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Review

# Lignin genetic engineering for improvement of wood quality: Applications in paper and textile industries, fodder and bioenergy production

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ABSTRACT

Lignin, a complex racemic phenolic heteropolymer present in plant cell walls, plays crucial role in the adaptive strategies of vascular plants. But from agroindustrial perspective, lignin exerts a negative impact on the utilization of plant biomass in pulp and paper industry, textile industry, forage digestibility and production of biofuel. In this direction, lignin manipulation by genetic engineering approaches serves as a promising strategy. The researches on lignin biosynthesis, especially monolignol biosynthesis, have demonstrated that alteration of lignin content and composition can be attained to acquire economic and environmental benefits. Thus, transgenic plants with modified lignin content and composition can cope with large shifts in *p*-hydroxyphenyl/guaiacyl/syringyl lignin ratios and modified lignin can serve as improved feedstock for production of paper, biofibers, biofuels and forage. This review provides an overview of lignin genetic engineering in plants to yield new insights into the lignin biosynthetic pathway and quality amelioration of wood for efficient pulping, ease of forage digestibility, and production of biofiber and biofuel.



**Contents**



## 1. Introduction

Lignin, a complex aromatic heteropolymer, is a major constituent of the secondary cell walls of all vascular plants. It plays essential roles in strengthening and impermeabilization of the cell walls and constitutes mechanical and chemical barriers against pathogens (Rastogi and Dwivedi, 2003, 2008; Vinardell et al., 2008). Lignification of plant cell wall is developmentally regulated; however, its deposition is also induced by various abiotic and biotic stresses, such as metabolic stress, wounding, pathogen infection as well as changes in cell wall structure (Caño-Delgado et al., 2003; Tronchet et al., 2010). It is primarily synthesized and deposited in the secondary cell walls of xylem vessels, tracheary elements, phloem fibers and periderm (Lewis et al., 1999; Rogers and Campbell, 2004).

Lignin is derived by oxidative polymerization of three cinnamyl alcohols, *i.e.* monolignols (namely, *p*-coumaryl, coniferyl and sinapyl

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alcohols), which differ in their degree of methoxylation and form *p*-hydroxy phenyl (H), monomethylated guaiacyl (G) and dimethylated syringyl (S) units, respectively (Boerjan et al., 2003; Boudet, 1998; Cesarino et al., 2012; Chen et al., 2006; Chiang, 2005, 2006; Dixon and Reddy, 2003; Dixon et al., 2001; Douglas, 1996; Goujon et al., 2003b; Harakava, 2005; Harris and DeBolt, 2010; Heitner et al., 2010; Humphreys and Chapple, 2002; Humphreys et al., 1999; JinHua et al., 2007; Li and Chapple, 2010; Liu, 2012; Morreel et al., 2004; Ralph et al., 2004; Ralph and Brunow, 2006; Rastogi and Dwivedi, 2008; Sederoff et al., 1994; Vanholme et al., 2010a; Weisshar and Jenkins, 1998; Whetten and Sederoff, 1991, 1995; Whetten et al., 1998). Monolignol biosynthesis occurs in cytoplasm involving a series of steps forming a 'metabolic grid', in which some enzymes display broad substrate specificities and some are multifunctional (Fig. 1). In brief, the phenylpropanoid pathway for the biosynthesis of monolignols commences with the deamination of phenylalanine derived from the shikimate biosynthetic pathway occurring in the plastid. Then several reactions including side chain reduction, aromatic ring hydroxylation/methoxylation and conversion of the side chain carboxyl to an alcohol group occur (Liu, 2012). Genome sequencing, genome array expression profiling and transcriptome profiling data have provided useful information concerning different gene families encoding lignin biosynthesis pathway enzymes (Bonawitz and Chapple, 2010; Bosch et al., 2011; Casu et al., 2007; Courtial et al., 2013; Ko and Han, 2004; Minic et al., 2009). Through combination of sequence homology or specific transcript-profiling techniques, it is now possible to isolate genes that are more specific for secondary cell wall formation (Bedon and Legay, 2011). Three enzymes of the monolignol biosynthesis pathway, namely, C4H, C3H and F5H, are membrane-bound cytochrome P450 enzymes and are active at the cytosolic side of endoplasmic reticulum. The monolignols, synthesized in the cytoplasm, are then translocated to cell wall by a still unclear mechanism (Alejandro et al., 2012; Kaneda et al., 2011; Liu et al., 2011; Miao and Liu, 2010; Vanholme et al., 2010a; Wang et al., 2012; Whetten and Sederoff, 1995). According to one model, monolignol glucosides, formed by UDP-glucosyltransferase, are transported to plant cell wall and hydrolyzed by coniferin $\beta$-glucosidase. According to the second model, monolignols are transported to the plasma membrane by Golgi-derived vesicles. However, significant evidences for these two models are lacking and at present the roles of ATP-binding cassette-like (ABC) transporters or unaided diffusion of monolignols across the plasma membrane are being demonstrated. In the cell wall, polymerization of

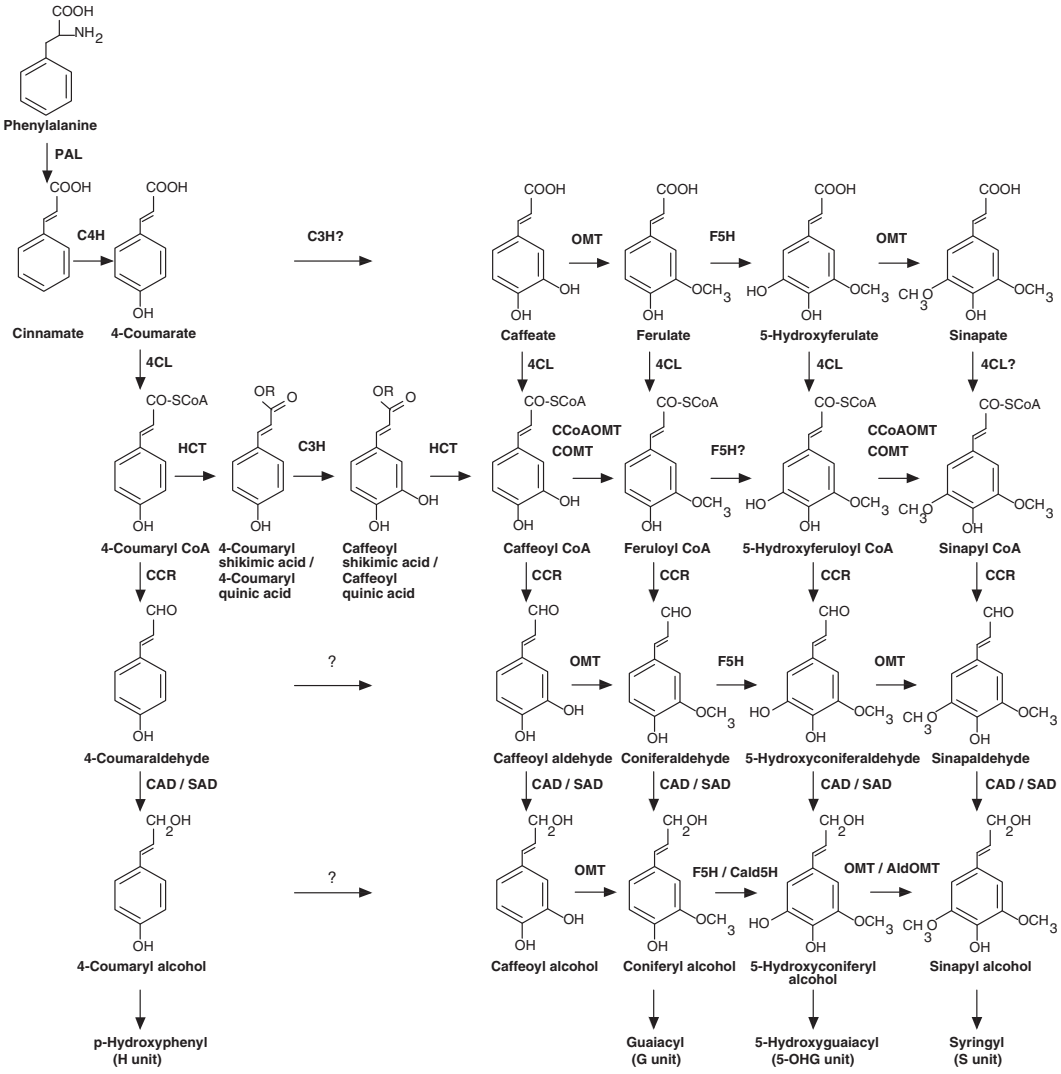


**Fig. 1.** Biosynthetic route towards three monolignols, namely, *p*-coumaryl, coniferyl and sinapyl alcohols [PAL: Phenylalanine ammonia-lyase; C4H: Cinnamate 4-hydroxylase; C3H: *p*-Coumarate 3-hydroxylase or *p*-Coumaroyl CoA 3-hydroxylase or *p*-Coumaroyl shikimate/quinate 3-hydroxylase; OMT: *O*-methyltransferase; HCT: *p*-Hydroxy cinnamoyl-CoA: shikimate/quinate *p*-hydroxy cinnamoyl transferase; CCoAOMT: Caffeoyl coenzyme A *O*-methyltransferase; F5H: Ferulate 5-hydroxylase; 4CL: 4-Coumarate: coenzyme A ligase; CCR: Cinnamoyl coenzyme A reductase; Cald5H: Coniferaldehyde 5-hydroxylase; AldOMT: 5-Hydroxy coniferaldehyde *O*-methyltransferase; SAD: Sinapyl alcohol dehydrogenase; CAD, Cinnamyl alcohol dehydrogenase;?: Yet unconfirmed reactions].