The First ACC Synthase Gene From Pine: Studies Of Its Relationship To Wood Quality

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ABSTRACT

The plant hormone, ethylene, is known to take part in numerous plant responses, including senescence, ripening, and wounding, and evidence suggests it has a role in wood development. In particular, ethylene may signal for the production of the undesirable reaction wood, known as compression wood, that forms on the underside of branches and leaning stems in gymnosperms. Silvicultural practices can alter amounts of compression wood within managed tree stands, but little is known about the genetics underlying the production of compression wood. To investigate the molecular underpinnings of compression wood formation, cDNA libraries were constructed from differentiating xylem tissue from both a normal, straight ~15 year old tree and a ~7 year old tree that had been purposely bent and staked so that its trunk is roughly parallel to the ground. A third cDNA library was made using mRNA from elongating pine shoots that had been treated for two hours with 1 mM indole acetic acid (IAA). From these libraries, we recovered a partial cDNA for 1-aminocyclopropane-1-carboxylate (ACC) synthase, the enzyme catalyzing the first committed step of the ethylene biosynthetic pathway. In this report we describe preliminary results regarding the expression of this ACC synthase gene. Probes from this gene are being used to screen libraries for other ACC synthase family members that are specifically expressed in bent stems. Heterologous expression in *Escherichia* coli and subsequent enzymatic characterization will be used to compare the activities of gymnosperm and angiosperm ACC synthases.

INTRODUCTION

Compression wood is formed in gymnosperms in response to shear stress and is, thus, a form of reaction wood (Timmel, 1986). It may be formed in response to wind perturbation, snow loading, or various other mechanical stresses, such as those occurring in leaning stems and on the underside of branches. Compression wood is found more frequently in juvenile wood compared to mature wood, and is characterized by an eccentric pattern growth rings, increased radial growth in the compressed area, and reddish wood color (Fig 1). These phenotypic changes reflect in anatomical, as well as physical and chemical changes that make the wood less desirable to the forest products industry. For example, shortened compression wood fibers contain more lignin and free phenolic materials, which the fibers more difficult to pulp and bleach, and the angle of cellulose microfibrils is increased in compression wood fibers, which contributes greatly to the dimensional instability of solid wood products.

Ethylene was first recognized as a plant growth regulator by Neljubow, who demonstrated it to be responsible for the classic “triple response” -- decreased elongation, increased lateral growth, and epinasty (altered gravitropic response) -- in etiolated pea seedlings growth and reduced longitudinal growth as might be expected if ethylene was being produced in

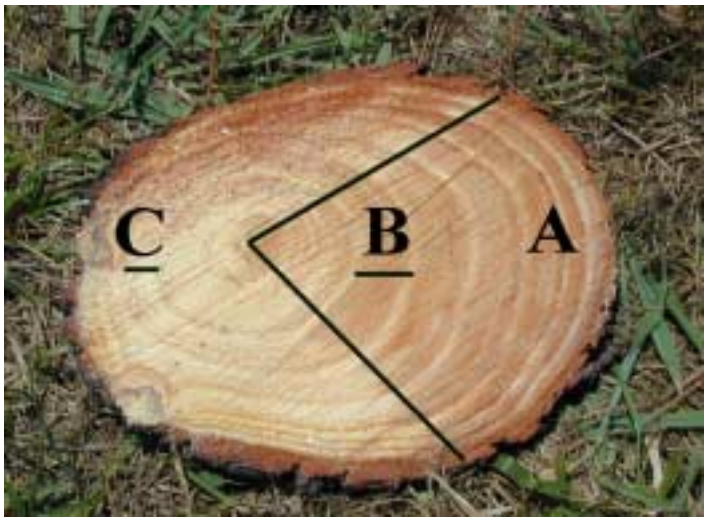

Figure 1. Cross-section through a zone of compression wood in loblolly pine. What was the underside of the stem is clearly demarcated by the darker (reddish) colored wood (A). The eccentric nature of the annual growth rings is highlighted by the bars depicted beneath the lettering (B) and (C).

(as reviewed in Taiz and Zeiger, 1998). Compression wood is characterized enhanced radial response to shear stress. In fact, the relationship between ethylene formation and tension wood, the reaction wood formed in angiosperm trees responding to bending stresses, has been known for some time (Sinnott 1952, Robitalle and Leopold 1974). Savidge *et al.* (1983) found elevated levels of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), in compression wood tissue, while Telewiski and Jaffe (1986) demonstrated that mechanical perturbation of tree stems led to ethylene emission, and the formation of wood with compression wood-like characteristics (increased spiral grain and increased lignin deposition in tracheids).

ACC synthase (S-adenosyl-L-methioadenosine lyase, EC 4.4.1.14), a low abundance, highly labile enzyme that catalyzes the first step in the ethylene biosynthetic pathway, is the primary regulator of ethylene production in most plants. More than 75 full-length sequences for ACC synthase genes are currently listed in Genbank, and while a few were isolated from woody plants (e.g. apple, mango, peach, orange, and kiwi), none are from gymnosperms. In most plants, ACC synthases comprise a large gene family (Fluhr and Mattoo, 1996), and the various members of the family are thought to play differing physiological roles within the plant (e.g. Liang *et al.*, 1992). In mung bean, Botella *et al.* (1992) isolated three different ACC synthase cDNAs from etiolated hypocotyls, and expression of one of these (AIM-1) was rapidly (≤10 min) induced by bending stress (Botella *et al.*, 1995). To date this is the only report of an ACC synthase gene that is responsive to shear stresses.

This study is directed toward isolation and characterization of ACC synthase gene(s) from *Pinus taeda*, particularly genes that respond to bending stress and may therefore be involved in controlling compression wood formation.

METHODS

Three types of loblolly pine tissue were used for RNA extraction – non-lignified xylem from a mature, vertical trunk; non-lignified xylem from the underside of a bent juvenile tree, and

elongated pine shoots incubated for 2 hr at room temperature in a solution of 1mM indole acetic acid (IAA).

Total RNA was extracted from the various tissues essentially using the method described by Alosi (http://dendrome.ucdavis.edu/Protocols/rna_from_needles.html). cDNA libraries were constructed from this RNA using the SMART cDNA library construction kit (CLONTECH, Palo Alto, CA.) according to the manufacturer's protocols.

For expression profiling of the ACC synthase gene in different tissues, cDNA was synthesized using Superscript reverse transcriptase (Life Technologies, Rockville, MD) primed with an oligo-dT primer. Prior to cDNA synthesis, total RNA was t treated with DNAse to eliminate genomic DNA contamination. For each RT-PCR reaction, 5ng of cDNA template was used.

RESULTS

Starting with sequence from an unannotated EST in the loblolly pine EST database (www.cbc.umn.edu/researchprojects/pine/ifg.loblolly) having apparent homology to other ACC synthases a cDNA fragment was isolated from the IAA-treated pine shoot library. This sequence was extended to the 3' terminus using 3' RACE (Life Technologies Rockville, MD) such that the longest clone currently available comprises about one-third of the expected full-length cDNA (Fig 6). Efforts to clone a full-length cDNA are still underway; however, from the available sequence information this appears to be the first ACC synthase described for a conifer. Preliminary results suggest that ACC synthase gene expression is elevated in compression wood (Fig. 3).

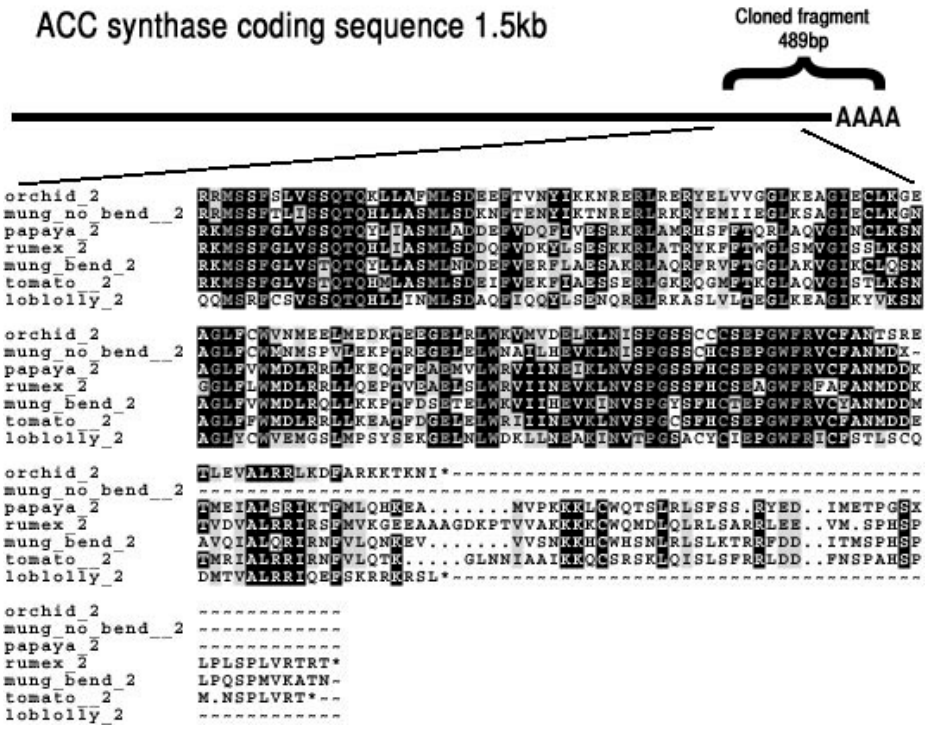

Figure 2. DNA sequence alignment of ACC synthases from several plant species. Sequence of a partial ACC synthase cDNA from loblolly pine (bottom line) is compared to equivalent regions of cDNAs from orchid, mung bean, papaya, sorrel (Rumex) and tomato.

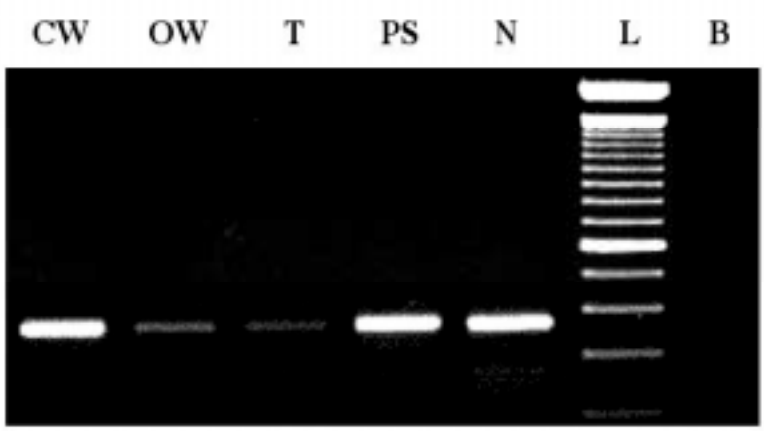

Figure 3. Differential expression of the ACC synthase gene in various loblolly pine tissues. Semi-quantitative RT-PCR was used to detect ACC synthase gene expression levels in non-lignified xylem from compression wood (CW), opposite wood (OW), and vertical wood (T), as well as pine shoots with (PS) and without (N) IAA.

CONCLUSIONS

A cDNA fragment from loblolly pine appears to represent the first ACC synthase described for a gymnosperm; however, verification will require expression and characterization of an active enzyme from a full-length clone. The gene appears to have elevated expression in compression wood, a finding which is congruent with the hypothesis that ethylene is involved with compression wood formation. Although ethylene action is relatively well understood in angiosperms, this is not the case for conifers. We expect that this work will provide a better understanding of how trees respond to stress, and that the findings will be used to address problems related to compression wood formation in gymnosperms.

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