Summary and conclusion
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Structural and histochemical changes in cell wall during xylogenesis and within tree variation

- Present study shows that the ontogeny of cambium in *Leucaena* begins with procambial strand which develops from the residual meristem near the shoot apex. The development of procambial strand is preceded by the procambial trace which gave rise to protophloem elements first followed by protoxylem. Development of metacambium is marked by the differentiation of large lumen metaxylem tracheary elements and metaphloem containing sieve elements with distinct companion cell. The developmental sequence of primary phloem fibres (PPF) maturation, development of fascicular cambium and interfascicular cambium occurs at short distance from the base of second internode. This shows the lignification of PPF could be an early indicator of cessation of elongation growth in shoots of *Leucaena*. The fascicular cambium was demarcated from metacambium by the frequent anticlinal divisions and differentiation of tangential multiples of metaxylem elements. The development of fascicular cambium is subsequently followed by the periclinal divisions in the radially elongated interfascicular parenchyma cells and successive bridging of fascicular cambial cells. After joining of fascicular cambium as a continuous ring of cambium, the interfascicular parenchyma beneath the cambial cell layers close to the pith have shown SW deposition and lignification leading to the development of interfascicular fibres (IFF) and subsequently fusiform and ray initials in the cambium giving rise to secondary xylem and phloem elements.

- The vascular cambium of two year old stem shows actively dividing fusiform cells and mutiseriate rays. The frequent anticlinal divisions and elongation growth results in elimination of fusiform cambial cells from the cambial zone. The radial wall of FCCs are characterized by a relatively thick and appearance...
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The fusiform initials increase their number by pseudotransverse and lateral anticlinal division. The daughter cell thus formed after pseudotransverse division undergo intrusive growth at their vertical ends. The present study also demonstrates the loosening of cell walls during elongation growth of fusiform derivatives towards xylem resulting in disappearance of beaded structure from radial walls.

- The anatomical studies revealed that the major structural changes associated with xylogenesis include cell division, cell expansion and wall thickening. The loosening of cell wall and disassembly and reassembly of pectic polysaccharides appears to be major event associated with cell expansion. The secondary wall deposition and lignification first occur in the vessel elements followed by paratracheal parenchyma, rays and fibres.

- Histochemical studies in *Leucaena* stem revealed that during primary growth stage guaiacyl unit forms the major lignin monomer in the conducting elements. The sequential histochemical changes during xylogenesis following secondary growth include the disorganization of pectins from middle lamellae region, deposition of cellulosic secondary walls and lignification. The Maule’s reaction and fluorescence microscopy reveal that the first deposited lignin in the compound middle lamellae region found to be guaiacyl units and in the later stages vessels and parenchyma wall deposits more guaiacyl units while fibres showed co-polymerization of more syringyl units.

- The present study reveals that majority of the anatomical changes are gradual during transition from juvenile to adult wood, hence a clear demarcation of transition zone may be difficult to find out. However, the conversion of triseriate- to-multiseriate rays appears to be a distinct feature of wood maturation leading to adult wood formation in *Leucaena leucocephala*. Based on the anatomical features with the outer
wood of 15 year old main trunk, the periphery of the wood in the trunk and branch xylem studied are considered as an adult or mature wood.

- The anatomical structure and histochemistry of tension wood in *Leucaena* varies from opposite and normal wood. The major changes in tension wood include reduction in density and dimensions of vessels, wide fibres with thin lignified walls rich in guaiacyl units and reduction in ray dimensions.

**Effect of exogenous plant growth regulators on xylogenesis and lignification pattern**

- The exogenous application of plant growth regulators can profoundly influence the cambial activity and xylogenesis in decapitated shoots of *Leucaena leucocephala*. The hormones also have a significant effect on structure and dimensions of xylem elements and tissue composition in the xylem.

- Auxins are (both IAA and NAA) found to be the key regulators of differentiation leading to the development of narrow lumen vessels and tracheary elements.

- The exogenous feeding of GA3 induced differentiation of gelatinous fibres, deformed vessels, elongated thin walled fibres and a high fibre:vessel ratio and increase in the incorporation of guaiacyl lignin units into thin wall of xylem fibres. BAP application decreased the cambial activity and cell differentiation process resulting in development of thick walled fibres and vessel elements, and also delayed lignification of cell walls and promotes the incorporation of more syringyl lignin units into vessel and fibre wall.

- Exogenously applied ethephon in low concentration (10-100µl/l) enhanced cambial cell division with less xylem differentiation while moderate concentration (100-150 µl/l) enhanced both cambial activity and xylogenesis. However, high concentration of ethephon (200-250 µl/l) slowed down both the events. Ethephon
induces high ray frequency through transformation of fusiform cambial cells into ray initials.

- The application of combination of hormones showed both synergistic and antagonistic nature of interaction among different hormones. Combination of GA$_3$ and auxins induced cambial cell division and relatively fast differentiation of xylem. However, increasing concentrations of GA$_3$ over auxins induced differentiation of gelatinous fibres. The combination of high concentration of BAP either with auxins or with GA$_3$ slowed down cambial activity and xylem differentiation and promoted the incorporation of syringyl lignin units into vessel and fibre walls. On the other hand, moderate concentrations of BAP and GA$_3$ induced gelatinous fibres with thick gelatinous layer indicating the synergistic effect of both hormones. Ethephon showed synergistic interaction with IAA, BAP and GA$_3$. The combination with all three class of hormone enhances cambial activity and xylogenesis. The synergistic activity of ethephon was more towards GA$_3$ and BAP.

- Ethephon and its combination with other hormone were demonstrated to induce tension wood formation in the shoots of *Leucaena*. The tension wood severity was directly proportional to the concentration of ethephon and its interaction with BAP and GA$_3$. High concentration of ethephon and its combination with BAP demonstrated a potential role in modifying lignin monomeric composition in G-fibres and vessels by incorporation of more S-lignin units by delaying lignification.

**Within tree quantitative variation in the cell wall polymers**

- The chemical composition of juvenile and mature wood from branch and main trunk of *Leucaena* shows specific differences. The pattern of changes in the chemical composition during juvenile and adult wood growth phase of branch and main trunk occurs in similar manner.
The juvenile wood is characterized by relatively more holocellulose and α-cellulose content, less lignin and low S/G ratio compared to that of mature wood. The S/G ratio and Klason lignin are positively correlated as the mature wood shows high lignin content and S/G ratio. Both the juvenile and mature wood in main trunk showed more quantity of holocellulose, α-cellulose and S/G ratio than of branch indicating high pulp value for the trunk wood xylem than branches.

The chemical composition of tension wood in *Leucaena* vary in comparison to opposite and normal wood. The chemical compositional changes in the tension wood includes more α-cellulose and holocellulose, reduction in hemicelluloses content especially xylose, mannose, uronic acids, high galactose and rhamnose, low lignin content and S/G ratio compared to opposite wood.

**Ultrastructural changes in the cell wall polymers during xylogenesis**

- The present study unravel the ultrastructural changes in the cell walls during wood formation with special reference to changes in cell wall polysaccharides and lignin. PATAg staining revealed that loosening of wall materials in the middle lamellae followed by the expansion of xylem derivatives. Vessels showed more radial and tangential expansion while other axial elements showed more radial expansion.

- Ruthenium red staining revealed that the disorganization of pectic polysaccharides in CCML and CML region during cell expansion and its reappearance after the initiation of secondary wall deposition. Fibres were characterized by the relatively thick S₂ wall layer compared to that of parenchyma cells. KMnO₄ staining reveals the lignification pattern during xylogenesis.

- Lignification initiates at CCML region and spreads to CML region after a deposition of major part of S₂ layer and
subsequently spreads to inner layers of SW with the progressive deposition of wall layers.

- The present study indicates that the intercellular spaces formed at CCML region of fibres are filled with pectins after initiation of SW deposition and lignification initiated at the cell corners. Both fibres and axial parenchyma showed more lignin distribution in CML, outer region of S2 layer and S3 layer while S1 and major part of S2 layers are characterized by relatively less lignin distribution.

- The present study also indicates that the inhomogenous lignin distribution in the cell corner middle lamellae might be related to the corresponding inhomogeneity of pectins in this region.

- The living nature and the occurrence of starch grains in the thick walled septate fibres shows the taxonomic importance and functional diversity of xylem fibres in *Leucaena*.

- Delignification experiment shows that mild alkali treatment is effective in removal of lignin from secondary walls of wood fibres of *Leucaena* indicating vulnerability of lignin in the fibre wall to mild concentration of chemical agents which is a desirable feature from the perspective of paper and pulp industry.

- The scanning electron microscopy illustrates the morphology of different wood elements in normal and tension wood of *L. leucocephala*.

- Ultrastructure of tension wood shows that a thick gelatinous layer in fibres which replaces S3 and major part of S2 layer of the secondary wall.

- Present study also highlights the need of detailed investigation on the temporal and spatial distribution of different cell wall chemical constituents at their monomeric level to understand relationship between matrix polysaccharides and lignin in the xylem fibres of *Leucaena leucocephala*. 
In conclusion, the present study demonstrated anatomical, ultrastructural, histochemical and biochemical changes in the wood cell walls with special reference to pattern and heterogeneity of lignification, within tree variation in structure and chemical composition and the effect of exogenous plant growth regulators on xylogenesis and lignification pattern in *Leucaena leucocephala*. Looking into the commercial importance of *Leucaena*, the present study highlights the following important findings related to its better utilization.

The anatomical studies revealed that the wood attains its mature characteristics after 5 years of radial growth. On the other hand, biochemical studies showed that both the juvenile and mature wood in the main trunk have more quantity of holocellulose, α-cellulose and S/G ratio compared to those of branch wood. The results obtained from juvenile and mature wood indicate that the main trunk xylem after reaching 5 years of radial growth may be better for high pulp value for paper industry. Furthermore, the structural and biochemical studies on tension wood showed that the fibres with high tension wood severity due to replacement of major part of S_2 and complete S_3 layer by gelatinous layer along with chemical compositional changes such as high α-cellulose content, low hemicelluloses and lignin content indicates tension wood of *Leucaena* could be desirable wood variant for paper industry.

Exogenously applied plant growth regulators shown to have significant effect on cell wall structure and its chemical constituents. For instance, BAP treatment increased secondary wall thickness (more cellulose) and incorporated more S-lignin units (easily removable lignin units during pulp making process) in fibre cell wall. GA_3 and ethephon induced G-fibres with high tension wood severity (thick gelatinous layer rich in cellulose). These results highlight the importance of further studies on hormonal regulation of xylogenesis involving physiological and molecular methods to produce superior wood from *Leucaena* for paper industry.