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Review

Tension Wood as a Model System to Explore the Carbon Partitioning between Lignin and Cellulose Biosynthesis in Woody Plants

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Tension wood, a specialized tissue developed in the upper side of the leaning stem and drooping branches of angiosperm, is an attractive experimental system attractive for exploring the development and the biochemical pathways of the secondary cell wall formation, as well as the control mechanism of the carbon flux into lignin, cellulose, and hemicellulose. However, the mechanism underlying the induction and the development of the tension wood is largely unknown. Recently, several researchers suggested the possible roles of the plant growth hormones including auxin, gibberellin, and ethylene mainly based on the expression pattern of the genes in this specialized tissue. In addition, expressed sequence tag of *Poplar* and *Eucalyptus* provide global view of the genetic control underlying the tension wood formation. However, the roles of the majority of the identified genes have not yet been clearly elucidated. The present review summarized current knowledge on the biosynthesis of tension wood to provide a brief synopsis of the molecular mechanism underlying the development of the tension wood.

Key words: *tension wood, lignin, cellulose, secondary xylem*

Anatomical Characteristics of Tension Wood

Displacement of stems and branches by wind or mechanical stress in the woody species results in the formation of a reaction wood. In general, tissues differ greatly depending upon whether the woody plant is a gymnosperm or an angiosperm, with the former giving rise to a compression wood and the latter to a tension wood [Timell, 1986]. Tension wood developed upper side of the leaning stems and drooping branch where tensile stress developed [Scafield, 1973; Hellgren *et al*., 2004]. The formation of the reaction wood in response to the altered gravitational stimuli is believed to be the mechanism developed by the tilted stem and the branches to recover their original upright positions [Hellgren *et al*., 2004; Timell, 1986]. This mechanism is usually, but not always, associated with the eccentric growth towards the upper side of the leaning stems and branches (Fig. 1a) [Timell, 1986]. Anatomically, the tension wood is characterized by a reduction in size and frequency of the vessels and by an increase in the proportion of the fibers

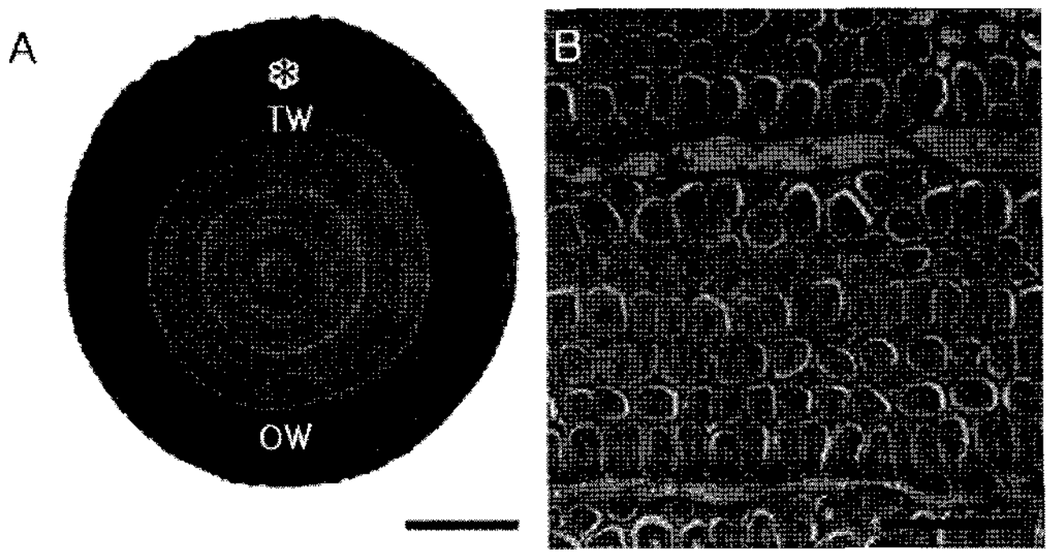

Fig. 1. Cross sections of *Fraxinus mandshurica* var. *japonica* stem (A) and *Quercus mongolica* var. *grosseserrata* stem (B) after exogenous application of gibberellin. (A) Growth promotion with a wide growth ring is clearly visible at the point of gibberellin application (*). Scale bar = 1 cm. (B) Transverse section of *Quercus mongolica* var. *grosseserrata* tension wood shows fibers with thickened inner layers of the cell wall. Scale bar = 100 μm. TW, tension wood; OW, opposite wood. Pictures were obtained from Ryo Funada (Funada *et al*., 2008).

(Fig. 1b) with the latter differing in structure (Fig. 2) and composition from those of the normal wood [Wardrop and Dadswell, 1955]. The fiber in the tension wood is characterized by thick cell walls and a gelatinous (G) layer, which is not found in the normal wood fibers.

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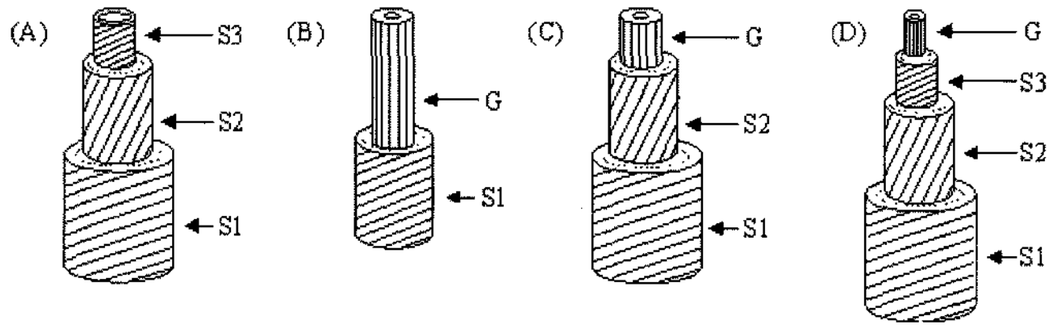

Fig. 2. Schematic models for the cell wall structures of fibers in normal wood (A) and tension wood (B, C, and D), redrawn from those of Wardrop and Dadswell [1955]. Solid lines indicate cellulose microfibril orientation. (A) Normal fiber does not develop G layer. (B) G-layer develops where S2 and S3 layers developed normally. (C) S3 layer could be replaced with G-layer. (D) G-layer could be form as the innermost layer next to the S3 layer (D). S1, outermost layer of secondary wall; S2, middle layer of secondary cell wall; S3, innermost layer adjacent to the cell lumen; G, gelatinous layer.

Although the G layer has variable thickness, it is often quite massive, normally replacing the innermost secondary cell wall layer, the S3 layer (Fig. 2). However, it can also replace both the S2 (middle layer of secondary cell wall) and the S3 layers or be added to the inside of the S3 layer (Fig. 2). The occurrence of the gelatinous (G) fibers is the most typical feature of the tension wood, even though they do not form in certain species and are quite rarely observed in others [Côté *et al*., 1969]. The G-layer was believed to be composed entirely or almost entirely of cellulose [Norberg and Meier, 1966; Cronshow and Morey, 1965; Scurfield and Wardrop, 1963], whose microfibrils are oriented parallel or nearly parallel to the longitudinal axis and have a high degree of crystallinity [Scurfield, 1973; Côté *et al*. 1969; Mia, 1968]. However, the nature of stimulus and mechanism controlling the altered cellulose microfibril orientation in the tension wood has not yet been fully studied.

Role of Plant Hormones in Tension Wood Formation

Tension wood formation was induced not only by the gravitational stimuli but also by mechanical stresses such as those experienced during the clinostat and the centrifuge treatments, or by bending [Wordrop, 1964; Westing, 1965; Westing, 1968]. In addition, many plant growth hormones were suggested to be involved in the formation of the tension wood. For example, auxin redistribution has been regarded to play an essential role in the tension wood formation, because more auxins were detected on the underside of the leaning stems in the dicots [Wardrop 1964; Westing 1965; Westing, 1968]. Moreover, the treatment of auxin transport inhibitor apparently induced the tension wood in the vertical dicot stems by reducing the auxin concentration [Cronshow and Morey, 1965; Morey and Cronshaw, 1968; Kennedy and Farrar, 1965]. However, a recent study by Hellgren *et al*. (2004) showed that the formation of the tension wood resulted in no obvious alteration of the IAA balance around the stem upon gravitational induction [Hellgren *et al*., 2004], suggesting that the redistribution of auxin may not be an essential regulator for the development of the tension wood. However, several Aux/IAA gene families were differentially expressed between the tension and the opposite wood [Moyle *et al*., 2002; Hellgren *et al*., 2004], implying that the auxin affects the signaling pathway of the tension wood formation [Hellgren *et al*., 2004].

Other plant growth hormones such as gibberellin, ethylene, and cytokinins were not regarded to play any roles in the tension wood formation for the long time, because their exogenous applications did not affect the formation of the reaction wood [Pharis *et al*., 1972; Wareing *et al*., 1964; Hejnowicz and Tomaszewski, 1969; Wilson and Archer, 1977]. However, a recent work by Funada *et al*. [2008] clearly demonstrated that the exogenous application of gibberellin to the vertical stems of four angiosperm species induced the tension wood formation where gibberellin was applied (Fig. 4). Ethylene was also proposed to be involved in the formation of the tension wood, because the gene coding the ACC oxidase of poplar displayed an asymmetric expression between the tension and the opposite woods [Andersson-Gunnerås *et al*., 2003]. In addition, its expression was mediated by the gravitational stimuli, which is known to be an important signal for initiation of the tension wood development [Andersson-Gunnerås *et al*., 2003]. Transcript analysis provided the evidence that ethylene is an important regulator of the tension wood formation due to the finding that the gene encoding amino-cyclo-propane (ACC) oxidase was highly overexpressed in the tension wood [Pilate *et al*., 2004]. Taken together, the plant hormones are now regarded as essential regulators of the tension wood formation. However, the molecular mechanisms underlying the hormonal control of the tension wood formation are yet largely unknown.

Biosynthesis of Cellulose in Tension Wood

The content of cellulose in the tension wood is much higher than in the opposite wood [Timell, 1986]. In addition, the tension wood develops a cellulose-enriched gelatinous layer (G-layer) next to the lumen of the fiber (Fig. 2) [Norberg and Meier, 1966; Cronshow and Morey, 1965; Scurfield and Wardrop, 1963], which is generally not found in the normal fiber. The G-layers have been

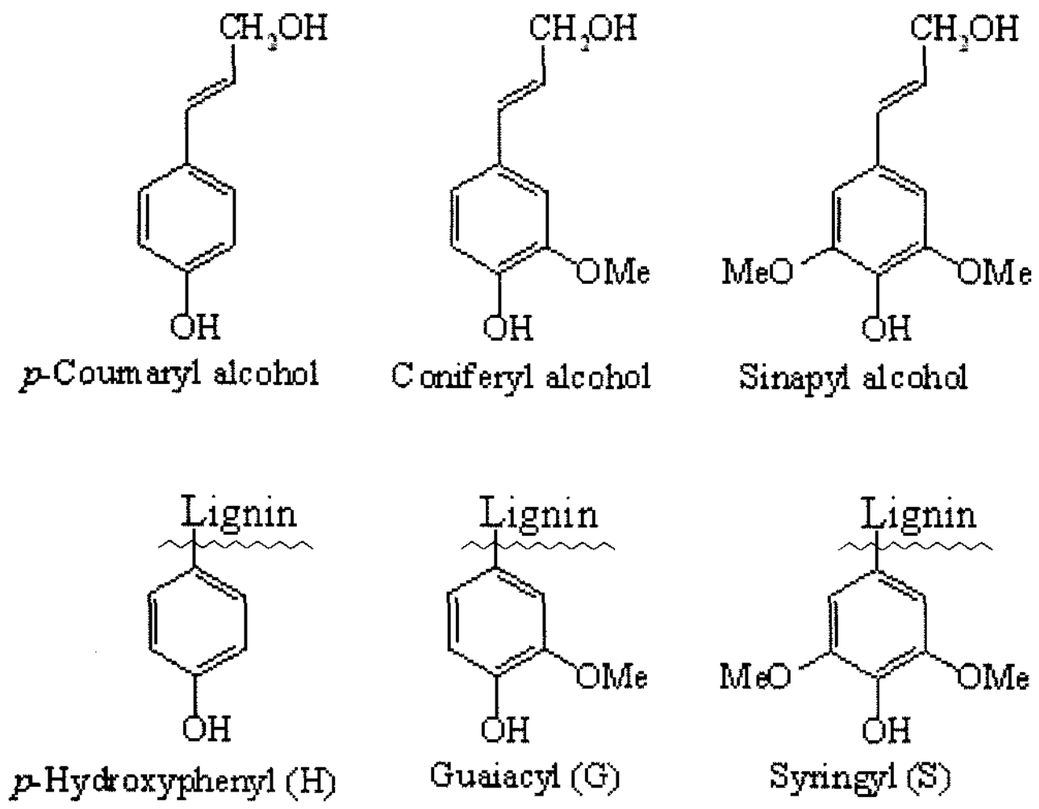

Fig. 3. The monolignols, *p*-coumaryl, coniferyl, and sinapyl alcohols, and the aromatic *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units in lignin macromolecule.

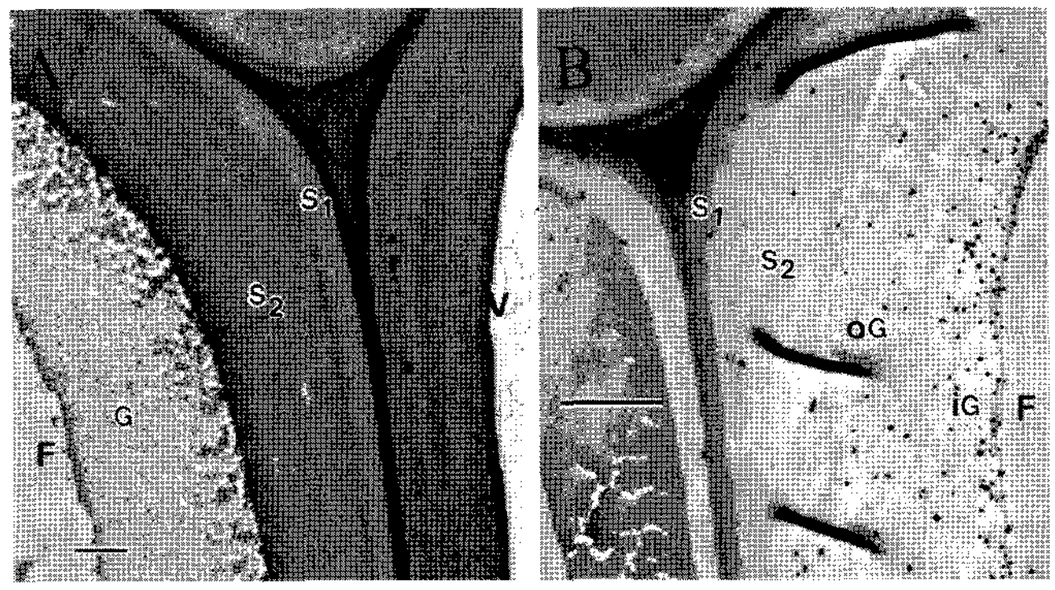

Fig. 4. Transmission electromicrograph of the tension wood fibers with gelatinous layer developed (A) and immunolocalization using an S-antiserum raised against dehydrogenative polymer prepared by peroxidase-catalyzed polymerization of sinapyl alcohol in tension wood fiber (B) of *Populus deltoides*. Innermost gelatinous layer (G-layer) was intensively labeled with immunonogold specific to syringyl units in lignin. (B) Scale bar = 0.5 μm for (A), 1.0 μm for (B). F, fiber; V, vessel; S1, outermost layer of secondary wall; S2, middle layer of secondary cell wall; S3, innermost layer adjacent to the cell lumen; G, gelatinous layer. Pictures were taken from Joseleau *et al.* [2004].

often described as a pure cellulose layer with highly crystalline microfibrils orientated nearly parallel to the longitudinal axis [Wardrop, 1965]. In aspen, *PtrKOR* (Korrigan endoglucanase, *CesA*) mRNA was extensively localized to where the tension wood formed, whereas the *PtrKOR* expression was significantly suppressed in the opposite wood [Bhandari *et al.*, 2006]. In addition, four cellulose synthase genes, *PtrCesA1*, *PtrCesA2*, *PtrCesA3*, and *PtrKOR* were coexpressed with the highly crystalline cellulose in the tension wood fiber as demonstrated by *in situ* hybridization technique [Bhandari *et al.*, 2006], implying the roles of the cellulose synthase [*CesA*] gene in the tension wood formation. In addition, the *CesA* gene expression was highly upregulated in response to the tensile stress [Bhandari *et al.*, 2006]. Analysis of the differentially expressed genes in the tension wood implied that the tension wood formation involves reprogramming of the carbohydrate metabolism, that is, the increased activities for the cellulose biosynthesis and the pectin degradation [Anderson-Gunnerås *et al.*, 2006]. In addition, the transcriptome analysis suggested that the overall carbon flux to the biosynthetic pathway of the cell wall matrix carbohydrates significantly decreased in the tension wood of the poplar [Anderson-Gunnerås *et al.*, 2006].

Recent analysis of the poplar EST library indicated that the fasciclin-like arabinogalactan protein gene family was significantly up-regulated in the tension wood [Anderson-Gunnerås *et al.*, 2006]. For example, the expression level of fasciclin-like arabinogalactan protein of the poplar (*PtFLA12 K, PtFLA12E*, and *PtFLA12V*) was approximately 18 times highly in the tension wood than that in the opposite wood at transcription level [Anderson-Gunnerås *et al.*, 2006]. Because the animal fasciclin-like arabinogalactan protein was shown to play a role in the cell adhesion and communication, the significant increase of the fasciclin-like arabinogalactan protein transcript could be correlated with the signaling pathway of the tension wood formation. However, the roles of the fasciclin-like arabinogalactan protein in the tension wood have not been uncovered.

Lignin Biosynthesis During Tension Wood Formation

The lignin content of the tension wood significantly decreases by about 20% of the cell wall dry weight compared with those of the normal and the opposite woods [Timell, 1969; Donaslon, 2001]. In general, the angiosperm lignins mainly contain coniferyl and sinapyl units (Fig. 3) roughly in equal proportions, together with a small amount of interunits derived from the *p*-coumaryl alcohol. In the tension wood fiber, however, the syringyl unit is predominantly detected in the secondary cell wall of the fiber cell type [Terashima *et al.*, 1998; Joseleau *et al.*, 2004]. Although decreases in the lignin content and guaiacyl unit (G in Fig. 3) of the tension wood lignin have previously been reported, little is known about the lignin modification associated with the tension wood development.

The G-layer has often been described as being mostly composed of pure cellulose, based on the results of the

histochemical staining and sugar analysis obtained by means of the ultrasonic treatment [Pilate *et al.*, 2004]. Ruel *et al.* [1999] studied the distribution of the lignin subunits in *Eucalyptus gunnii* and revealed that the S2 layer of the secondary cell wall of the tension wood has a lower amount of the guaiacyl-syringyl subunits than those of the opposite and the normal woods. *In situ* hybridization of *Populus deltoides* tension wood further demonstrated that the syringyl units of the lignin were detected in the G-layer of the tension wood fiber (Fig. 4) [Joseleau *et al.*, 2004], which opened the possibility of a partial lignification of the G-layer with the syringyl unit, rather than being in a pure cellulose state.

Differential regulation of the genes in the shikimic acid and the phenylpropanoid pathways were observed, especially at the early stages [Pilate *et al.*, 2004; Anderson-Gunnerås *et al.*, 2006]. Anderson-Gunnerås *et al.* [2006] showed that the transcripts of shikimate kinase, chorismate synthase, prephenate dehydratase, and phenylalanine ammonia lyase (accession number PU02719) significantly decreased in the tension wood. They also noticed that transcripts of the genes in the down-stream enzymes of caffeoyl-CoA, i.e., caffeoyl-CoA *O*-methyltransferase (CCoAOMT2, accession number PU02461), cinnamoyl-CoA oxidoreductase (CCR1, accession number PU0003), ferulate-5-hydroxylase (F5H, PU01148), and caffeic acid *O*-methyl transferase (COMT1, PU02638) greatly decreased in the tension wood [Anderson-Gunnerås *et al.*, 2006]. The transcriptional down-regulation of these genes clearly explains the reduced amount of coniferyl alcohol-derived guaiacyl units (G in Fig. 3) in the G-layer of the tension wood fiber. Interestingly, the expression of genes involved in the glycosylation of monolignol also greatly decreased in the tension wood [Anderson-Gunnerås *et al.*, 2006], implying that the glycosylation of the monolignols during the process of monolignol transport to the lignifying cell wall was inhibited, which, in turn, explains the reduced extent of the lignification in the tension wood. Genes involved in the monolignol polymerization, laccase and peroxidase, were also greatly down-regulated in the tension wood [Pilate *et al.*, 2004]. Therefore, the lignin biosynthesis in the tension wood appears to be regulated at multiple points including the monolignol biosynthesis, monolignol glycosylation, transportation to the cell wall, monolignol coupling for lignin polymerization, and specific deposition within the secondary cell wall.

Interestingly, Akiyama *et al.* [2003] investigated the ratio of *erythro* and *threo* forms of β-*O*-4 structures of the tension wood lignin in the yellow poplar. They demonstrated that the content of the *erythro* form of β-*O*-4-linked lignin was higher than that of the *threo* form of lignin in the tension wood, implying a stereoselective regulation of the lignin polymerizatyion during the tension wood formation. Taken together, the lignin biosynthesis during the tension wood formation appeared to be very tightly regulated, spatially and temporally, as well as stereochemically.

Discussion

The inducible formation of the tension wood together with the altered cellulose and the lignin biosynthesis provide an excellent system to dissect the mechanism of cellulose biosynthesis and down-regulation of the lignin biosynthesis during the secondary xylem formation in the woody tissues. However, the genes involved in this process have only recently started to be reported [Anderson-Gunnerås *et al.*, 2006; Paux *et al.*, 2005], with most of them not yet functionally characterized. However, the transcriptome analyses of *Pouplus* and *Eucalyptus* tension woods provided a more global view of the carbon flux redirected from cellulose-, hemicellulose-, and lignin-rich sub-layers of the normal secondary cell wall into the cellulose-rich G-layers with the lignin modification during the tension wood formation. Transcript profiling of the tension wood also highlighted the candidate genes that could be important regulators responsible for the characteristic features of the tension wood [Paux *et al.*, 2005]. The functional characterization of these genes will greatly help to expand the understanding of the carbon partitioning between the cell wall carbohydrates and the lignin biosynthesis, thereby providing tools for the metabolic engineering of the lignocellulosic material as a desirable biofuel.

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