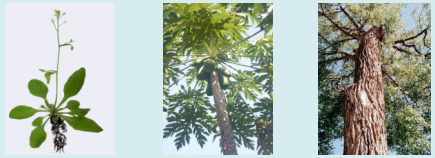
Lignin biosynthesis genes in the papaya genome and comparative analysis with orthologs in *Arabidopsis* and poplar

Andrea R. Gschwend [A], Qingyi Yu [B], Shaobin Hou [C], Robert E. Paull [D], Maqsudul Alam [C], and Ray Ming [A]

(A) Department of Plant Biology, University of Illinois at Urbana-Champaign (B) Hawaii Agriculture Research Center,
(C) Advanced Studies in Genomics, Proteomics and Bioinformatics, University of Hawaii (D) Department of Tropical Plant and Soil Sciences, University of Hawaii

Introduction

Lignin is an abundant cell wall polymer, which aids in plant defense and structural support (Raes et al. 2003). The biosynthesis pathway consists of ten central enzymes which ultimately results in guaiacyl and syringyl lignin. *Arabidopsis* has 18 genes likely to be involved in lignin biosynthesis, whereas poplar has 15 bona fide poplar lignin genes (Raes et al. 2003, Ehlting et al. 2005, Hamberger et al. 2007). *Carica papaya*, a polygamous, perennial, herbaceous crop fruit tree, has a single stem of intermediate size compared to *Arabidopsis,* which is an annual plant that lacks secondary growth, and poplar, a perennial that has secondary growth (**Figure 1**). In this study, 32 papaya lignin candidate genes, identified by Ming et al (2008), are further explored to gain a better understanding of the genes involved in lignin biosynthesis.



Arabidopsis thaliana *Carica papaya* *Populus trichocarpa*

Figure 1. Distinctive morphology of *Arabidopsis*, papaya, and poplar

Methods

RT-PCR

1. Designed primers from genes to span at least one intron.
2. Tested apical meristem, leaf, stem, and root tissues 2, 4, 6, and 8 weeks after germination for expression.

Phylogenetic Analysis

1.Constructed phylogenetic trees for each lignin gene family

Results

• Of the 32 papaya lignin candidate genes, 19 were validated by papaya expressed sequence tags (ESTs)

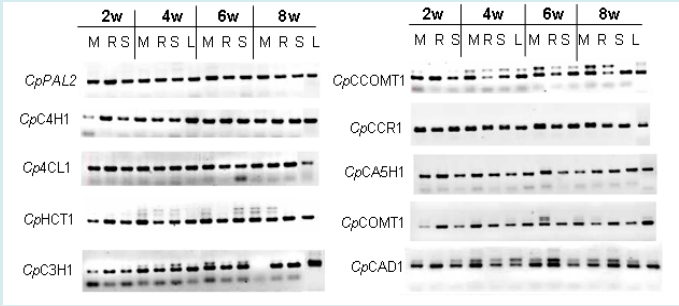


Figure 2. RT-PCR expression profiles of select lignin biosynthesis candidate genes

• All of the papaya candidate lignin genes were expressed in at least one tissue and time period, except for *CpC3H2*, CpC5H3, and CpC5H4, which showed no expression. Out of the remaining 29 candidate genes, 21 were expressed in all the tissues across all the time periods.

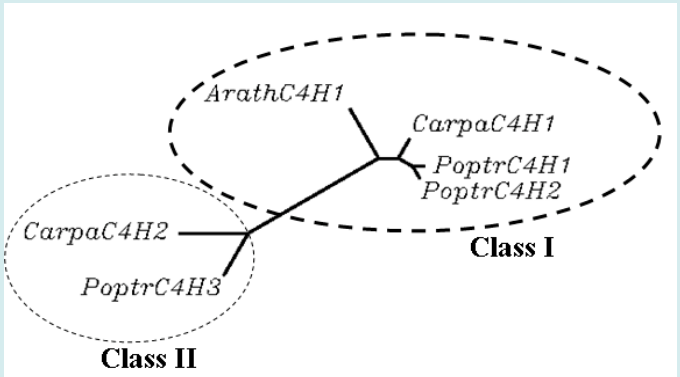


Figure 3. Unrooted phylogenetic tree of the *C4H* gene families of *Carica papaya*, *Arabidopsis thaliana*, and *Populus trichocarpa*

• Out of the 29 expressed papaya lignin candidate genes, 17 were clustered closely, on the phylogenetic tree, to the bona fide lignin biosynthesis genes of *Arabidopsis* and poplar, but the role of *CpC4H2* is uncertain (see below).

• The role of the class II *C4H* genes in lignin production is still unclear, especially since both *Arabidopsis* and poplar lack bona fide class II *C4H* genes. Without further investigation, *CpC4H2* can not be considered a bona fide lignin biosynthesis gene.

Gene Name	Protein Name
PAL1	phenylalanine amonnia lyase
PAL2	
PAL3	
C4H1	Trans-cinnamate 4-hydroxylase
4CL1	4-Coumarate:CoA Ligase
4CL2	
4CL3	
4CL4	
C3H1	*p*-Coumaroyl shikimate 3'-hydroxylase/Coumaroyl 3-hydroxylase
CA5H1	Ferulate 5-hydroxylase
CA5H2	
CCR1	Cinnamoyl-CoA reductase
CAD1	Cinnamyl alcohol dehydrogenase
CCOMT2	Trans-caffeoyl-CoA 3-*O*-methyltransferase
COMT1	Caffeic acid 3-*O*-methyltransferase
HCT1	Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyltransferase

Table 1. Bona fide papaya lignin biosynthesis genes, based on phylogenetic and expression analyses

Conclusions

The results of these studies suggest 16 genes are most likely involved in lignin biosynthesis in *Carica papaya*

Acknowledgements

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