CHARACTERIZATION OF LACCASE GENES FROM SWEETGUM (LIQUIDAMBAR STYRACIFLUA L.)

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Abstract. A unique feature distinguishing vascular plants from lower plants is the lignification of their xylem cell walls. Laccase appears to be one of the enzymes involved in the final steps of lignin biosynthesis. In order to more fully elucidate the role of laccase in wood formation, efforts were made to clone the laccase genes from sweetgum, a southern hardwood species which is gaining in commercial importance. RNA from sweetgum cambium was used as template for the cloning of laccase genes via reverse transcription-polymerase chain reaction (RT -PCR). A 360 bp fragment was amplified using primers to a ligated anchor and a conserved copperbinding domain. The translated sequence was 42 % identical to the N-terminal 84 amino acids of an A cer pseudoplatanus laccase. Laccase mRNA was detected in xylem of sweetgum via northern blot analysis. Southern blot analysis of genomic DNA with 300 bp laccase cDNA fragment showed that sweetgum laccase was derived from a single gene. Using sequence from this cDNA fragment, a 457 bp genomic DNA fragment was cloned. This 457 bp fragment comprised 28 bp of downstream sequence and 429 bp of upstream sequence relative to a putative translational start site, and included proposed TATA and CAAT boxes. The prospective sweetgum laccase promoter region revealed homologies with several regulatory elements involved in the phenylpropanoid pathways of other species. Among these were two elements containing the consensus sequence ACCTA (Box L) and one element containing the sequence CCGT (Box A), both of which are putative cis-acting elements in the promoters of the cinnamate 4-hydroxylase (C4H) and phenylalanine ammonia-lyase (PAL) genes from A rabidopsis.

Key words: Laccase, promoter, RT-PCR, regulatory element, sweetgum