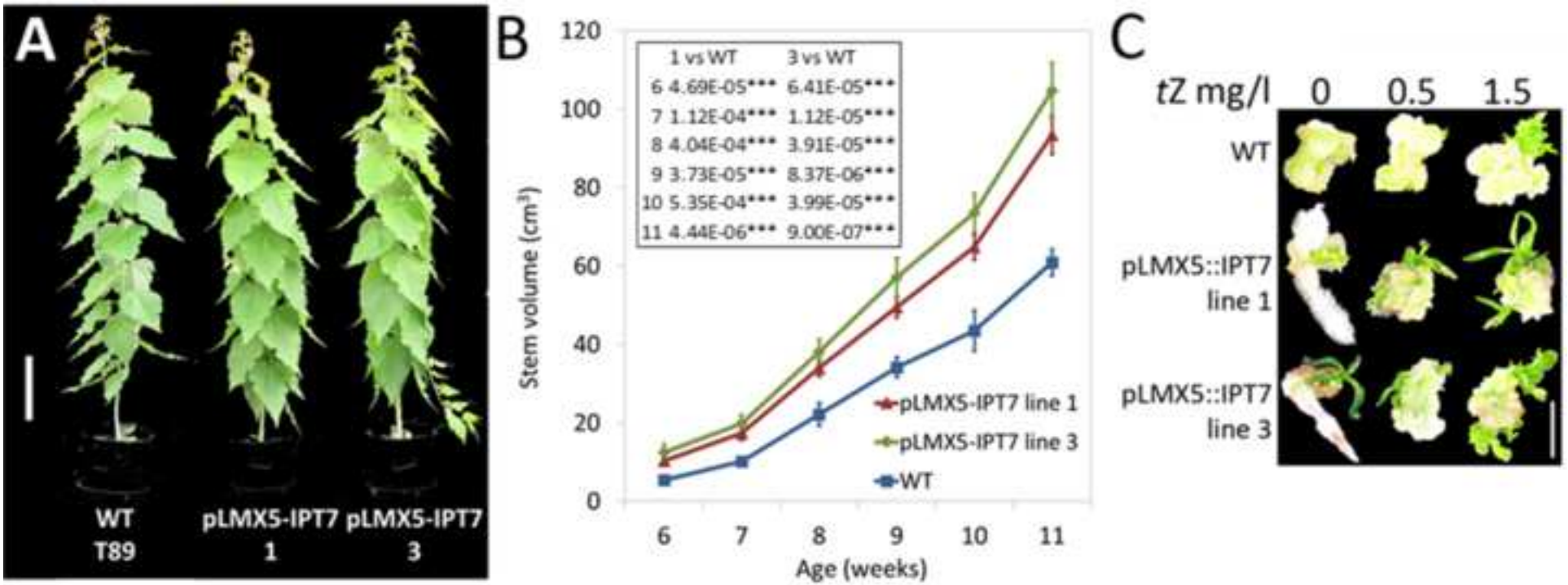

Figure 4

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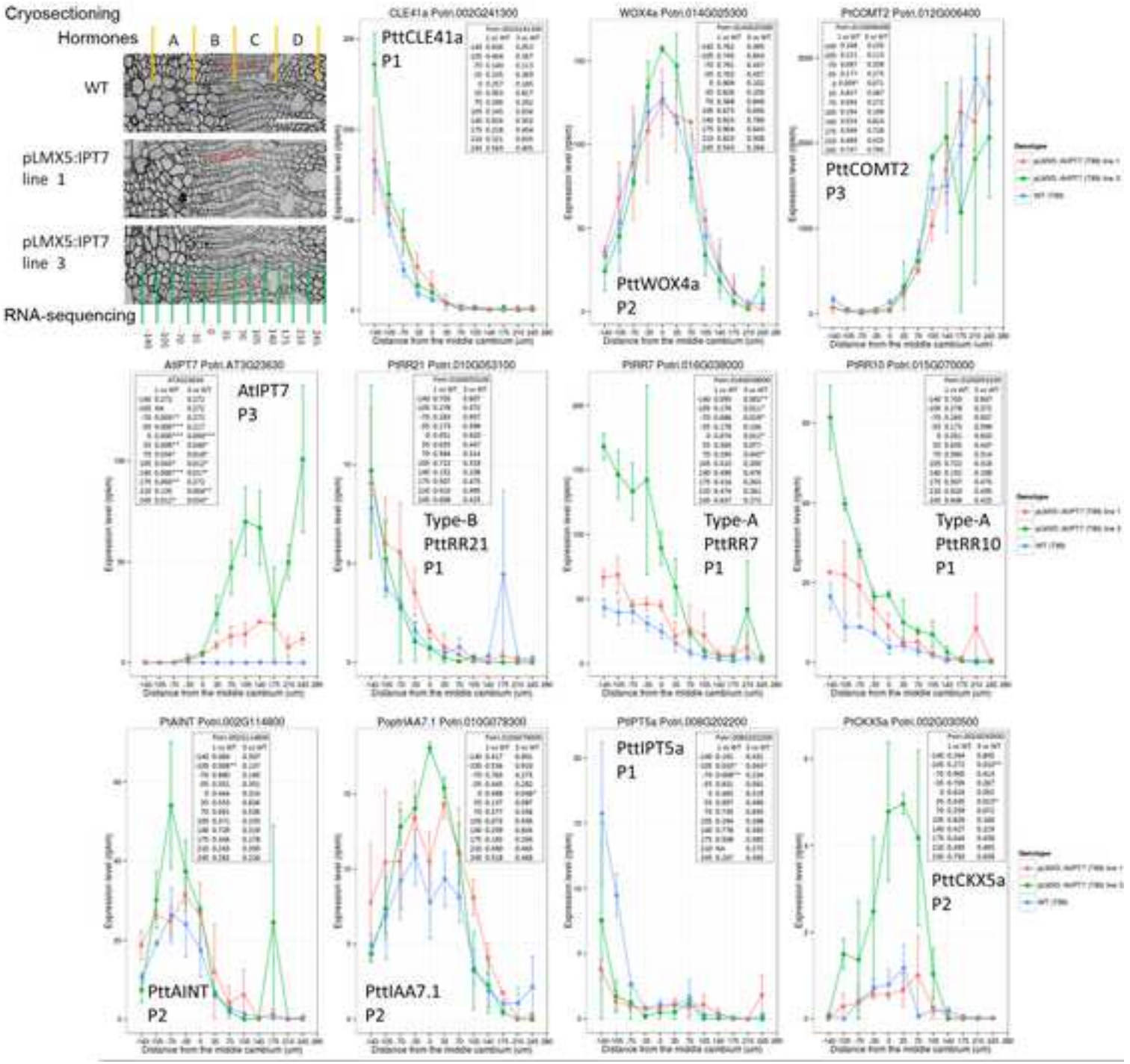

Figure 3

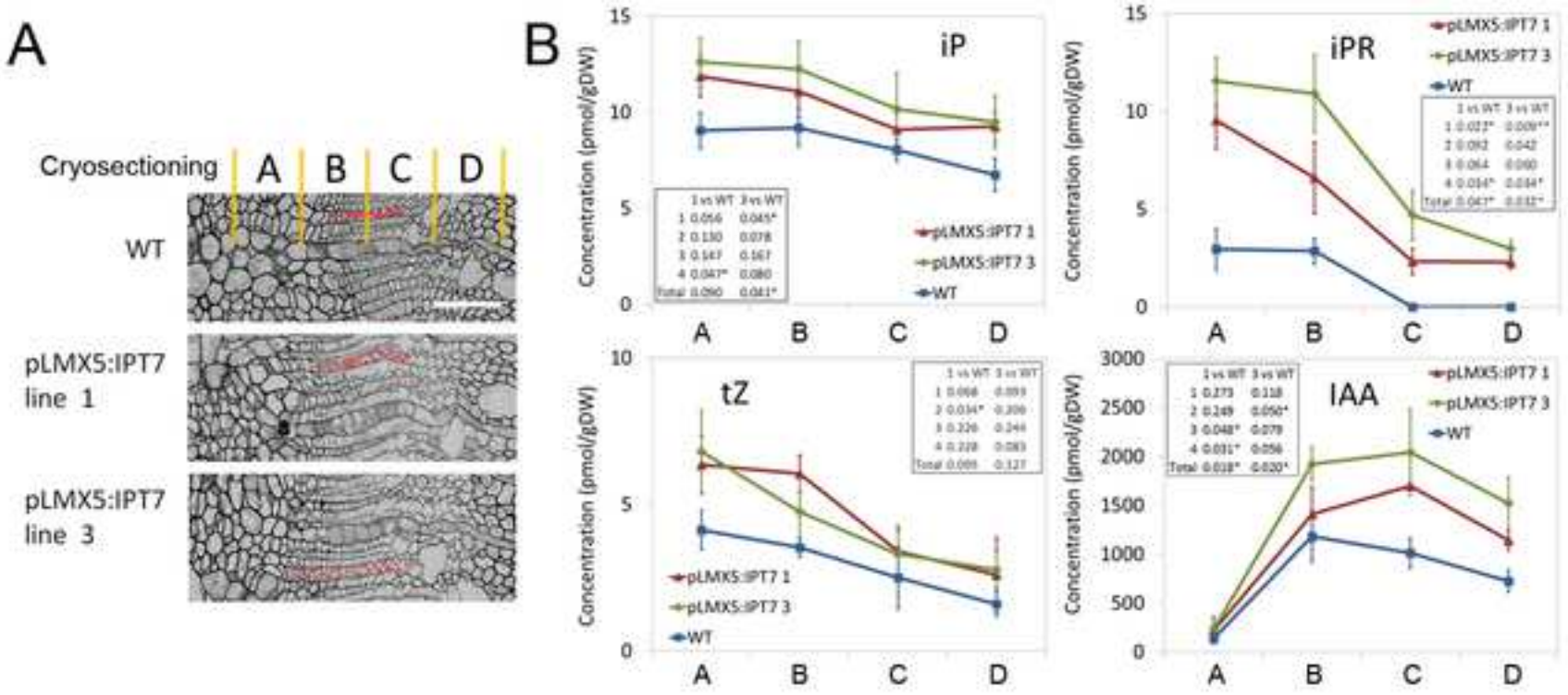

Table S1 Excel file with *p* -values from Student's t-test for the expression profiles in Figure S4 (* *p* ≤ 0.

	PtCLE41a		
	Potri.002G241300		
	1 vs WT	3 vs WT	
-140	0.836	0.053	-140
-105	0.404	0.367	-105
-70	0.140	0.113	-70
-35	0.105	0.269	-35
0	0.257	0.185	0
35	0.903	0.817	35
70	0.286	0.242	70
105	0.145	0.836	105
140	0.826	0.502	140
175	0.238	0.494	175
210	0.321	0.835	210
245	0.569	0.405	245

	PtCRE1b		
	Potri.010G102900		
	1 vs WT	3 vs WT	
-140	0.845	0.080	-140
-105	0.711	0.125	-105
-70	0.912	0.532	-70
-35	0.953	0.441	-35
0	0.368	0.290	0
35	0.962	0.438	35
70	0.802	0.405	70
105	0.417	0.264	105
140	0.736	0.909	140
175	0.447	0.444	175
210	0.212	0.711	210
245	0.069	0.579	245

	PtRR21		
	Potri.010G053100		
	1 vs WT	3 vs WT	
-140	0.703	0.607	-140
-105	0.278	0.372	-105
-70	0.263	0.937	-70
-35	0.173	0.599	-35
0	0.051	0.920	0
35	0.635	0.447	35
70	0.584	0.314	70
105	0.722	0.318	105
140	0.152	0.108	140
175	0.507	0.475	175
210	0.910	0.495	210
245	0.608	0.425	245

	PtRR16		
	Potri.010G105600		
	1 vs WT	3 vs WT	
-140	0.977	0.280	-140
-105	0.194	0.124	-105
-70	0.770	0.168	-70
-35	0.732	0.866	-35
0	0.811	0.051	0
35	0.726	0.578	35
70	0.306	0.282	70
105	0.445	0.127	105
140	0.389	0.695	140
175	0.186	0.911	175
210	0.832	0.945	210
245	0.963	0.212	245

	PtRR10		
	Potri.015G070000		
	1 vs WT	3 vs WT	
-140	0.235	0.008 **	-140
-105	0.197	0.008 **	-105
-70	0.176	0.001 ***	-70
-35	0.208	0.002 **	-35
0	0.190	0.010 *	0
35	0.688	0.280	35
70	0.302	0.045 *	70
105	0.982	0.148	105
140	0.524	0.285	140
175	0.178	0.897	175
210	0.286	0.327	210
245	0.428	0.240	245

	PtRR1		
	Potri.008G193000		
	1 vs WT	3 vs WT	
-140	0.211	0.144	-140
-105	0.497	0.058	-105
-70	0.758	0.723	-70
-35	0.003 **	0.016 *	-35
0	0.697	0.099	0
35	0.253	0.431	35
70	0.722	0.424	70
105	0.411	0.244	105
140	0.293	0.292	140
175	0.333	0.311	175
210	0.909	0.790	210
245	0.979	0.567	245

PtIPT2

Potri.009G147600

	1 vs WT	3 vs WT	
-140	0.592	0.128	-140
-105	0.370	0.719	-105
-70	0.346	0.693	-70
-35	0.739	0.049	-35
0	0.383	0.031	0
35	0.621	0.865	35
70	0.142	0.375	70
105	0.061	0.775	105
140	0.623	0.758	140
175	0.094	0.219	175
210	0.459	0.817	210
245	0.211	0.187	245

PtABCG14

Potri.004G236500

	1 vs WT	3 vs WT	
-140	0.164	0.174	-140
-105	0.523	0.086	-105
-70	0.095	0.146	-70
-35	0.220	0.114	-35
0	0.437	0.199	0
35	0.942	0.703	35
70	0.900	0.976	70
105	0.756	0.277	105
140	0.069	0.426	140
175	0.488	0.620	175
210	0.612	0.541	210
245	0.424	0.695	245

PtIAA27.1

Potri.006G161400

	1 vs WT	3 vs WT	
-140	0.843	0.317	-140
-105	0.739	0.263	-105
-70	0.612	0.321	-70
-35	0.584	0.588	-35
0	0.981	0.871	0
35	0.819	0.556	35
70	0.932	0.292	70
105	0.939	0.755	105
140	0.968	0.751	140
175	0.280	0.233	175
210	0.987	0.450	210
245	0.921	0.313	245

PtIAA28.1

Potri.018G057000

	1 vs WT	3 vs WT	
-140	0.134	0.230	-140
-105	0.551	0.265	-105
-70	0.779	0.233	-70
-35	0.376	0.075	-35
0	0.411	0.330	0
35	0.907	0.393	35
70	0.294	0.288	70
105	0.693	0.186	105
140	0.075	0.068	140
175	0.421	0.473	175
210	0.383	0.707	210
245	0.223	0.682	245

PtTDR-1a

Potri.003G107600

	1 vs WT	3 vs WT	
-140	0.307	0.478	-140
-105	0.964	0.586	-105
-70	0.105	0.163	-70
-35	0.482	0.439	-35
0	0.482	0.354	0
35	0.568	0.356	35
70	0.970	0.386	70
105	0.401	0.687	105
140	0.714	0.424	140
175	0.771	0.442	175
210	0.667	0.922	210
245	0.206	0.124	245

PtWOX4a

Potri.014G025300

	1 vs WT	3 vs WT	
-140	0.762	0.495	-140
-105	0.745	0.843	-105
-70	0.791	0.437	-70
-35	0.702	0.457	-35
0	0.909	0.102	0
35	0.826	0.100	35
70	0.368	0.848	70
105	0.673	0.656	105
140	0.924	0.786	140
175	0.904	0.643	175
210	0.823	0.508	210
245	0.543	0.266	245

PtLOG5a

Potri.002G012500

PtCKX5a

Potri.002G030500

	1 vs WT	3 vs WT				
-140	0.495	0.495	-140	0.264	0.845	-140
-105	0.484	0.972	-105	0.272	0.010 *	-105
-70	0.457	0.461	-70	0.965	0.414	-70
-35	0.442	0.452	-35	0.705	0.267	-35
0	0.395	0.426	0	0.624	0.052	0
35	0.385	0.487	35	0.545	0.013 *	35
70	0.372	0.242	70	0.259	0.072	70
105	0.449	0.426	105	0.826	0.166	105
140	0.431	0.462	140	0.427	0.219	140
175	0.448	0.568	175	0.644	0.438	175
210	0.300	0.323	210	0.495	0.495	210
245	0.472	0.363	245	0.750	0.638	245

PtIAA12.2
Potri.008G172400

PtIAA12.1
Potri.010G065200

	1 vs WT	3 vs WT				
-140	0.276	0.149	-140	0.634	0.939	-140
-105	0.537	0.646	-105	0.754	0.413	-105
-70	0.395	0.058	-70	0.314	0.003	-70
-35	0.564	0.731	-35	0.453	0.559	-35
0	0.766	0.806	0	0.614	0.671	0
35	0.516	0.987	35	0.664	0.030	35
70	0.931	0.757	70	0.735	0.210	70
105	0.865	0.668	105	0.962	0.821	105
140	0.443	0.933	140	0.500	0.431	140
175	0.987	0.567	175	0.828	0.413	175
210	0.506	0.957	210	0.577	0.807	210
245	0.113	0.165	245	0.394	0.549	245

PtPIN6a
Potri.005G187500

PtPIN1a
Potri.012G047200

	1 vs WT	3 vs WT				
-140	0.203	0.226	-140	0.595	0.989	-140
-105	0.116	0.518	-105	0.366	0.282	-105
-70	0.921	0.394	-70	0.202	0.515	-70
-35	0.426	0.772	-35	0.799	0.946	-35
0	0.762	0.456	0	0.904	0.911	0
35	0.058	0.820	35	0.885	0.516	35
70	0.869	0.862	70	0.465	0.627	70
105	0.820	0.121	105	0.324	0.690	105
140	0.482	0.555	140	0.962	0.635	140
175	0.669	0.250	175	0.322	0.725	175
210	0.385	0.505	210	0.660	0.477	210
245	0.418	0.844	245	0.282	0.193	245

PtAUX5LAX7
Potri.004G172800

PtAUX8LAX4
Potri.002G087000

	1 vs WT	3 vs WT				
-140	0.088	0.511	-140	0.732	0.724	-140

-105	0.888	0.665	-105	0.434	0.388	-105
-70	0.126	0.059	-70	0.875	0.226	-70
-35	0.376	0.630	-35	0.432	0.354	-35
0	0.917	0.965	0	0.537	0.412	0
35	0.363	0.666	35	0.326	0.303	35
70	0.750	0.930	70	0.142	0.161	70
105	0.265	0.448	105	0.614	0.406	105
140	0.707	0.581	140	0.844	0.955	140
175	0.338	0.491	175	0.815	0.481	175
210	0.321	0.897	210	0.526	0.495	210
245	0.168	0.101	245	0.944	0.470	245

05; ** $p \leq 0.01$; *** $p \leq 0.001$).

PtHK3a			**PtHK3b**			**PtHP8a**		
Potri.001G057400			**Potri.003G171000**			**Potri.013G028300**		
1 vs WT	3 vs WT		1 vs WT	3 vs WT		1 vs WT	3 vs WT	
0.415	0.061	-140	0.386	0.021 *	-140	0.431	0.683	
0.517	0.404	-105	1.000	0.712	-105	0.754	0.334	
0.758	0.143	-70	0.252	0.252	-70	0.889	0.441	
0.917	0.026 *	-35	0.173	0.147	-35	0.897	0.437	
0.143	0.818	0	0.135	0.026	0	0.036 *	0.103	
0.334	0.585	35	0.876	0.522	35	0.405	0.116	
0.467	0.746	70	0.084	0.147	70	0.357	0.544	
0.538	0.885	105	0.945	0.808	105	0.209	0.376	
0.591	0.522	140	0.492	0.879	140	0.971	0.329	
0.758	0.654	175	0.495	0.492	175	0.190	0.429	
0.396	0.883	210	0.365	0.595	210	0.317	0.981	
0.658	0.929	245	0.144	0.435	245	0.299	0.694	

PtRR15			**PtRR13**			**PtRR23**		
Potri.008G135500			**Potri.010G001000**			**Potri.006G188000**		
1 vs WT	3 vs WT		1 vs WT	3 vs WT		1 vs WT	3 vs WT	
0.900	0.186	-140	0.662	0.656	-140	0.396	0.486	
0.803	0.270	-105	0.285	0.274	-105	0.070	0.541	
0.236	0.208	-70	0.825	0.072	-70	0.097	0.607	
0.193	0.387	-35	0.252	0.603	-35	0.120	0.125	
0.285	0.907	0	0.930	0.075	0	0.745	0.783	
0.953	0.183	35	0.328	0.108	35	0.587	0.341	
0.144	0.131	70	0.130	0.139	70	0.288	0.569	
0.464	0.081	105	0.413	0.603	105	0.776	0.351	
0.851	0.756	140	0.222	0.094	140	0.774	0.429	
0.260	0.912	175	0.194	0.865	175	0.184	0.250	
0.563	0.502	210	0.379	0.628	210	0.572	0.957	
0.823	0.397	245	0.485	0.653	245	0.498	0.310	

PtRR3			**PtIPT5a**			**PtIPT5b**		
Potri.002G082200			**Potri.008G202200**			**Potri.010G030500**		
1 vs WT	3 vs WT		1 vs WT	3 vs WT		1 vs WT	3 vs WT	
0.613	0.452	-140	0.191	0.431	-140	0.178	0.343	
0.236	0.900	-105	0.033	0.043	-105	0.281	0.283	
0.182	0.103	-70	0.008	0.234	-70	0.754	0.285	
0.072	0.529	-35	0.931	0.091	-35	0.212	0.062	
0.395	0.537	0	0.465	0.319	0	0.755	0.403	
0.884	0.889	35	0.997	0.490	35	0.495	0.495	
0.353	0.978	70	0.735	0.835	70	0.829	0.495	
0.139	0.308	105	0.294	0.288	105	NA	NA	
0.253	0.678	140	0.778	0.383	140	NA	0.272	
0.213	0.484	175	0.508	0.385	175	0.302	0.343	
0.113	0.620	210	NA	0.272	210	0.495	0.495	
0.242	0.462	245	0.267	0.495	245	0.266	0.357	

PtENT6
Potri.004G032300

1 vs WT	3 vs WT
0.058	0.061
0.297	0.676
0.806	0.324
0.337	0.328
0.561	0.052
0.267	0.429
0.728	0.173
0.394	0.495
0.348	0.318
0.758	0.543
NA	0.272
0.903	0.362

PtIAA26.2
Potri.001G190300

	1 vs WT	3 vs WT
-140	0.293	0.162
-105	0.306	0.045
-70	0.269	0.007
-35	0.256	0.019
0	0.277	0.000
35	0.339	0.075
70	0.364	0.122
105	0.275	0.259
140	0.316	0.141
175	0.597	0.610
210	0.472	0.450
245	0.411	0.527

PtIAA27.3
Potri.001G186100

	1 vs WT	3 vs WT
-140	0.694	0.458
-105	0.423	0.774
-70	0.962	0.091
-35	0.683	0.497
0	0.709	0.589
35	0.864	0.636
70	0.780	0.311
105	0.521	0.702
140	0.773	0.636
175	0.568	0.578
210	0.423	0.860
245	0.834	0.243

PtIAA28.2
Potri.006G236200

1 vs WT	3 vs WT
0.653	0.026
0.755	0.490
0.451	0.013
0.484	0.255
0.233	0.189
0.778	0.448
0.663	0.314
0.985	0.227
0.380	0.356
0.495	0.281
0.925	0.495
0.410	0.245

PtYUCCA3
Potri.002G207400

	1 vs WT	3 vs WT
-140	0.659	0.377
-105	0.554	0.308
-70	0.085	0.255
-35	0.328	0.367
0	0.291	0.308
35	0.291	0.283
70	0.471	0.762
105	0.395	0.259
140	0.502	0.324
175	0.668	0.338
210	0.495	0.982
245	0.289	0.327

PtPIN5b
Potri.013G087000

	1 vs WT	3 vs WT
-140	0.240	0.227
-105	0.224	0.197
-70	0.297	0.151
-35	0.389	0.342
0	0.495	0.620
35	0.248	0.248
70	0.226	0.226
105	0.325	0.325
140	0.771	0.258
175	0.495	0.495
210	0.460	0.495
245	0.634	0.633

PtANT
Potri.002G114800

1 vs WT	3 vs WT
0.084	0.307
0.008 **	0.137
0.880	0.160
0.551	0.351
0.444	0.324
0.553	0.826
0.691	0.536
0.371	0.233
0.729	0.319
0.346	0.278
0.243	0.350
0.292	0.226

PtCRE1a
Potri.008G137900

	1 vs WT	3 vs WT
-140	0.250	0.828
-105	0.519	0.291
-70	0.195	0.386
-35	0.063	0.927
0	0.410	0.280
35	0.674	0.962
70	0.496	0.412
105	0.337	0.937
140	0.666	0.886
175	0.747	0.169
210	0.306	0.245
245	0.582	0.111

PtRR5
Potri.001G027000

	1 vs WT	3 vs WT
-140	0.766	0.168
-105	0.623	0.140
-70	0.972	0.266
-35	0.617	0.263
0	0.572	0.733
35	0.584	0.339
70	0.667	0.313
105	0.561	0.312
140	0.295	0.671
175	0.295	0.295
210	0.902	0.668
245	0.776	0.597

PtCKX7
Potri.006G221000

PtIAA16.2
Potri.013G041400

PtIAA9
Potri.002G108000

	1 vs WT	3 vs WT
	0.213	0.016 *
	0.276	0.133
	0.317	0.167
	0.645	0.956
	0.483	0.440
	0.476	0.108
	0.840	0.824
	0.102	0.646
	0.787	0.392
	0.635	0.522
	0.679	0.667
	0.590	0.450

	1 vs WT	3 vs WT
-140	0.884	0.626
-105	0.144	0.184
-70	0.031	0.996
-35	0.074	0.130
0	0.459	0.811
35	0.892	0.101
70	0.417	0.149
105	0.178	0.470
140	0.221	0.584
175	0.138	0.387
210	0.046	0.280
245	0.243	0.381

	1 vs WT	3 vs WT
-140	0.259	0.619
-105	0.014	0.063
-70	0.496	0.303
-35	0.487	0.839
0	0.481	0.713
35	0.814	0.485
70	0.971	0.614
105	0.615	0.181
140	0.346	0.682
175	0.167	0.761
210	0.261	0.885
245	0.475	0.731

PtIAA19.1
Potri.001G177500

	1 vs WT	3 vs WT
	0.879	0.547
	0.815	0.849
	0.596	0.068
	0.848	0.851
	0.564	0.092
	0.794	0.118
	0.980	0.577
	0.846	0.336
	0.772	0.301
	0.630	0.657
	0.751	0.691
	0.245	0.299

PtIAA7.1
Potri.010G078300

	1 vs WT	3 vs WT
-140	0.417	0.601
-105	0.536	0.910
-70	0.763	0.275
-35	0.445	0.282
0	0.498	0.048
35	0.137	0.087
70	0.377	0.336
105	0.073	0.936
140	0.259	0.845
175	0.183	0.294
210	0.490	0.465
245	0.518	0.468

PtYucca2
Potri.006G243400

	1 vs WT	3 vs WT
-140	0.132	0.661
-105	0.528	0.777
-70	0.978	0.500
-35	0.604	0.221
0	0.824	0.210
35	0.422	0.147
70	0.597	0.490
105	0.317	0.708
140	0.514	0.233
175	0.616	0.369
210	0.495	0.907
245	0.016	0.015

PtPIN6b
Potri.002G072200

	1 vs WT	3 vs WT
	0.515	0.183
	0.956	0.710
	0.752	0.265
	0.042	0.196
	0.674	0.908
	0.388	0.751
	0.421	0.759
	0.322	0.879
	0.710	0.456
	0.944	0.270
	0.368	0.311
	0.395	0.364

PtPIN1d
Potri.016G035300

	1 vs WT	3 vs WT
-140	0.462	0.929
-105	0.741	0.597
-70	0.685	0.435
-35	0.726	0.664
0	0.872	0.255
35	0.842	0.570
70	0.416	0.516
105	0.658	0.133
140	0.339	0.774
175	0.971	0.932
210	0.155	0.828
245	0.211	0.180

PtPIN3a
Potri.010G112800

	1 vs WT	3 vs WT
-140	0.057	0.047
-105	0.956	0.810
-70	0.385	0.764
-35	0.257	0.382
0	0.410	0.418
35	0.314	0.326
70	0.513	0.358
105	0.685	0.397
140	0.664	0.597
175	0.208	0.731
210	0.301	0.421
245	0.053	0.398

PtAUX3LAX2
Potri.010G191000

	1 vs WT	3 vs WT
	0.342	0.394

PtAUX1LAX5
Potri.006G098300

	1 vs WT	3 vs WT
-140	0.013 *	0.551

PtAUX4LAX6
Potri.008G066400

	1 vs WT	3 vs WT
-140	0.263	0.666

0.968	0.547	-105	0.769	0.660	-105	0.609	0.936
0.224	0.184	-70	0.418	0.368	-70	0.355	0.085
0.819	0.057	-35	0.439	0.955	-35	0.334	0.288
0.515	0.381	0	0.385	0.297	0	0.346	0.771
0.657	0.190	35	0.357	0.929	35	0.649	0.142
0.674	0.336	70	0.344	0.830	70	0.227	0.046
0.018	0.686	105	0.356	0.831	105	0.093	0.157
0.197	0.767	140	0.559	0.967	140	0.219	0.867
0.911	0.530	175	0.062	0.592	175	0.544	0.814
0.624	0.497	210	0.442	0.438	210	0.486	0.952
0.552	0.440	245	0.798	0.313	245	0.957	0.529

PtHP1b

Potri.010G027100

	1 vs WT	3 vs WT	
-140	0.412	0.980	
-105	0.576	0.840	
-70	0.701	0.302	
-35	0.251	0.693	
0	0.803	0.282	
35	0.396	0.837	
70	0.489	0.249	
105	0.698	0.707	
140	0.304	0.090	
175	0.776	0.888	
210	0.784	0.713	
245	0.761	0.023	*

PtRR7

Potri.016G038000

	1 vs WT	3 vs WT
-140	0.095	0.002
-105	0.176	0.011
-70	0.686	0.018
-35	0.178	0.136
0	0.074	0.012
35	0.565	0.077
70	0.295	0.045
105	0.310	0.200
140	0.496	0.478
175	0.316	0.263
210	0.474	0.281
245	0.837	0.273

PtIPT9

Potri.001G376600

	1 vs WT	3 vs WT
-140	0.784	0.140
-105	0.351	0.057
-70	0.433	0.590
-35	0.879	0.138
0	0.920	0.700
35	0.757	0.882
70	0.579	0.383
105	0.340	0.488
140	0.734	0.770
175	0.274	0.469
210	0.357	0.870
245	0.715	0.091

PtIAA27.2

Potri.003G051300

	1 vs WT	3 vs WT
-140	0.276	0.915
-105	0.328	0.329
-70	0.542	0.987
-35	0.630	0.361
0	0.349	0.358
35	0.482	0.327
70	0.690	0.203
105	0.204	0.796
140	0.922	0.745
175	0.896	0.090
210	0.351	0.574
245	0.228	0.231

PtPIN8b

Potri.004G124200

	1 vs WT	3 vs WT
-140	0.657	0.542
-105	0.360	0.528
-70	0.266	0.563
-35	0.445	0.886
0	0.890	0.823
35	0.754	0.495
70	0.401	0.401
105	0.266	0.266
140	0.495	0.495
175	NA	NA
210	NA	0.272
245	0.382	0.495

PtLOG1

Potri.009G010800

	1 vs WT	3 vs WT
-140	0.449	0.323
-105	0.365	0.189
-70	0.179	0.137
-35	0.297	0.316
0	0.093	0.072
35	0.194	0.859
70	0.129	0.655
105	0.787	0.858
140	0.286	0.227
175	0.417	0.290
210	0.495	0.277
245	0.465	0.354

PtIAA3.1

Potri.005G053800

	1 vs WT	3 vs WT
-140	0.345	0.436
-105	0.065	0.577
-70	0.771	0.062
-35	0.873	0.392
0	0.201	0.881
35	0.561	0.148
70	0.238	0.507
105	0.328	0.615
140	0.218	0.858
175	0.270	0.634
210	0.032	0.241
245	0.196	0.101

PtPIN1b
Potri.015G038700

	1 vs WT	3 vs WT
-140	0.218	0.858
-105	0.992	0.330
-70	0.148	0.300
-35	0.469	0.437
0	0.741	0.773
35	0.205	0.962
70	0.892	0.780
105	0.362	0.052
140	0.288	0.599
175	0.264	0.494
210	0.232	0.638
245	0.455	0.680

PtAUX6LAX3
Potri.009G132100

		1 vs WT	3 vs WT
*	-140	0.153	0.636
	-105	0.980	0.856
	-70	0.175	0.260
	-35	0.095	0.476
	0	0.559	0.398
	35	0.074	0.483
	70	0.689	0.792
	105	0.736	0.599
	140	0.841	0.424
	175	0.520	0.413
	210	0.326	0.330
	245	0.375	0.928

*

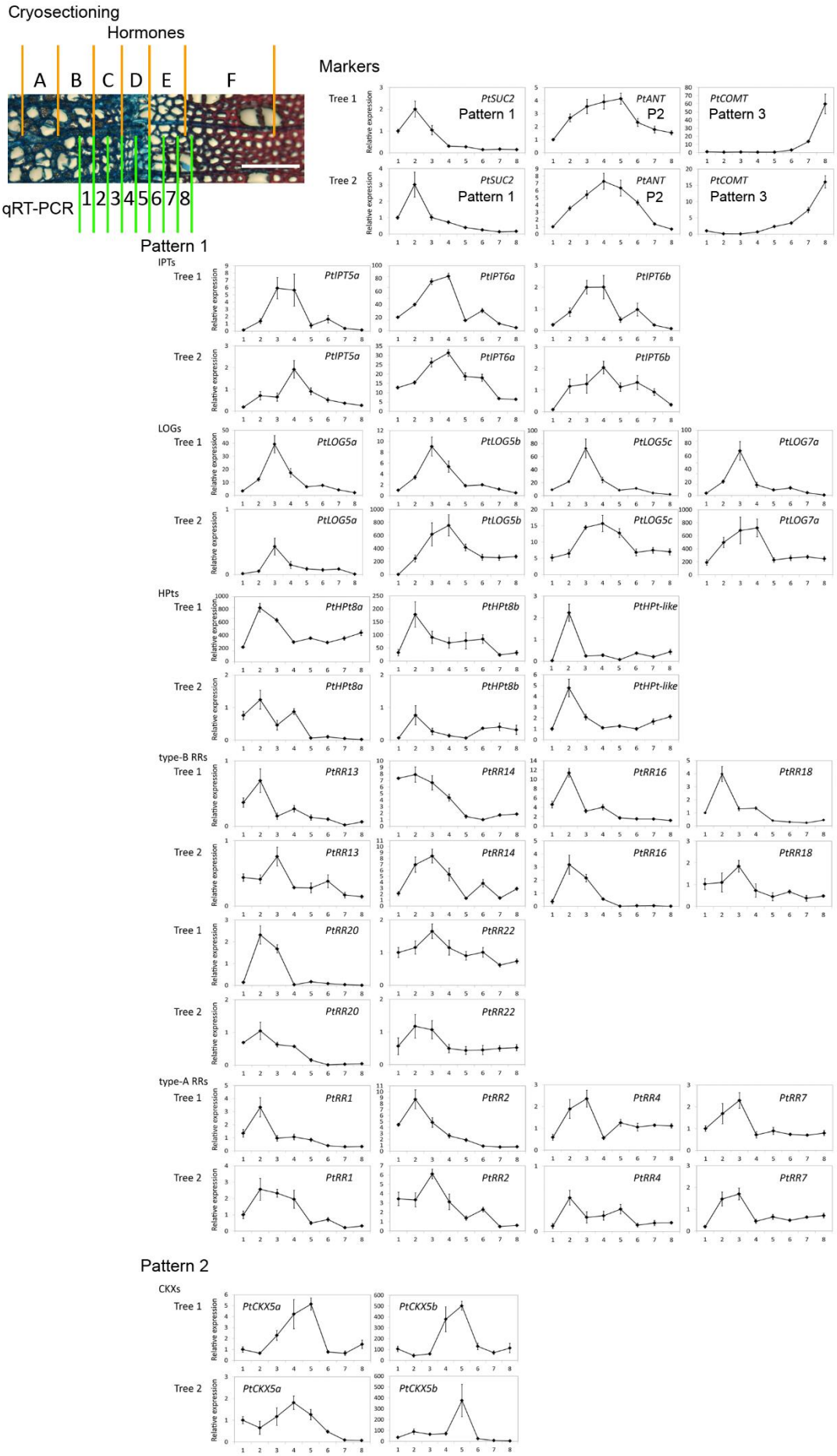
Cryosectioning
Hormones
A
B
C
D
E
F
qRT-PCR
1 2 3 4 5 6 7 8
Markers
Tree 1
Tree 2
PtSUC2
Pattern 1
PtANT
P2
PtCOMT
Pattern 3
Relative expression
Pattern 1
IPTs
PtIPT5a
PtIPT6a
PtIPT6b
LOGs
PtLOG5a
PtLOG5b
PtLOG5c
PtLOG7a
HPts
PtHPt8a
PtHPt8b
PtHPt-like
type-B RRs
PtRR13
PtRR14
PtRR16
PtRR18
PtRR20
PtRR22
type-A RRs
PtRR1
PtRR2
PtRR4
PtRR7
Pattern 2
CKXs
PtCKX5a
PtCKX5b

Figure S1

Figure S1 Expression of cytokinin signaling and homeostasis genes across the cambial zone of *Populus trichocarpa* stem; related to Figure 1. Expression of *IPT*s, *LOG*s, *CKX*s, *HPt*s, type-A and type-B response regulators was studied by RT-qPCR in eight 50 μm cryosections representing tissues from developing phloem (section 1) to the developing xylem (section 8). Scale bar 200 μm. The level of auxin signaling was studied through an auxin primary response gene *PtIAA3*. Tissue identity of cryosections was verified through marker gene analysis. Two trees were analyzed; expression is given relative to the expression level of one gene family member in the developing phloem (cryosection 1). Error bars SE.

Based on the profiling data, three distinct gene expression patterns were identified. All cytokinin signaling and biosynthetic genes with a robust and reproducible expression profile were most abundant in the phloem side of cambial meristem (Pattern 1, verified by the phloem marker gene *PtSUC*). This was in contrast to the putative auxin primary response gene *PtIAA3*, which had its highest expression in the middle of cambium zone. This Pattern 2 was coinciding with the domain where cell divisions take place, and the cambial marker gene *PtANT* was peaking. A similar expression profile was observed for the cytokinin catabolic *CKX*s. None of the cytokinin genes resembled the xylem peaking Pattern 3 specified by the developing xylem marker gene *PtCOMT2*.

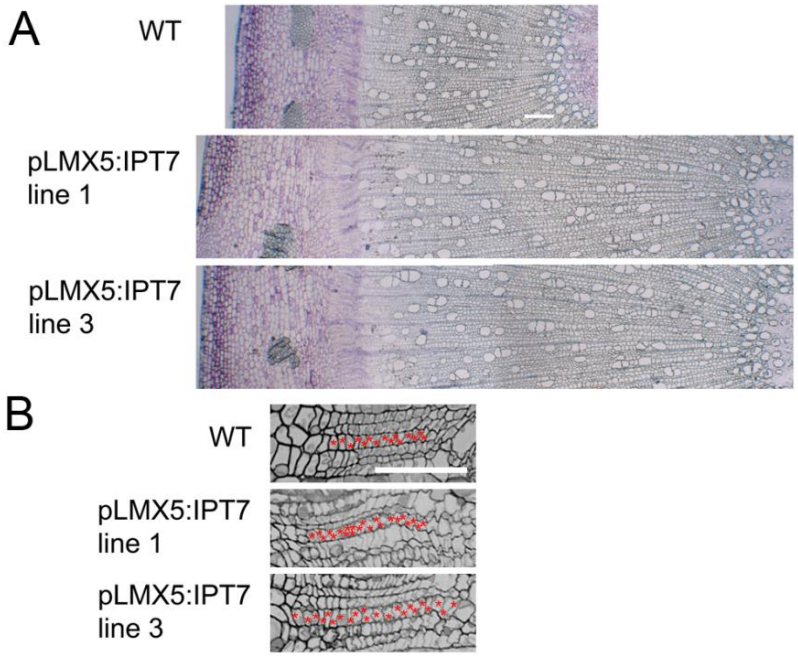

Figure S2 Anatomy of the 20th internode of three-month old WT and *pLMX5:AtIPT7* line 1 and 3 trees; related to Figures 2 and 3. **A)** Stem diameter was increased in the transgenic trees as compared to the WT trees. Scale bar 200 µm. **B)** Number of meristematic cells (marked by red asterisks) was increased in the transgenic trees. Scale bar 100 µm.

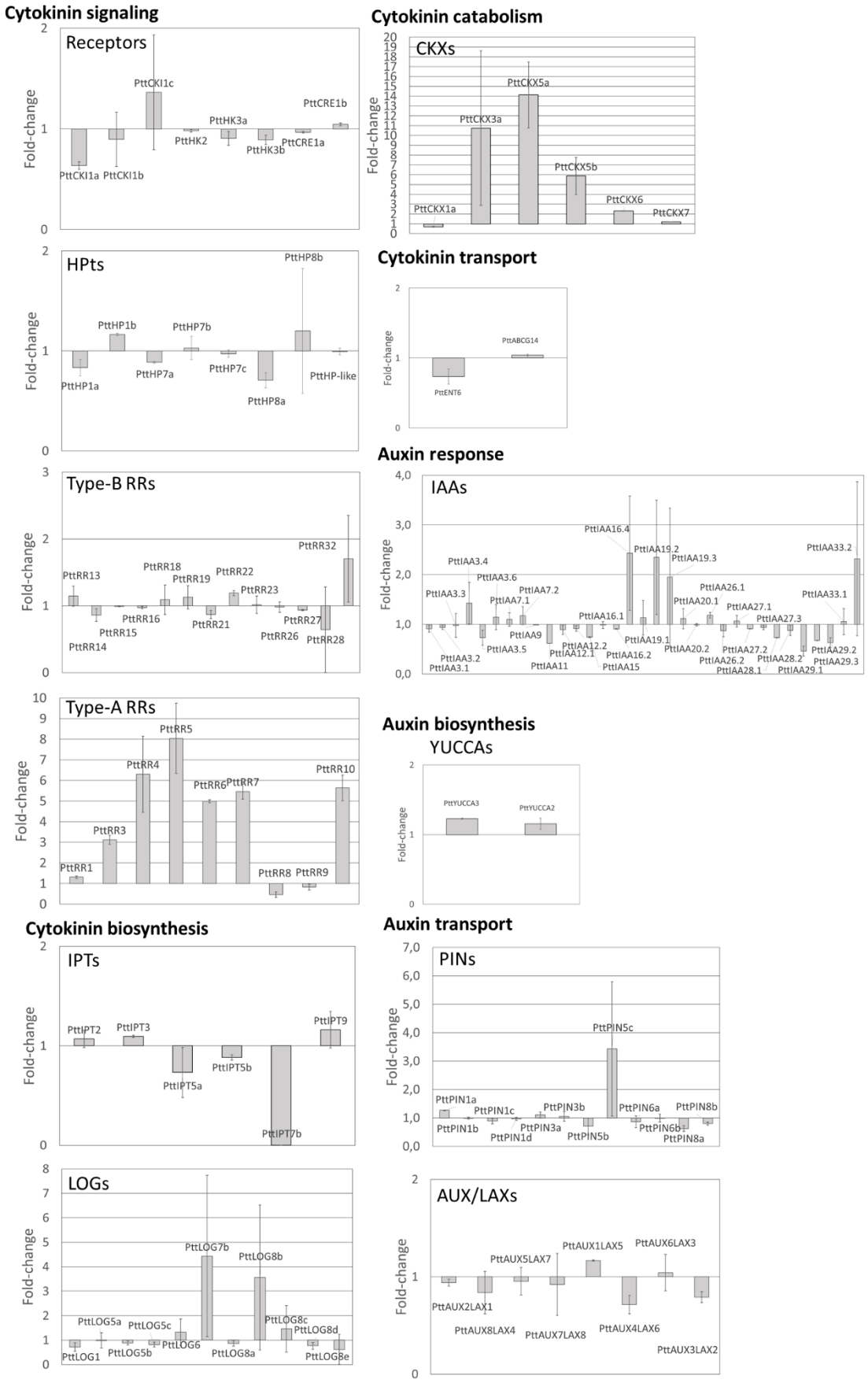

Figure S3 Induction of cytokinin and auxin signaling, homeostasis and transport gene expression in *P. tremula* × *tremuloides* stem discs treated 1 h with 100 nM cytokinin; related to Figure 4. Fold change was calculated between the RNA-sequencing data (rpkm) from the cytokinin treated and control samples; fold change value 1 denotes no difference in expression level between the samples.

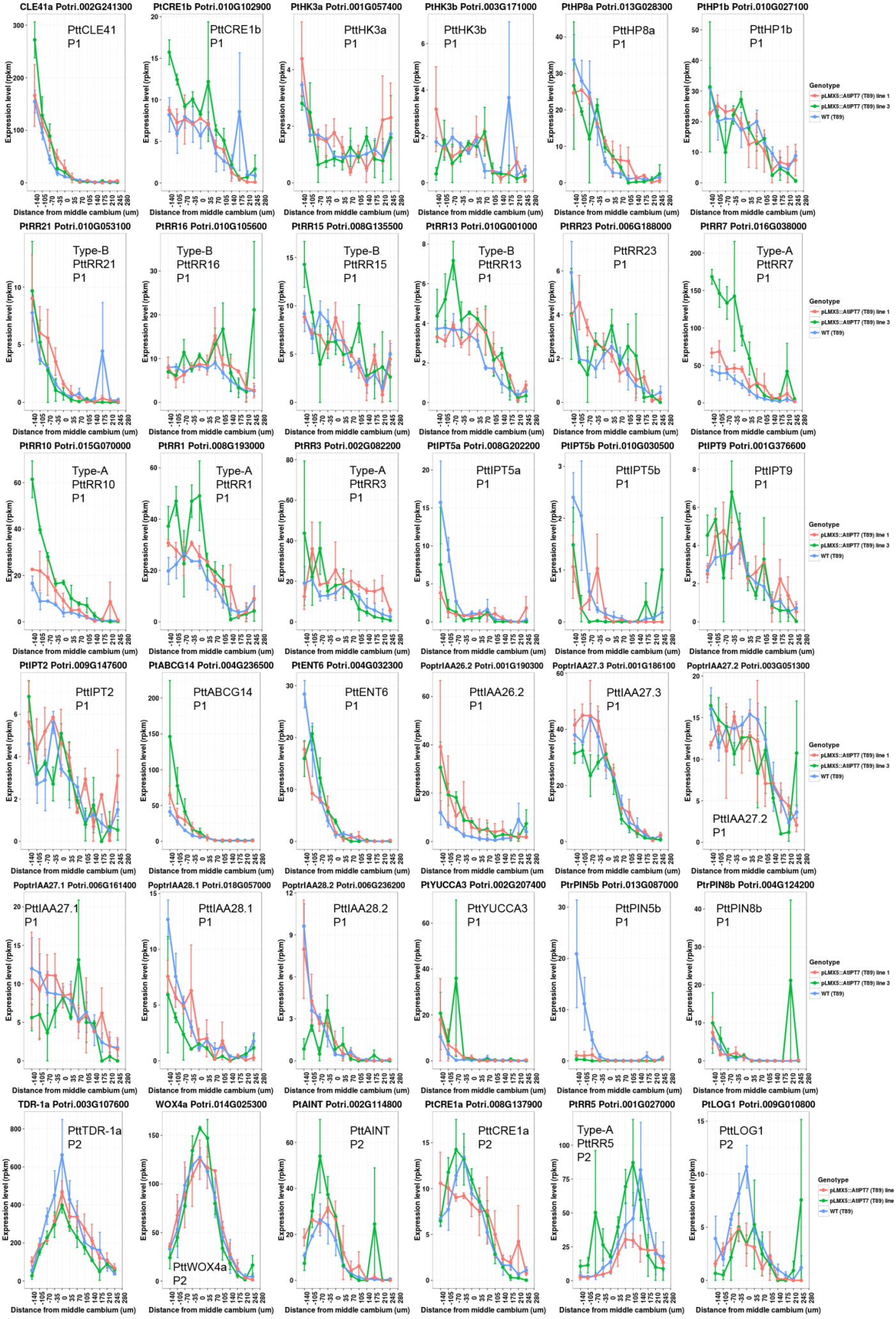

Figure S4 (continues on the next page)

Figure S4 continued

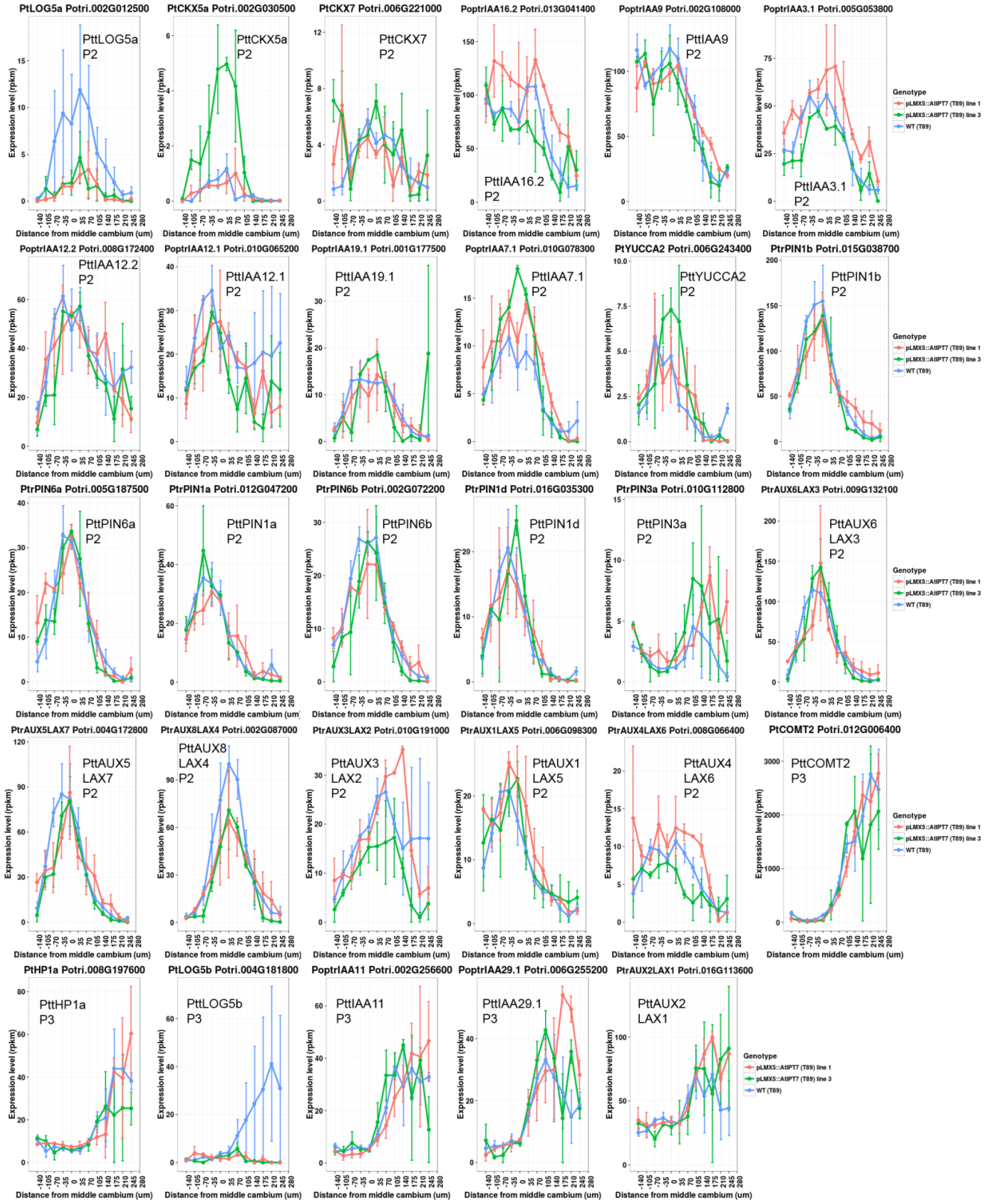

Figure S4 Expression profiles of the cytokinin and auxin signaling, homeostasis and transport genes across the cambial zone of *P. tremula* × *tremuloides* WT and *pLMX5:AtIPT7* line 1 and 3 trees; related to Figure 4. *p*-values from Student's t-test are given in Table S1 (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$). Cytokinin signaling: receptors, *HPt*s, type-B *RR*s and type-A *RR*s; biosynthesis: *IPT*s and *LOG*s; catabolism: *CKX*s, transport: *PttABCG14*, *PttENT6*; auxin response: *IAA*s; biosynthesis: *YUCCA*s; auxin transport: *PIN*s and *AUX/LAX* genes. **Pattern 1 (P1):** Expression profiles of the developing phloem peaking genes (verified by phloem marker gene *PttWOX4a*); most cytokinin signaling genes belong to this category. **Pattern 2 (P2):** Profiles of the genes with expression maxima in the middle of the cambial zone (verified by cambium marker genes *PttTDR-1a* and *PttWOX4a*); most auxin response genes are defined by this profile. **Pattern 3 (P3):** Expression profiles of the developing xylem peaking genes (verified by xylem marker gene *PttCOMT2*); only a few cytokinin or auxin signaling genes follow this profile.

Table S1 Excel file with *p*-values from Student's t-test for the expression profiles in Figure S4 (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$).

Supplemental Experimental Procedures

RT-qPCR gene expression analysis

Primers used in qRT-PCR studies.

Gene	Locus	FWD Primer	REW Primer
PtIPT2	**Potri.009G147600**	TCACTGCTAAGGACTTTCGGG	TCCCAATGCTGAGAACAGGAA
PtIPT3	**Potri.014G139300**	ACAAGTCGGATGCTTGATATCCCT	GGCTTCTGGGACTTAGCATGT
PtIPT5a	**Potri.008G202200**	GATGTGTCACTACCTCTACTCCAC	CCTCATCAATCAAGCCTGCTCTT
PtIPT5b	**Potri.010G030500**	TGGACGTGTCACTCCCAA	CTCATCAATCAAGCCTGCTTCC
PtIPT6a	**Potri.008G121500**	TTAGGGAGGTGATGACGGTG	CATGCTTGGCTCCATCACATC
PtIPT6b	**Potri.010G123900**	CACGTGTCAGCTGGCAAAG	TCCGTAGCATCCACTCTTTGC
PtIPT7a	**Potri.004G150900**	GGTTTACAAGGGCCTTGACATG	CACACCTCGGCGTTCAAT
PtIPT7b	**Potri.008G033300**	CACTAACTTTCTCCAGGAAGATGGG	AGGCAAACCACTAGCATCCA
PtIPT9	**Potri.001G376600**	GCAGGGAGCAATGGGATTTT	ACCCTCATCAAGAAGCCATTTG
PtLOG1	**Potri.009G010800**	AGAAATGGCCCGCAATTCT	CCATAGCCACCTGGTAACGC
PtLOG5a	**Potri.002G012500**	GAGATGGCCCGTCATTCTGA	TCCATACCCACCTGGTAAGGC
PtLOG5b	**Potri.004G181800**	ACTGCTACAGGGATGCTGCC	TTTTCGCCACCAGCTCTTG
PtLOG5c	**Potri.005G248900**	ACCAGGTGGGTATGGAACCTT	GCCCAAGTGATGACCTCCAA
PtLOG5d	**Potri.009G141500**	ATGGCTAGACATTCCGATGCC	GGTCCCATAGCCACCAGGTAA
PtLOG6	**Potri.016G090500**	TGGTGGTCGTCATGTTATTGGA	TCTCGAGGCATGAGCGTCTT
PtLOG7a	**Potri.005G248900**	TGGAGGTCATTACATGGGCC	AACCCACAGGCTTGTCGTG
PtLOG7b	**Potri.006G204800**	AAGCCAGCTACCAGGAAGCTG	TCTCAACCAGTTCCTTGGCC
PtLOG8a	**Potri.001G265300**	GCAGAAATGGCAAAACATGCT	ATAACCACCAGGAAGGGCAAT
PtLOG8b	**Potri.001G005400**	AAGGCAGAGATGGCTCGGA	CCCTCCAGGAAGAGCAATGA
PtLOG8c	**Potri.003G219300**	AAAAGATATTCAGTGATGCAGCCC	TTTCAACCAGCTCCCTTCCA
PtLOG8d	**Potri.006G127400**	AGTCTTCAGCGATGCCGC	TTCCGCTTCACCAGTTCATCT
PtLOG8e	**Potri.009G060300**	GAAATGGCAAAACATGCCG	TCCATAACCGCCAGGAAGG
PtCKX1a	**Potri.006G047900**	TGGGCCACAAATTAACAACATTT	TCCCTTGCCTGTGACAACCT
PtCKX1b	**Potri.016G044100**	GGTCCCATCCTCATCTATCCG	TGTTCGGTTATCCCACTTGGA
PtCKX3a	**Potri.006G152500**	CGGCCCTCAGATTAGCAATG	CCTTTCCCGGTAACAACATCC
PtCKX3b	**Potri.007G066100**	AAGGATTGCTTTAAGGTCGGC	AATGCTCGAGACCACCTAACTTTT
PtCKX5a	**Potri.002G030500**	CATCACAAGGGCTAGAATTGCA	CACCTCACCCTTTGAGGAGCT
PtCKX6	**Potri.003G203600**	TTGGCATCATAACTCGGGC	CCATATCAGGTGCTGGTTCCA
PtHP1a	**Potri.008G197600**	AGCAGCTCCAGCATAGGTGC	AAAGGCGATGCAGTCGTTTT
PtHP1b	**Potri.010G027100**	GGCTTCTGAGTGATCTCACCTTT	GCGGAAAGCGATGCAATCA
PtHP4a	**Potri.001G189900**	TAGGCAGGTTGCTCTCACA	CTGAATCCCTGTAGTGCAATGAGA
PtHP4b	**Potri.001G465000**	CCCTGATGATTTTACCTTCGTCC	AATCCCCTTCATGGCAAAATCC
PtHP4d	**Potri.009G146300**	CCCAAAGTTATCGACCGAGCT	AGCATATCGGAGCTCACGACTA
PtHP6a	**Potri.001G191900**	GCGAGCTTTGGAGTTGCTG	ATGCAGTGGGTACCTGACA
PtHP6b	**Potri.003G032400**	CTTGAGAGCTTTGGAGCTGCTA	TTTCAATGCTGCGGTGCG
PtHP7a	**Potri.006G098200**	GACAGTATACCGATTTCACTACCC	GAAGAGAGACACAACCTCCAC
PtHP7b	**Potri.014G136200**	CCTGGTTTTGTGGTGGAAGTG	TGCTGCACCAATGCTAGAACTA
PtHP7c	**Potri.016G113500**	GATTTCCTAGCTCCTCTCTACCG	CCACCTGTTTGAAGTCCACAAC
PtHP8a	**Potri.013G028300**	ACAGGAAAATTGATGCCCATG	TGCTGGAGCTGCTACCCTTC
PtHP8b	**Potri.005G040400**	ACCCAGATTTCGTAGTTGAACTG	CTTTGGCTAGCTCATTGATAAGCT
PtRR1	**Potri.008G193000**	TGTCATCAGAGAACGTGGTGG	CTCCTTCCTCCAAACATCGG
PtRR2	**Potri.008G193000**	TCTTGTAAAGTGACAGCGGTGG	AAGCAGTTTTAGAGCTCCCCATC
PtRR3	**Potri.002G082200**	TCTTCGTCGTCCCAATCACC	GATCAAATTTACTTTCATCCCCTCTT
PtRR4	**Potri.003G197500**	CAGGTCACAGCAGTGGATTCA	CAAACCCAAAAACTCCAAGGC
PtRR5	**Potri.001G027000**	TCAAGTCACGGCAGTGGATTC	AAGCCCAAAAACTCCAAGGC
PtRR6	**Potri.006G041100**	ATCGGGAAGTGGAAGTGAATCTT	TGCCGGGCATACAGTAATCTG
PtRR7	**Potri.016G038000**	ACCAGATGTTTGGAGGAGGGA	TCTTACTGGCTTCAAGAAAAATTCC
PtRR8	**Potri.019G058900**	AAACTGGTGGAAAGACTGCTCAA	CGCAGTAGTCACCTTGCAGG
PtRR9	**Potri.019G133600**	CAATCACGGTGACTTGAAGGTG	CGCATGCAATAGTCTGTGATGA
PtRR10	**Potri.015G070000**	CATCTGAGAACATCTTGGCTCG	CTGCCCCTTCTTCTAAACACCTAT
PtRR11	**Potri.019G058900**	ATCAGTGCATGGAGGGAGGA	GCTGAAGAGGCTTCAGCAAGAA
PtRR13	**Potri.010G001000**	GAAGCAGCTAGCCGACTTGC	TCATTTAGTATCTGGCCCTGAGC
PtRR14	**Potri.008G181000**	AAGGTGACCACATGCGGTTT	CTCACGAAGCAGGTTCAAAGC
PtRR15	**Potri.008G135500**	TTGGGTTGGAAATGGACCTC	TTCTCCCATCAGCAGACATCATA
PtRR16	**Potri.010G105600**	GTTGGGTTGGAAATGGACCTT	TCTCCCATCAGCAGACATCATAA
PtRR17	**Potri.012G133800**	AGGGCTAGACAAGGCTGTTCC	ACCGGTTTTTGCTGGCCT
PtRR18	**Potri.006G262100**	ATCAGAAGTTTGTCGCCGCT	GAACAGCCTTGTCAAGACCCAT
PtRR19	**Potri.018G111300**	GGCAAGCCATTTGCAGAAA	CCCACAACTTAGTCTTTTGAGGTAAA
PtRR20	**Potri.015G136000**	ATACAGGAACAAACCCTCCGG	GTGAGGCCATTAGGGACACTT
PtRR21	**Potri.010G053100**	TGATGAATGTGCCGCGTTT	TCTGCAAATGGCTAGCGACA
PtRR22	**Potri.018G021300**	GATGAGGACGAGGACGAG	TGTCAACGCCCAATTGATTAAC
PtRR23	**Potri.006G188000**	CATGTTGTCAGCGAACGGTG	TGAGTGATCCCCTTCATCACAAG

Phenotypic analyses of transgenic tree lines

Stem diameter (together with total height) of 6-11 week old trees was measured once per week at three points (10 cm above soil level, at the middle and 2 cm below apex). Stem volume was calculated by the formula of frustum (sum of basal to middle and middle to apex) V= $\frac{\pi h}{3}(r^2 + rR + R^2)$.

Data analysis using custom R scripts

Count data processing has been done as shown in the example R script presented here.

#An example script for processing the count data. In this script the three wildtype samples are processed.
#The pLMX5:AtIPT7 samples were processed identically using corresponding sample tables included in this supplement.
#Make a workdir and subdirs for each sample. Place the annotation gff (Ptrichocarpa_210_v3.0.gene_exons_IPT.gff3)
#into your workdir e.g./home/username/EMTAB4631 and /home/username/EMTAB4631/4A etc.

```
source("http://bioconductor.org/biocLite.R")
library(Rsamtools)
library(GenomicFeatures)
library(GenomicAlignments)
library(BiocParallel)

wrkdir <- "YOURWORKDIRFOR4A" # location of sample 4A bam-files
setwd(wrkdir)
dir <- getwd()
gfffile <- file.path(dir, "../Ptrichocarpa_210_v3.0.gene_exons_IPT.gff3") #gff-file located at previous directory
(txdb <- makeTxDbFromGFF(gfffile, format = "gff3", circ_seqs = character()))
(ebg <- exonsBy(txdb, by="gene"))
csvfile <- file.path(dir, "4A_sample_table.csv")
(sampleTable <- read.csv(csvfile,row.names = 1))
filenames <- file.path(dir, paste0(sampleTable$Run, ".bam"))
file.exists(filenames)
bamfiles <- BamFileList(filenames, yieldSize = 2000000)
seqinfo(bamfiles[1])
register(SerialParam())
se <- summarizeOverlaps(features=ebg, reads=bamfiles, mode="Union",ignore.strand=TRUE)
(colData(se) <- DataFrame(sampleTable))
colSums(assay(se))
permil_counts_4A <- assay(se)*1000000/colSums(assay(se))[col(assay(se))]

wrkdir <- "YOURWORDIRFOR4B" # location of sample 4B bam-files
setwd(wrkdir)
dir <- getwd()
csvfile <- file.path(dir, "4B_sample_table.csv")
(sampleTable <- read.csv(csvfile,row.names = 1))
filenames <- file.path(dir, paste0(sampleTable$Run, ".bam"))
file.exists(filenames)
bamfiles <- BamFileList(filenames, yieldSize = 2000000)
seqinfo(bamfiles[1])
register(SerialParam())
se <- summarizeOverlaps(features=ebg, reads=bamfiles, mode="Union",ignore.strand=TRUE)
(colData(se) <- DataFrame(sampleTable))
colSums(assay(se))
permil_counts_4B <- assay(se)*1000000/colSums(assay(se))[col(assay(se))]

wrkdir <- "YOURWORDIRFOR6B" # location of sample 6B bam-files
setwd(wrkdir)
dir <- getwd()
csvfile <- file.path(dir, "6B_sample_table.csv")

(sampleTable <- read.csv(csvfile,row.names = 1))
filenames <- file.path(dir, paste0(sampleTable$Run, ".bam"))
file.exists(filenames)
bamfiles <- BamFileList(filenames, yieldSize = 2000000)
seqinfo(bamfiles[1])
register(SerialParam())
se <- summarizeOverlaps(features=ebg, reads=bamfiles, mode="Union",ignore.strand=TRUE)
```

```
(colData(se) <- DataFrame(sampleTable))
colSums(assay(se))
permil_counts_6B <- assay(se)*1000000/colSums(assay(se))[col(assay(se))]


wrkdir <- "YOURWORKDIR" #Your workdir containing annotation and sample subdirs
setwd(wrkdir)
Gene_lengths <- sum(width(ebg))

rpkm_4A <- permil_counts_4A*1000/Gene_lengths
rpkm_4B <- permil_counts_4B*1000/Gene_lengths
rpkm_6B <- permil_counts_6B*1000/Gene_lengths
data_array_WT <- array(c(rpkm_4A,rpkm_4B,rpkm_6B), dim = c(dim(rpkm_4A),3))
mean_rpkm_WT <- apply(data_array_WT,c(1,2),mean)
SD_rpkm_WT <- apply(data_array_WT,c(1,2),sd)
SE_mean_rpkm_WT <- SD_rpkm_WT/sqrt(3)
```

Sample tables required for count data processing

The following sample table files are required when count data is processed as presented in the R script example.

1_2A_sample_table.csv

```
"","SampleName","tree","frac","Run","avgLength","Experiment","Sample","Biosample"
"1-2A-F3","1-2A-F3","1-2A","3","1-2A-F3","78.52","1-2A-F3","1-2A-F3","1-2A-F3"
"1-2A-F4","1-2A-F4","1-2A","4","1-2A-F4","78.33","1-2A-F4","1-2A-F4","1-2A-F4"
"1-2A-F5","1-2A-F5","1-2A","5","1-2A-F5","78.17","1-2A-F5","1-2A-F5","1-2A-F5"
"1-2A-F6","1-2A-F6","1-2A","6","1-2A-F6","78.68","1-2A-F6","1-2A-F6","1-2A-F6"
"1-2A-F7","1-2A-F7","1-2A","7","1-2A-F7","78.87","1-2A-F7","1-2A-F7","1-2A-F7"
"1-2A-F8","1-2A-F8","1-2A","8","1-2A-F8","78.18","1-2A-F8","1-2A-F8","1-2A-F8"
"1-2A-F9","1-2A-F9","1-2A","9","1-2A-F9","78.99","1-2A-F9","1-2A-F9","1-2A-F9"
"1-2A-F10","1-2A-F10","1-2A","10","1-2A-F10","78.36","1-2A-F10","1-2A-F10","1-2A-F10"
"1-2A-F11","1-2A-F11","1-2A","11","1-2A-F11","76.11","1-2A-F11","1-2A-F11","1-2A-F11"
"1-2A-F12","1-2A-F12","1-2A","12","1-2A-F12","78.63","1-2A-F12","1-2A-F12","1-2A-F12"
"1-2A-F13","1-2A-F13","1-2A","13","1-2A-F13","77.98","1-2A-F13","1-2A-F13","1-2A-F13"
"1-2A-F14","1-2A-F14","1-2A","14","1-2A-F14","77.26","1-2A-F14","1-2A-F14","1-2A-F14"
"1-2A-F15","1-2A-F15","1-2A","15","1-2A-F15","79.11","1-2A-F15","1-2A-F15","1-2A-F15"
"1-2A-F16","1-2A-F16","1-2A","16","1-2A-F16","77.77","1-2A-F16","1-2A-F16","1-2A-F16"
"1-2A-F17","1-2A-F17","1-2A","17","1-2A-F17","78.31","1-2A-F17","1-2A-F17","1-2A-F17"
"1-2A-F19","1-2A-F19","1-2A","19","1-2A-F19","78.14","1-2A-F19","1-2A-F19","1-2A-F19"
"1-2A-F20","1-2A-F20","1-2A","20","1-2A-F20","78.81","1-2A-F20","1-2A-F20","1-2A-F20"
"1-2A-F21","1-2A-F21","1-2A","21","1-2A-F21","78.53","1-2A-F21","1-2A-F21","1-2A-F21"
"1-2A-F22","1-2A-F22","1-2A","22","1-2A-F22","78.42","1-2A-F22","1-2A-F22","1-2A-F22"
"1-2A-F23","1-2A-F23","1-2A","23","1-2A-F23","79.45","1-2A-F23","1-2A-F23","1-2A-F23"
"1-2A-F24","1-2A-F24","1-2A","24","1-2A-F24","78.83","1-2A-F24","1-2A-F24","1-2A-F24"
"1-2A-F25","1-2A-F25","1-2A","25","1-2A-F25","78.61","1-2A-F25","1-2A-F25","1-2A-F25"
```

12_4_sample_table.csv

```
"","SampleName","tree","frac","Run","avgLength","Experiment","Sample","Biosample"
"12_4_F5","12_4_F5","12_4","5","12_4_F5","78.30","12_4_F5","12_4_F5","12_4_F5"
"12_4_F6","12_4_F6","12_4","6","12_4_F6","79.57","12_4_F6","12_4_F6","12_4_F6"
"12_4_F7","12_4_F7","12_4","7","12_4_F7","80.65","12_4_F7","12_4_F7","12_4_F7"
```

"12_4_F8","12_4_F8","12_4","8","12_4_F8","64.46","12_4_F8","12_4_F8","12_4_F8"
"12_4_F9","12_4_F9","12_4","9","12_4_F9","68.90","12_4_F9","12_4_F9","12_4_F9"
"12_4_F10","12_4_F10","12_4","10","12_4_F10","78.50","12_4_F10","12_4_F10","12_4_F10"
"12_4_F11","12_4_F11","12_4","11","12_4_F11","77.00","12_4_F11","12_4_F11","12_4_F11"
"12_4_F12","12_4_F12","12_4","12","12_4_F12","77.22","12_4_F12","12_4_F12","12_4_F12"
"12_4_F13","12_4_F13","12_4","13","12_4_F13","77.36","12_4_F13","12_4_F13","12_4_F13"
"12_4_F14","12_4_F14","12_4","14","12_4_F14","77.72","12_4_F14","12_4_F14","12_4_F14"
"12_4_F15","12_4_F15","12_4","15","12_4_F15","77.52","12_4_F15","12_4_F15","12_4_F15"
"12_4_F16","12_4_F16","12_4","16","12_4_F16","78.94","12_4_F16","12_4_F16","12_4_F16"
"12_4_F17","12_4_F17","12_4","17","12_4_F17","78.96","12_4_F17","12_4_F17","12_4_F17"
"12_4_F18","12_4_F18","12_4","18","12_4_F18","79.05","12_4_F18","12_4_F18","12_4_F18"
"12_4_F19","12_4_F19","12_4","19","12_4_F19","79.13","12_4_F19","12_4_F19","12_4_F19"
"12_4_F20","12_4_F20","12_4","20","12_4_F20","79.54","12_4_F20","12_4_F20","12_4_F20"
"12_4_F21","12_4_F21","12_4","21","12_4_F21","79.42","12_4_F21","12_4_F21","12_4_F21"
"12_4_F22","12_4_F22","12_4","22","12_4_F22","79.33","12_4_F22","12_4_F22","12_4_F22"
"12_4_F23","12_4_F23","12_4","23","12_4_F23","78.65","12_4_F23","12_4_F23","12_4_F23"

1C_sample_table.csv:

"","SampleName","tree","frac","Run","avgLength","Experiment","Sample","Biosample"
"1C-F8","1C-F8","1C","8","1C-F8","74.65","1C-F8","1C-F8","1C-F8"
"1C-F9","1C-F9","1C","9","1C-F9","77.43","1C-F9","1C-F9","1C-F9"
"1C-F10","1C-F10","1C","10","1C-F10","78.45","1C-F10","1C-F10","1C-F10"
"1C-F11","1C-F11","1C","11","1C-F11","78.49","1C-F11","1C-F11","1C-F11"
"1C-F12","1C-F12","1C","12","1C-F12","78.02","1C-F12","1C-F12","1C-F12"
"1C-F13","1C-F13","1C","13","1C-F13","76.37","1C-F13","1C-F13","1C-F13"
"1C-F14","1C-F14","1C","14","1C-F14","77.49","1C-F14","1C-F14","1C-F14"
"1C-F15","1C-F15","1C","15","1C-F15","77.73","1C-F15","1C-F15","1C-F15"
"1C-F16","1C-F16","1C","16","1C-F16","77.65","1C-F16","1C-F16","1C-F16"
"1C-F17","1C-F17","1C","17","1C-F17","78.19","1C-F17","1C-F17","1C-F17"
"1C-F18","1C-F18","1C","18","1C-F18","78.78","1C-F18","1C-F18","1C-F18"
"1C-F19","1C-F19","1C","19","1C-F19","79.81","1C-F19","1C-F19","1C-F19"
"1C-F20","1C-F20","1C","20","1C-F20","77.63","1C-F20","1C-F20","1C-F20"
"1C-F21","1C-F21","1C","21","1C-F21","79.18","1C-F21","1C-F21","1C-F21"
"1C-F22","1C-F22","1C","22","1C-F22","73.04","1C-F22","1C-F22","1C-F22"
"1C-F23","1C-F23","1C","23","1C-F23","77.70","1C-F23","1C-F23","1C-F23"
"1C-F24","1C-F24","1C","24","1C-F24","75.87","1C-F24","1C-F24","1C-F24"

1E_sample_table.csv

"","SampleName","tree","frac","Run","avgLength","Experiment","Sample","Biosample"
"1E-F2","1E-F2","1E","2","1E-F2","76.16","1E-F2","1E-F2","1E-F2"
"1E-F3","1E-F3","1E","3","1E-F3","75.89","1E-F3","1E-F3","1E-F3"
"1E-F4","1E-F4","1E","4","1E-F4","76.33","1E-F4","1E-F4","1E-F4"
"1E-F5","1E-F5","1E","5","1E-F5","76.28","1E-F5","1E-F5","1E-F5"

"1E-F6","1E-F6","1E","6","1E-F6","75.43","1E-F6","1E-F6","1E-F6"
"1E-F7","1E-F7","1E","7","1E-F7","77.85","1E-F7","1E-F7","1E-F7"
"1E-F8","1E-F8","1E","8","1E-F8","76.49","1E-F8","1E-F8","1E-F8"
"1E-F9","1E-F9","1E","9","1E-F9","78.09","1E-F9","1E-F9","1E-F9"
"1E-F10","1E-F10","1E","10","1E-F10","78.09","1E-F10","1E-F10","1E-F10"
"1E-F11","1E-F11","1E","11","1E-F11","76.29","1E-F11","1E-F11","1E-F11"
"1E-F12","1E-F12","1E","12","1E-F12","77.26","1E-F12","1E-F12","1E-F12"
"1E-F13","1E-F13","1E","13","1E-F13","76.38","1E-F13","1E-F13","1E-F13"
"1E-F14","1E-F14","1E","14","1E-F14","76.18","1E-F14","1E-F14","1E-F14"
"1E-F15","1E-F15","1E","15","1E-F15","76.63","1E-F15","1E-F15","1E-F15"
"1E-F16","1E-F16","1E","16","1E-F16","76.64","1E-F16","1E-F16","1E-F16"
"1E-F18","1E-F18","1E","18","1E-F18","77.07","1E-F18","1E-F18","1E-F18"
"1E-F19","1E-F19","1E","19","1E-F19","77.63","1E-F19","1E-F19","1E-F19"
"1E-F20","1E-F20","1E","20","1E-F20","77.60","1E-F20","1E-F20","1E-F20"
"1E-F21","1E-F21","1E","21","1E-F21","77.48","1E-F21","1E-F21","1E-F21"
"1E-F22","1E-F22","1E","22","1E-F22","78.23","1E-F22","1E-F22","1E-F22"
"1E-F23","1E-F23","1E","23","1E-F23","77.51","1E-F23","1E-F23","1E-F23"
"1E-F24","1E-F24","1E","24","1E-F24","75.37","1E-F24","1E-F24","1E-F24"
4A_sample_table.csv
"","SampleName","tree","frac","Run","avgLength","Experiment","Sample","Biosample"
"4A_F9","4A_F9","4A","9","4A_F9","78.85","4A_F9","4A_F9","4A_F9"
"4A_F10","4A_F10","4A","10","4A_F10","77.03","4A_F10","4A_F10","4A_F10"
"4A_F11","4A_F11","4A","11","4A_F11","77.01","4A_F11","4A_F11","4A_F11"
"4A_F12","4A_F12","4A","12","4A_F12","78.27","4A_F12","4A_F12","4A_F12"
"4A_F13","4A_F13","4A","13","4A_F13","77.52","4A_F13","4A_F13","4A_F13"
"4A_F14","4A_F14","4A","14","4A_F14","77.84","4A_F14","4A_F14","4A_F14"
"4A_F15","4A_F15","4A","15","4A_F15","77.39","4A_F15","4A_F15","4A_F15"
"4A_F16","4A_F16","4A","16","4A_F16","77.64","4A_F16","4A_F16","4A_F16"
"4A_F17","4A_F17","4A","17","4A_F17","77.79","4A_F17","4A_F17","4A_F17"
"4A_F18","4A_F18","4A","18","4A_F18","77.92","4A_F18","4A_F18","4A_F18"
"4A_F19","4A_F19","4A","19","4A_F19","78.50","4A_F19","4A_F19","4A_F19"
"4A_F20","4A_F20","4A","20","4A_F20","78.41","4A_F20","4A_F20","4A_F20"
"4A_F21","4A_F21","4A","21","4A_F21","78.41","4A_F21","4A_F21","4A_F21"
"4A_F22","4A_F22","4A","22","4A_F22","76.29","4A_F22","4A_F22","4A_F22"
"4A_F23","4A_F23","4A","23","4A_F23","73.64","4A_F23","4A_F23","4A_F23"
"4A_F24","4A_F24","4A","24","4A_F24","77.99","4A_F24","4A_F24","4A_F24"
"4A_F25","4A_F25","4A","25","4A_F25","77.98","4A_F25","4A_F25","4A_F25"
"4A_F26","4A_F26","4A","26","4A_F26","79.82","4A_F26","4A_F26","4A_F26"
4B_sample_table.csv
"","SampleName","tree","frac","Run","avgLength","Experiment","Sample","Biosample"
"4B-F6","4B-F6","4B","6","4B-F6","80.31","4B-F6","4B-F6","4B-F6"

"4B-F7","4B-F7","4B","7","4B-F7","78.78","4B-F7","4B-F7","4B-F7"
"4B-F8","4B-F8","4B","8","4B-F8","79.32","4B-F8","4B-F8","4B-F8"
"4B-F9","4B-F9","4B","9","4B-F9","78.71","4B-F9","4B-F9","4B-F9"
"4B-F10","4B-F10","4B","10","4B-F10","79.05","4B-F10","4B-F10","4B-F10"
"4B-F11","4B-F11","4B","11","4B-F11","77.17","4B-F11","4B-F11","4B-F11"
"4B-F12","4B-F12","4B","12","4B-F12","79.33","4B-F12","4B-F12","4B-F12"
"4B-F13","4B-F13","4B","13","4B-F13","79.73","4B-F13","4B-F13","4B-F13"
"4B-F14","4B-F14","4B","14","4B-F14","78.11","4B-F14","4B-F14","4B-F14"
"4B-F15","4B-F15","4B","15","4B-F15","79.06","4B-F15","4B-F15","4B-F15"
"4B-F17","4B-F17","4B","17","4B-F17","78.19","4B-F17","4B-F17","4B-F17"
"4B-F18","4B-F18","4B","18","4B-F18","79.66","4B-F18","4B-F18","4B-F18"
"4B-F19","4B-F19","4B","19","4B-F19","80.28","4B-F19","4B-F19","4B-F19"
"4B-F20","4B-F20","4B","20","4B-F20","80.98","4B-F20","4B-F20","4B-F20"
"4B-F21","4B-F21","4B","21","4B-F21","78.99","4B-F21","4B-F21","4B-F21"
"4B-F22","4B-F22","4B","22","4B-F22","79.24","4B-F22","4B-F22","4B-F22"

6B_sample_table.csv

"","SampleName","tree","frac","Run","avgLength","Experiment","Sample","Biosample"
"6B_F2","6B_F2","6B","2","6B_F2","78.96","6B_F2","6B_F2","6B_F2"
"6B_F3","6B_F3","6B","3","6B_F3","79.33","6B_F3","6B_F3","6B_F3"
"6B_F4","6B_F4","6B","4","6B_F4","80.67","6B_F4","6B_F4","6B_F4"
"6B_F5","6B_F5","6B","5","6B_F5","79.86","6B_F5","6B_F5","6B_F5"
"6B_F6","6B_F6","6B","6","6B_F6","79.89","6B_F6","6B_F6","6B_F6"
"6B_F7","6B_F7","6B","7","6B_F7","80.10","6B_F7","6B_F7","6B_F7"
"6B_F8","6B_F8","6B","8","6B_F8","79.25","6B_F8","6B_F8","6B_F8"
"6B_F9","6B_F9","6B","9","6B_F9","80.07","6B_F9","6B_F9","6B_F9"
"6B_F10","6B_F10","6B","10","6B_F10","80.69","6B_F10","6B_F10","6B_F10"
"6B_F11","6B_F11","6B","11","6B_F11","78.34","6B_F11","6B_F11","6B_F11"
"6B_F12","6B_F12","6B","12","6B_F12","80.36","6B_F12","6B_F12","6B_F12"
"6B_F13","6B_F13","6B","13","6B_F13","80.80","6B_F13","6B_F13","6B_F13"
"6B_F14","6B_F14","6B","14","6B_F14","80.93","6B_F14","6B_F14","6B_F14"
"6B_F15","6B_F15","6B","15","6B_F15","81.31","6B_F15","6B_F15","6B_F15"
"6B_F16","6B_F16","6B","16","6B_F16","80.70","6B_F16","6B_F16","6B_F16"
"6B_F17","6B_F17","6B","17","6B_F17","80.86","6B_F17","6B_F17","6B_F17"
"6B_F18","6B_F18","6B","18","6B_F18","80.93","6B_F18","6B_F18","6B_F18"
"6B_F19","6B_F19","6B","19","6B_F19","80.47","6B_F19","6B_F19","6B_F19"