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Review article

Ferulic acid: a key component in grass lignocellulose recalcitrance to hydrolysis

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Summary

In the near future, grasses must provide most of the biomass for the production of renewable fuels. However, grass cell walls are characterized by a large quantity of hydroxycinnamic acids such as ferulic and *p*-coumaric acids, which are thought to reduce the biomass saccharification. Ferulic acid (FA) binds to lignin, polysaccharides and structural proteins of grass cell walls cross-linking these components. A controlled reduction of FA level or of FA cross-linkages in plants of industrial interest can improve the production of cellulosic ethanol. Here, we review the biosynthesis and roles of FA in cell wall architecture and in grass biomass recalcitrance to enzyme hydrolysis.

Introduction

The worldwide enrichment occurred after industrial revolution was fuelled with coal and petroleum (dos Santos *et al.*, 2011). But, as a finite resource, fossil fuels cannot sustain technological development indefinitely. New technologies that allow us to ensure a continuous economic development are needed (Escobar *et al.*, 2009). As the most abundant renewable raw material available (Gnansounou, 2010), plant biomass has a great potential to replace oil in a reasonable economic and technological development horizon (Buaban *et al.*, 2010; Poovaiah *et al.*, 2014). Grass residues including switchgrass, miscanthus, sugarcane and maize are potential sources of lignocellulose for the production of bioenergy (Burton and Fincher, 2012; Torres *et al.*, 2014).

To date, almost all ethanol produced in the world comes from food carbohydrates, such as sucrose and starch (Balat, 2011). After extraction of soluble carbohydrates, the residual biomass is underused as solid fuel to produce electricity (Goldemberg *et al.*, 2008) or as cattle feed (Rocha *et al.*, 2012). However, crop residues such as sugarcane bagasse are mainly constituted by polysaccharides. Once hydrolysed, monosaccharides can be partially fermented and, thus, contribute for increase of ethanol production (Buckeridge *et al.*, 2010), without competing with food production (Alvira *et al.*, 2010).

To produce cellulosic ethanol at competitive costs, several challenges need to be overcome. It is necessary to develop efficient enzymatic cocktails to understand how the enzymes act together, how to cope with catalytic inhibitors and also drastically reduce catalysts costs (Jørgensen *et al.*, 2007). This task is not trivial as recalcitrance of lignocellulose is the result of hundred million of years of co-evolution between plants and predators (Cheynier *et al.*, 2013; Malinovsky *et al.*, 2014). Among the adaptations against biological attack, we highlight the presence of hydroxycinnamates in primary and secondary cell walls of grasses and other commelinoids (Hatfield *et al.*, 1999; dos Santos *et al.*, 2006).

Produced in the phenylpropanoid pathway, ferulic acid (FA) is able to couple oxidatively with other FA and derivatives (Buanafina, 2009), lignin and structural proteins from cell wall (Carpita *et al.*, 2001). However, in contrast with the alcohol function of monolignols, hydroxycinnamic acids have a carboxylic group at the end of their propenyl group, which provides the ability to esterify hemicelluloses (Figure 1). Thus, FA is covalently linked to the lignin and proteins by ether bonds and with polysaccharides by ester bonds, acting as a universal connector between cell wall polymers. It performs a key role in cessation of cell growth, anchoring lignin in cell wall polysaccharides restricting the accessibility of plant pathogens (Lygin *et al.*, 2011).

The revolutionary potential of cellulosic ethanol for civilization justifies *per se* the importance of overcoming cell wall recalcitrance (dos Santos *et al.*, 2011). However, the relevance of this knowledge can reach far beyond the fuel industry with benefits which comes from plant physiology and wood science to paper industry and cattle feed. Herein, we reviewed the role of FA in type II primary and secondary cell wall architecture and properties highlighting its relevance in recalcitrance to enzyme hydrolysis.

Composition and architecture of cell wall

Lignocellulosic biomass is composed mainly by cellulose, hemicellulose, lignin, pectin, proteins and aromatic compounds. Nevertheless, there are relevant differences in the proportion of these constituents among plant species and tissues (Carpita *et al.*, 2001). Overall, such components account for over 90% of the total dry

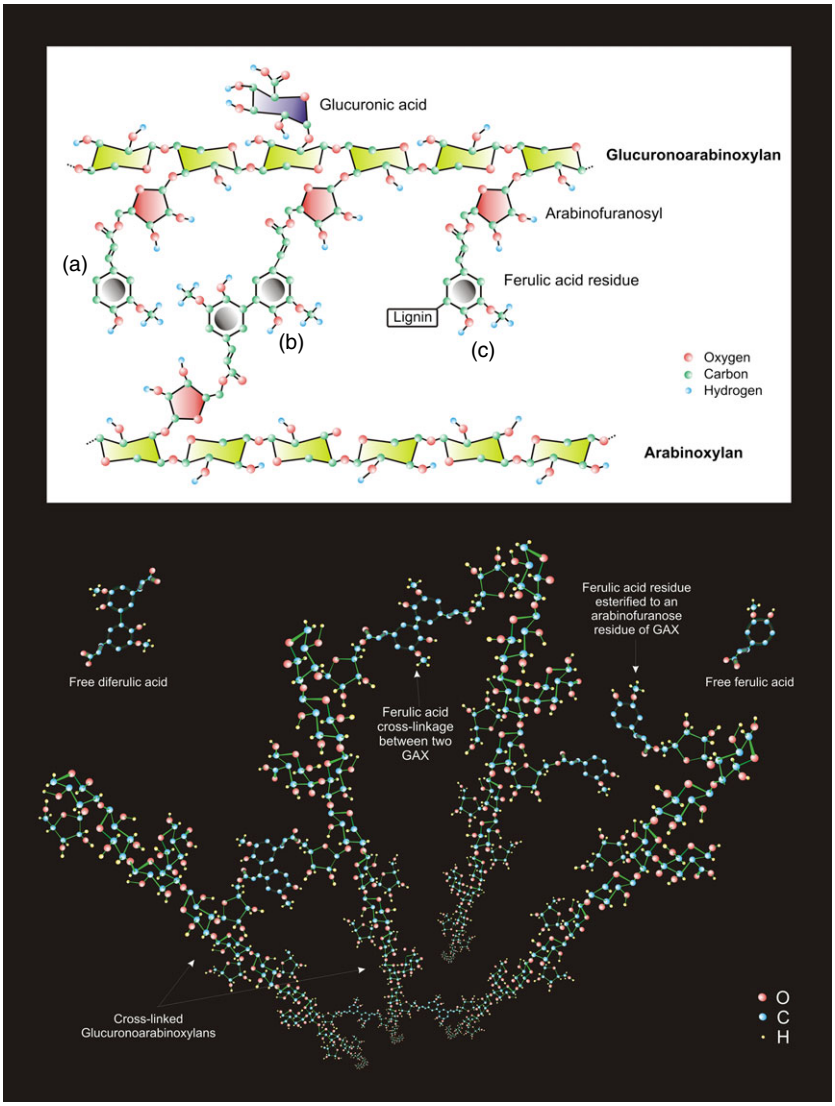

Figure 1 Above (white background): Feruloylated glucuronoarabinoxylan (FA-GAX). (a) ferulic acid residue esterified to the arabinofuranosyl residue of GAX; (b) diferulic acid cross-linking two FA-GAX; (c) ferulic acid residue anchoring lignin to the GAX. Below (black background): Perspective view of the cross-linkages among vicinal FA-GAX.

weight. Specific kinds of polysaccharides and the monomeric composition vary in different plant groups (Boerjan *et al.*, 2003; Buckeridge *et al.*, 2010). The main component of cell wall is cellulose, a homopolysaccharide consisting of a long and linear chain of β(1→4) linked glucose units. Each cellulose molecule is tightly bound to other molecules by means of multiple hydrogen bonds producing insoluble, rigid and crystalline microfibrils (Carpita and McCann, 2000). These cellulose fibres are impeded to collapse with each other by hemicelluloses. The richer topology of these frequently branched hemicelluloses allows them to link only occasionally with cellulose microfibrils. Intermittent pattern of free and bound regions from hemicellulose with cellulose results in the cross-linking of microfibrils (Carpita, 1996). The network of cellulose and hemicellulose molecules is still embedded in a matrix of pectin (Scheller and Ulvskov, 2010). These very complex heteropolysaccharides are branched with acidic sugars as glucuronic and galacturonic acids. They adsorb high amounts of water forming a gel which is involved in cell–cell adhesion, pore sizing, pH control and cation trapping (Buckeridge *et al.*, 2010).

Primary cell walls are classified into types I and II. The majority of dicot plants share a similar type I cell wall composition and architecture. Type I is found in dicots noncommelinoid angiosperms (e.g. aroids, alismatids and liliods) and gymnosperms. The main hemicellulose of type I cell walls is xyloglucan, which is composed by a glucose backbone branched with xylose which in turn may be substituted with galactose and fucose (Carpita and Gibeaut, 1993).

In commelinoid monocotyledons, a group of angiosperms including grass, palms, bromeliads and ginger have a type II cell wall, which contrasts with type I cell wall of other angiosperms (Carpita and Gibeaut, 1993; Vogel, 2008). A relevant characteristic of type II cell walls is the abundance of glucuronoarabinoxylan (GAX). It is composed by a core chain of xylan branched with arabinose and glucuronic acid (Dodd and Cann, 2009). The arabinose residues can be further esterified with feruloyl and *p*-coumaroyl residues (Figure 1). Produced in the phenylpropanoid pathway together with monolignols, feruloyl esterified to polysaccharides can oxidatively polymerize to produce dehydrodimer and oligomers that cross-link vicinal GAX. In commelinoids, FA-branched GAX (FA-GAX) is abundant in primary cell walls. This polysaccharide is believed to occur only in these species and performs a set of distinguished structural and physiological roles in their cell walls (Carpita *et al.*, 2001). In addition, Poales type II cell walls contain significant quantities of mixed-linkage glucans (β-glucans) present exclusively in this order (De Souza *et al.*, 2013; Smith and Harris, 1999).

In spite of being a rich source of energy itself, cell wall is a frontline cell defence against micro-organisms (Grabber *et al.*, 1998a). To get access to the energetic content of lignocellulosic biomass, micro-organisms must make use of catalytic tools to break the lignin–carbohydrate complex and cellulose crystallinity (Wyman, 1994). Only then, polysaccharides become available to hydrolysis and monosaccharide fermentation. Lignin is one of the hardiest cell wall barriers to overcome. Beyond being hard to digest, lignin fractions adsorb hydrolytic proteins reducing the access of enzymes to the polysaccharides (Huang *et al.*, 2011). In mature cells, lignin forms a highly hydrophobic matrix of C-C and C-O-C linked phenylpropanoids, mainly coniferyl, sinapyl and *p*-coumaroyl alcohols. Respective residues in lignin are dubbed guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) units. The amount of lignin as well as its monomeric composition is ontogenetic-tissue-species dependent. Lignin is deposited in secondary cell walls of schlerenchymatic tissues in fibre and vessels. It confers high hydrophobicity and mechanical resistance to cell walls required by xylem vessels to perform the capillary transport of water and for fibres support the massive habit of trees (Salvador *et al.*, 2013; Pedersen *et al.*, 2005; Zobiole *et al.*, 2010). In lignified secondary cell walls, the FA ester linked to GAX are nucleation sites for lignin polymerization through ether bounds, anchoring lignin to polysaccharide moiety (Carpita *et al.*, 2001; Renger and Steinhart, 2000).

Ferulic acid function, biosynthesis and cell wall esterification

Ferulic acid [(E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid] (Figure 2) was first isolated from *Ferula foetida* (devil's dung) in 1866 (Fazary and Ju, 2007) being more abundant in tissues as epidermis, xylem and sclerenchyma. Since its early discovery, FA has also been reported to exhibit a wide range of important biological (dos Santos *et al.*, 2008a) and therapeutic properties (Paiva *et al.*, 2013). These include anti-inflammatory, antibacterial, antidiabetic, anticarcinogenic, antiageing, and neuroprotective effects, which can be attributed to its antioxidant capacity (Paiva *et al.*, 2013; dos Santos *et al.*, 2008b). FA is highly abundant in the cell walls of all commelinoid orders: Arecales (palms), Commenlinales (water hyacinth), Poales (grasses) and Zingiberales (banana, ginger) (Smith and Harris, 2000). It is present in much smaller quantities in cell walls of most (probably all) dicots such as Caryophyllales (cactus) (Hartley and Harris, 1981), Solanales (tomato) (Keller *et al.*, 1996), Brassicaceae (*Arabidopsis*) (Chen *et al.*, 1998) and Apiales (Parsley and devil's dung) (Parr *et al.*, 1997). In the Chenopodioideae and other dicots, FA is found to be ester-linked to pectin (Ishii, 1997; dos Santos *et al.*, 2008b).

Although type II cell walls only are found in few orders clustered as commelinoid, these include all cereal crops and perennial grasses (Carpita and McCann, 2000) responding for most of the biomass produced in agriculture (dos Santos *et al.*, 2011). In type II cell walls, GAX intermittently interact with cellulose through hydrogen linkages cross-linking microfibrils (Lee *et al.*, 2008) which contributes with up to 40% of dry weight (Scheller and Ulvskov, 2010). Xyloglucans, pectins and structural proteins are minor compounds, while FA and *p*-coumaric acid abound. Ferulic acid is ester-linked to *O*-5 position of arabinose residue of GAX (Ishii, 1997).

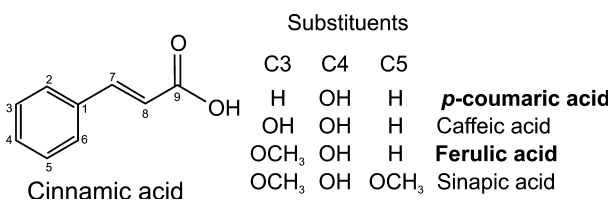

Figure 2 Chemical structure of cinnamic acid and substituents.

More recent researches on the phenylpropanoid pathway have shown that the traditional view of lignin biosynthesis is incorrect (Humphreys and Chapple, 2002). Although the hydroxylation and methylation reactions of the metabolic pathway were long thought to occur at the level of the free hydroxycinnamic acids, it now seems clear that the enzymes catalysing phenylpropanoid 3-hydroxylation and 3-*O*-methylation reactions use shikimate and coenzyme A (CoA) conjugates as substrates (Franke *et al.*, 2002a, 2002b; Guo *et al.*, 2001; Parvathi *et al.*, 2001; Schoch *et al.*, 2001; Sullivan and Zarnowski, 2010; Zhong *et al.*, 1998). Similarly, it is not completely elucidated how FA is synthesized in plants. Although a supposed free acid pathway has been suggested (Buanafina, 2009), the following evidences indicate that the synthesis of FA from *p*-coumaric acid seems unlikely *in vivo* (Figure 3): (i) The enzyme *p*-coumaroyl shikimate/quinate 3-hydroxylase (C3′H) does not directly hydroxylate *p*-coumaric acid to caffeic acid, but rather act on *p*-coumaroyl ester derivatives (Schoch *et al.*, 2006). The preferred substrate for C3H appears to be *p*-coumaroyl-shikimate, although it can hydroxylate other *p*-coumaroyl esters (Franke *et al.*, 2002; Schoch *et al.*, 2001). (ii). The 3-*O*-methyltransferase (COMT) has a clear preference for 5-hydroxyferulic acid than for caffeic acid (Louie *et al.*, 2010). In addition, down-regulation of COMT activity in transgenic alfalfa, maize and poplar plants produces significant effect on S lignin, but little effect on G lignin (see Louie *et al.*, 2010 and references therein). Thus, the current knowledge suggests that the mainstream phenylpropanoid is better described as illustrated in Figure 3. L-Phenylalanine is deaminated by phenylalanine ammonia-lyase (PAL) to produce *t*-cinnamic acid. This step is followed by hydroxylation of the aromatic ring, catalysed by cinnamate 4-hydroxylase (C4H), to give *p*-coumaric acid, the first phenolic compound produced in the pathway. The next step is the activation of the acid to a thioester via 4-coumarate:CoA ligase (4CL) to yield *p*-coumaroyl-CoA. This compound is transesterified to shikimate or quinate by action of *p*-hydroxycinnamoyl CoA:quinate/shikimate *p*-hydroxycinnamoyl-transferase (HCT). The ester is further hydroxylated in the C3 to produce caffeoyl-shikimate/quinate ester by *p*-coumaroyl shikimate/quinate 3-hydroxylase (C3′H). Caffeoyl-shikimate/quinate is transesterified back with CoA by HCT and *O*-methylated in the hydroxyl group in C3 by caffeoyl-CoA *O*-methyltransferase (CCoAOMT) to produce feruloyl-CoA, the activated form of FA (Chen *et al.*, 2000; Zhong *et al.*, 1998, 2000). Despite the potential relevance of CCoAOMT to feruloylation in grasses, there is no information about the effects of CCoAOMT down-regulation to feruloylation, lignification or digestibility in grasses. An additional pathway to produce FA was revealed by Nair and collaborators in 2004. The synthesis of monolignols uses FA-CoA as intermediate to produce coniferaldehyde in a reduction catalysed by cinnamoyl-CoA reductase (CCR). The aldehyde can be oxidized directly to FA by action of coniferyl aldehyde dehydrogenase (CALDH). The free form produced by CALDH can be directly exported to the cell wall where FA acts as a strong antioxidant and an UV protector. However, in order to be

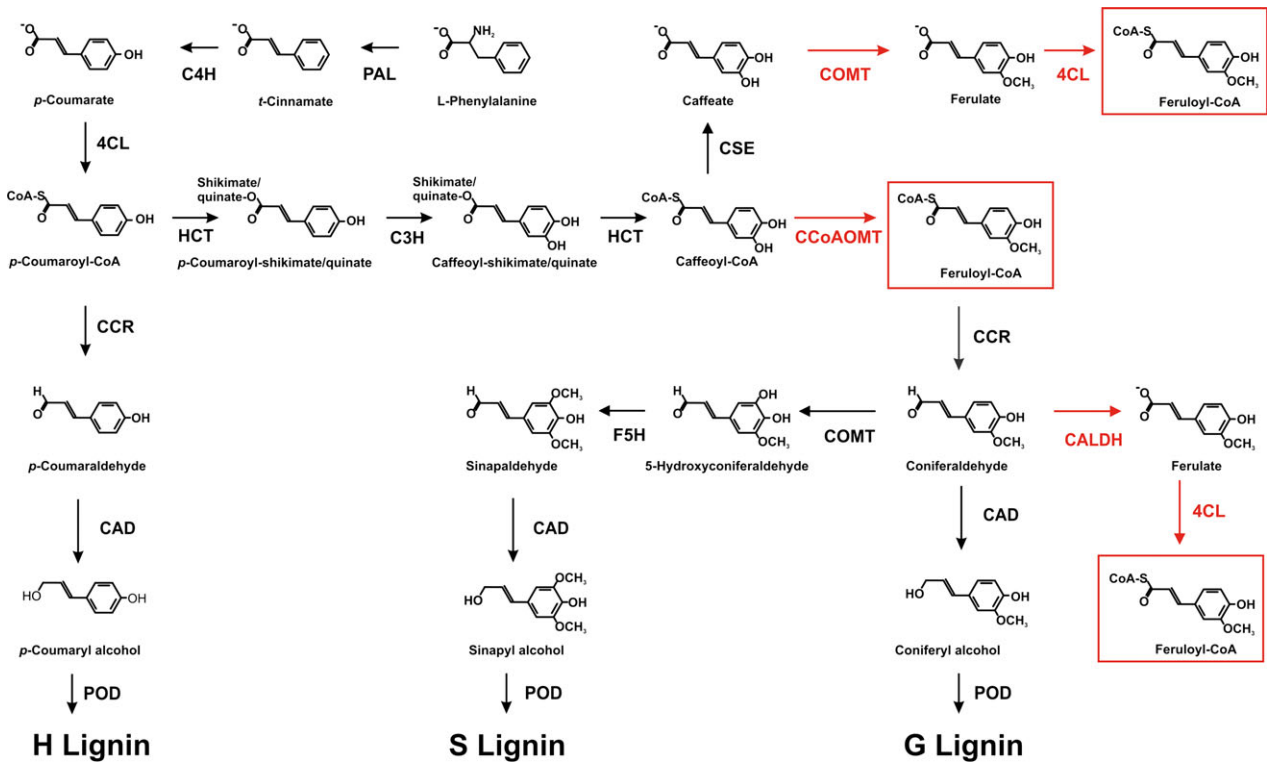

Figure 3 Imbrications between biosynthesis of monolignols and ferulic acid (as well as its coenzyme A-activated derivative). Steps in blue shadow are the mainstream for monolignol biosynthesis. Red arrows draw attention to enzymatic steps that produce ferulic acid (black squares) and feruloyl-CoA (red squares). PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, *p*-coumarate:CoA ligase; HCT, *p*-hydroxycinnamoyl-CoA:shikimate/quinate *p*-hydroxycinnamoyl transferase; C3′H, *p*-coumaroyl shikimate/quinate 3-hydroxylase; CCoAOMT, caffeoyl-CoA 3 *O* methyltransferase; CCR, cinnamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase; F5H, ferulate 5-hydroxylase; COMT, caffeic acid 3-*O*-methyltransferase; CALDH, coniferaldehyde dehydrogenase; POD, peroxidase. H, *p*-hydroxyphenyl; G, guaiacyl; S, syringyl.

esterified to GAX, the free form of FA must be first activated to its active form FA-CoA (Guo *et al.*, 2001; Schmitt *et al.*, 1991). The enzyme 4CL has been demonstrated to be responsible for catalysing the esterification of exogenous-free FA to CoA *in vivo*. In soybean, the 4CL reaction has been suggested as entrance point into the pathway for exogenously supplied FA, which contributed for monolignol biosynthesis (G lignin) and esterification of FA residues into cell wall polysaccharides (dos Santos *et al.*, 2008b). At this time, the possibility that a portion of the FA synthesized in grasses is also generated by other enzyme pathways cannot be excluded. At least in specific situations, it has been shown that thioesterases as HCT break ester linkages between hydroxycinnamic acids and shikimate, quinate or CoA (Hoffmann *et al.*, 2003).

There are little evidences about the mechanism by which FA is esterified into GAX. The simplest mechanism proposed is that GAX may be synthesized in the Golgi apparatus and then feruloylated by feruloyl transferases using FA-CoA transported from cytosol (Buanafina, 2009; Molinari *et al.*, 2013). However, a more complex mechanism is also possible. The reasoning behind the second mechanism is putative involvement of *BAHD acyl-CoA transferase* genes in feruloylation of glucuronoarabinoxylans (GAX) as proposed by Mitchell *et al.* (2007), based on a bioinformatics approach. Expressed sequence tags (ESTs) from grasses and dicots were compared to identify clades where genes were much more highly expressed in grasses than their homologs in dicots. One of the clades showing the greatest bias was within the *BAHD acyl-CoA transferase* gene family (carrying the PFAM PF02458 domain). St-Pierre and Luca (2000) named the *BAHD acyl-CoA transferase* family after the first four characterized members: (i) benzylalcohol *O*-acetyl transferase from *Clarkia breweri* (BEAT); (ii) anthocyanin *O*-hydroxycinnamoyl transferases from *Petunia*, *Senecio*, *Gentiana*, *Perilla* and *Lavandula* (AHCTs); (iii) anthranilate *N*-hydroxycinnamoyl/benzoyltransferase from *Dianthus caryophyllus* (HCBT); and (iv) deacetylvindoline 4-*O*-acetyltransferase from *Catharanthus roseus* (DAT) (D'Auria, 2006; Mitchell *et al.*, 2007; Tuominen *et al.*, 2011).

Experimental support for the involvement in GAX feruloylation was obtained from transgenic rice where the candidate genes were suppressed by RNAi, resulting in significant decreases in cell wall ferulate (Piston *et al.*, 2010). In another study, rice lines where one *BAHD* gene in the clade was up-regulated increased the amount of *p*-coumaroyl ester-linked to GAX (Bartley *et al.*, 2013). Taken together, these studies provide a strong circumstantial support for the hypothesis that *BAHD* genes are responsible for GAX feruloylation, although definitive evidence is still lacking. As *BAHD* proteins can be localized in the cytosol, another mechanism has been suggested to explain feruloylation as shown in Figure 4 (Molinari *et al.*, 2013). The precursor of GAX UDP-arabinose would be feruloylated to form FA-Ara-UDP which then enters into the Golgi apparatus. After, a glycosyl transferase introduces the FA-Ara into the nascent GAX chains (Figure 4). However, a rice *xax1* mutant had been identified to be unable to produce a specific Ara substitution in xylan. The mutant presented an unexpected decrease in feruloyl and coumaroyl esters content. No decrease in feruloyl esters would be expected

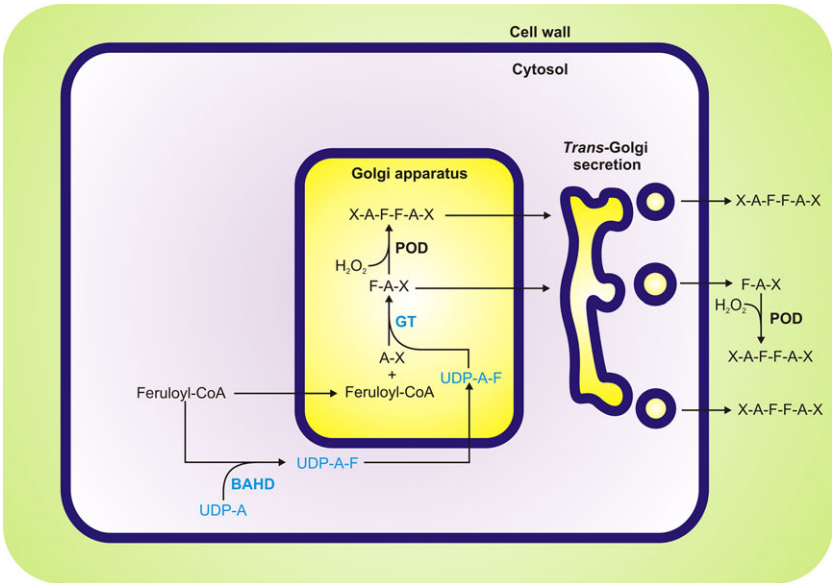

Figure 4 Blue text shows possible pathways for feruloylation based on cytosolic location of BAHD proteins implicated in feruloylation. A, arabinosyl; F, feruloyl group; F-A-X, feruloylated arabinoxylan; GT, putative glycosyl transferase; POD, peroxidase; UDP-A, UDP-arabinofuranose; X, xylan polymer. Below: Schematic representation of FA-GAX and FA-GAX cross-linked by dehydrodiferulic acid (Buanafina, 2009; Nair *et al.*, 2004; dos Santos *et al.*, 2008a; Umezawa, 2010).

if the substrate for feruloylation was UDP-feruloyl-arabinose (Chiniquy *et al.*, 2012).

The last steps in both mechanisms occur in the Golgi apparatus, and the feruloylated glucuronoarabinoxylan (FA-GAX) is exported to apoplast by exocytosis (Anders *et al.*, 2012; Buanafina, 2009; Burton and Fincher, 2012). The involvement of esterification models involving transport of FA from RE pathway has been partially discarded. [^{14}C]Ferulated polysaccharides were observed both in protoplasm and apoplast when wheat roots were incubated with *trans*-[U-^{14}C]cinnamic acid or L-[1-^{14}C]arabinose. The appearance of [^{14}C]ferulated polysaccharides occurred even in the presence of the lactone antibiotic brefeldin A, which is used to suppress the transport between RE and Golgi apparatus (Mastrangelo *et al.*, 2009).

It is well established that oxidative coupling of polysaccharides through ester-linked feruloyl groups might occur both within the protoplast in the Golgi apparatus (Mastrangelo *et al.*, 2009; dos Santos *et al.*, 2008b; Umezawa, 2009), and after their secretion into the apoplast. This process is catalysed by multiple cell wall bounds and putative Golgi resident isoperoxidases, which use hydrogen peroxide as substrate in a mechanism similar to that of lignin polymerization (Fry, 1986; Hatfield *et al.*, 1999; Lindsay and Fry, 2008).

The apoplastic polymerization among FA residues esterified to GAX has been related with cessation of cell wall growth and defence-related mechanisms. Ferulic acid residues couple when they are oxidatively activated by radicals produced by peroxidases or external oxidizing agents, such as chemical oxidants or UV radiation (Mastrangelo *et al.*, 2009; dos Santos *et al.*, 2008b; Umezawa, 2010).

The level of FA in the cell wall is related to biomass recalcitrance

Cross-linking of grass cell wall components, especially through FA and dehydrodimer diferulate esterified to GAX, affects many cell wall properties, such as adherence, extensibility, accessibility and biodegradability (Bunzel, 2010; Ralph *et al.*, 1998). Besides its relevance in plant physiology and crop protection, these properties are of great interest in food and agricultural chemistry, food technology and nutritional sciences (Barros-Rios *et al.*, 2012). An additional consequence of FA dehydrodimer cross-linkages of GAX is the reduction of digestibility of cell wall polysaccharides by polysaccharidases, a major limitation for conversion of biomass in ethanol (Chen and Dixon, 2007; Grabber *et al.*, 2009; Jung *et al.*, 2013). Several *in vitro* enzyme digestion assays suggest that cell wall digestibility and FA level are negatively correlated (Grabber *et al.*, 1998a,b; Iiyama *et al.*, 1994; Kamisaka *et al.*, 1990). FA dehydrodimer cross-linkages can reinforce the cell wall against action of cellulases, xylanases, pectinases (Akin *et al.*, 1993; Damásio *et al.*, 2012; Wojtaszek, 1997) and laccases from pathogens (Arora and Sharma, 2009; Sterjiades *et al.*, 1993). In sugarcane bagasse, approximately 50% of the FA content is ester-linked to hemicelluloses (Harris and Trethewey, 2010; Xu *et al.*, 2005), and all ether and dehydrodimer cross-links involve ester links that can be cleaved by alkalis and feruloyl esterases (Fazary and Ju, 2007; Harris and Trethewey, 2010).

Enhancement of enzymatic digestibility using feruloyl esterases

Synergy between cellulases, hemicellulases and accessory enzymes with carboxylic ester hydrolases potentiates the saccharification process. Among the carboxylic ester hydrolases, the feruloyl esterases deserves special attention (Wong, 2006). The term feruloyl esterase denotes a group with several close-related enzymes able to catalyse the cleavage of ester linkage between hydroxycinnamoyl substrates and polysaccharides. Due to their varied degree of substrate specificity, they can be named as feruloyl esterase, cinnamoyl hydrolases, *p*-coumaroyl esterases or hydroxycinnamoyl esterases (Koseki and Fushinobu, 2009).

It has been demonstrated that bacteria and fungi secrete feruloyl esterases to hydrolyse the ester bond between FA and polysaccharides (Topakas *et al.*, 2007; Wong, 2006). Feruloyl esterases are able to release phenolic acids and its bio-products from biomass and are important tools for understanding structural differences and pattern of FA esterification in plant biomass (Wong *et al.*, 2013; Zhang *et al.*, 2013). Damásio *et al.* (2012) demonstrated that FA and *p*-coumaric acid can be enzymatically extracted from sugarcane bagasse using a feruloyl esterase from

Aspergillus clavatus. In pulp and paper process, application of feruloyl esterase contributes to water removal from pulp (Record *et al.*, 2003; Sigoillot *et al.*, 2008).

Several studies have demonstrated that feruloyl esterases show synergy with xylanases, cellulases, pectinases and accessory enzymes in the degradation of cell wall (Faulds *et al.*, 2006; Selig *et al.*, 2008; Wong *et al.*, 2013). Gottschalk *et al.* (2010) reported a synergic action between cellulase, xylanase, β-glucosidase and feruloyl esterase from *Trichoderma reesei* and *Aspergillus awamori* in the hydrolysis of sugarcane bagasse. The capacity of feruloyl esterase to hydrolyse ester bonds between carbohydrates residues and phenolic compounds is thought to enhance the access of hydrolases to the polysaccharides (Dyk and Pletschke, 2012; Faulds *et al.*, 2006; Wong, 2006). This synergy reduces the amount of enzyme necessary to achieve saccharification and the bioethanol production costs from lignocellulose biomass (Tabka *et al.*, 2006).

Digestion enhancement by down-regulation of enzymes from phenylpropanoid pathway

Li *et al.* (2008) reported that different studies have obtained different findings on the impact of down-regulation of certain gene on lignin content and digestibility. Some studies suggest that down-regulation of genes early in the phenylpropanoid pathway, such as PAL, C4H, HCT and C3′H, reduced strongly lignin content and biomass (Chen and Dixon, 2007; Li *et al.*, 2008; Poovaiah *et al.*, 2014), while down-regulation of F5H or COMT reduced the lignin S/G ratio, but has a smaller effect on lignin (Li *et al.*, 2008) or digestibility (Reddy *et al.*, 2005). Chen and Dixon (2007) investigated six alfalfa lines independently down-regulated for C4H, HCT, C3′H, F5H, CCoAOMT or COMT. Alfalfa lines suppressed in C4H, HCT and C3′H showed the lowest lignin level (<50%) with enzymatic saccharification efficiencies that were almost double that the controls. On the other hand, lines suppressed in COMT, CCoAOMT and F5H produced little effect on lignin content and digestibility efficiencies. In contrast with data produced by Chen and Dixon (2007), in alfalfa, Zhong *et al.* (2000) found that poplar transgenic lines with up 80% reduction in CCoAOMT presented up to 40% reduction in lignin content.

Yet, down-regulation of COMT in maize resulted in biochemical alterations of lignin content and increased cell wall digestibility (Piquemal *et al.*, 2002). Suppression of COMT by RNA interference also decreased biomass recalcitrance up to 23%, without reducing the production of sugarcane biomass (Jung *et al.*, 2013). The down-regulation reduced up to 6% the lignin content; however, it was not able to reduce content of FA in the cell wall.

Down-regulation of enzymes from phenylpropanoid pathway may affect feruloylation, but they are known to produce effects on lignin content, digestibility and in plant growth and development (Boerjan *et al.*, 2003; Li *et al.*, 2008; Verma and Dwivedi, 2014). Jung and Phillips (2010) have identified and demonstrated a putative mutation in maize seedling that reduces the content of ferulate ester and ether cross-linking in the cell wall and increases the biomass digestibility without affecting plant growth and yield.

The enzyme CCoAOMT catalyses the synthesis of FA-CoA, an intermediate of monolignols biosynthesis, but also, the active for required for polysaccharide feruloylation. The probable participation of CCoAOMT on feruloylation makes it an interesting target for down-regulation in grasses to improve saccharification. Nair *et al.* (2004) identified a gene in *Arabidopsis thaliana* mutant that encodes a functional aldehyde dehydrogenase (ALDH). It oxidizes both sinapaldehyde and coniferaldehyde to sinapic acid and FA, respectively. The authors described other plant species with ability to synthesize FA from coniferaldehyde in dicots (*A. thaliana*, *Nicotiana tabacum* and *Raphanus sativus*), monocots (*Zea mays*), gymnosperm (*Pinus strobus*) and pteridophyte (*Ceratopteris richardii*). The 4CL has demonstrated to be active *in vivo* on exogenous ferulic acid, improving both polysaccharide feruloylation and G lignin (dos Santos *et al.*, 2008a). So, 4CL is a plausible candidate to perform activation of FA to FA-CoA. If so, CALDH can be another interesting target to gene suppression in order to control feruloylation and biomass digestibility.

Conclusions

Herein, we reviewed the role of FA in the plant cell wall architecture and physiology. A better understanding of FA metabolism, its esterification to the cell wall polysaccharides and the controlled coupling mechanisms will contribute to our understanding on plant physiology, but also to develop new technologies to control cell the wall properties. Such know-how will impact in several areas, from improvement of cattle feed and organoleptic properties of vegetables to production of paper and cellulosic ethanol, a technology that promises to revolutionize the way civilization to obtain energy.

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