Summary

The present study was aimed at understanding lignin biosynthesis pathway in *Leucaena leucocephala*. Choice of *L. leucocephala* was based on the fact that this hard wood tree is used exclusively in India for pulp and paper production with contribution of around 25%. No study has been done so far in this regard anywhere. CCoAOMT being a key enzyme responsible for diversion of flux towards formation of G lignin was chosen as target gene as it may be helpful for development of transgenic *L. leucocephala* plants with desired characters suitable for Indian pulp and paper industry.

The findings of present study are as follows: CCoAOMT in *L. leucocephala* is gene family of possibly 3 members. Two of the possible three, CCoAOMT1 and CCoAOMT2 genomic gene clones, 1292 bp each were isolated. Their NCBI GenBank database accession numbers are DQ517929 and DQ517930 respectively. The genes show 92.7% nucleotide sequence similarity with each other. Nucleotide sequence similarity with CCoAOMT genes from other plants is between 70-90%.

CCoAOMT1 and CCoAOMT2 genomic clones comprised of five exons and four introns. Deduced coding sequence of both the genomic CCoAOMT1 and CCoAOMT2 is of 735 nucleotides. Two 5’ upstream (313 and 319 bp) and one 3’ downstream (265 bp) nucleotide sequence of CCoAOMT1 and CCoAOMT2 were isolated by Genome walking. The isolated 3’ downstream UTR is from CCoAOMT2 gene.

Two cDNA clones of CCoAOMT1 and CCoAOMT2, 735 bp each, were isolated. Their NCBI GenBank database accession numbers are DQ431233 and DQ431234. They show 97% nucleotide and 99% deduced amino acid sequence similarity with each other. Nucleotide sequence similarity with CCoAOMT cDNA gene clones from other plants was 70-90%.

Deduced amino acid sequences of CCoAOMT1 and CCoAOMT2 genes show presence of SAM binding domain I (LIDLVKVGGVI), domain II (VAPPDAPLRKYV) and domain III (ALAVDPRIEI) which are also present in other plant CCoAOMTs. Two 57 bp long 5’UTR associated with both the CCoAOMT1 and CCoAOMT2 cDNA genes were isolated using 5’ RACE. Similarly two 3’UTRs of 189 bp and 176 bp associated with the two CCoAOMT1 and CCoAOMT2 cDNA genes respectively were isolated by 3’ RACE.

Distance tree results group the CCoAOMT1 and CCoAOMT2 genes from *L. leucocephala* with CCoAOMTs of other Fabaceae members.
The CCoAOMT gene was expressed in *E. coli* BL21 (DE3) and protein purified from inclusion bodies using Ni-chelated affinity column. Polyclonal antibodies were raised against purified CCoAOMT protein in rabbit. CCoAOMT specific polyclonal IgG were purified using Affi-gel 15 affinity matrix.

Transverse sections of different plant parts of different age stained with phloroglucinol-HCL show increase in number of differentiating xylem cells as well as their stain intensity indicated progression of lignification with age. When these transverse sections were visualization under polarized light, phloroglucinol-HCL stained tissues showed different colour in different plant parts of different ages, suggesting chemical compositional changes in lignin with tissue age.

CCoAOMT was immunolocalized in xylem and fibers suggesting its presence at the sites of extensive lignification. The semiquantitative and QPCR results showed that both the CCoAOMT1 and CCoAOMT2 genes were expressed in tandem in all tissues. The genes are, however, differentially expressed with the tissue age.

Analysis of the two CCoAOMT1 (ProC1) and CCoAOMT2 (ProC2) promoter nucleotide sequences was done using MatInspector 2.2 revealed presence of different cis-regulatory elements involved in CCoAOMT gene regulation. Tobacco plants transformed with cassettes where promoters ProC1 and ProC2 drive GFP gene were recovered. GFP was visualized in xylem and fibers in leaf mid rib, shoot and root. In root GFP was visualized in tissues other than xylem suggesting involvement of other regulatory elements in the two promoters. The above GFP expression and visualization results confirmed that the two promoters were able to drive GFP expression in the aerial plant organs in a tissue specific fashion. The gene expression in the roots was across all tissue types. However, the GFP expression was higher in epiblema and pericycle cells as compared to the other tissue.