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Review

What Makes the Wood? Exploring the Molecular Mechanisms of Xylem Acclimation in Hardwoods to an Ever-Changing Environment

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Received: 28 February 2019; Accepted: 23 April 2019; Published: 25 April 2019

Abstract: Wood, also designated as secondary xylem, is the major structure that gives trees and other woody plants stability for upright growth and maintains the water supply from the roots to all other plant tissues. Over recent decades, our understanding of the cellular processes of wood formation (xylogenesis) has substantially increased. Plants as sessile organisms face a multitude of abiotic stresses, e.g., heat, drought, salinity and limiting nutrient availability that require them to adjust their wood structure to maintain stability and water conductivity. Because of global climate change, more drastic and sudden changes in temperature and longer periods without precipitation are expected to impact tree productivity in the near future. Thus, it is essential to understand the process of wood formation in trees under stress. Many traits, such as vessel frequency and size, fiber thickness and density change in response to different environmental stimuli. Here, we provide an overview of our current understanding of how abiotic stress factors affect wood formation on the molecular level focussing on the genes that have been identified in these processes.

Keywords: wood formation; abiotic stress; nutrition; gene regulation; tree

1. Introduction: The Xylem Keeps the Stream of Life Flowing

The main function of the xylem besides granting plant stability is to ensure long-distance water transport driven by water transpiration from the leaves to the atmosphere [1,2]. Therefore, xylem vessels are connected to each other in order to form a large conduit system throughout the plant. During vessel formation, the cell wall between two adjacent vessel cells is degraded to build a continuum. A measure for the water transport capacity within the xylem is xylem hydraulic conductance, which is, among other factors, determined by the size of the xylem vessels. Vessels with higher diameter facilitate faster water transport, thereby resulting in a higher hydraulic conductance. The volumetric flow rate is proportional to the fourth power of the vessel radius according to the Hagen-Poiseuille law [3,4]. Thus, a small reduction in vessel lumen already results in a large reduction of hydraulic conductivity. A severe danger for plant viability is a disruption of this water flow, called embolism, which primarily occurs at the bordered pits [5,6]. When the conduits are filled with air or vapor instead of xylem sap, cavitation stops further water flux. This process is considered as one of the most life-threatening phenomena for plants [3,7]. Cavitation results in non-functional water conduits, which leads to a reduction in hydraulic conductivity and stomatal closure, thereby reduced photosynthetic activity, which finally can lead to the plant´s death [5,8]. It is therefore crucial for plants to develop mechanisms to prevent cavitation by modulating their xylem structure. The major adjustment is to form vessels with smaller diameter

to reduce the risk of cavitation. An additional mechanism is to increase vessel stability, achieved by increasing vessel cell wall thickness [9]. As a result, vessel lumina decrease [9]. To counteract the reduction in hydraulic conductivity per vessel plants can increase the number of vessels in their xylem [9–11]. In this review, we will summarize the current knowledge on the molecular regulation of xylem acclimation in angiosperms with the focus on *Populus* species and identify knowledge gaps.

2. Anatomy and Molecular Biology of Wood Formation

2.1. What Makes the Stem?

Growth depends on the presence of cells, which are able to undergo division and differentiation processes. These cells are designated as stem cells [12]. In plants, four layers of stem cell harboring tissues are present: the root apical meristem, the shoot apical meristem, the vascular meristem (cambium) and the cork cambium. While the apical meristems are primarily responsible for longitudinal growth, which is not within the scope of this review, they also contribute to radial growth to a certain extent during primary growth. During primary growth of the stem, the stem cell layer deriving from the shoot apical meristem is designated as pro-cambium, which gives rise to the proto-xylem [13]. The procambial cells divide asymmetrically either into proto-phloem or proto-xylem precursor cells. Proto-phloem precursor cells further differentiate into sieve elements, phloem fibers, phloem parenchyma cells and companion cells, while proto-xylem precursor cells further diversify into xylem vessels, xylem fibers and ray parenchyma cells. The wood generated by the activity of the pro-cambium is designated as proto-xylem. An excellent overview on wood formation during primary growth has been published by Furuta and colleagues [14].

Wood is produced from the activity of vascular cambium that is composed of meristematic initials, which either differentiate to either phloem or xylem precursor cells. Xylem precursor cells further diversify into vessels, fibers and parenchyma cells in order to build up the xylem in angiosperms (Figure 1).

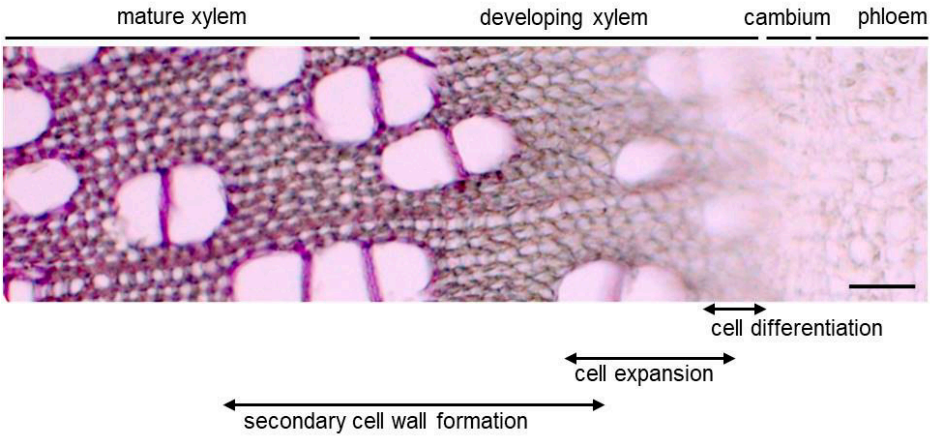

Figure 1. Cross-section of a 3 month old poplar stem illustrating the different steps of wood formation. Cross-section has been stained with Phloroglucinol/HCl. Purple color indicates lignification. Bar = 50 µm.

Secondary growth is what makes up the girth of the stem by generating the majority of the wood. The correct botanical denomination of this secondary wood is meta-xylem. It is also comprised of the three major components, vessels, fibers and parenchyma cells formed by the activity of the cambium. It should be noted that vessels are often surrounded by small living cells, the paratracheal parenchyma, which has important functions in the transport of compounds for example in *Populus tremula* x *tremuloides*, *Fraxinus excelsior* and *Acer pseudoplatanus* [15].

A major difference between the proto- and meta-xylem is the deposition of the secondary cell wall (SCW) compounds leading to structural differences: the SCW of proto-xylem vessels is build up in a

helical shape, which allows for continuous growth as the SCW can be elongated during longitudinal growth [16]. The SCW of meta-xylem is deposited directly onto the primary cell wall resulting in a rigid structure around the vessel that is no longer able to expand.

The SCW is a complex structure, which is primarily composed of cellulose, hemicelluloses and lignin with addition of pectin and cell wall proteins. The composition of the SCW varies within the plant kingdom, e.g., in angiosperms SCWs contain 40%–50% cellulose, 20%–30% hemicelluloses and 25%–30% lignin [17]. Cellulose is a linear homopolymer of β-1,4-linked D-anhydroglucose molecules forming cellulose chains of varying length. These cellulose chains are then connected via Van-der-Waals interactions and hydrogen-bonds to form cellulose micro fibrils [18]. In contrast to cellulose, hemicellulose is chemically complex, consisting of polysaccharide heteropolymers of hexose and pentose sugars with a β-1,4-linked backbone of xyloglucans, xylans, mannans and glucomannans with a vast variety of side chains [19]. The main function of hemicellulose is the interconnection of cellulose micro fibrils and lignin [20]. Lignin provides rigidness and recalcitrance to the cell wall, with strong impact on wood properties by adding extra strength and water impermeability [21]. Lignin is built up by three phenylpropanoid compounds, also designated as monolignols: coniferyl alcohol (G), sinapyl alcohol (S) and p-coumaryl alcohol (H) [22] with G and S subunits being the main building blocks for lignin in angiosperms [23,24]. The combination of these monolignols via radical coupling constitutes a complex polymer, the composition of which varies with developmental and environmental stimuli. Interestingly, the lignin composition is also specific for different cell types, e.g., the lignin in fibers differs significantly from those of vessel elements as it shows a higher content of S-subunits [25].

Wood formation is, thus, characterized by a succession of four major steps, including cell division, cell expansion (elongation and radial enlargement), cell wall thickening (involving biosynthesis and deposition of cellulose, hemicelluloses, lignin and cell wall proteins), and finally programmed cell death [26].

2.2. Molecular Mechanism of Wood Formation

Our understanding of the molecular mechanisms of xylogenesis has massively increased in the last two decades. Several detailed reviews about the molecular mechanisms of wood formation have been published [13,16,26,27]. Here, we will shortly summarize the main aspects important for understanding the impact of environmental stimuli that alter wood formation (Figure 2). Wood formation is tightly controlled by two classes of transcription factors (TFs), namely the NAC family and the MYB family. Using *Arabidopsis thaliana* as a model system, several members of these two TF families have been shown to work in a hierarchical manner to regulate the transcription of genes necessary for wood formation [26]. The first level master switches all belong to the class of NAC TFs and their activation is crucial for the cell identity of wood cells. For example, SND1, NST1 and NST2 are responsible for fiber development, while VASCULAR-RELATED NAC DOMAIN 7 (VND7) controls differentiation of the proto-xylem and VASCULAR-RELATED NAC DOMAIN 6 (VND6) of the meta-xylem [28,29]. Several orthologs of these *A. thaliana* genes have been identified in *Populus trichocarpa* (Table 1). It is somewhat confusing that these master switch TFs are not termed VNDs, as in Arabidopsis but were named WOOD-related NAC DOMAIN (WND) transcription factors, yet they also belong to the family of NAC TFs and are closely related to the *A. thaliana* genes [30]. The expression of these master switches is modulated by fine-tuning factors like the HD-Zip transcription factors PtrAtHB.11 and PtrATHB.12 or PtrE2FC.1 [31,32]. The master switch WNDs further activate a group of MYB master switch TFs (PtrMYB2, PtrMYB3, PtrMYB20, PtrMYB21, [33,34]), which either directly regulate the biosynthesis of cellulose, hemicellulose, and lignin or lead to the activation of down-stream MYC and NAC TFs that promote or decrease the expression of cell wall biosynthesis genes (Table 1, Figure 2).

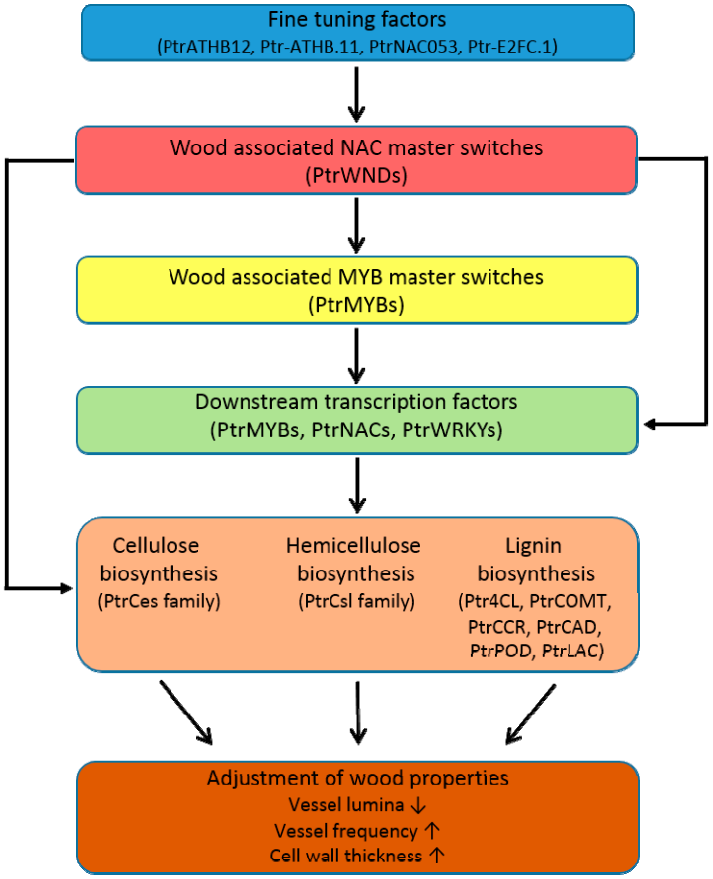

Figure 2. Schematic representation of the molecular mechanisms and gene families leading to xylem formation and acclimation under various stresses.

Cell wall biosynthesis requires the production of cellulose strands by cellulose synthases (CesA), which are homologs of prokaryotic *celA* genes [35] and were firstly characterized in the plant kingdom in *A. thaliana* [35]. The CesA proteins are localized across the plasma membrane using intracellular UDP-glucose to generate cellular strands towards the outside. Meanwhile, a whole *CesA* gene family comprised of 18 genes, of which four show xylem specific expression patterns, was discovered in *P. trichocarpa* [36,37]. A recent study revealed that during xylem formation, two different types of CesA complexes are necessary for cell expansion and cell wall thickening in Arabidopsis, indicating that CesA complex composition is dynamically changing [38]. Since these processes also occur in trees, it is likely that a similar mechanism is also present in woody plants, although there is no experimental evidence up to now.

The cell wall of the meta-xylem in trees is heavily lignified. Lignin biosynthesis is a very complex process starting with aromatic amino acids, mainly phenylalanine [39]. The main classes of dedicated enzymes required to produce lignin building units are 4-coumarate:CoA ligases (4CL), caffeic acid *O*-methyltransferases (COMT), cinnamoyl-CoA reductases (CCR), and cinnamyl alcohol dehydrogenases (CAD) [22]. Monolignols are transported from the cytosol into the cell wall [40], where they are polymerized by peroxidases (POD) and laccases (LAC), yielding lignin. However, only very few monolignol transporters have been identified by now, such as the Arabidopsis p-coumaryl alcohol transporter AtABCG29 [41,42].

As outlined above hemicelluloses are also very complex molecules. Therefore, it is not surprising that a variety of enzymes is necessary for their synthesis. These enzymes are summarized as Cellulose Synthase-Like (CSL) proteins [43], of which 30 have been identified in poplar [37]. Four CSL genes (*PtrCSLA1*, *PtrCSLA2*, *PtrCSLA5*, *PtrCSLD6*) are predominantly expressed in the xylem [37].

Table 1. List of orthologs of *Arabidopsis thaliana* wood-formation associated transcription factors present in *Populus trichocarpa*.

Gene Function	Potri ID	Populus Gene Name	AGI ID	Arabidopsis Gene Name
Fine-tuning factors	Potri.001G188800	*Ptr-ATHB.12*	At1G52150	*AtHB15/AtCNA/AtICU4*
	Potri.003G050100	*Ptr-ATHB.11*	At1G52150	*AtHB15/AtCNA/AtICU4*
	Potri.001G197000		AT3G13890	*AtMYB26*
	no annotated ortholog		At3G32090	*AtWRKY12*
	Potri.002G023400	*Ptr-E2FC.1/E2Fc*	At1G47870	*AtE2Fc*
	Potri.001G061200	*PtrNAC053*	AT5G13180	*AtVNI2/AtANAC083*
Master regulators (NAC)	no annotated ortholog		At1G32770	*AtNST3/AtSND1/AtANAC012*
	Potri.001G448400	*PtrWND1B/NAC063/PtVNS11*	At2G46770	*AtANAC043/AtNST1*
	Potri.002G178700	*PtrWND2B/NAC061/PtVNS10*	AT2G46770	*AtANAC043/AtNST1*
	Potri.011G153300	*PtrWND1A/NAC068/PtVNS12*	AT2G46770	*AtANAC043/AtNST1*
	Potri.014G104800	*PtrWND2A/NAC065/PtVNS09*	AT2G46770	*AtANAC043/AtNST1*
	Potri.015G127400	*PtrWND3A/NAC050/PtVNS05*	AT1G12260	*AtANAC007/AtNAC007/ATVND4*
	Potri.012G126500	*PtrWND3B/NAC037/PtVNS06*	AT1G12260	*AtANAC007/AtNAC007/ATVND4*
	Potri.001G120000	*PtrWND4A/NAC038/PtVNS03*	AT1G12260	*AtANAC007/AtNAC007/ATVND4*
	Potri.003G113000	*PtrWND4B/NAC046/PtVNS04*	AT1G12260	*AtANAC007/AtNAC007/ATVND4*
	Potri.007G014400	*PtrWND5A/NAC025/PtVNS01*	AT2G18060	*AtANAC037/AtVND1*
	Potri.005G116800	*PtrWND5B/NAC039/PtVNS02*	AT2G18060	*AtANAC037/AtVND1*
	Potri.013G113100	*PtrWND6A/NAC055/PtVNS07*	AT1G71930	*AtANAC030/AtVND7*
	Potri.019G083600	*PtrWND6B/NAC060/PtVNS08*	AT1G71930	*AtANAC030/AtVND7*
	Potri.015G002900	*PtrNAC147*	AT1G71930	*AtANAC030/AtVND7*
	no annotated ortholog		At3G61910	*AtNST2*
	Potri.004G107200		At5G62380	*AtVND6*
	Potri.004G107400		At5G62380	*AtVND6*
	Potri.005G082700	*PtrNAC144*	At5G62380	*AtVND6*
	Potri.006G231300		At5G62380	*AtVND6*
	Potri.014G163600		At5G62380	*AtVND6*
	no annotated ortholog		At4G36160	*AtVND2/AtANAC076*
	no annotated ortholog		At5G66300	*AtVND3/AtANAC105*
	no annotated ortholog		At1G62700	*AtVND5*
Second Level Regulators (MYB)	Potri.001G258700	*PtrMYB2*	At5G12870	*AtMYB46*
	Potri.009G053900	*PtrMYB21*	At5G12870	*AtMYB46*
	Potri.001G267300		At3G08500	*AtMYB83*
	Potri.009G061500		At3G08500	*AtMYB83*

Table 1. *Cont.*

Gene Function	Potri ID	Populus Gene Name	AGI ID	Arabidopsis Gene Name
Third Level Regulators	Potri.004G049300		AT4G28500	*AtSND2/AtANAC073*
	Potri.007G135300		AT4G28500	*AtSND2/AtANAC073*
	Potri.011G058400		AT4G28500	*AtSND2/AtANAC073*
	Potri.017G016700		AT4G28500	*AtSND2/AtANAC073*
	no annotated ortholog		AT1G28470	*AtSND3*
	no annotated ortholog		At1G63910	*AtMYB103*
	Potri.002G073500		At1G17950	*AtMYB52*
	Potri.005G186400	*PtrMYB158/PtrMYB.50*	At1G17950	*AtMYB52*
	Potri.007G134500	*PtrMYB161/PtrMYB.43*	At1G17950	*AtMYB52*
	Potri.012G039400	*PtrMYB167/PtrMYB.41*	At1G17950	*AtMYB52*
	Potri.015G033600	*PtrMYB090/PtrMYB.38*	At1G17950	*AtMYB52*
	no annotated ortholog		At3G48920	*AtMYB45*
	no annotated ortholog		At1G16490	*AtMYB58*
	Potri.005G096600		At1G79180	*AtMYB63*
	Potri.007G067600		At1G79180	*AtMYB63*
	Potri.019G118900		At1G79180	*AtMYB63*
	Potri.015G129100		At4G22680	*AtMYB85*
	Potri.001G112200	*PtrKNAT7.1*	At1G62990	*AtKNAT7*

This rough overview on the regulatory mechanisms and main processes that are required for the main cell wall compounds provides only a glimpse into the complexity of the underlying processes. The composition of wood is important for its further use, e.g., as construction material, where durable material is necessary and for paper making, where fibers of a certain length are favorable, or for secondary biofuel production where easily accessible cellulose is needed. Therefore, it is not only important to know how wood is being produced, but also to understand how wood properties are changed in response to environmental cues.

3. Abiotic Stresses Affecting Wood Formation

3.1. Drought Severely Changes Xylem Anatomy

Drought has a major impact on wood and wood formation processes. Since the xylem is the tissue that enables water transport throughout the whole tree, it is pertinent to keep the xylem architecture intact and to acclimate it to changing water supply to prevent embolism [44,45]. The effects of drought on xylogenesis have been studied on several angiosperm species over the last decade. Poplar, in particular, was intensely studied [46,47]. Cambial cell layers are reduced under drought compared to well-watered plants [10,48]. When growth is still possible under water-limited conditions, poplars, regardless of whether they originate from dry or moist habitats, show reduced vessel lumina and an increased number of vessels compared to non-stressed plants (*Populus* x *canescens*, [49]; *Populus euphratica*, [11]; different *Populus nigra* genotypes originating from dry and moist areas, [10]), Figure 3). Moreover, Schreiber and colleagues reported a strong correlation between vessel diameter and cavitation resistance in five hybrid poplar clones in Alberta, Canada [50]. However, these acclimatory anatomical changes are not only confined to water-spending trees species like poplar but also to more drought tolerant species such as oak depending on the level of acclimation (*Quercus pubescens* > *Quercus robur* > *Quercus petraea*, [51]). The importance of this safety strategy is further corroborated by a study on dead trees performed in Italian forests [52]. Here, the authors compared the wood anatomy of dead trees to that of surviving trees of the same age. They found that dead trees had formed wide early vessels and no vessels with reduced lumen during dry periods in contrast to trees that survived this period [52].

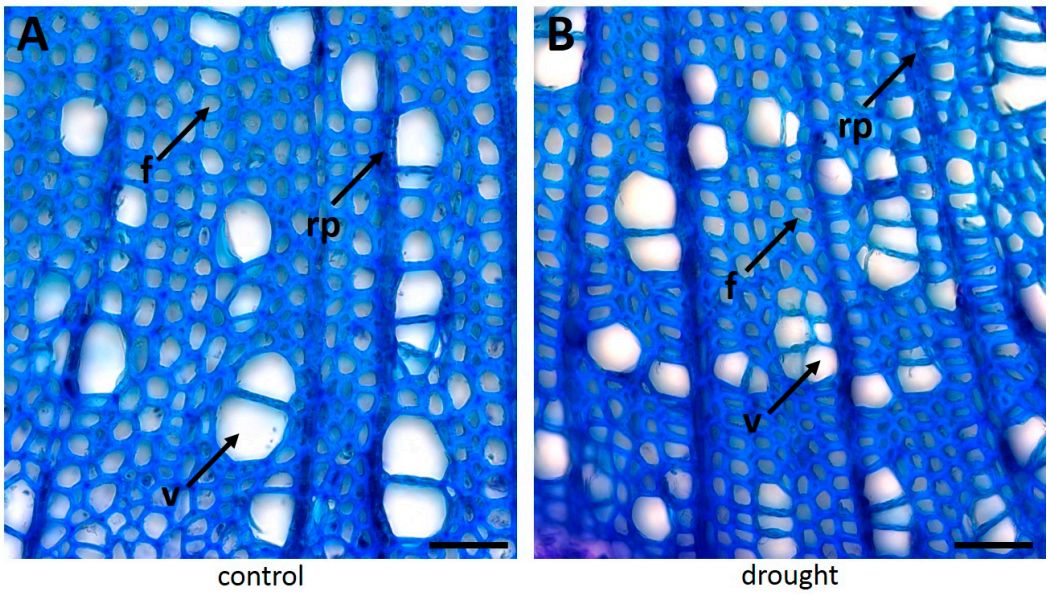

Figure 3. Comparison of non-stressed (**A**) and drought-stressed (**B**) xylem tissue of *Populus tremula* x *tremuloides*. B shows vessels with reduced size but increased vessel frequency. v: vessel cells, f: fiber cells, rp: ray parenchyma. Bar = 50 µm.

Taken together, these studies suggest that angiosperms have developed a common mechanism to acclimate their xylem to increasing drought. The xylem, as stated earlier, is formed by vessels and fibers, with the vessels forming the pipe system that facilitates water transport [26]. Under drought the plant has to fortify the vessels by thickening the secondary cell wall to prevent cavitation, which results in a reduced water transportation rate due to diminished vessel lumina [11]. To compensate the loss in vessel lumen, more vessels are formed to restore the water transportation rate that is necessary for tree growth (Figure 3). Improving the resistance against drought-induced xylem cavitation is a crucial acclimation mechanism of plants to dry environments [8,53]. It is notable that these alterations resemble those of seasonal acclimation of wood to shorter day lengths and cold, which lead to the formation of tree rings. Tree rings are a result of changes in the activity of xylem building processes [54]. Interestingly, Arend and Fromm observed in 2007 that trees responded to drought with smaller vessel lumina only during the main growth season but not in fall, when late wood with small lumina was formed [48]. Still, controlled experiments to disentangle temperature and day lengths effects on wood anatomy and the impact of drought under these conditions are lacking.

3.2. Drought Leads to Major Transcriptional Remodelling

The molecular mechanism behind the reduction of vessel volume and cell wall strengthening has not yet been unravelled. One important tool to tackle this question is the analysis of wood transcriptomes to identify key genes in this acclimation processes. Wildhagen and colleagues (2018) found an altered expression of genes involved in cell wall polysaccharide biosynthesis (e.g., xyloglucan endotransglucosylase/hydrolases, UDP-XYL synthase 6, expansin A) in three poplar genotypes. This finding suggests that denser wood under drought is achieved by regulation of cellulose and hemicellulose biosynthesis; notably the lignin content was unaltered [10]. These changes unexpectedly resulted in a higher saccharification potential of wood formed under drought compared to wood of well-irrigated poplars [10]. If these changes were also responsible for the reduction in vessel volume has not been clarified yet. Vessel expansion depends on the activity of potassium (K^+) uptake transporters (e.g., PtrKUP1) and outward rectifying K^+ channels (e.g., PTORK) [55]. In agreement with a high requirement for K^+ to regulate turgor pressure, the cambium contains the highest K^+ concentrations [56]. Furthermore, KUPs show seasonal regulation with high expression levels in wood [57]. Since drought limits K^+ acquisition from the soil [58], it is possible that changes in the osmotic control influence vessel size. However, this suggestion is currently speculative as an impact of drought on K^+ channel transcription levels has only been reported for an ortholog of the Arabidopsis *KUP2* which is positively correlated with fiber lumen under drought in *P. nigra* [10]. In addition, the concentration of soluble non-structural carbohydrates, sucrose in particular, has a major impact on vessel expansion. It has been shown that sucrose accumulation in the cambium correlates with cambial activity and cell expansion phase during xylogenesis in *Populus* x *canadensis* Mönch 'I-214' [59]. Interestingly, transgenic *Populus tremula* x *alba* suppressing the expression of the vacuolar sucrose efflux transporter PtaSUT4, are more susceptible to moderate drought compared to wild type plants. This suggests that sucrose also plays an important role in drought acclimation in addition to seasonal wood formation [60].

In addition to its influence on wood anatomy, drought has also major impact on the genes putatively involved cell wall biosynthesis. For instance, in *P. trichocarpa*, 119 genes related to cell wall organization, carbohydrate metabolism, and lipid metabolism were significantly differentially expressed. Among these genes four master switch regulators (*PtrWND1*, *PtrWND1B*, *PtrWND2A*, *PtrWND2B*) which are orthologs of the Arabidopsis ANAC043 (Table 1), as well as the secondary level regulators *PtrMYB2*, *PtrMYB21* and Potri.009G061500 (an ortholog of Arabidopsis *AtMYB83*, Table 1) were downregulated [61], indicating major changes in the transcription of cell wall biosynthesis genes. Additionally, 29 laccases-encoding genes were also found to be differentially regulated [61]. Except for one laccase all other laccases were downregulated under drought [61]. These findings corroborate major changes in cell wall composition, though biochemical analyses were not conducted.

It is clear that the supply with energy and precursors of cell wall components, especially carbohydrates, has to be sustained under drought since earlier studies showed strong correlations between growth and cambial carbohydrate concentrations [62]. However, transcriptome analyses also identified many genes involved in lipid metabolism [10,61]. There is now accumulating evidence for the role lipids in SCW formation: metabolomic and transcriptomic analyses revealed links between decreasing concentrations of sterols and fatty acids with increasing transcript levels of genes involved in β-oxidation and the glyoxylate cycle required for the production of carbohydrate precursors for SCW [63,64]. Transcriptome analysis of Eucalyptus xylem revealed a putative UDP-glucose:sterol glucosyltransferase, which produces sitosterol-cellodextrin. This molecule serves as a primer the elongation of β-1,4-glucan strands by the cellulose synthases [63,65]. These lipoglucan primers are discussed as being responsible for elongation by a special CesA isoform [66], forming a possible indirect link to channel carbon from lipids towards cellulose.

Other players, whose role in regulating drought acclimation has not yet been understood, are micro RNAs (miRNAs), which are widely known to function as regulatory molecules in gene expression under stress conditions [67,68]. A recent study identified five up- and seven down-regulated miRNAs in *P. trichocarpa* under drought stress, which in total regulate 72 targets according to degradome analysis. Among these were four UDP-glucosyl transferases, which transfer sugars to target molecules and have been shown to build up β-1,4-glucan from UDP-activated glucose [69,70].

It is important to mention that trees can recover from xylem embolism to a certain extent. To re-establish the water flow in the xylem it is necessary to refill the vessels [45]. Although this process is not yet fully understood, there is substantial evidence that paratracheal parenchyma cells also designated as vessel-associated cells (VACs) play an important role in this process [71]. As stated earlier, VACs are living cells surrounding the xylem vessels, which makes them ideal candidates to facilitate the refilling process [15]. To refill the vessels with water, a change in the osmotic gradient between VACs and vessel cells is indispensable. The most important osmotic compound in this regard is most likely sucrose [72]. Experimental evidence has been provided by mimicking xylem embolism in *P. trichocarpa* [72], which resulted in enhanced expression of α-amylases (*PtrAMY1*, *PtrAMY2*, *PtrAMY3*) leading to a reduction in starch content in the VACs [72]. Simultaneously, the expression of sucrose transporters (*PtrSUT2a* and *PtrSUT2b*) as well as aquaporins (*PtrPIP1.2*, *PtrPIP1.4*, *PtrPIP1.5*, *PtrPIP2.2*) was upregulated. These findings support that upon xylem embolism, starch is degraded to sucrose, which is transported from the VACs to the vessel cells leading to enhanced water uptake of the vessel cells via aquaporins. Upregulation of aquaporins of the PIP1 and PIP2 family in VACs has been observed in several tree species (*P. trichocarpa* [72–74], *Populus* x '*Okanese*' [75], *Juglas regia* [76]), substantiating the hypothesis of VAC-mediated refilling of vessels upon embolism. It is still under discussion as to whether the VACs store sufficient carbohydrates for this process or if they are replenished by carbohydrate export from the phloem during embolism recovery [77].

3.3. Phytohormones Mediate Xylem Changes under Drought

Although transcriptomic studies have strongly enhanced our knowledge on plant drought responses and growth regulation, they cannot elucidate the underlying signals. In this regard phytohormones such as cytokinins, auxin, abscisic acid (ABA) and many others play crucial roles. The major plant hormone mediating drought stress is ABA [78,79]. Although drought has drastic effects on xylem formation, it is still unclear if ABA plays a direct role in regulating wood formation process. Circumstantial evidence suggests a role of ABA in fall for late wood formation because the concentration of ABA in the cambium increases in fall when cells with small lumina and thick cell walls are produced [80]. Reduction of stem radial growth under drought stress is accompanied by a strong increase in cambial ABA concentrations [81]. Therefore, ABA might play a negative role in secondary xylem development similar to that found for its function regulating bud dormancy [82], but no direct evidence on cellular or molecular level has been reported so far. An alternative suggestion was that enhanced ABA concentrations in fall might influence stomatal aperture, thereby leading to a decline

in carbon fixation and consequently in a reduction in stem growth [83]. It is also possible that ABA interacts with the activity of auxin inhibited xylem initiation [84]. To understand the effect of ABA on wood formation in different environments it is necessary to get more insight on the molecular basis of wood formation and to identify the genes in ABA signaling which may affect the regulation of wood cell development. The first evidence for this idea was found in Arabidopsis, where ABA-dependent upregulation of the vessel specific master switch genes VND2, VND4 and VND6 was observed resulting in enhanced xylem differentiation [85].

Another important class of phytohormones involved in drought acclimation are cytokinins, trans-zeatin, kinetin and 6-benzylaminopurine in particular. Cytokinins are generally referred to as growth promoting phytohormones by initiating cell division (reviewed by [86]). Together with ethylene and auxin, cytokinins regulate cambial activity. In poplar, overexpression of the Arabidopsis *CYTOKININ OXIDASE 2*, an enzyme involved in cytokinin catabolism, resulted in non-detectable levels of the cytokinin *trans*-zeatin and its storage form zeatin-*O*-glucoside and lead to a reduced number of cambial cells, resulting in impaired wood formation [87]. Moreover, cytokinin activity was high in primary meristems supporting its function in early wood formation processes [88] and reduced in the cambial zone of drought stressed poplar, indicating a regulatory function of cytokinins under drought stress [89]. There is now increasing evidence that cytokinins interact with jasmonic acid. A reverse genetic approach showed that application of 10 µM methyl-jasmonic acid promotes xylem development in Arabidopsis roots [90]. In addition, exogenous application of jasmonic acid reduces the cytokinin responses in regard to xylem formation. A follow-up study provided evidence that drought stress also induces xylem development by increasing jasmonic acid responses and diminishing cytokinin responses [91]. These results imply that cytokinin activity is a negative regulator of xylem development under drought stress while jasmonic acid promotes xylogenesis. The molecular mechanism how cytokinins mediate xylem development is not yet fully understood, but it was shown that external application of 50 ng/mL kinetin downregulates the expression of the vessel specific master switch genes *VND6 and VND7*, which are important for proto- and meta-xylem differentiation [28]. Cytokinins are known to induce cell wall loosening expansion genes [92,93]. Application of 6-benzylaminopurine has a negative effect on cell wall thickness [94]. These findings corroborate the function of cytokinins as negative regulators of secondary xylem, yet there is still plenty of research necessary to identify the role of specific cytokinins in these processes.

Cytokinin levels in plants are affected by nitrogen supply and are implicated in stress responses mboxciteB95-forests-465031,B96-forests-465031. It is possible that they also mediate nitrogen effects in wood. Under nitrogen starvation, wood properties resemble those of wood produced under drought with smaller vessel lumina and thicker fiber cell walls [97]. Under these conditions, secondary cell wall related transcription factors, like members of *MYB*, *bHLH*, *WRKY* and *WD40* gene families, are up-regulated, leading to increased cell wall formation [97–99]. In contrast, under high nitrogen availability the secondary cell walls of fibers and vessels are thinner and the vessel lumina are expanded [97,99,100].

Notably, changes in nitrogen supply affected ABA and JA [101], which are known to mediate stress responses. Synergistic effects of drought stress and nitrogen availability, which both influence wood formation [101–105] have been noted [106–108]. For example, when water and nitrogen are sufficiently available, water supply increases nitrogen uptake, leading to larger vessel lumina and in turn to higher susceptibility to drought stress [109–111]. SCW-related genes are not suppressed by drought under high nitrogen supply, only under nitrogen limitation [112]. Consequently, water and nitrogen deprivation has a negative synergistic effect on secondary growth compared to either limitation alone [112,113]. These examples show that drought stress impacts on xylem development at different levels, clearly as an osmotic stress as demonstrated by PEG studies but also via its influence on tree nutrition. To generate or select more drought-resistant trees, it will be necessary to disentangle the complex hormonal network with emphasis on the mechanism of phytohormone accumulation, the

transition of active to inactive forms and the turnover to identify the key mechanisms how water limitation affects xylem anatomy and cell wall composition.

3.4. Salt Severely Affects Wood Formation

Survival and productivity of trees are affected by salt through different processes, mainly related to osmotic stress, nutritional imbalances or ion toxicity [114,115]. High salt concentration within the plant itself can be toxic, resulting in the inhibition of many physiological and biochemical processes. Saline environments restrict the ability of plant to take up water and, therefore, reduce plant growth [116]. Uptake of salt facilitates water uptake and has been identified as a tolerance mechanism in poplars, *P. euphratica* in particular, which are acclimated to salt deserts [117]. Hence, the xylem originating from salinity affected meristem also exhibits modifications in its anatomical and chemical aspects [118]. It has been shown that salt stressed poplars have a reduced number of cell layers in cambial zone compared to control plants, indicating growth reduction [119]. Similar to drought-stressed poplars, vessel lumina of salt-exposed trees are reduced [119,120] and the vessel frequency is increased [121]. The sensitivity of these anatomical alterations to salt exposure depends on the salt-sensitivity of the species. For example, the salt-sensitive poplar species *P.* x *canescens* reacts strongly to moderate NaCl levels, while the salt-tolerant *P. euphratica* shows diminished cambial activity and decreased vessel lumina only after long-term exposure to higher salinity levels, i.e., 150 mM NaCl [120,121]. Among other reasons, diminished nutrient supply, in particular lower calcium and potassium supply caused decreased xylem radial growth under salt stress [119].

Salinity impairs the tree water status and may increase tension in the water-conducting system, promoting cavitation and subsequently, embolisms [118]. However, trees can reinforce the wall strength of conducting cells so as to prevent against vessel collapse under osmotic stress [122]. This phenomenon is also found in poplar vessel cell walls, which show a significant increase in strength when exposed to salinity [120]. Furthermore, increased vessel frequencies can at least partly compensate negative effects on hydraulic efficiency [121]. Cell wall reinforcement under salt stress goes along with drastic alterations in major wood compounds such as cellulose, hemicelluloses, and lignin [121]. The transcriptional changes found under these conditions were opposite to those found during tension wood formation in poplar [121]. Therefore, the type of wood developed under salinity was termed “pressure wood” [121].

Since the anatomical changes in secondary xylem under salt and drought stress might be of similar nature, the question arises if there are similar hormones involved in these processes. The activity of the vascular cambium largely determines the rate of wood formation and is strictly controlled by the interplay of several phytohormones [123]. Early studies demonstrate that a concentration gradient of auxin across the cambial zone regulates cell division and expansion of the xylem elements [124–126]. Osmotic stress in plants is known to influence auxin transport [127]. Using an auxin responsive reporter gene construct (*GH3:GUS*), an impact of salt on auxin activity was shown in poplar wood [128]. Associated with wood alteration, free-auxin levels decrease in developing xylem under salt stress. In contrast to salt susceptible grey poplar (*P.* x *canescens*), the salt tolerant *P. euphratica* shows only little reduction in auxin and moreover, an increase in indole-3-acetic acid-amido conjugates, which can be used as a source of auxin [120]. Overexpression of auxin amidohydrolase (ILL3) from poplar in Arabidopsis rendered the plants more salt tolerant [120], indicating an important function of auxin in salt acclimation. The signaling pathways show how auxin and other plant hormones affect gene regulation remains elusive.

Recent transcriptome comparisons of salt treated and non-stressed eucalypt trees uncovered responses of a large number of transcription factors (TFs) including members of the MYB TF family [129] to salt. Upregulation genes encoding transcription factors (*EgrMYB20*, *EgrMYB47* and *EgrMYB36*) involved as master switches in secondary xylem development were observed in several eucalyptus genotypes tested, in particular under the higher (125 mM) salt treatment [130]. *BplMYB46*, a MYB gene from *Betula platyphylla* (birch), has also been reported to be involved in both abiotic stress tolerance and secondary wall biosynthesis [131]. *BplMYB46* is predominantly expressed in stems and its expression

is induced by NaCl [131]. In *BplMYB46* overexpressing lines a positive effect on lignin and cellulose content but a negative effect on hemicellulose content was found. Correspondingly, the expression of genes related to lignin and cellulose biosynthesis (phenylalanine ammonia lyase (PAL), caffeoyl-CoA O-methyltransferase (CCoAOMT), 4-coumarate-coa ligase (4CL), POD, Laccase (LAC), cinnamoyl-CoA reductase (CCR), cellulose synthase (CesA) was significantly upregulated, and that of genes related to hemicellulose biosynthesis (e.g., fragile fiber (FRA) and irregular xylem (IRX)) was significantly down-regulated in *BplMYB46* overexpressing lines. Hence, *BplMYB46* functions in linking stress responses and cell wall properties [131]. Further functional analyses by forward and reverse genetic approaches are required to understand the acclimatory responses of trees to salt stress in more detail. While a number of candidate genes has already been studied for their involvement in enhancing salt tolerance in leaves or roots [47], systematic genetic studies targeting at enhanced xylem salt resistance are lacking.

4. Conclusions

Trees respond to environmental stress factors like drought and salt with the adjustment of xylem formation primarily to maintain the hydraulic function of the wood. These responses are strongly affected by nutrients, especially by major cations (K^+, Ca^{2+}) and by nitrogen availability. Interestingly, the anatomical changes in response to different environmental cues are quite similar and characterized by a reduction in xylem vessel lumina and fortification of secondary cell walls in vessels and fibers. However, the molecular regulations that act between the initial stimulus and the output, i.e., modified wood, are just poorly understood. It has become apparent that phytohormones play a crucial role in mediating those stress stimuli by modulating xylogenesis related genes like the NAC and MYB master switch regulators, which lead to stress specific gene transcription. Yet, this review shows that our picture of the processes leading to wood acclimation is far from being complete. In order to put the puzzle together, it will be important to not only continue investigating specific stimulus responses, but also to integrate the information of all processes to get a complete picture of wood stress acclimation. Transcriptome analyses helped us a lot in getting a better picture of the processes during stress acclimation, yet they mostly focus on gene clusters build by GO term or KEGG analyses. It will be crucial to exploit this powerful resource more in-depth in the future to get to the key genes involved in those regulatory mechanisms. Identification and functional characterization of candidate genes improving stress tolerance and/or growth under nutrient-limiting conditions will promote the generation of new genotypes either by improving trees by genetic engineering or by smart breeding. Significant advances have been made over recent years to build up an effective toolbox for the characterization of candidate genes, especially in poplar. While overexpression of candidate genes has been well established for years, the generation of loss-of-function mutants has always been difficult. However, recent advances in the CRISPR/Cas9 technology have provided researchers with a powerful tool to investigate gene function and to generate genetically modified trees.

Author Contributions: C.E., S.S., L.K., D.Y., G.J.S., A.K. and A.P. wrote the draft manuscript, C.E. and A.P. reviewed, edited and finalized the manuscript.

Funding: We are grateful for financial support in the frame of WATBIO (Development of improved perennial biomass crops for water stressed environments), which is a collaborative research project funded from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No. 311929. S.S. acknowledges financial support by the DAAD, D.Y. by the C.S.C., and A.K. and G.J.S. by the graduate programme MaFO-Holz funded by the Lichtenberg programme of the country Lower Saxony.

Acknowledgments: We thank Merle Fastenrath for technical support with the anatomical studies used to prepare the figures in this review.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Boer, A.H.D.; Volkov, V. Logistics of water and salt transport through the plant: Structure and functioning of the xylem. *Plant Cell Environ.* **2003**, *26*, 87–101. [CrossRef]
2. Myburg, A.A.; Lev-Yadun, S.; Sederoff, R.R. Xylem Structure and Function. In *Encycolpedia of Life Science*; American Cancer Society: London, UK, 2013; ISBN 978-0-470-01590-2.
3. Tyree, M.T.; Sperry, J.S. Vulnerability of Xylem to Cavitation and Embolism. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.* **1989**, *40*, 19–36. [CrossRef]
4. Sperry, J.S.; Hacke, U.G.; Pittermann, J. Size and function in conifer tracheids and angiosperm vessels. *Am. J. Bot.* **2006**, *93*, 1490–1500. [CrossRef] [PubMed]
5. Alder, N.N.; Sperry, J.S.; Pockman, W.T. Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. *Oecologia* **1996**, *105*, 293–301. [CrossRef] [PubMed]
6. Choat, B.; Cobb, A.R.; Jansen, S. Structure and function of bordered pits: New discoveries and impacts on whole-plant hydraulic function. *New Phytol.* **2008**, *177*, 608–626. [CrossRef]
7. Zwieniecki, M.A.; Holbrook, N.M. Confronting Maxwell's demon: Biophysics of xylem embolism repair. *Trends Plant Sci.* **2009**, *14*, 530–534. [CrossRef] [PubMed]
8. Awad, H.; Barigah, T.; Badel, E.; Cochard, H.; Herbette, S. Poplar vulnerability to xylem cavitation acclimates to drier soil conditions. *Physiologia Plantarum* **2010**, *139*, 208–288. [CrossRef]
9. Rita, A.; Cherubini, P.; Leonardi, S.; Todaro, L.; Borghetti, M. Functional adjustments of xylem anatomy to climatic variability: Insights from long-term *Ilex aquifolium* tree-ring series. *Tree Physiol.* **2015**, *35*, 817–828. [CrossRef]
10. Wildhagen, H.; Paul, S.; Allwright, M.; Smith, H.K.; Malinowska, M.; Schnabel, S.K.; Paulo, M.J.; Cattonaro, F.; Vendramin, V.; Scalabrin, S.; et al. Genes and gene clusters related to genotype and drought-induced variation in saccharification potential, lignin content and wood anatomical traits in *Populus nigra*. *Tree Physiol* **2018**, *38*, 320–339. [CrossRef] [PubMed]
11. Bogeat-Triboulot, M.-B.; Brosché, M.; Renaut, J.; Jouve, L.; Thiec, D.L.; Fayyaz, P.; Vinocur, B.; Witters, E.; Laukens, K.; Teichmann, T.; et al. Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. *Plant Physiol.* **2007**, *143*, 876–892. [CrossRef] [PubMed]
12. Aichinger, E.; Kornet, N.; Friedrich, T.; Laux, T. Plant Stem Cell Niches. *Annu. Rev. Plant Biol.* **2012**, *63*, 615–636. [CrossRef] [PubMed]
13. Plomion, C.; Leprovost, G.; Stokes, A. Wood Formation in Trees. *Plant Physiol.* **2001**, *127*, 1513–1523. [CrossRef] [PubMed]
14. Furuta, K.M.; Hellmann, E.; Helariutta, Y. Molecular control of cell specification and cell differentiation during procambial development. *Annu. Rev. Plant Biol.* **2014**, *65*, 607–638. [CrossRef] [PubMed]
15. Słupianek, A.; Kasprowicz-Maluśki, A.; Myśkow, E.; Turzańska, M.; Sokołowska, K. Endocytosis acts as transport pathway in wood. *New Phytol.* **2018**. [CrossRef]
16. Růžička, K.; Ursache, R.; Hejátko, J.; Helariutta, Y. Xylem development – from the cradle to the grave. *New Phytol.* **2015**, *207*, 519–535. [CrossRef]
17. Déjardin, A.; Laurans, F.; Arnaud, D.; Breton, C.; Pilate, G.; Leplé, J.-C. Wood formation in Angiosperms. *Comptes Rendus Biologies* **2010**, *333*, 325–334. [CrossRef] [PubMed]
18. McFarlane, H.E.; Döring, A.; Persson, S. The cell biology of cellulose synthesis. *Annu. Rev. Plant Biol.* **2014**, *65*, 69–94. [CrossRef] [PubMed]
19. Pauly, M.; Gille, S.; Liu, L.; Mansoori, N.; de Souza, A.; Schultink, A.; Xiong, G. Hemicellulose biosynthesis. *Planta* **2013**, *238*, 627–642. [CrossRef]
20. Scheller, H.V.; Ulvskov, P. Hemicelluloses. *Annu. Rev. Plant Biol.* **2010**, *61*, 263–289. [CrossRef]
21. Voxeur, A.; Wang, Y.; Sibout, R. Lignification: Different mechanisms for a versatile polymer. *Curr. Opin. Plant Biol.* **2015**, *23*, 83–90. [CrossRef]
22. Liu, Q.; Luo, L.; Zheng, L. Lignins: Biosynthesis and Biological Functions in Plants. *Int. J. Mol. Sci.* **2018**, *19*, 335. [CrossRef] [PubMed]
23. Yamamoto, E.; Bokelman, G.H.; Lewis, N.G. Phenylpropanoid Metabolism in Cell Walls. In *Plant Cell Wall Polymers*; ACS Symposium Series; American Chemical Society: Washington, DC, USA, 1989; Volume 399, pp. 68–88. ISBN 978-0-8412-1658-7.

24. Zhao, Q. Lignification: Flexibility, biosynthesis and regulation. *Trends Plant Sci.* **2016**, *21*, 713–721. [CrossRef]
25. Barros, J.; Serk, H.; Granlund, I.; Pesquet, E. The cell biology of lignification in higher plants. *Ann. Bot.* **2015**, *115*, 1053–1074. [CrossRef] [PubMed]
26. Schuetz, M.; Smith, R.; Ellis, B. Xylem tissue specification, patterning, and differentiation mechanisms. *J. Exp. Bot.* **2013**, *64*, 11–31. [CrossRef]
27. Ye, Z.-H.; Zhong, R. Molecular control of wood formation in trees. *J. Exp. Bot.* **2015**, *66*, 4119–4131. [CrossRef]
28. Kubo, M.; Udagawa, M.; Nishikubo, N.; Horiguchi, G.; Yamaguchi, M.; Ito, J.; Mimura, T.; Fukuda, H.; Demura, T. Transcription switches for protoxylem and metaxylem vessel formation. *Genes Dev.* **2005**, *19*, 1855–1860. [CrossRef]
29. Yamaguchi, M.; Mitsuda, N.; Ohtani, M.; Ohme-Takagi, M.; Kato, K.; Demura, T. Vascular-related NAC-domain 7 directly regulates a broad range of genes for xylem vessel differentiation. *BMC Proc.* **2011**, *5*, O37. [CrossRef]
30. Zhong, R.; Lee, C.; Ye, Z.-H. Functional characterization of poplar wood-associated NAC domain transcription factors. *Plant Physiol.* **2010**, *152*, 1044–1055. [CrossRef] [PubMed]
31. Mariconti, L.; Pellegrini, B.; Cantoni, R.; Stevens, R.; Bergounioux, C.; Cella, R.; Albani, D. The E2F family of transcription factors from *Arabidopsis thaliana*. *J. Biol. Chem.* **2002**, *277*, 9911–9919. [CrossRef]
32. Du, J.; Miura, E.; Robischon, M.; Martinez, C.; Groover, A. The *Populus* class III HD ZIP transcription factor *POPCORONA* affects cell differentiation during secondary growth of woody stems. *PLoS ONE* **2011**, *6*, e17458. [CrossRef]
33. McCarthy, R.L.; Zhong, R.; Fowler, S.; Lyskowski, D.; Piyasena, H.; Carleton, K.; Spicer, C.; Ye, Z.-H. The poplar MYB transcription factors, PtrMYB3 and PtrMYB20, are involved in the regulation of secondary wall biosynthesis. *Plant Cell Physiol.* **2010**, *51*, 1084–1090. [CrossRef] [PubMed]
34. Zhong, R.; McCarthy, R.L.; Haghighat, M.; Ye, Z.-H. The poplar MYB master switches bind to the SMRE site and activate the secondary wall biosynthetic program during wood formation. *PLoS ONE* **2013**, *8*, e69219. [CrossRef] [PubMed]
35. Pear, J.R.; Kawagoe, Y.; Schreckengost, W.E.; Delmer, D.P.; Stalker, D.M. Higher plants contain homologs of the bacterial *celA* genes encoding the catalytic subunit of cellulose synthase. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12637–12642. [CrossRef] [PubMed]
36. Djerbi, S.; Lindskog, M.; Arvestad, L.; Sterky, F.; Teeri, T.T. The genome sequence of black cottonwood (*Populus trichocarpa*) reveals 18 conserved cellulose synthase (*CesA*) genes. *Planta* **2005**, *221*, 739–746. [CrossRef] [PubMed]
37. Suzuki, S.; Li, L.; Sun, Y.-H.; Chiang, V.L. The Cellulose Synthase Gene Superfamily and Biochemical Functions of Xylem-Specific Cellulose Synthase-Like Genes in *Populus trichocarpa*. *Plant Physiol.* **2006**, *142*, 1233–1245. [CrossRef] [PubMed]
38. Watanabe, Y.; Schneider, R.; Barkwill, S.; Gonzales-Vigil, E.; Hill, J.L., Jr.; Samuels, A.L.; Persson, S.; Mansfield, S.D. Cellulose synthase complexes display distinct dynamic behaviors during xylem transdifferentiation. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E6366–E6374. [CrossRef] [PubMed]
39. Polle, A.; Janz, D.; Teichmann, T.; Lipka, V. Poplar genetic engineering: Promoting desirable wood characteristics and pest resistance. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 5669–5679. [CrossRef]
40. Liu, C.-J.; Miao, Y.-C.; Zhang, K.-W. Sequestration and transport of lignin monomeric precursors. *Molecules* **2011**, *16*, 710–727. [CrossRef]
41. Alejandro, S.; Lee, Y.; Tohge, T.; Sudre, D.; Osorio, S.; Park, J.; Bovet, L.; Lee, Y.; Geldner, N.; Fernie, A.R.; Martinoia, E. AtABCG29 Is a Monolignol Transporter Involved in Lignin Biosynthesis. *Curr. Biol.* **2012**, *22*, 1207–1212. [CrossRef]
42. Miao, Y.-C.; Liu, C.-J. ATP-binding cassette-like transporters are involved in the transport of lignin precursors across plasma and vacuolar membranes. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 22728–22733. [CrossRef]
43. Sandhu, A.P.S.; Randhawa, G.S.; Dhugga, K.S. Plant cell wall matrix polysaccharide biosynthesis. *Mol. Plant* **2009**, *2*, 840–850. [CrossRef]
44. Savi, T.; Bertuzzi, S.; Branca, S.; Tretiach, M.; Nardini, A. Drought-induced xylem cavitation and hydraulic deterioration: Risk factors for urban trees under climate change? *New Phytol.* **2015**, *205*, 1106–1116. [CrossRef]
45. Nardini, A.; Savi, T.; Trifilò, P.; Gullo, M.A.L. Drought stress and the recovery from xylem embolism in woody plants. In *Progress in Botany*; Progress in Botany; Springer: Cham, Switzerland, 2017; Volume 79, pp. 197–231. ISBN 978-3-319-71412-7.
46. Fischer, U.; Polle, A. *Populus* responses to abiotic stress. In *Genetics and Genomics of Populus*; Plant Genetics and Genomics: Crops and Models; Springer: New York, NY, USA, 2010; pp. 225–246. ISBN 978-1-4419-1540-5.

47. Polle, A.; Chen, S.L.; Eckert, C.; Harfouche, A. Engineering drought resistance in forest trees. *Front. Plant Sci.* **2019**, *9*. [CrossRef]
48. Arend, M.; Fromm, J. Seasonal change in the drought response of wood cell development in poplar. *Tree Physiol.* **2007**, *27*, 985. [CrossRef] [PubMed]
49. Beniwal, R.S.; Langenfeld-Heyser, R.; Polle, A. Ectomycorrhiza and hydrogel protect hybrid poplar from water deficit and unravel plastic responses of xylem anatomy. *Environ. Exp. Bot.* **2010**, *69*, 189–197. [CrossRef]
50. Schreiber, S.G.; Hacke, U.G.; Chamberland, S.; Lowe, C.W.; Kamelchuk, D.; Bräutigam, K.; Campbell, M.M.; Thomas, B.R. Leaf size serves as a proxy for xylem vulnerability to cavitation in plantation trees. *Plant Cell Environ.* **2016**, *39*, 272–281. [CrossRef]
51. Fonti, P.; Heller, O.; Cherubini, P.; Rigling, A.; Arend, M. Wood anatomical responses of oak saplings exposed to air warming and soil drought. *Plant Biol.* **2013**, *15*, 210–219. [CrossRef]
52. Colangelo, M.; Camarero, J.J.; Borghetti, M.; Gazol, A.; Gentilesca, T.; Ripullone, F. Size matters a lot: Drought-affected italian oaks are smaller and show lower growth prior to tree death. *Front. Plant Sci.* **2017**, *8*. [CrossRef]
53. Fichot, R.; Barigah, T.S.; Chamaillard, S.; Le Thiec, D.; Laurans, F.; Cochard, H.; Brignolas, F. Common trade-offs between xylem resistance to cavitation and other physiological traits do not hold among unrelated *Populus deltoides* x *Populus nigra* hybrids: Xylem resistance to cavitation and water relations in poplar. *Plant Cell Environ.* **2010**, *33*, 1553–1568. [CrossRef] [PubMed]
54. Rathgeber, C.B.K.; Cuny, H.E.; Fonti, P. Biological Basis of Tree-Ring Formation: A Crash Course. *Front. Plant Sci.* **2016**, *7*. [CrossRef] [PubMed]
55. Langer, K.; Ache, P.; Geiger, D.; Stinzing, A.; Arend, M.; Wind, C.; Regan, S.; Fromm, J.; Hedrich, R. Poplar potassium transporters capable of controlling K^+ homeostasis and K^+-dependent xylogenesis. *Plant J.* **2002**, *32*, 997–1009. [CrossRef]
56. Wind, C.; Arend, M.; Fromm, J. Potassium-Dependent Cambial Growth in Poplar. *Plant Biol.* **2004**, *7*, 30–37. [CrossRef]
57. Larisch, C.; Dittrich, M.; Wildhagen, H.; Lautner, S.; Fromm, J.; Polle, A.; Hedrich, R.; Rennenberg, H.; Müller, T.; Ache, P. Poplar wood rays are involved in seasonal remodeling of tree physiology. *Plant Physiol.* **2012**, *160*, 1515–1529. [CrossRef] [PubMed]
58. Danielsen, L.; Polle, A. Poplar nutrition under drought as affected by ectomycorrhizal colonization. *Environ. Exp. Bot.* **2014**, *108*, 89–98. [CrossRef]
59. Giovannelli, A.; Emiliani, G.; Traversi, M.L.; Deslauriers, A.; Rossi, S. Sampling cambial region and mature xylem for non structural carbohydrates and starch analyses. *Dendrochronologia* **2011**, *29*, 177–182. [CrossRef]
60. Xue, L.-J.; Frost, C.J.; Tsai, C.-J.; Harding, S.A. Drought response transcriptomes are altered in poplar with reduced tonoplast sucrose transporter expression. *Sci. Rep.* **2016**, *6*. [CrossRef] [PubMed]
61. Tang, S.; Dong, Y.; Liang, D.; Zhang, Z.; Ye, C.-Y.; Shuai, P.; Han, X.; Zhao, Y.; Yin, W.; Xia, X. Analysis of the drought stress-responsive transcriptome of black cottonwood (*Populus trichocarpa*) using deep RNA sequencing. *Plant Mol. Biol. Rep.* **2015**, *33*, 424–438. [CrossRef]
62. Deslauriers, A.; Giovannelli, A.; Rossi, S.; Castro, G.; Fragnelli, G.; Traversi, L. Intra-annual cambial activity and carbon availability in stem of poplar. *Tree Physiol.* **2009**, *29*, 1223–1235. [CrossRef]
63. Paux, E.; Tamasloukht, M.; Ladouce, N.; Sivadon, P.; Grima-Pettenati, J. Identification of genes preferentially expressed during wood formation in *Eucalyptus*. *Plant Mol. Biol.* **2004**, *55*, 263–280. [CrossRef]
64. Druart, N.; Johansson, A.; Baba, K.; Schrader, J.; Sjödin, A.; Bhalerao, R.R.; Resman, L.; Trygg, J.; Moritz, T.; Bhalerao, R.P. Environmental and hormonal regulation of the activity-dormancy cycle in the cambial meristem involves stage-specific modulation of transcriptional and metabolic networks. *Plant J.* **2007**, *50*, 557–573. [CrossRef]
65. Doblin, M.S.; Kurek, I.; Jacob-Wilk, D.; Delmer, D.P. Cellulose biosynthesis in plants: From genes to rosettes. *Plant Cell Physiol.* **2002**, *43*, 1407–1420. [CrossRef]
66. Peng, L.; Kawagoe, Y.; Hogan, P.; Delmer, D. Sitosterol-β-glucoside as primer for cellulose synthesis in plants. *Science* **2002**, *295*, 147–150. [CrossRef]
67. Leung, A.K.L.; Sharp, P.A. MicroRNA functions in stress responses. *Mol. Cell* **2010**, *40*, 205–215. [CrossRef]
68. Sunkar, R.; Li, Y.-F.; Jagadeeswaran, G. Functions of microRNAs in plant stress responses. *Trends Plant Sci.* **2012**, *17*, 196–203. [CrossRef] [PubMed]
69. Shuai, P.; Liang, D.; Zhang, Z.; Yin, W.; Xia, X. Identification of drought-responsive and novel *Populus trichocarpa* microRNAs by high-throughput sequencing and their targets using degradome analysis. *BMC Genomics* **2013**, *14*, 233. [CrossRef] [PubMed]

70. Brown, C.; Leijon, F.; Bulone, V. Radiometric and spectrophotometric in vitro assays of glycosyltransferases involved in plant cell wall carbohydrate biosynthesis. *Nat. Protoc.* **2012**, *7*, 1634–1650. [CrossRef]
71. Secchi, F.; Pagliarani, C.; Zwieniecki, M.A. The functional role of xylem parenchyma cells and aquaporins during recovery from severe water stress. *Plant Cell Environ.* **2017**, *40*, 858–871. [CrossRef] [PubMed]
72. Secchi, F.; Zwieniecki, M.A. Sensing embolism in xylem vessels: The role of sucrose as a trigger for refilling. *Plant Cell Environ.* **2011**, *34*, 514–524. [CrossRef] [PubMed]
73. Secchi, F.; Maciver, B.; Zeidel, M.L.; Zwieniecki, M.A. Functional analysis of putative genes encoding the PIP2 water channel subfamily in *Populus trichocarpa*. *Tree Physiol.* **2009**, *29*, 1467–1477. [CrossRef]
74. Secchi, F.; Zwieniecki, M.A. Patterns of PIP gene expression in *Populus trichocarpa* during recovery from xylem embolism suggest a major role for the PIP1 aquaporin subfamily as moderators of refilling process. *Plant Cell Environ.* **2010**, *33*, 1285–1297. [CrossRef] [PubMed]
75. Almeida-Rodriguez, A.M.; Hacke, U.G. Cellular localization of aquaporin mRNA in hybrid poplar stems. *Am. J. Bot.* **2012**, *99*, 1249–1254. [CrossRef] [PubMed]
76. Sakr, S.; Alves, G.; Morillon, R.; Maurel, K.; Decourteix, M.; Guilliot, A.; Fleurat-Lessard, P.; Julien, J.-L.; Chrispeels, M.J. Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiol.* **2003**, *133*, 630–641. [CrossRef] [PubMed]
77. Nardini, A.; Lo Gullo, M.A.; Salleo, S. Refilling embolized xylem conduits: Is it a matter of phloem unloading? *Plant Sci.* **2011**, *180*, 604–611. [CrossRef] [PubMed]
78. Fujita, Y.; Fujita, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. ABA-mediated transcriptional regulation in response to osmotic stress in plants. *J. Plant Res.* **2011**, *124*, 509–525. [CrossRef] [PubMed]
79. Yoshida, T.; Fernie, A.R. Remote Control of Transpiration via ABA. *Trends Plant Sci.* **2018**, *23*, 755–758. [CrossRef] [PubMed]
80. Lachaud, S. Participation of auxin and abscisic acid in the regulation of seasonal variations in cambial activity and xylogenesis. *Trees-Struct. Funct.* **1989**, *3*, 125–137. [CrossRef]
81. Luisi, A.; Giovannelli, A.; Traversi, M.L.; Anichini, M.; Sorce, C. Hormonal responses to water deficit in cambial tissues of *Populus alba* L. *J. Plant Grow. Regul.* **2014**, *33*, 489–498. [CrossRef]
82. Tylewicz, S.; Petterle, A.; Marttila, S.; Miskolczi, P.; Azeez, A.; Singh, R.K.; Immanen, J.; Mähler, N.; Hvidsten, T.R.; Eklund, D.M.; et al. Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. *Science* **2018**, *360*, 212–215. [CrossRef]
83. Sorce, C.; Giovannelli, A.; Sebastiani, L.; Anfodillo, T. Hormonal signals involved in the regulation of cambial activity, xylogenesis and vessel patterning in trees. *Plant Cell Rep.* **2013**, *32*, 885–898. [CrossRef]
84. Popko, J.; Haensch, R.; Mendel, R.R.; Polle, A.; Teichmann, T. The role of abscisic acid and auxin in the response of poplar to abiotic stress. *Plant Biol.* **2010**, *12*, 242–258. [CrossRef]
85. Jensen, M.K.; Kjaersgaard, T.; Nielsen, M.M.; Galberg, P.; Petersen, K.; O'Shea, C.; Skriver, K. The *Arabidopsis thaliana* NAC transcription factor family: Structure–function relationships and determinants of ANAC019 stress signalling. *Biochem. J.* **2010**, *426*, 183–196. [CrossRef]
86. Keshishian, E.A.; Rashotte, A.M. Plant cytokinin signalling. *Essays Biochem.* **2015**, *58*, 13–27. [CrossRef]
87. Nieminen, K.; Immanen, J.; Laxell, M.; Kauppinen, L.; Tarkowski, P.; Dolezal, K.; Tähtiharju, S.; Elo, A.; Decourteix, M.; Ljung, K.; et al. Cytokinin signaling regulates cambial development in poplar. *PNAS* **2008**, *105*, 20032–20037. [CrossRef]
88. Paul, S.; Wildhagen, H.; Janz, D.; Teichmann, T.; Hänsch, R.; Polle, A. Tissue- and cell-specific cytokinin activity in *Populus* x *canescens* monitored by *ARR5::GUS* reporter lines in summer and winter. *Front. Plant Sci.* **2016**, *7*. [CrossRef]
89. Paul, S.; Wildhagen, H.; Janz, D.; Polle, A. Drought effects on the tissue- and cell-specific cytokinin activity in poplar. *AoB Plants* **2018**, *10*. [CrossRef]
90. Jang, G.; Chang, S.H.; Um, T.Y.; Lee, S.; Kim, J.-K.; Choi, Y.D. Antagonistic interaction between jasmonic acid and cytokinin in xylem development. *Sci. Rep.* **2017**, *7*. [CrossRef]
91. Jang, G.; Choi, Y.D. Drought stress promotes xylem differentiation by modulating the interaction between cytokinin and jasmonic acid. *Plant Signal. Behav.* **2018**, *13*. [CrossRef]
92. Brenner, W.G.; Ramireddy, E.; Heyl, A.; Schmülling, T. Gene regulation by cytokinin in Arabidopsis. *Front Plant Sci.* **2012**, *3*. [CrossRef]
93. Nafisi, M.; Fimognari, L.; Sakuragi, Y. Interplays between the cell wall and phytohormones in interaction between plants and necrotrophic pathogens. *Phytochemistry* **2015**, *112*, 63–71. [CrossRef]

94. Jung, K.W.; Oh, S.-I.; Kim, Y.Y.; Yoo, K.S.; Cui, M.H.; Shin, J.S. *Arabidopsis* histidine-containing phosphotransfer factor 4 (AHP4) negatively regulates secondary wall thickening of the anther endothecium during flowering. *Mol. Cells* **2008**, *25*, 294–300.
95. Wilkinson, S.; Kudoyarova, G.R.; Veselov, D.S.; Arkhipova, T.N.; Davies, W.J. Plant hormone interactions: Innovative targets for crop breeding and management. *J. Exp. Bot.* **2012**, *63*, 3499–3509. [CrossRef] [PubMed]
96. Reguera, M.; Peleg, Z.; Abdel-Tawab, Y.M.; Tumimbang, E.B.; Delatorre, C.A.; Blumwald, E. Stress-induced cytokinin synthesis increases drought tolerance through the coordinated regulation of carbon and nitrogen assimilation in rice. *Plant Physiol.* **2013**, *163*, 1609–1622. [CrossRef] [PubMed]
97. Euring, D.; Bai, H.; Janz, D.; Polle, A. Nitrogen-driven stem elongation in poplar is linked with wood modification and gene clusters for stress, photosynthesis and cell wall formation. *BMC Plant Biol.* **2014**, *14*, 391. [CrossRef] [PubMed]
98. Van den Broeck, H.C.; Maliepaard, C.; Ebskamp, M.J.M.; Toonen, M.A.J.; Koops, A.J. Differential expression of genes involved in C1 metabolism and lignin biosynthesis in wooden core and bast tissues of fibre hemp (*Cannabis sativa* L.). *Plant Sci.* **2008**, *174*, 205–220. [CrossRef]
99. Camargo, E.L.O.; Nascimento, L.C.; Soler, M.; Salazar, M.M.; Lepikson Neto, J.; Marques, W.L.; Alves, A.; Teixeira, P.J.P.L.; Mieczkowski, P.; Carazzolle, M.F.; et al. Contrasting nitrogen fertilization treatments impact xylem gene expression and secondary cell wall lignification in Eucalyptus. *BMC Plant Biol.* **2014**, *14*. [CrossRef] [PubMed]
100. Plavcová, L.; Hacke, U.G.; Almeida-Rodriguez, A.M.; Li, E.; Douglas, C.J. Gene expression patterns underlying changes in xylem structure and function in response to increased nitrogen availability in hybrid poplar. *Plant Cell Environ.* **2013**, *36*, 186–199. [CrossRef] [PubMed]
101. Luo, J.; Zhou, J.; Li, H.; Shi, W.; Polle, A.; Lu, M.; Sun, X.; Luo, Z.-B. Global poplar root and leaf transcriptomes reveal links between growth and stress responses under nitrogen starvation and excess. *Tree Physiol.* **2015**, *35*, 1283–1302. [CrossRef] [PubMed]
102. Benson, M.L.; Myers, B.J.; Raison, R.J. Dynamics of stem growth of *Pinus radiata* as affected by water and nitrogen supply. *For. Ecol. Manag.* **1992**, *52*, 117–137. [CrossRef]
103. Dobbertin, M. Tree growth as indicator of tree vitality and of tree reaction to environmental stress: A review. *Eur. J. For. Res.* **2006**, *125*, 89. [CrossRef]
104. Novaes, E.; Osorio, L.; Drost, D.R.; Miles, B.L.; Boaventura-Novaes, C.R.D.; Benedict, C.; Dervinis, C.; Yu, Q.; Sykes, R.; Davis, M.; et al. Quantitative genetic analysis of biomass and wood chemistry of *Populus* under different nitrogen levels. *New Phytol.* **2009**, *182*, 878–890. [CrossRef] [PubMed]
105. Galle, A.; Esper, J.; Feller, U.; Ribas-Carbo, M.; Fonti, P. Responses of wood anatomy and carbon isotope composition of *Quercus pubescens* saplings subjected to two consecutive years of summer drought. *Ann. For. Sci.* **2010**, *67*, 809. [CrossRef]
106. Liu, Z.; Dickmann, D.I. Abscisic acid accumulation in leaves of two contrasting hybrid poplar clones affected by nitrogen fertilization plus cyclic flooding and soil drying. *Tree Physiol.* **1992**, *11*, 109–122. [CrossRef]
107. Liu, Z.; Dickmann, D.I. Responses of two hybrid *Populus* clones to flooding, drought, and nitrogen availability. I. Morphology and growth. *Can. J. Bot.* **1992**, *70*, 2265–2270. [CrossRef]
108. Ibrahim, L.; Proe, M.F.; Cameron, A.D. Interactive effects of nitrogen and water availabilities on gas exchange and whole-plant carbon allocation in poplar. *Tree Physiol.* **1998**, *18*, 481–487. [CrossRef] [PubMed]
109. Mazzoleni, S.; Dickmann, D.I. Differential physiological and morphological responses of two hybrid *Populus* clones to water stress. *Tree Physiol.* **1988**, *4*, 61–70. [CrossRef]
110. Harvey, H.P.; Van Den Driessche, R. Nutrition, xylem cavitation and drought resistance in hybrid poplar. *Tree Physiol.* **1997**, *17*, 647–654. [CrossRef]
111. Zimmermann, M.H. Hydraulic architecture of some diffuse-porous trees. *Can. J. Bot.* **2011**, *56*, 2286–2295. [CrossRef]
112. Euring, D. Nitrogen responsive wood formation in poplar (*Populus* sp.). In *Chapter 3: N-Responsive Network*; Georg-August-Universität Göttingen: Göttingen, Germany, 2014.
113. Tarighaleslami, M.; Zarghami, R.; Mashhadi, M.; Boojar, A.; Oveysi, M. Effects of drought stress and different nitrogen levels on morphological traits of proline in leaf and protein of corn seed (*Zea mays* L.). *Am.-Euras. J. Agric. Environ. Sci.* **2012**, *12*, 49–56.
114. Chen, S.; Polle, A. Salinity tolerance of *Populus*. *Plant Biol.* **2010**, *12*, 317–333. [CrossRef] [PubMed]

115. Polle, A.; Chen, S. On the salty side of life: Molecular, physiological and anatomical adaptation and acclimation of trees to extreme habitats. *Plant Cell Environ.* **2015**, *38*, 1794–1816. [CrossRef] [PubMed]
116. Munns, R.; Tester, M. Mechanisms of Salinity Tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [CrossRef] [PubMed]
117. Ottow, E.A.; Brinker, M.; Teichmann, T.; Fritz, E.; Kaiser, W.; Brosché, M.; Kangasjärvi, J.; Jiang, X.; Polle, A. *Populus euphratica* displays apoplastic sodium accumulation, osmotic adjustment by decreases in calcium and soluble carbohydrates, and develops leaf succulence under salt stress. *Plant Physiol.* **2005**, *139*, 1762–1772. [CrossRef]
118. Lautner, S. Wood formation under drought stress and salinity. In *Cellular Aspects of Wood Formation*; Fromm, J., Ed.; Springer: Berlin, Germany, 2013; Volume 20, pp. 187–202. ISBN 978-3-642-36490-7.
119. Escalante-Pérez, M.; Lautner, S.; Nehls, U.; Selle, A.; Teuber, M.; Schnitzler, J.-P.; Teichmann, T.; Fayyaz, P.; Hartung, W.; Polle, A.; et al. Salt stress affects xylem differentiation of grey poplar (*Populus* x *canescens*). *Planta* **2009**, *229*, 299–309. [CrossRef]
120. Junghans, U.; Polle, A.; Düchting, P.; Weiler, E.; Kuhlman, B.; Gruber, F.; Teichmann, T. Adaptation to high salinity in poplar involves changes in xylem anatomy and auxin physiology. *Plant Cell Environ.* **2006**, *29*, 1519–1531. [CrossRef] [PubMed]
121. Janz, D.; Lautner, S.; Wildhagen, H.; Behnke, K.; Schnitzler, J.-P.; Rennenberg, H.; Fromm, J.; Polle, A. Salt stress induces the formation of a novel type of 'pressure wood' in two *Populus* species. *New Phytol.* **2012**, *194*, 129–141. [CrossRef]
122. Hacke, U.G.; Sperry, J.S. Functional and ecological xylem anatomy. *Perspect. Plant Ecol. Evol. Syst.* **2001**, *4*, 97–115. [CrossRef]
123. Bhalerao, R.P.; Fischer, U. Environmental and hormonal control of cambial stem cell dynamics. *J. Exp. Bot.* **2017**, *68*, 79–87. [CrossRef]
124. Uggla, C.; Moritz, T.; Sandberg, G.; Sundberg, B. Auxin as a positional signal in pattern formation in plants. *PNAS* **1996**, *93*, 9282–9286. [CrossRef]
125. Uggla, C.; Mellerowicz, E.J.; Sundberg, B. Indole-3-Acetic Acid Controls Cambial Growth in Scots Pine by Positional Signaling. *Plant Physiol.* **1998**, *117*, 113–121. [CrossRef]
126. Nilsson, J.; Karlberg, A.; Antti, H.; Lopez-Vernaza, M.; Mellerowicz, E.; Perrot-Rechenmann, C.; Sandberg, G.; Bhalerao, R.P. Dissecting the Molecular Basis of the Regulation of Wood Formation by Auxin in Hybrid Aspen. *Plant Cell* **2008**, *20*, 843–855. [CrossRef]
127. Sheldrake, A.R. Effects of Osmotic Stress on Polar Auxin Transport in Avena Mesocotyl Sections. *Planta* **1979**, *145*, 113–117. [CrossRef] [PubMed]
128. Teichmann, T.; Bolu-Arianto, W.H.; Olbrich, A.; Langenfeld-Heyser, R.; Gobel, C.; Grzeganek, P.; Feussner, I.; Hansch, R.; Polle, A. GH3::GUS reflects cell-specific developmental patterns and stress-induced changes in wood anatomy in the poplar stem. *Tree Physiol.* **2008**, *28*, 1305–1315. [CrossRef]
129. Oh, J.E.; Kwon, Y.; Kim, J.H.; Noh, H.; Hong, S.-W.; Lee, H. A dual role for MYB60 in stomatal regulation and root growth of *Arabidopsis thaliana* under drought stress. *Plant Mol. Biol.* **2011**, *77*, 91–103. [CrossRef]
130. Sixto, H.; González-González, B.D.; Molina-Rueda, J.J.; Garrido-Aranda, A.; Sanchez, M.M.; López, G.; Gallardo, F.; Cañellas, I.; Mounet, F.; Grima-Pettenati, J.; et al. *Eucalyptus* spp. and *Populus* spp. coping with salinity stress: An approach on growth, physiological and molecular features in the context of short rotation coppice (SRC). *Trees* **2016**, *30*, 1873–1891. [CrossRef]
131. Guo, H.; Wang, Y.; Wang, L.; Hu, P.; Wang, Y.; Jia, Y.; Zhang, C.; Zhang, Y.; Zhang, Y.; Wang, C.; Yang, C. Expression of the MYB transcription factor gene *BplMYB46* affects abiotic stress tolerance and secondary cell wall deposition in *Betula platyphylla*. *Plant Biotechnol. J.* **2017**, *15*, 107–121. [CrossRef] [PubMed]