Plant Science 236 (2015) 272–282

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Cell wall modifications triggered by the down-regulation of Coumarate 3-hydroxylase-1 in maize

Silvia Fornalé[a], Jorge Rencoret[b], Laura Garcia-Calvo[c], Montserrat Capellades[a], Antonio Encina[c], Rogelio Santiago[d], Joan Rigau[a], Ana Gutiérrez[b], José-Carlos del Río[b], David Caparros-Ruiz[a,*]

[a] Centre for Research in Agricultural Genomics (CRAG) Consorci CSIC-IRTA-UAB-UB Edifici CRAG Campus de Bellaterra de la UAB, 08193 Cerdanyola del Valles, Barcelona, Spain
[b] Instituto de Recursos Naturales y Agrobiología de Sevilla, CSIC, P.O. Box 1052, 41080-Seville, Spain
[c] Área de Fisiología Vegetal, Universidad de León, 24071 León, Spain
[d] Agrobiología Ambiental, Calidad de Suelos y Plantas (UVIGO) (unidad asociada a la Misión Biológica de Galicia, CSIC), Dpto. Biología Vegetal y Ciencias del Suelo, Facultad de Biología, Universidad de Vigo, Campus As Lagoas Marcosende, 36310, Vigo, Spain

ARTICLE INFO

Article history:
Received 19 March 2015
Received in revised form 9 April 2015
Accepted 10 April 2015
Available online 28 April 2015

Keywords:
Cell wall polysaccharides
Degradability
Flavonoids
Lignin
Maize

ABSTRACT

Coumarate 3-hydroxylase (C3H) catalyzes a key step of the synthesis of the two main lignin subunits, guaiacyl (G) and syringyl (S) in dicotyledonous species. As no functional data are available in regards to this enzyme in monocotyledonous species, we generated C3H1 knock-down maize plants. The results obtained indicate that C3H1 participates in lignin biosynthesis as its down-regulation redirects the phenylpropanoid flux: as a result, increased amounts of *p*-hydroxyphenyl (H) units, lignin-associated ferulates and the flavone tricin were detected in transgenic stems cell walls. Altogether, these changes make stem cell walls more degradable in the most *C3H1*-repressed plants, despite their unaltered polysaccharide content. The increase in H monomers is moderate compared to C3H deficient Arabidopsis and alfalfa plants. This could be due to the existence of a second maize C3H protein (C3H2) that can compensate the reduced levels of C3H1 in these *C3H1*-RNAi maize plants. The reduced expression of *C3H1* alters the macroscopic phenotype of the plants, whose growth is inhibited proportionally to the extent of *C3H1* repression. Finally, the down-regulation of C3H1 also increases the synthesis of flavonoids, leading to the accumulation of anthocyanins in transgenic leaves.

1. Introduction

In plants, 20–30% of photosynthetically fixed carbon is directed toward lignin and other phenylpropanoid compounds, being lignin the second most abundant organic polymer after cellulose [1].

Lignin is a heterogeneous phenolic biopolymer formed by the oxidative coupling of three main hydroxycinnamyl alcohols (monolignols): *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, forming the *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin subunits, respectively [1–3]. Recently, it has been described that monolignols can be acylated through the action of different acyl-transferases prior to their polymerization [4–6].

Lignin is produced by the phenylpropanoid pathway (Fig. 1), together with a broad range of specialized metabolites such as flavonoids, hydroxycinnamic acids and esters, tannins, suberin, cutin and stilbenes [7] and many of them, including flavonoids and stilbenes, possess extraordinary antioxidant activity and therefore a putative health-protecting function [8,9].

The synthesis of monolignols starts from phenylalanine and consists of coordinated reactions of deamination, hydroxylation, methylation, acylation and reduction and the genes involved in this process are well established [2,7,10,11] (Fig. 1).

Once produced, lignin is irreversibly deposited into the cell wall where it interacts covalently with hemicelluloses, generating a strong network that limits the access to cell wall sugars. These interactions reduce the digestibility of the lignocellulosic biomass for its use as forage, reduce the efficiency of the industrial processing for the pulp and paper industry and increase its recalcitrance

* Corresponding author. Tel.: +34 935636600; fax: +34 935636601.
E-mail addresses: silvia.fornale@cragenomica.es (S. Fornalé), jrencoret@irnase.csic.es (J. Rencoret), lgarc@unileon.es (L. Garcia-Calvo), montse.capellades@cragenomica.es (M. Capellades), a.encina@unileon.es (A. Encina), rsantiago@mbg.csic.es (R. Santiago), joan.rigau@cragenomica.es (J. Rigau), anagu@irnase.csic.es (A. Gutiérrez), delrio@irnase.csic.es (J.-C. del Río), david.caparros@cragenomica.es (D. Caparros-Ruiz).

http://dx.doi.org/10.1016/j.plantsci.2015.04.007
0168-9452/

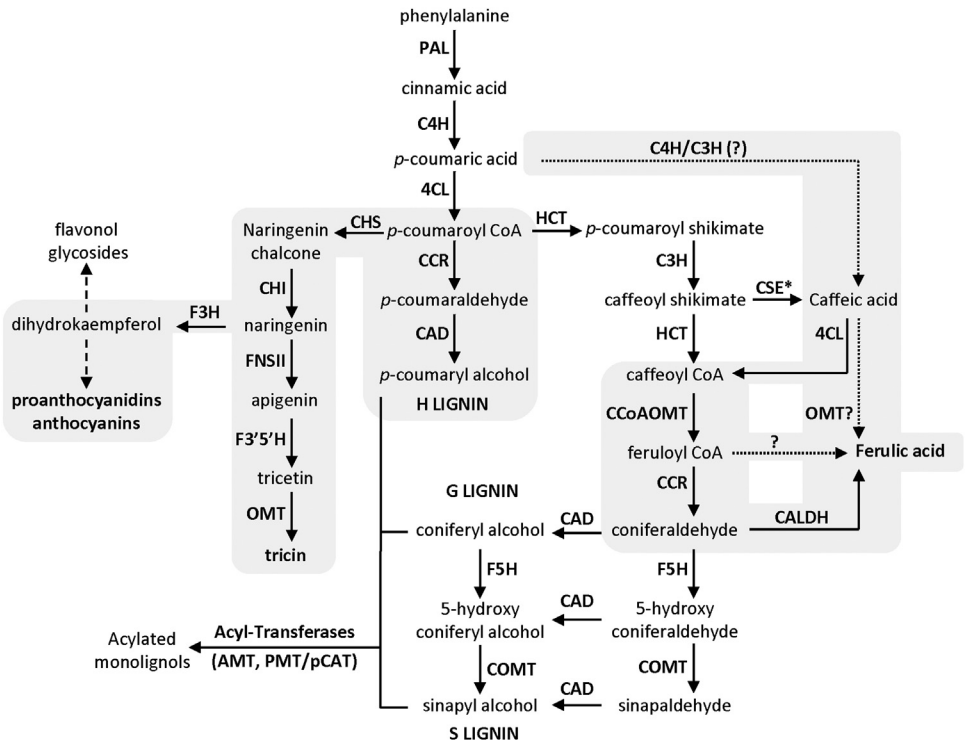

Fig. 1. The phenylpropanoid pathway. PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; CCR, cinnamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase; HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyltransferase; C3H, 4-coumarate 3-hydroxylase; CSE, caffeoyl shikimate esterase; COMT, caffeic acid O-methyltransferase; CCoAOMT, caffeoyl-CoA O-methyltransferase; F5H, ferulate-5-hydroxylase, HCALDH, hydroxycinnamaldehyde dehydrogenase; AMT, acetyl-CoA:monolignol transferase; PMT, p-coumarate:monolignol transferase; pCAT, p-coumaroyl CoA:hydroxycinnamyl alcohol transferase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; FNSII, flavone synthase II; F3′5′H, flavonoid 3′,5′-hydroxylases; pCA-Lignin, p-coumarate linked to the lignin polymer. The metabolic steps required to produced flavonoids from dihydrokaempferol are represented by single broken arrows. Grey dotted arrows represent putative pathways through which ferulic acid can be synthesized. The enzymatic steps involved in the production of three main lignin-related metabolites (p-coumaryl alcohol, ferulic acid and tricin) and flavonoids that are over-accumulated in C3H1-RNAi plants are shown under a grey background (only the possible pathways that could produce ferulic acid without the involvement of C3H are marked). Enzymes with asterisks refer to enzymatic steps that have been described in Arabidopsis thaliana but still not described in maize or in any grass plant species.

for bioethanol production [12,13]. Many studies showed that the chemical and physical properties of the lignin polymer are affected by the relative amount of the three main subunits [1,12,14] and an increasing amount of transgenic plants in which the expression of monolignol biosynthetic genes have been altered have been characterized [7,15,16].

Within the lignin pathway, three cytochrome P450 enzymes, the general phenylpropanoid cinnamate 4-hydroxylase (C4H) and the lignin-specific *p*-coumarate 3-hydroxylase (C3H) and ferulate 5-hydroxylase (F5H) catalyze the corresponding hydroxylations of the aromatic ring (Fig. 1). Recent studies showed that at least C4H and C3H co-localize in the endoplasmic reticulum, forming protein complexes that interact with the soluble HCT and 4CL. These findings suggest the existence of a cluster of membrane proteins acting as a scaffold for further looser associations of soluble partners, leading to the creation of dynamic metabolons that drive the synthesis of a specific monolignol [17,18].

The role of C3H in lignification has been clearly demonstrated by the characterization of two *Arabidopsis thaliana C3H* mutants [19–21]. A point mutation in the *C3H* coding sequence of the *ref8* mutant is sufficient to produce a strong impact on plant growth and inhibited the formation of G and S lignin monomers [20], but the T-DNA insertion mutant *cyp98A3* was found to have an even greater impact [21]. In the latter, the complete lack of C3H results in a strong reduction of lignin that is almost exclusively composed of H monomers (95%), a reduced cell expansion, altered cell wall sugar composition and decreased levels of crystalline cellulose. In addition, the authors reported a higher accumulation of flavonoids, indicating the occurrence of a cross-talk between hydroxycinnamic acid/lignin and flavonoid pathways [21]. Two additional CYP98A genes (*AtC3H2* and *AtC3H3*) have been identified in the *A. thaliana* genome [10] but are more divergent and constitute a separate class and do not appear to hydroxylate shikimate and quinate esters of *p*-coumaric acid [22].

Heavily *C3H*-downregulated alfalfa plants (*Medicago sativa*), a forage legume, have been generated and characterized [23,24]. Despite the only 5% residual C3H activity, these plants did not show severe growth and phenotypic impairments. Nevertheless, the huge increase in H lignin units and the reduction of the total lignin content highly increase the *in vitro* digestibility of their cell walls [23]. Similarly, one of the characterized *C3H*-repressed poplar plants [25,26] showed that *C3H* down-regulation leads to 45% reduction of lignin but with a 20% accumulation of H monomers that occurs at expenses of the G subunits [26].

Thus, the existing studies on *C3H* down-regulation performed in dicotyledonous species (Arabidopsis, poplar and alfalfa) revealed striking differences in the extent of the perturbations produced on lignin biosynthesis. As the only data available on the effects of C3H repression came from dicotyledonous plants, we addressed this issue in the monocotyledonous maize plant. Like other grasses, the structural organization of maize cell wall is significantly different from those of dicotyledonous plants in terms of lignin (with higher amounts of H subunits), higher hydroxycinnamates content [27,28] and hemicellulose composition [27,29]. In addition, it has been recently shown that the flavone tricin is also a structural component of the lignin polymer in grasses [30,31].