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REVIEW

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Suberin and hemicellulose in sugarcane cell wall architecture and crop digestibility: A biotechnological perspective

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Abstract

Sugarcane is a highly efficient biomass producer used in the last decades for bioethanol and bioelectricity production, as well as for animal feeding. Together with lignin, suberin is a major factor for the low sugarcane biomass digestibility by ruminants. The lipid–phenolic biomolecular composition and the ultrastructure of suberin and associated waxes confer them extraordinary properties of hydrophobicity, flexibility, and anti-microbial resistance, responsible for the low digestibility of suberized tissues. Additionally, hemicelluloses cross-linked with suberin and lignin also contribute appreciably to cell wall recalcitrance. In this review, a main focus was given to suberin and secondly to hemicellulose and how they may interfere with ruminant digestibility. Suberin and hemicellulose deposition and biomolecular composition are genetically regulated, showing a close regulatory interplay with phenylpropanoid and fatty acid biosynthesis pathways, cutin disruption, and stress and defense responses. Understanding the bulk of transcription factors network and hormonal regulatory mechanisms will allow accurate biotechnological approaches to the production of more feasible forage sugarcane.

KEYWORDS

cell wall architecture, digestibility, forage sugarcane, functional genomics, grass biomass, hemicellulose, suberin

1 | INTRODUCTION

Grasses are a very large and diverse family of plants that include several of the most relevant agronomic crops, constituting an essential source of feed and food feedstock and, in recent decades, also the main source of lignocellulosic biomass for bioethanol generation. The vegetative tissues of grass forage crops, together with leguminous, are the fundamental fraction of feedstock for animal production. An important source of carbon for animal nutrition consists precisely in the wide network of polysaccharides biopolymers, such as cellulose and hemicelluloses, structural to the cell walls of forage crops. However, with the breeding and genetic improvement of livestock for meat and milk production, the ability of forage crops to supply a wide range of animals' requirements for energy and nutrients is no longer feasible. The low performance of forage crops as feeding supply for high-producing livestock is due mainly to two factors: the low digestibility and the high proportion of cell walls, that together, difficult the intake of forage crops by the ruminants and therefore the plant nutrients availability (Jung & Allen, 1995). In an artificial model system using maize cell suspensions, it was observed that induced lignification of primary cell walls delayed and decreased

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hemicellulose and cellulose fermentation by bovine rumen microflora (Grabber et al., 2008). This study also demonstrated that the proportion of ferulate–lignin cross-linking had a concomitant inhibitory effect on cell wall digestibility, but the same was not observed for varying ratios of lignin monolignols that did not alter the rate of maize cell wall carbohydrates fermentation (Grabber et al., 2008). However, the influence of monolignol composition on biomass digestibility was observed in other studies (Dien et al., 2009; Jung, Fouad, Vermerris, Gallo, & Altpeter, 2012). In sugarcane, the cell wall digestibility was correlated with chemical composition of the stem tissue of more than 30 sugarcane hybrids (*Saccharum* spp.) and it was also demonstrated that the high lignin content of the cell wall corresponded to lower digestibility, a parameter equally influenced, but to a lesser extent, by the amount of etherified hydroxycinnamic acids ferulic acid and *p*-coumaric acid (Chong, Bonnett, & O'Shea, 2016; Daniel et al., 2016). As also observed by Chong et al. (2016), cell wall digestibility of 34 different sugarcane hybrids was not correlated with the relative amount of lignin monolignols monomers but with total lignin content. Plant breeding and biotechnological manipulation of cell wall architecture in forage crops have focused in manipulating the cell wall biopolymers themselves to increase their digestibility but also in the reduction of the proportion of recalcitrant cell wall biopolymers, such as lignin, and the overall cell wall extent, specifically in grass crops, in order to improve grass forage intake and nutrient concentration (Jung & Allen, 1995; Wang et al., 2016).

Sugarcane is a grass crop of great economic interest in the production of sugar and also in bioethanol and electricity generation (Bottcher et al., 2013; Dantas, Legey, & Mazzone, 2013; Vermerris, 2011), with an area harvested of near 26 millions ha worldwide in 2017 (FAOSTAT, 2017). This crop is a particularly efficient producer of biomass. In 2017, it was registered a world average yield of roughly 71 t/ha and a global production of 1841 millions tons (FAOSTAT, 2017). Nowadays, sugarcane is under strong biotechnological development due to its potential for lignocellulosic ethanol generation. Forage sugarcane exemplifies a high biomass producer crop with low nitrogen requirements and easily cultivated during dry winters of subtropical regions, being used for years in countries like Brazil as supplementary feeding forage for livestock and constituting an important green forage supply during winter (Anjos, Silva, & Campana, 2008). In sugarcane, the stem forms much of the forage content. On the other side, tropical climates usually allow forages to grow the entire year meaning that sugarcane availability for feeding forage consists actually of mature stems, which due to their high content in lignin present low digestibility and reduced physical disruption during intake by ruminants, and thus may have an inferior nutritive quality.

2 | PLANT BIOMASS DIGESTIBILITY—CONCEPT

Plants are characterized by an elaborated and multiscale set of chemical and physical barriers that hamper the degradation and/or disintegration of their biomass caused by abiotic agents or by microorganisms such as pathogens, soil-decomposing microbes, and the herbivore digestive tract microflora. Plant biomass digestibility consists in the hydrolysis of cell wall polysaccharides by microbial cellulolytic enzymes, which efficiency depends largely on the complex chemical interaction of the cell wall biopolymers matrix with the cellulose fibrils (Ding et al., 2012). Cell wall digestibility is a complex and quantitative trait influenced by several causes, namely lignin and suberin content and cross-linking to hemicelluloses, porosity, surface area, lignin monomer ratio, and cellulose crystallinity and degree of polymerization that difficult cellulolytic enzymes access to cell wall polysaccharides (Pu, Hu, Huang, Davison, & Ragauskas, 2013). Saccharification yield refers to the amount of monomeric sugars released by the enzymatic hydrolysis of the cell wall polysaccharides. On the other hand, biomass recalcitrance refers to the resistance of the plant cell walls to be dismantled to monomeric sugars and results from the broaden set of ultrastructural and biomolecular features of the plant material, the hydrolytic properties of the catalysts agents used to break down the cell wall, and the interactions established between these hydrolytic catalysts and the plant biomass (Ding et al., 2012). The concept of biomass recalcitrance has been centered in the plant material resistance to hydrolysis by fermentative microbial enzymes; however, diverse chemical catalysts and heat, or combination of these catalysts, have been equally used in the context of industrial conversion pathway of lignocellulosic biomass for biofuels generation (McCann & Carpita, 2015).

3 | THE CELL WALL AS THE MAIN RECALCITRANCE FACTOR

The plant cell wall digestibility of forage plants is a cornerstone factor for animal productivity. The plant cell wall is a layered structure of different intricate matrices of biopolymers that juxtapose gradually during cell growth and subsequent cell maturation. The primary cell wall forms during cell elongation and is composed mainly by the polysaccharide biopolymers cellulose, hemicelluloses and pectins, and also proteins and phenolic acids, but no lignin. Some specialized cells, particularly bundle sheaths and fibers surrounding vascular bundles in stems and leaf blades of C4 grass crops, develop internally to the primary cell wall a suberin lamella of varying thickness. After cell elongation finishes, secondary cell wall expands gradually toward the cell lumen.

Cellulose is more abundant than hemicelluloses in the polysaccharide matrix of the secondary cell wall and pectins are little or not present. Secondary cell walls are strongly lignified. The microflora of the reticulorumen compartment of ruminants is able to hydrolyze cellulose and hemicelluloses from plant cell walls, releasing sugars that are easily absorbed as nutrients (Jung & Allen, 1995). However, the other two biopolymers of plant cell walls, lignin and suberin, are poorly digested by the reticulorumen microflora and limit the access of the ruminants microorganisms to the cell wall polysaccharide matrix, compromising forage crops digestibility (Wilson & Mertens, 1995). Noteworthy, the lignin biopolymer starts to be incorporated in the beginning of the secondary cell wall formation in a specific spatial pattern. Lignin deposition initiates in the middle lamella and is orientated inward through the primary and secondary walls toward the lumen of the cell, implicating that the polysaccharides recently deposited in the lumen side of the secondary cell wall are not lignified. The cellulose fibrils and hemicelluloses from the lumen side of cell walls, exposed by the thorough chewing of the ruminants, are exactly the starting point for reticulorumen microflora degradation of forage biomass. Curiously, in grass forage crops, the main limitation to the digestion of secondary cell walls is mainly due to the increasing inaccessibility by the reticulorumen microflora to the lumen surface of the cell wall. There are two major factors contributing to counteract the microflora access to the secondary wall. On one side, the lignified and very poorly digested primary wall/middle lamella structure is the key factor of cell wall recalcitrance by making difficult the disruption of forage tissues and cells by animal chewing (Wilson & Hatfield, 1997). On the other, in certain types of cells such as the sclerenchyma fibers and the bundle sheath surrounding vascular bundles, the bulk of the lumen is occupied by the secondary wall thickening, leaving little surface area exposed to degradation by reticulorumen microflora. Besides, in grass forage crops the primary and secondary wall lignification and secondary wall thickening is generalized to practically all cell types in vegetative tissues (Wilson & Hatfield, 1997).

The presence of a suberin layer in the primary cell walls of forage crops, as C4 subtropical grass crops, represents an extra barrier to cell wall digestibility. The analysis of bovine reticulorumen-digested leaf blade samples of different C4 grass crops by transmission electron microscopy (TEM) revealed that the presence of a suberin lamellae in bundle sheath parenchyma cell walls has refrained the degradation of these cells (as low as 25% after 128 hr incubation) and has avoided the hydrolysis of the starch granules localized within. TEM examination also demonstrated a lower colonization of the bundle sheath cell walls of C4 grass leaf blade by the reticulorumen microflora (Wilson & Hattersley, 1983). Like lignification and secondary wall thickening, in C4 grass crops such as sugarcane, suberization is widespread to the majority of cell walls in the stems (Table 1; Rae, Perroux, & Grof, 2005).

Other important causal agents of cell wall recalcitrance are the polysaccharides biopolymers themselves, particularly hemicelluloses (Table 1). Hemicelluloses act as support for cellulose microfibrils, providing robustness and strength to the cell wall. Sugarcane hemicellulose is composed of arabinoxylan (40%), xyloglucan (8%), mixed-linkage β-glucans (10%), and mannan traces (de Souza, Leite, Pattathil, Hahn, & Buckeridge, 2013), forming a second domain in the sugarcane cell wall (Buckeridge, dos Santos, Tiné, & de Souza, 2016). These polysaccharides have a complex branching pattern and may interact with each other as well as with the cellulose domain. Xylan, the second most abundant polysaccharide in the cell wall, is composed of a β-1,4 linear chain bound to xylosyl residues that are frequently replaced by sugar chains, such as glucuronic acid (GlcA) and methylglucuronic acid (MeGlcA; Buckeridge et al., 2016). The level of oligosaccharide branch organization of hemicelluloses and the ratio of acetylation, methylation, and feruloylation restrict the hydrolysis of the main chain by cellulolytic enzymes. This restriction is even stronger at regions of interaction between polysaccharides polymers or with others cell wall biopolymers (Pauly et al., 2013). Furthermore, during plant development occurs an increase in the concentration of phenolics in the cell wall that is responsible for the cross-linking of lignin and suberin biopolymers with the polysaccharide matrix, strengthening the overall structure of the cell wall and rendering it even less digestible (Jung & Allen, 1995). Ferulic acid and *p*-coumaric acid have a carboxylic group at the end of the propenyl group that establishes an ester bond with arabinoxylans hemicelluloses and also with lignin monolignols and suberin fatty acids (de Oliveira et al., 2015; Graça et al., 2015). By forming hydroxycinnamates ester dimers, or higher complexity oligomers, ferulic acid reinforces the cross-link inside the carbohydrate fraction of the cell wall, as well as, within suberin lamella. Additionally, hydroxycinnamic acids form covalent ether bonds with lignin monolignols and cell wall proteins, increasing the mechanical resistance and the recalcitrance of the overall cell wall (de Oliveira et al., 2015; Ralph, 2010). In the cell wall of sugarcane, as other taller grasses with large diameter stem, such as corn and sorghum, the more abundant hydroxycinnamic acid is the *p*-coumaric acid and not ferulic acid (Costa, Masarin, Bonifácio, Milagres, & Ferraz, 2013; Xu et al., 2005). *p*-Coumaric acid accumulation in grass crops cell walls has been correlated with lignin deposition and poor cell wall digestibility (Lygin et al., 2011; Zhang et al., 2011). Throughout the development of these plants, the accumulation of *p*-coumaric acid mainly in the secondary cell wall may favor the more linear and less reticulated lignin matrix observed in grasses, since *p*-coumaric acid preferentially bounds to lignin monolignols only through an ester linkage, especially sinapyl alcohol (Ralph, 2010).

TABLE 1 Hemicellulose and suberin wax composition of several species of *Saccharum spp*. and other grasses

	Hemicelluloses monomers (%)			Suberin waxes (g/100 g rind DW[a])	References
	Glucose	Xylose	Arabinose		
Sugarcane					
Saccharum officinarum	47.6	48.7	2.4	3.50[b]	Portilla Llerena (2016)
Saccharum barberi	46.4	49.2	3.9	—	Portilla Llerena (2016)
Saccharum robustum	50.1	46.4	2.9	—	Portilla Llerena (2016)
Saccharum spontaneum	50.7	45.8	2.8	8.00[b]	Portilla Llerena (2016)
Saccharum cv.	59.80	33.50	2.40	0.93–1.65	de Souza et al. (2013), Asikin et al. (2012)
Miscanthus					
Miscanthus sinensis	52.84	30.94	3.26	0.69	Van der Weijde et al. (2017), Attard et al. (2016)
Miscanthus sacchariflorus	70.6	24.2	5.3	1.81	Visser and Pignatelli (2001)
M. × *giganteus*	37.39–42.9	18.26–22.0	1.45	0.52	Wang et al. (2017), Zhang, Wyman, Jakob, and Yang (2012), Attard et al. (2016)
Switchgrass					
Panicum virgatum	43.7–46.1	22.8–24.6	2.1–2.3	0.45	Hu et al. (2010), Tulloch and Hoffman (1980)
Maize					
Zea mays	36.8	22.2	5.5	0.61	Galbe and Zacchi (2007), Zhao et al. (2007)
Rice					
Oryza sativa	38.66	22.93	3.94	0.82[c]	Wu et al. (2018), Zhao et al. (2007)
Wheat					
Triticum aestivum	34.9–37.8	22.8	2.37	0.57[c]	Zhang et al. (2012), Tozluoglu, Özyürek, Çöpür, and Özdemir (2015), Zhao et al. (2007)

[a]Unless state otherwise. [b]The values estimated by the weight difference of the sample before and after wax extraction using a Soxhlet apparatus with a mixture of hexane and methanol (20:1 v/v), following Asikin et al. (2012). Rind was hand peeled with a blade, grounded in liquid nitrogen, thoroughly washed with Milli-Q water, and freeze-dried for 3 days before wax extraction. [c]Total amount of wax extracted from straw.

Remarkably, the regions of higher lignification in sugarcane, namely the strongly recalcitrant rind, are characterized by low nano-porosity of the cell wall (Maziero et al., 2013). The tissue architecture imposed by *p*-coumaric acid acylation of lignin and probably also of the aromatic fraction of the suberin lamella may constitute a major physicochemical factor limiting the degradation by rumen microflora of the sugarcane forage.

In sugarcane stem, the high levels of hydroxycinnamic acids in the cell walls coincide with an enrichment in arabinoxylans, lignin, and suberin, particularly in the rind and cortex, the most peripheric tissues that present great recalcitrance. The corn fiber gum extracted from the corn pericarp and formed mainly by arabinoxylans hemicelluloses is characterized by high levels of hydroxycinnamic acids, namely ferulic acid and *p*-coumaric acid, and lipids, including the ω-hydroxy fatty acids C16 (palmitic acid) and C18:1 (oleic acid), triacylglycerols, and fatty acyl and hydroxycinnamates esters (Yadav, Moreau, & Hicks, 2007). Moreover, the extraction of the lipid portion from the corn fiber gum was only possible by strong alkaline hydrolysis, which may imply the cross-linking of corn gum fatty acids with arabinoxylans most probably through ferulic acid esters (Yadav et al., 2007). In the internode of several sugarcane hybrids occurs a centrifuge gradient of cell wall arabinoxylans, with a high concentration at the rind and cortex particularly at the vascular bundle fibers (Costa, Vega-Sánchez, Milagres, Scheller, & Ferraz, 2016), tissues strongly suberized and lignified. These tissues are characterized by a great recalcitrance, showing a higher resistance to enzymatic digestibility. Conversely, the

cell walls at the pith of the sugarcane hybrids internodes have a higher concentration of another more amorphous hemicellulose, the mixed-linkage glucans, and reduced quantities of lignin and suberin (Costa et al., 2013, 2016). This reinforces the notion that hydroxycinnamates esters strengthen the cross-link between suberin fatty acids and arabinoxylans, and also with lignin, anchoring the suberin lamella in the interior of the cell wall (Graça et al., 2015), in a similar process to what has been described to the cross-linking between arabinoxylans and lignin (de Oliveira et al., 2015; Ralph, 2010).

4 | VARIATION IN DIGESTIBILITY AMONG *SACCHARUM* SPP.

Saccharum spp. are Poaceae from the tribe Andropogoneae, subtribe Saccharinae, group that comprises also two other bioenergy crops, *Sorghum* and *Miscanthus*. The modern hybrid cultivars of the sugarcane crop are highly polyploid and aneuploid and have been developed through interspecific hybridization of the sucrose-accumulating species *Saccharum officinarum* L. and the wild species *Saccharum spontaneum* L. with small contribution from *Saccharum robustum*, *Saccharum sinense*, *Saccharum barberi,* and the genera *Erianthus* and *Miscanthus* (Paterson, Moore, & Tew, 2013). Phenotypically, *Saccharum* spp. main parental species are very divergent. *S. officinarum* L. has thick stalks with high sucrose levels and lower fiber content. On the other hand, *S. spontaneum* L. has a very variable anatomy, forming from short bushes without stalks to very tall thin stalks with low sucrose content but high fiber amounts. Notably, *S. spontaneum* L. contributed to crucial agronomic traits to the modern commercial sugarcane hybrids, namely increased tillering and improved disease resistance and stress tolerance. The genomes of the modern sugarcane cultivars are composed largely by chromosomes derived from *S. officinarum* L., 70%–80%, with an important portion inherited entirely from *S. spontaneum*, 10%–20%, and with around 10% of the sugarcane hybrids genomes constituted by recombinant chromosomes from these two parental species (Paterson et al., 2013).

In several countries, sugarcane has been used to feed animals, mainly cattle, as a nutritional supplement. Since the main use of sugarcane as a crop has been the production of sugar and bioethanol, most of the genetic material used for forage breeding is selected among existing varieties of *Saccharum* spp. (Anjos et al., 2008). Although high sucrose is a desirable trait, digestibility is the main target for selecting varieties of sugarcane for foraging as no relationship has been found between sucrose content and digestibility (Freitas et al., 2006). In Brazil, sugarcane is used to feed cattle during the dry season that coincides with the winter when culm maturation is complete and the sucrose content is highest (Bottcher et al., 2013; Landell et al., 1999). Other advantage over other grasses is that combining precocious and late varieties, regarding sucrose accumulation, animals can be feed during the entire dry season. The main nutritional limitation of sugarcane is the low content of protein what is usually corrected by supplying urea as a source of nitrogen (Anjos et al., 2008).

4.1 | Cell wall architecture among *Saccharum* spp.

In *Saccharum* spp., a C4 grass monocot, the vascular bundles are disseminated throughout all stem diameter with the remaining space filled by parenchyma cells. The cortex parenchyma at the periphery of the internode is composed by small thick-wall cells but toward the center, or pith, the parenchyma cells are highly vacuolated with thin walls, forming the storage parenchyma as stem matures. At the center of the internode, there are few but small vascular bundles and at the periphery the vascular bundles are larger and more profuse (Figure 1). The vascular bundles are surrounded by a sheath of sclerenchyma fibers called mestoma that gradually becomes heavily lignified (Bottcher et al., 2013). Externally to the mestoma is formed a parenchyma sheath more or less wide. During stem development in *Saccharum* spp., mestoma and parenchyma sheaths become more extensive at expense of neighboring parenchyma cells. Noteworthy, *Saccharum* spp. stem maturation is characterized by the suberization of these mestoma and parenchyma sheaths (Rae et al., 2005) and also of the storage parenchyma in older stem internodes. Furthermore, the cortex outer cell layers immediately below the epidermis form the hypodermis, a tissue composed by thick-wall, lignified and suberized cells. In some *Saccharum* spp. genotypes, below the hypodermis a few cell layers of chlorophyllic tissue are present. The epidermis is composed by a single layer of heterogeneous cells, long rectangular cells punctuated by single or pairs of short cells of two types, cork cells and silica cells (Roth, 1968). Cork cells are suberized and thin-wall cells. Furthermore, the epidermis of sugarcane is characterized by longitudinal fissures filled by a suberized plug known as corky cracks or patches, depending on the size, that initially are inconspicuous and become more prominent and abundant with internode maturation. The profusion of corky patches is genotype-dependent in *Saccharum* spp. (Moore, 1987). The epidermis synthetizes to the exterior the cuticle and waxes. Noteworthy, the rind of *S. officinarum* internodes retains the ability to undergo suberization in response to injury. Four days after superficial mechanical wounding of the internode, the parenchyma cells of the rind that surround the lesion show suberin deposition in the cell wall. The wound-induced suberization of the parenchyma sheath around the vascular bundles in the vicinity of the lesion site has also occurred.

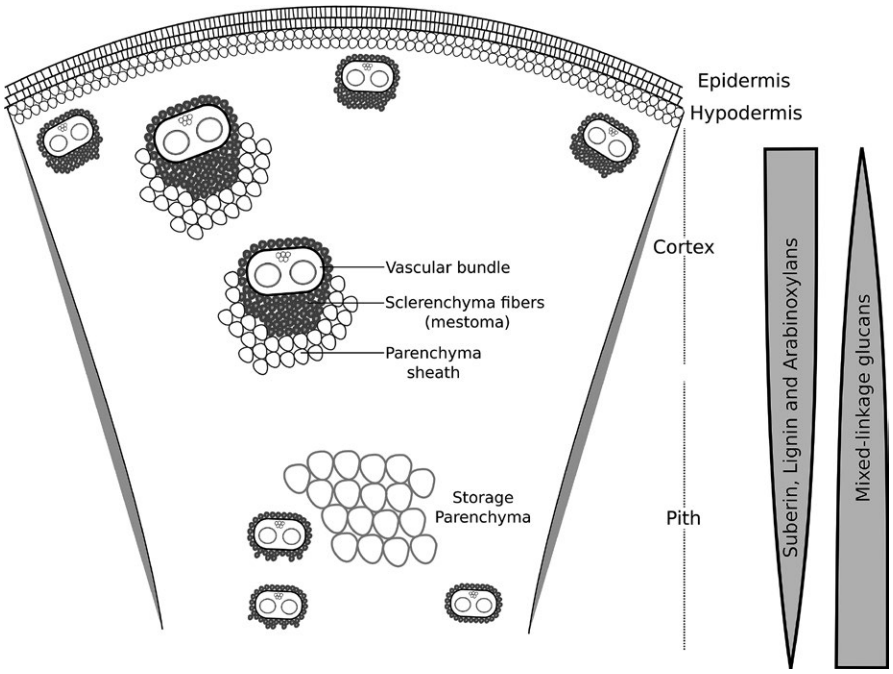

FIGURE 1 Cross section scheme of *Saccharum* spp. internode. The principal tissues of the internode are represented

Concomitantly, *S. officinarum* orthologous genes of suberin biosynthetic pathway genes *CYP86A1* and *ABCG2* are transcriptionally induced by wounding (Figueiredo and Mazzafera, unpublished data). The conspicuous differences in the development and morphology among the *Saccharum* spp. genotypes were associated with different patterns of cell wall biopolymers distribution, namely lignin, hemicelluloses, and suberin. *S. spontaneum* is characterized by higher lignin content and percentage of lignified fiber, and thicker fiber cell walls compared to one of the more cultivated modern hybrids, RB867515 (RB) (Poelking et al., 2015). In the sugarcane hybrid RB, lignification in the intermediate internodes occurs mainly at the protoxylem, at the metaxylem, and at the first layers of mestoma fibers, and the epidermis, while in *S. spontaneum* the lignification comprises also a larger number of fibers layers, the parenchyma bundle sheath as well as cortical parenchyma cells. With maturation, the lignification of cell wall extends to the bulk of cortical parenchyma and to the pith in *S. spontaneum* (Poelking et al., 2015). In a recent study comparing the four *Saccharum* wild-type species, *S. officinarum*, *S. spontaneum*, *S. robustum,* and *S. barberi*, it was shown that saccharification levels of young internodes were equivalent in the four species but were considerably lower in mature internodes of *S. spontaneum* and *S. robustum* (Portilla Llerena, 2016; Portilla Llerena and Mazzafera, unpublished data). Noteworthy, cellulose content is higher in *S. spontaneum* and *S. robustum* and its accumulation increases through internode development in these species. Curiously, hemicellulose deposition is higher in younger internodes in all four species, with *S. officinarum* showing a lower content than the other three *Saccharum* species (Figure 2).

Concerning suberin distribution in the stem of *Saccharum* spp., the comparative histochemical study of *S. officinarum* and *S. spontaneum* indicates that the mestoma sclerenchyma fibers and parenchyma sheath surrounding the vascular bundles are suberized in both species but suberization of cortical parenchyma initiates earlier in *S. spontaneum*, at the 5th internode (Figure 3). In mature internodes, cortical and storage parenchyma deposit suberin at the cell wall in both *Saccharum* species, with *S. spontaneum* presenting denser cell walls. Furthermore, *S. spontaneum* maintains through internode development a well-organized two-layered hypodermis with thick suberized cell walls. Hypodermis and the outermost cortex cells are also suberized in *S. officinarum* but present thinner cell walls.

5 | GENETIC MODIFICATIONS OF SUGARCANE CELL WALL ARCHITECTURE TOWARD BETTER DIGESTIBILITY

The recent development of different "omics" strategies, including genomics, transcriptomics, and metabolomics, allowed the characterization of the genetic and biochemical elements involved in the formation and deposition of lignin and suberin in the cell wall and ultimately responsible for the high recalcitrance of these biopolymers. The chemical linkages with cellulose and hemicellulose have also been suggested to play a significant role in the cell wall recalcitrance. Among the cell wall polymers, lignin has been extensively studied and genetic modified plants for reduced content or altered lignin composition from several species have shown improved saccharification. Most of these studies were justified based on the demand for second generation

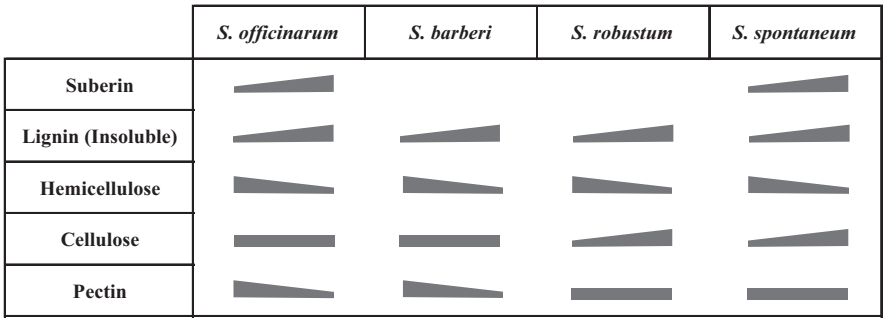

FIGURE 2 Schematic accumulation of the cell wall biopolymers during stem maturation in plants of *Saccharum officinarum*, *Saccharum barberi*, *Saccharum robustum*, *Saccharum spontaneum*. Data are not quantitative and represent variation between second/third and eight internodes (Data provided by Portilla Llerena, 2016)

bioethanol, as a strategy to control greenhouse gas emission and decrease global warming. Several comprehensive reviews have extensively covered lignin in other crops (Mottiar, Vanholme, Boerjan, Ralph, & Mansfield, 2016; Wang, Dudareva, Morgan, & Chapple, 2015). To our knowledge, much less is known about hemicelluloses and suberin. Hemicellulose fermentation by microorganisms is still a bottleneck in the bioethanol lignocellulosic industry. The available processes focus the C6 sugars recovered from cellulose and C5 sugars are separate and cofermented with sucrose-rich cane juice and molasses in the first-generation fermenters (Losordo et al., 2016). By its lipid nature, suberin is probably lost during the hot-acid pretreatments during the bagasse processing for the bioethanol lignocellulosic generation. However, both biopolymers have the potential to be diverted to several applications, from biofuels and industrial oils to nutraceutical and pharmaceutical applications (Figure 4) (Asikin et al., 2012; Attard et al., 2015; del Río et al., 2015; Li-Beisson, Nakamura, & Harwood, 2016; Luo et al., 2019; Sedlmeyer, 2011).

5.1 | Hemicelluloses

Cell wall polysaccharides are divided into three groups: cellulose, consisting entirely of β-(1→4)-linked glucan chains; pectins, heterogeneous polysaccharides containing a large amount of galacturonic acid residues; and hemicelluloses, the remaining cell wall polysaccharides having β-(1→4)-linked backbones of glucose, mannose, or xylose (Scheller & Ulvskov, 2010). In grasses, the primary cell walls are thin, aqueous, and flexible and composed mainly by hydroxycinnamic acids (like ferulic and *p*-coumaric acids), pectin and cellulosic microfibrils embedded in a hemicellulose matrix containing mixed-linkage glucans (MLGs) and glucuronoarabinoxylans (GAXs). A step forward, the secondary cell wall contains only traces of pectin and MLGs, showing reduced water content. The secondary cell wall is characterized by cellulosic fibrils presenting higher amounts of layers embedded in GAXs cross-linked to lignin and suberin biopolymers most probably through the action of hydroxycinnamic acids (Vogel, 2008). The principal hemicellulose types present in terrestrial plants are xyloglucans (XyG), xylans, mannans, and glucomannans, while β-(1→3,1→4)-glucans are restricted mainly to grasses. Compared with lignin, much less is known about hemicelluloses biosynthetic pathway genes and limited success has been obtained engineering xylan and other hemicelluloses metabolic pathway aiming to improve cell wall digestibility. Notwithstanding, changes in hemicelluloses content require greater knowledge of cell wall dynamics and of the key genes controlling plant development (Carpita, 2011).

Xyloglucans are characteristics of land plants and also of Charophycean green algae (Domozych, Sørensen, & Willats, 2009). XyG is forming a gelatinous layer abundant in primary walls of Spermatophytes, except for grasses, constituted by a 1,4-β-glucan backbone branched by 1,6-α-xylosyl residues. Grass and other Commelinid monocots primary wall are different from dicots because of the low content, at least four times lower, of XyG. Primary cell walls of grasses are described as Type II due to the high content of GAXs instead of XyG, characteristics of type I cell walls (Carpita & Gibeaut, 1993). XyG play a key role in the dynamic loosening and tightening of cellulose microfibrils during cell growth and differentiation (Popper & Fry, 2003). Furthermore, the occurrence of fucose (Fuc) in XyG is related to various developmental contexts and the study of Fuc-deficient mutants in *Arabidopsis thaliana* suggests that XyG fucosylation facilitates xyloglucan–cellulose interactions or intervenes in modulating growth regulation (Vanzin et al., 2002). In grasses and other Commelinid monocots, the XyG present occur as XXGn (X = xylose, G = glucose, F = fucose, L = galactose) and G core motifs, and in small proportions as XXFG units (Hsieh & Harris, 2009), with the less substituted XXGG structure being the prevalent form in the cell walls of grasses. XyG oligomers like XXLG and XLLG can promote cell expansion (Eckardt, 2008), and the breakdown products of XyG were demonstrated to counteract auxin-induced cell expansion. In grasses, XyG is potentially fucosylated during synthesis and fucose is removed during XyG deposition in the cell wall.

Xylan is the major hemicellulose in secondary cell walls of angiosperms (Scheller & Ulvskov, 2010) and is characterized by polysaccharides with β-(1→4)-linked xylose residues backbone. Common substitutions are α-(1→2)-linked glucuronosyl (GlcA) and 4-O-methyl glucuronosyl (MeGlcA) residues, called glucuronoxylans. Grass xylans are substituted

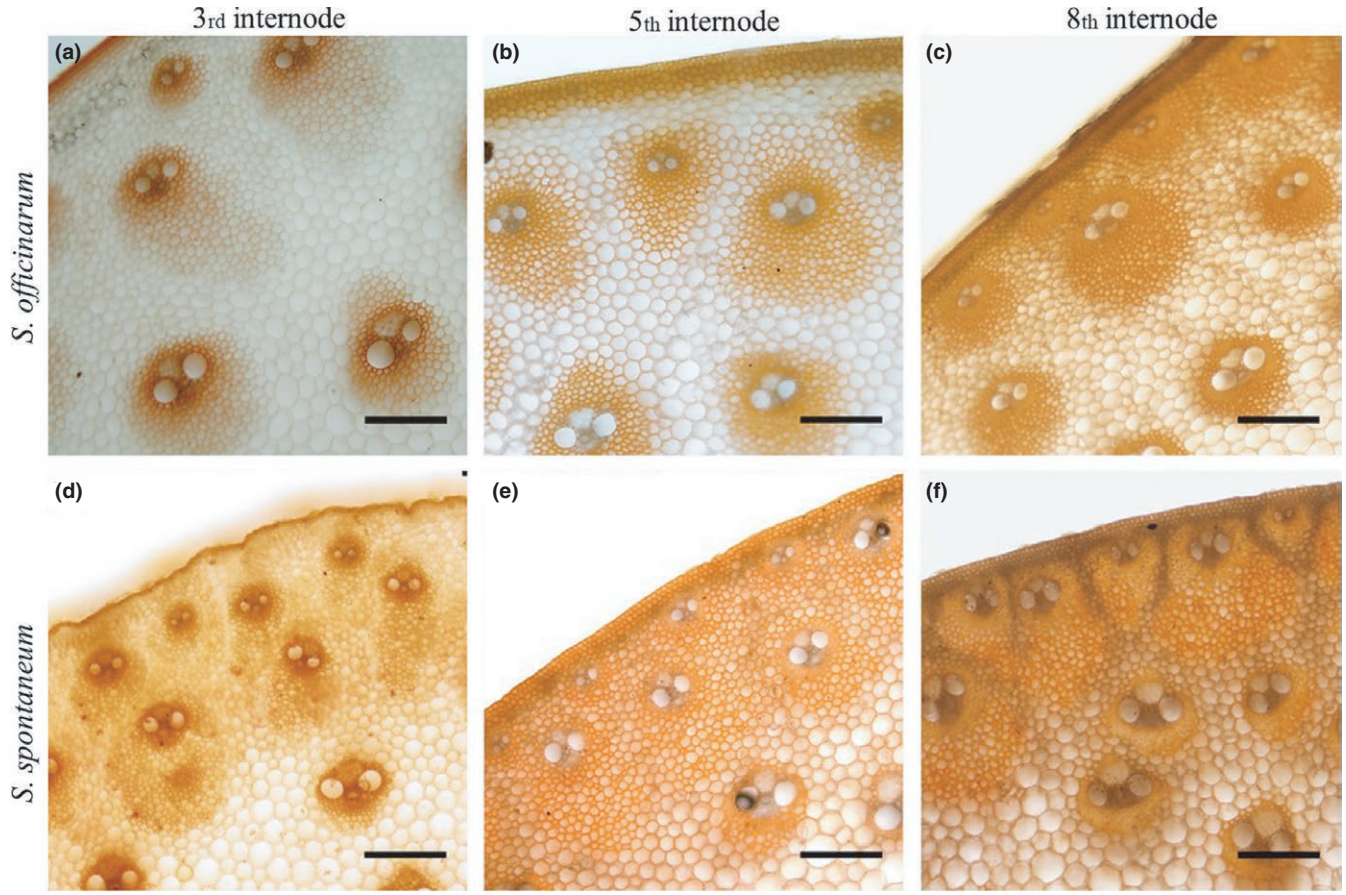

FIGURE 3 Suberin staining with Sudan IV of internode transverse sections of two sugarcane species. (a) *Saccharum officinarum* 3rd internode (young). (b) *Saccharum officinarum* 5th internode. (c) *Saccharum officinarum* 8th internode (mature). (d) *Saccharum spontaneum* 3rd internode (young). (e) *Saccharum spontaneum* 5th internode. (f) *Saccharum spontaneum* 8th internode (mature). Scale bar = 100 μm

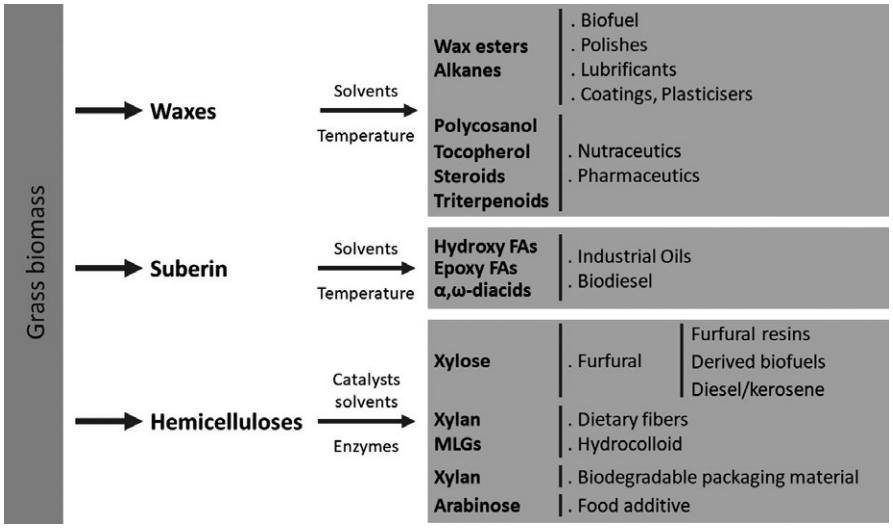

FIGURE 4 Major applications of suberin-associated waxes, suberin and hemicellulose derivatives extracted from grass biomass

to GAXs with O-acetyl substituents and both α-1,3 or/and α-1,2-linked arabinofuranosyl (Araf) and α-1,2-linked GlcA/MeGlcA. The Araf residue on grass GAXs may be further decorated by α-(1,3)-linked Araf or β-(1,2)-linked xylose substituents (Ebringerová, Hromádková, & Heinze, 2005; Pauly et al., 2013). Most grass xylans are acetylated to various degrees compromising their solubility and digestibility. The analysis of 79 *Miscanthus* accessions with heterogeneous cell

wall composition and disparate biomass digestibility pointed arabinose substitution degree of xylan as the key factor to improve biomass saccharification upon alkaline pretreatment (Li et al., 2013). Arabinose in xylan is partially associated with cellulose amorphous regions, perturbing cellulose crystallinity, a major biomass recalcitrance factor (Li et al., 2013). The cellulose macrofibrils are less attacked by enzyme cocktails, due to their lower cellulase accessibility per area when compared to microfibrils, and the resistance to hydrolysis is related to polymer interaction reinforced by the presence of acetyl esters and ferulic acid (Buckeridge et al., 2016; Pauly et al., 2013). Correlation between Xyl/Araf content and cellulose crystallinity is also observed in rice (Li et al., 2015), suggesting Xyl/Araf ratio as an indicator for biomass enzymatic recalcitrance in grasses. The analysis of 36 cell wall mutants of rice corroborated that cellulose crystallinity negatively determined biomass enzymatic digestion upon chemical pretreatments, and also agronomic traits such as lodging resistance (Li et al., 2015). Two mutant lines, *Osfc17* and *Osfc30*, displayed increased levels of hemicellulosic Araf levels but normal plant growth and high enzymatic saccharification, promoted by Araf interlinking to cellulose β-1,4-glucans, compromising its crystallinity (Li et al., 2015). An important feature of grass xylans is the presence of ferulic acid esters attached to the O-5 position of some of the Araf residues (Hatfield, Marita, & Kenneth, 2008). Esters of p-coumaric acid are also abundant in grass cell walls. Remarkably, higher amount of ferulic acid and *p*-coumaric acids are characteristics from grasses, being present as unbound acids, esterified to GAX, and ester- and ether-linked to lignin and suberin in cell wall (de Oliveira et al., 2015). Recently, a BAHD acyl-CoA transferase, *SvBAHD01,* was reported to interfere with arabinoxylans feruloylation in *Setaria viridis*. RNAi silencing of *SvBAHD01* resulted in approximately 60% reduction in arabinoxylans feruloylation in stems and doubled arabinose acylated by *p*-coumarate, with no alteration in total lignin content (de Souza et al., 2018). This silenced *SvBAHD01* RNAi line showed no effect on total biomass production but biomass saccharification efficiency increased 40%–60% (de Souza et al., 2018). Curiously, silencing *BAHD01* ortholog gene in *Brachypodium distachyon* had a much lesser effect in arabinoxylans feruloylation, maybe due to gene redundancy (de Souza et al., 2018).

Curiously, it has not been identified in grasses a conserved oligosaccharide in the reducing end of xylan (common in dicots and conifers) although the presence of the genes responsible for this feature is present (Verbruggen et al., 1998). In rice, overexpression of *OsARAF* decreased 20%–25% of the arabinose content and increased 28%–34% of the glucose content, enhancing 46%–70% of the saccharification yield without penalties (Sumiyoshi et al., 2013). Suppressed expression of *OsUAM1* reduced 6%–44% of the Araf content and 25%–80% of ferulic acid and *p*-coumaric acid amounts in rice (Konishi et al., 2011). On the same way, *PvUAM1* silencing in switchgrass reduced the levels of Araf and increased cellulose and lignin content as a compensation mechanism, maintaining saccharification levels (Willis et al., 2016). In wheat (*Triticum aestivum*), *TaGT43_2* and *TaGT47_2* are homologs of *A. thaliana IRX9* and *IRX10* (*IRREGULAR XYLEM9* and *10*, respectively) and promote biosynthesis of arabinoxylans (Lovegrove et al., 2013). Two homologs of *GT47*: *GRMZM2G100143* and *GRMZM2G05825* are differentially expressed in maize internodes and are related to xylan metabolism (Bosch, Mayer, Cookson, & Donnison, 2011). Mutants from grass-specific GT61 family members have reduced Araf side chains substitutions and lower levels of feruloylation and hydroxycinnamic acids cross-linking that resulted in enhanced saccharification of these mutants biomass (Anders et al., 2012; Chiniquy et al., 2012). Approximately one-third of grass cell wall genes do not have orthologs in *A. thaliana;* however, transcriptomic and metabolomic studies in several grasses shed light over the biosynthetic pathway of hemicelluloses and the impact in biomass saccharification (Carpita & McCann, 2008). The transcriptome of the endosperm of hexaploid wheat indicates that members from GT43/47/61 glycosyltransferase families have conserved functions in monocots (Pellny et al., 2012). The *SAC1* gene was identified in a mutant screening that allowed the identification of plants with increased saccharification (Marriott et al., 2014). Genome mapping has suggested that this gene encodes a glycosyltransferase from the GT61 glycosyltransferase family, involved in Araf transfer onto xylan (Anders et al., 2012). sac1-silenced plants have lower levels of ferulic acid (that is involved in the cross-linking between arabinoxylan and lignin), lower crystalline cellulose content, and higher frequency of lignin G and H units, features that are known to decrease cell wall recalcitrance. In fact, saccharification levels in *sac1* plants were up to 67.03% higher than in WT plants. Interestingly, the *sac1* mutants showed no impairment in the physical properties of the stem (Marriott et al., 2014). The *XAX1* gene also encodes a protein from the GT61 glycosyltransferase family with β-1,2-xylosyl transferase activity, allowing substitution of arabinosyl residues for xylosyl residues in xylan with concomitant loss of the ferulic and *p*-coumaric acids residues attached to the arabinosyl residues (Chiniquy et al., 2012). This, in turn, would reduce diferulic cross-links, increasing the access of cellulases to cellulose microfibrils. In fact, *xax1* mutants showed a 62% increase in the total sugar release compared to the wild type upon treatment with commercial enzyme cocktail (Chiniquy et al., 2012). *GUX1* and *GUX2* genes code for glucuronyltransferases (glucuronic acid substitution of xylan 1 and 2, respectively) and are responsible for the substitution of α(1,2)-linked D-GlcA and 4-O-MeGlcA on xylan of secondary cell walls (Mortimer et al., 2010). Mutations in *GUX1* or

GUX2 reduce GlcA and MeGlcA substitutions in xylan by two-thirds and one-third, respectively. Bromley et al. (2013) suggested *GUX2* is a good candidate for biomass improvement studies in grasses. Interestingly, mutants of *IRX10* and *IRX14*, genes also involved in xylan amount reduction, have presented penalties such as vessels collapsing and dwarfism (Brown et al., 2007; Chen et al., 2013). These adverse phenotypes were not observed in *gux1* and *gux2* mutants (Mortimer et al., 2010). Recently, Lyczakowski et al. (2017) showed that glucose and xylose released in *gux1/2* and *gux1/2/3 A. thaliana* mutants were 30% and 70% higher than wild-type plants during saccharification and that the ethanol production was double. Lastly, Costa et al. (2016) analyzed six hybrids of sugarcane for hemicellulose and lignin distribution in pith, interface, and rind regions and concluded that acetylated GAXs and lignin are critical for recalcitrance and that contrarily high MLGs and highly substituted GAX contributes for less recalcitrance.

Interestingly, overexpression of the transcription factors (TF) *PvKN1* (knotted 1-like) and *PvERF001* (AP2/ERF) in switchgrass enhanced saccharification and altered expression of hemicellulose biosynthetic genes and others (Wuddineh et al., 2015, 2016). An extensive list of TF related to regulation of the cell wall is briefly described by Bhatia, Gallagher, Gomez, and Bosch (2017). Many of them interact with lignin, cellulose, and hemicellulose contents such as *OsMYB46*, *ZmMYB46*, *PvMYB46A*, and several NAC members in *Oryza sativa* and *Zea mays SWIN1/3/7/25*.

Mixed-linkage glucans are broadly present in primary cell walls and associated with cell expansion and vasculature and sclerenchyma in mature tissues (Carpita & McCann, 2010; Vega-Sánchez, Verhertbruggen, Scheller, & Ronald, 2013). MLGs are soluble and present low recalcitrance with better glucose hydrolysis, becoming a strategy for cell wall fermentation approaches (Burton & Fincher, 2009). In sugarcane, MLGs (around 15% (w/w) of the cell wall) occur in the innermost regions of the internodes, regions showing also low lignin content (Costa et al., 2016). The *cslf6*-silencing mutant has reduced MLG content in rice and barley. On the contrary, *CSLF* overexpression in barley is lethal to most individuals due to the excessive accumulation of β-(1→3,1→4)-glucans (Burton et al., 2011). The β-(1→3,1→4)-glucans have not been found in dicots (Smith & Harris, 1999) but are common in grasses (Kiemle et al., 2014), and the genes responsible for β-(1→3,1→4)-glucans biosynthesis were already identified in grasses (Burton & Fincher, 2012). The structural associations of MLGs with other cell wall constituents need to be further investigated (Kiemle et al., 2014), with some studies concerning strategies around manipulation of MLGs metabolism in switchgrass (Shen et al., 2013), for high biofuel production sugarcane (de Souza et al., 2013), maize (Li, Pattathil, Hahn, & Hodge, 2014), and Miscanthus (da Costa et al., 2017) being recently reported.

Mannans are the major hemicellulose in the secondary cell wall of gymnosperms, However, mannans have been thoroughly studied as seed storage compounds and they are also found in variable amounts in all plants cell walls. The mannans backbones may consist of four classes: mannan, glucomannan, galactomannan, and galactoglucomannan (Domozych et al., 2012). Mannan and galactomannan backbone is made of β-1,4-linked mannose, glucomannan, and galactoglucomannan, containing mannose and glucose units in their backbone linked together (Scheller & Ulvskov, 2010). They are often acetylated. Lack or perturbance of mannans biosynthesis may cause lethal phenotype in *A. thaliana cslA7* mutants due absence of glucomannan synthase in seeds (Goubet et al., 2003). Curiously, a triple mutant for glucomannan synthase genes that do not have detectable glucomannan levels in *A. thaliana* stems does not show an obvious phenotype (Goubet et al., 2009).

The conversion of plant biomass into fermentable sugars for bioethanol production depends on cell wall composition, content, and access to the cell wall polysaccharides. Hemicelluloses represent a high amount of potential fermentable sugar and modification *in planta* can increase the bioethanol production. Revising the literature, grasses and sugarcane are not yet well exploited but some strategies can lead to further studies: (a) overexpressed barley *CslF* (*HvClsF6*) results in a threefold to fourfold increase in MLGs in the leaf tissue and a higher hexose content (Burton et al., 2011); (b) *O. sativa CslF6* was characterized in tobacco and *A. thaliana* increasing 13% of MLGs and 6% of glucan content (Vega-Sánchez et al., 2015); (c) in switchgrass, the removal of xylan increased almost 100% of the glucose yield which is indicated as recalcitrance factor (DeMartini et al., 2013) and similarly was found in *Miscanthus* (Xu et al., 2012); (d) the loss of xylans, MLGs, XyGs, and pectin in pretreatment represent the reduction of around 12%–33% of the potential biomass to produce 2G ethanol (Pauly & Keegstra, 2008); (e) the arabinoxylans present in cell walls of sugarcane contains decorations with acetyl groups that can interfere in enzymatic cleavage (de Souza et al., 2013); (f) galactomannans and mannans are associated with increased biomass saccharification (Tavares, de Souza, & Buckeridge, 2015); and (g) 50% of the ferulic acid content is ester-linked to hemicelluloses in sugarcane and is involved in cell wall recalcitrance (Harris & Trethewey, 2010). The efficiency of bioenergy production from plants depends on our demand for sustainable sources of energy. Different approaches have been conducted for cell wall modifications aiming higher biomass production, lower financial costs, and environmental friendly (Table 2).

5.2 | Suberin

Lipophilic barriers such as the suberin lamellae, the cuticle, and associated waxes avoid water loss through transpiration

and regulate the movement of water, solutes, and gases throughout the plant. Furthermore, the hydrophobic and ultrastructural features of these lipophilic barriers confer them an extraordinary mechanical resistance, namely against pathogen infections and herbivory. The genes associated with the suberin biosynthetic pathway have been globally uncovered by transcriptomic analysis of naturally suberin-rich tissues such as oak tree (*Quercus suber* L.) cork, potato (*Solanum tuberosum* L.) periderm, or apple (*Malus* × *domestica*) russeting (Landgraf et al., 2014; Legay et al., 2015; Soler et al., 2007). The function of several individual genes in the biosynthesis of the suberin biopolymer has been demonstrated by forward and reverse genetics with isolation of numerous mutant lines, mainly *A. thaliana* and *S. tuberosum*, displaying impaired suberin lamella phenotypes (Beisson, Li, Bonaventure, Pollard, & Ohlrogge, 2007; Compagnon et al., 2009; Domergue et al., 2010; Franke et al., 2009; Kosma et al., 2014; Molina, Li-Beisson, Beisson, Ohlrogge, & Pollard, 2009; Serra, Soler, Hohn, Franke, et al., 2009; Serra, Soler, Hohn, Sauveplane, et al., 2009; Yadav et al., 2014). Curiously, it is recurrent among mutant lines displaying defective suberin phenotypes a loss of water impermeability by specialized tissues, namely *A. thaliana* seed tegument and root endodermis, and potato tuber periderm (Table 2). Another powerful tool to highlight suberin compositional key features is metabolic profiling of suberin-rich tissues and mutants lines displaying defective suberin phenotypes (Graça et al., 2015; Molina, Bonaventure, Ohlrogge, & Pollard, 2006; Schreiber, Franke, Hartmann, Ranathunge, & Steudle, 2005).

Suberin is a biopolymer composed of long and very long C16–C26 fatty acid derivatives, cross-linked to each other by ester covalent bonds with glycerol and hydroxycinnamates, namely ferulic acid and *p*-coumaric acid, forming acylglycerols. A specific feature of suberin is that C18 fatty acids are unsaturated or mid-chain substituted by epoxy or diol groups. On the other hand, suberin shows a high concentration of very long $C \geq 20$ fatty acids (Pollard, Beisson, Li, & Ohlrogge, 2008). Noteworthy, the epoxy fatty acids and the very long $C \geq 20$ fatty acids that compose the aliphatic fraction of suberin have the propriety to aggregate in stable crystalline structures highly hydrophobic and organized in an impermeable, flexible, and mechanical resistant matrix that repels water adherence and flow through the suberin lamella. In sugarcane internode stem, the hydraulic architecture comprising vascular bundles circumscribed by a fiber mestoma sheath and a parenchymatic sheath, both heavily suberized and lignified, confers an extraordinary capacity to accumulate sucrose and other solutes in the culm. In this plant, sucrose storage is constrained to the fundamental parenchyma; sucrose values in xylem and phloem apoplast are virtually zero (Welbaum, Meinzer, Grayson, & Thornham, 1992). In mature sugarcane internodes, sucrose accumulates not only inside parenchyma cells but also in the apoplast. It is believed that the presence of sucrose in the apoplast promotes the equilibrium in the overall water potential that would be much higher in the symplast otherwise (Meinzer & Moore, 1988; Moore & Cosgrove, 1991). The suberization and lignification of mestoma and parenchyma sheaths are key factors to avoid sucrose from flowing back to the vascular bundles (Bihmidine, Hunter, Johns, Koch, & Braun, 2013). On the other hand, overall suberization of storage parenchyma cells in the mature sugarcane internodes contributes for these cells to support the high osmotic potential generated by the elevated sucrose concentration inside the cells. Connected with highly suberized tissues is the deposition of nonpolymeric suberin-associated waxes composed primarily by alkyl-hydroxycinnamates composed by ferulic acid, *p*-coumaric acid, or caffeic acid esterified to saturated fatty alcohols C16–C32; saturated free fatty acids C16–C30 and fatty alcohols conjugated in wax esters; very long $C \geq 20$ monoacylglycerols; sterols; and alkanes and their mid-chain keto or hydroxy derivatives (Kosma, Rice, & Pollard, 2015). Suberin-associated waxes may participate in the elaboration of physical barriers that complement the impermeability ability conferred by the suberin lamella. It is our belief that particularly in sugarcane stem internode, epidermis cork cells and the hypodermis beneath are preferential places for suberin and associated waxes synthesis that contribute to the formation of the corky patches plugs frequently observed at the surface of sugarcane internodes. The progressive accumulation of sucrose in the symplast but also in the apoplast that happens with stem differentiation as a sink organ imposes strong radial forces at the periphery of the rind of the sugarcane internodes that result in external fissures. Corky patches are an alternative strategy of sugarcane, a monocot unable of secondary growth, to seal these fissures with a flexible plug of suberin and associated waxes resistant to desiccation and pathogen attack, in order to simultaneously cope with disruption and dehydration at the internodes surface.

Several key enzymes required for suberin and associated waxes biosynthesis have been described recently (Beisson et al., 2007; Domergue et al., 2010; Franke et al., 2009; Landgraf et al., 2014; Molina et al., 2009; Pollard et al., 2008; Serra, Soler, Hohn, Franke, et al., 2009; Serra, Soler, Hohn, Sauveplane, et al., 2009; Yadav et al., 2014). The steps in suberin biosynthesis with high impact in tissue permeability and mechanical resistance are the ω-hydroxylation of the fatty acid precursors C16 and C18 and oxidation of α,ω-dicarboxylic acids by mono-oxygenases CYP86A1 (cytochrome P450) on one side and on the other the feruloylation of acylglycerols, fatty acids, and fatty alcohols by a large group of HD-type acyltransferase (BAHD family) that perform the acyl transfer between CoA-activated hydroxycinnamic acid derivatives and hydroxylated aliphatics (Pollard et al., 2008; Ranathunge, Schreiber, & Franke, 2011). The silencing of *CYP86A33* in potato compromised suberin

TABLE 2 Main genes involved in suberin and hemicellulose biosynthesis and regulation

Biopolymer	Species	Gene		Phenotype		References
Suberin synthesis	*A. thaliana* Potato	*CYP86A*	*horst* *Stcyp86a33* (RNAi)	Reduce aliphatic suberin, C18:1 ω-hydroxyacid, α,ω-diacids	Permeability ↑	Höfer et al. (2008), Serra, Soler, Hohn, Sauveplane, et al., 2009
	Potato	*StFHT*	*fht* (RNAi)	Reduce alkyl ferulates, esterified ferulic acid and C18:1 ω-hydroxyacid. Increase free feruloyl-CoA	Permeability ↑ Stiffness ↑	Serra et al. (2010)
	Potato	*StKCS6*	*kcs6* (RNAi)	Reduce very long fatty acids C ≥ 26	Permeability –	Serra et al. (2014)
	A. thaliana	*AtFAR1* *AtFAR4* *AtFAR5*	*far1far4far5* (T-DNA/amiRNA)	Reduce C18:0–C22:0 fatty alcohols, suberin-associated root waxes	Permeability ↑	Vishwanath et al. (2013)
	Rice	*RCN1/OsABCG5*	*rcn1-1* *rcn1-2* (M2)	Reduce C28–C30 fatty acids and ω-hydroxylic acids. Affect root growth in waterlogged soils.	Permeability ↑	Shiono et al. (2014)
Suberin regulation	Apple	*MdMYB93*	*MdMYB93* (transient expression in leaves of *N. benthamiana)*	Ectopic deposition of lipid polyester, soluble-free lipid and phenylpropanoids. Ectopic expression of suberin biosynthetic gene.		Legay et al. (2016)
	A. thaliana	*AtMYB41*	*p35S:At MYB41* (OE)	Ectopic deposition of lipid polyester, suberin-associated waxes and phenylpropanoids in leaves. Ectopic expression of suberin biosynthetic gene.		Kosma et al. (2014)
	A. thaliana	*AtMYB107* *AtMYB9*	*myb107* *myb9* (T-DNA)	Reduce aliphatic and aromatic suberin monomers and epicuticular waxes in the seeds. Lower germination under osmotic and salt stress.	Permeability ↑	Lashbrooke et al. (2016)
	Potato	*StNAC103*	*nac103* (RNAi)	Increase in suberin and wax load. Upregulation of suberin biosynthetic genes.		Verdaguer et al. (2016)
	Rice	*LGF1/HSD1*	*drp7* (M2)	Reduce the proportion of primary alcohols C30 in epicuticular waxes.	Hydrophobicity ↓	Kurokawa et al. (2018)

(*Continues*)

TABLE 2 (Continued)

Biopolymer	Species	Gene		Phenotype		References
Hemicellulose synthesis	Barley	*HvCslF6*	*p35S:Hv CslF6* (OE)	Increase MLG, sixfold. Higher hexose content.		Burton et al. (2011)
	Brachypodium	*BdSac1*	*BdSac1* (M2)	Reduce ferulic acid, cellulose. High lignin G, H	Yield – Digestibility ↑	Marriott et al. (2014)
	Rice	*OsARAF*	*pZmUbi1:Os ARAF* (OE)	Reduce Ara, increase glu	Yield – Digestibility ↑	Sumiyoshi et al. (2013)
		OsIRX10	*Osirx10* (RGT6229D)	Reduce xyl/ara. Affect growth	Yield ↓ Digestibility ↑	Chen et al. (2013)
		OsUAM	*uam* (RNAi)	Reduce *Araf*, ferulic, coumaric acid. Affect growth		Konishi et al. (2011)
		OsXAT2 *OsXAT3* *OsIRX9*	*Osfc17* *Osfc30*	Increase ara level and reduce cellulose crystallinity. High plant lodging resistance	Yield – Digestibility ↑	Li et al. (2015)
		OsXAX1	*axa1* (T-DNA)	Reduce xyl, ferulic, coumaric acid	Yield ↓ Digestibility ↑	Chiniquy et al. (2012)
	Setaria viridis	*SvBAHD01*	*bahd01* (RNAi)	Reduce AX feruloylation and increase arabinose acylated by *p*-coumarate.	Yield – Digestibility ↑	de Souza et al. (2018)
	Switchgrass	*PvUAM1*	*uam1* (RNAi)	Reduce Ara, increase cellulose, lignin.	Yield – Digestibility -	Willis et al. (2016)
	Wheat	*TaGT43* *TaGT47*	*gt43*/*gt47* (RNAi)	Reduce AX, cell wall thickness	Yield – Digestibility -	Lovegrove et al. (2013)
Hemicellulose degradation	Maize	*XynB*	*pUbi3:XynB* (OE)	Affect growth	Yield – Digestibility ↑	Shen et al. (2012)
Hemicellulose regulation	Switchgrass	*PvERF001*	*pZmUbi1:Pv ERF001* (OE)		Yield ↑ Digestibility ↑	Wuddineh et al. (2015)

Notes. Ara: arabinose; Araf: arabinofuranosyl residues; AX: arabinoxylans; glu: glucose; xyl: xylose.
↑: higher than wt; ↓: lower than wt; –: no significant differences to wt.

deposition and lamellar structure in the periderm, showing a reduced capacity of fatty acid chain terminal hydroxylation and cross-linking, and consequent disruption of the biosynthesis of acylglycerols that has resulted in greater concentration of free alkyl chains (Serra, Chatterjee, Figueras, Molinas, & Stark, 2014; Serra, Soler, Hohn, Sauveplane, et al., 2009). This transgenic line demonstrated enhanced water permeability that may be largely attributed to the significative diminution of suberin fatty acids and to the disturbance in suberin ultrastructure (Serra, Soler, Hohn, Sauveplane, et al., 2009). *CYP86A1* activity may be crucial in the regulation of the sophisticated balance between the proportion of the different suberin monomers and, this way, impact the ultrastructure of suberin by intervene in the equilibrium between suberin amorphous and crystalline nanostructures, resulting in variations in hydrophobicity, flexibility, and overall impermeability of the cell wall. In fact, *CYP86A33* RNAi periderms are characterized by a shift in hydrophilic–hydrophobic balance compared to the wild type, showing a lower fraction of hydrophobic aliphatic chain–chain interactions and conferring higher affinity to water interaction to the RNAi periderms (Serra et al., 2014). Strikingly, *CYP86A1* partial inhibition seems to divert suberin fatty acid precursors to the production of waxes that have a lower impermeability capacity, since the quantity of waxes rose by twofold in RNAi lines (Serra, Soler, Hohn, Sauveplane, et al., 2009). Alternatively, silencing of the HD-type acyltransferase *FHT* (suberin feruloyl transferase) in potato does not disturb the lamellar structure of suberin but reduces significantly the content in alkyl ferulates and also in both esterified ferulic acid and the main aliphatic suberin monomer linked to this hydroxycinnamate in potato periderm, the C18:1 ω-hydroxyacid (Serra et al., 2010, 2014). The unesterified free feruloyl-CoA, resulting from the suppressed activity of FHT, is diverted to a pool of soluble phenolics such as feruloyl-putrescine that may contribute to a more intricate and strong cross-linking of the suberin aromatic and aliphatic domains, conferring a more brittle texture to this biopolymer (Serra et al., 2014). Remarkably, the *FHT* RNAi periderm is characterized by lower tensile strength and greater stiffness than wild type and consequently is more breakable and susceptible to open fissures, and 15-fold more permeable to water (Serra et al., 2010). Curiously, the elongation of fatty acid aliphatic chains $C \geq 26$ performed by β-ketoacyl-CoA synthases (KCS) was reported to have a minor effect in water permeability of potato periderm. The silencing line *StKCS6*-RNAi showed no alteration in molecular composition and structure to wild type (Graça et al., 2015; Serra et al., 2014). However, in tomato (*S. lycopersicum* L.), another Solanaceae, mutant lines for the same gene with reduced very long fatty acids $C \geq 30$, showed that the reduction of intracuticular aliphatics in the fruit epidermis cause a fourfold increase in permeability, with a small contribution from the epicuticular waxes (Vogg et al., 2004). The importance of intracuticular waxes as the main transpiration barrier was further demonstrated for the leaves of other plants (Zeisler-Diehl, Müller, & Schreiber, 2018). Noteworthy, *KCS6* is induced in response to NaCl treatment in *A. thaliana*, indicating an osmotic protective role for suberin very long-chain fatty acids (Kosma et al., 2009).

Due to suberin aromatic-polyester nature, suberin biosynthetic pathway is profusely intricate with both the phenylpropanoid and the lipid metabolomic pathways. So, it is important to understand the factors and biochemical pathways that favor specific phenylpropanoids pools and classes of lipids at specific tissues in order to design genetic and metabolomic engineering strategies in order to improve suberin and associated waxes deposition on bioenergy plants like sugarcane. Recently, several key enzymes have been determined to be responsible for the remobilization of storage phenolic compounds such as chlorogenic acids and coumaroyl-quinic acids toward secondary metabolism, including, to this matter, lignin and suberin production (Valiñas, Lanteri, Have, & Ten Andreu, 2015). In potato, hydroxycinnamoyl transferase (HCT), caffeoyl-CoA *O*-methyltransferase (CCoAOMT), and COMT1 use coumaroyl-quinic acids and chlorogenic acid as precursors to the production of feruloyl-CoA, directing this metabolomic pathway to suberin production (Valiñas et al., 2015). Curiously, in sugarcane internodes, the main phenolic compounds are chlorogenic and coumaric acids with significative smaller contents of ferulic and caffeic acids (Bottcher et al., 2013; Costa et al., 2013). HCT may have a crucial role in the dynamic interplay between the *p*-coumaroyl-CoA and chlorogenic acids pools, controlling the amount of CoA-activated *p*-coumaric acid derivative available for the assembly of suberin and lignin in sugarcane. Complementary, the conjugated action of HCT, CCoAOMT, and COMT1 may divert a fraction of chlorogenic acids to the production of feruloyl-CoA that in sugarcane is a small portion of the hydroxycinnamic acid derivatives found in suberin and lignin. Finally, it is worth mention that the aliphatic monomers of suberin and associated waxes have different physicochemical properties and may promote a differential interaction between suberin fatty acids and other cell wall polymers (Buschhaus & Jetter, 2011). The more polar aliphatic monomers such as primary alcohols and diol and epoxy fatty acids interact more strongly with the suberin matrix. Alkanes and free fatty acids, less polar constituents, form a weaker network with the suberin matrix (Buschhaus & Jetter, 2011). In suberin and associated waxes, the primary alcohols and the alkanes derive from the same pool of very long-chain C20–C36 fatty acid precursors, depending on the subsequent biochemical modifications (Kunst & Samuels, 2009). The primary alcohol pathway implies very long-chain fatty acid acyl reduction while the alkane pathway involves very long-chain fatty acid decarbonylation. In rice leaves, *LGF1*/*HSD1*, a hydroxysteroid dehydrogenase that may intervene in fatty alcohol

acyl-CoA reductases (*FAR*) genes expression, defines the content of primary alcohols C30 and consequently the proportion between alcohols C30 and aldehydes C30, precursors of alkanes, available for waxes biosynthesis (Kurokawa et al., 2018). Primary alcohols C30 are determinant to the formation of profuse epicuticular waxes platelets on rice leaves surface and promotion of strong superficial hydrophobicity responsible for retention of a gas film in flooded leaves that permit gas exchanges underwater and photosynthesis during submergence of rice plants (Kurokawa et al., 2018).

Suberin and associated waxes deposition are restrict to specific tissues and occur at defined developmental stages or in response to biotic or abiotic stresses, suggesting a strong transcriptional regulation of the suberin biosynthetic pathway (Pollard et al., 2008). In cork oaks (*Q. suber*), cork layer development is accompanied by expression of genes with stress response and defense functions, pointing the cork layer as a barrier against external pressures. Noteworthy, the cork quality development depends on the branch of phenylpropanoid pathway favored; the route of flavonoid and free phenolic compounds occurs preferentially in cork of bad quality while lignin and suberin production predominates in good quality cork (Teixeira, Fortes, Bai, Pinheiro, & Pereira, 2018). The type of cork development results from differential gene reprogramming activating stress protective mechanisms through transcriptional pathways that triggers heat-shock proteins and regulatory proteins like the transcriptions factors families MYB (V-myb myeloblastosis viral oncogene homolog protein) and NAC (NAM, ATAF, CUC2) during the development of a good quality thick cork layer. On the contrary, stress protection in bad quality cork results from transcriptional activation of free phenolic compounds and a decrease in suberin and lignin production, resulting in deposition of a stiffer shielded layer probably with protective function against ultraviolet radiation (Teixeira et al., 2018). On the other hand, the transcriptome comparison of the thick well-organized periderm of cork oaks (*Q. suber*) and the bark of the close species holm oak (*Quercus ilex*) has shown that genes from phenylpropanoid metabolism and suberin and fatty acid biosynthesis were largely enriched in cork oak, reflecting a higher production of suberin in these trees periderms. Genes involved in isoprenoids such as triterpenes and sterols participating in suberin-associated waxes generation were also upregulated in cork oak (Boher et al., 2018). This intricate stress-related and spatiotemporal induction of suberization in plants implies transcriptional control by different sets of regulatory proteins. Several MYB transcription factors have been implicated in lipid metabolism in plants, particularly in suberin and cutin extracellular deposition. Is worth mention that suberin polymer and nonpolymeric suberin-associated waxes biosynthesis is activated by the same MYB transcription factors in apple, MdMYB93 and MdMYB52, and in *A. thaliana*, AtMYB41 (Kosma et al., 2014; Legay et al., 2015, 2017). The ectopic expression of the suberin master regulators MdMYB93 and AtMYB41 in *Nicotiana benthamiana* leaves, and in *A. thaliana* for AtMYB41, leads to the generation of a typical suberin lamellae and associated waxes composed by the major suberin monomers molecular types accompanied by remobilization of phenylpropanoids and enhanced expression of the suberin biosynthetic genes *GPAT5, CYP86A1,* and *CYP86B1* (Kosma et al., 2014; Legay et al., 2016). These data indicate that the same regulatory and metabolomic processes govern simultaneously the biosynthesis of suberin polymer and suberin-associated waxes. Curiously, AtMYB41 induces suberin and associated waxes accumulation in *A. thaliana* root endodermis specifically in response to abiotic stress (Kosma et al., 2014) and it was not found to be expressed during developmental suberization of periderm (Legay et al., 2015; Rains, de Silva, & Molina, 2018; Soler et al., 2007;). The developmental regulation of suberization has been shown to be performed by MdMYB93 and MdMYB52, involved in apple russeting, and by AtMYB107 and AtMYB9, master regulators of *A. thaliana* seed coat suberization (Gou et al., 2017; Lashbrooke et al., 2016; Legay et al., 2016, 2017). These last four MYB transcription factors are grouped in the very close phylogenetic subgroups 10 and 24 of the MYB family (Dubos et al., 2010). Noteworthy, more than one MYB transcription factor seems to participate in the regulation of the suberin biosynthetic pathway in a given tissue, regulating both the aliphatic and the phenylpropanoid pathways, and coordinating the transport and cell wall polymerization of suberin monomers. Curiously, some MYB transcription factors function as repressors of suberin biosynthesis. The cork oak ortholog of the *A. thaliana AtMYB4*, eucalyptus (*Eucalyptus grandis*) *EgMYB1,* and poplar (*Populus trichocarpa*) *PtoMYB156*, described as repressor of phenylpropanoid and lignin metabolism (Jin et al., 2000; Legay et al., 2010; Yang et al., 2017), has a reduced expression in cork, antagonist to the expression of the phenylpropanoid metabolism genes *PAL*, *C4H,* and *4CL* (Boher et al., 2018). Moreover, the analysis of suberin-enriched/defective tissues showed also the differential distribution of secondary metabolites such as waxes, protoanthocyanidins, flavonols or triterpenes and phenylpropanoids, implicating a broad spectrum of regulated genes for suberin-related MYBs (Lashbrooke et al., 2016; Legay et al., 2017). Additionally, transcriptomic analysis of several periderms revealed the elevated expression of NAC and WRKY superfamilies members during the suberization process (Legay et al., 2015; Rains et al., 2018; Soler, Serra, Fluch, Molinas, & Figueras, 2011; Soler et al., 2007). NAC and WRKY superfamily are involved in plant development regulation and response to biotic and abiotic stresses; however, their signaling pathways are poorly characterized. NAC regulators comprise both transcriptional activators and repressors (Lashbrooke et al., 2016). Curiously, in potato tuber periderm, silencing of *StNAC103* induced accumulation

of suberin and wax, which indicates *StNAC103* as a putative repressor of the suberization process (Verdaguer et al., 2016).

Fruit russeting is a well-known phenomenon characterized by the filling of microcracks that appear in the fruit skin with a layer of corky material, in order to manage water loss and pathogens attacks at the fruit surface. The occurrence of fissures may be due to the inability of the epidermis and cuticle, a stiff rigid layer, to expand at the necessary rate to cope with fruit cortex growth, mainly during early developmental stages. Remarkably, apple skin russeting is a genetic trait. A major quantitative trait loci (QTL) controlling this mechanism and involved in cuticle organization and suberin plug deposition have been described for the apple variety "Renetta Grigia di Torriana" (Falginella et al., 2015). A very important aspect is that transcriptomic analysis of differentially russeted apple varieties showed a reduced expression of cutin and cuticular waxes biosynthetic genes and simultaneously an increased expression of suberin biosynthetic genes on russeted apple varieties (Legay et al., 2015). The same transcriptional profile was observed comparing russeted and nonrusseted patches of the semirusseted apple variety "Cox Orange Pippin" (Legay et al., 2017). Strikingly, suberin-type alkyl-hydroxycinnamates, product of the esterification of hydroxycinnamic acids with primary alcohols, resulted from the enhanced expression of suberin-related genes *KCS2* and *KCS4* and *FAR5* in russeted patches (Legay et al., 2017). Curiously, apple orthologous genes *CER1* and *CER3* responsible for alkane biosynthesis were upregulated in nonrusseted skin patches of the semirusseted apple variety, indicating the importance of alkanes and further secondary alcohols and ketone biosynthetic pathway in the formation of apple skin cuticular waxes. Parallel results were also found in tomato *DCR* (defective in cuticular ridges; BAHD acyltransferase) RNAi silencing lines (Lashbrooke et al., 2016) that have the fruit skin severely impaired, showing the formation of a coating of superficial corky material. This phenotype was accompanied by an increase in suberin monomer molecular types, including ferulate, and a shift toward transcript expression of suberin biosynthetic genes GPAT5 and ASFT (Lashbrooke et al., 2016). These data suggest that the biosynthetic pathways of suberin and cutin might be under a close regulatory interplay with developmental or stress induced cutin disruption or deficiency being substituted by suberized patches establishment.

Plant hormones function as chemical signals in the perception of environmental factors and response to stress conditions. During cork development in oaks, abscisic acid (ABA)-related genes and ABA-linked MYB transcription factors were upregulated. Moreover, genes involved in ethylene, auxins, and jasmonates biosynthesis had also enhanced expression during cork formation, indicating that these four hormones may participate in abiotic stress response, cell wall differentiation, and regulation of secondary metabolism involved in suberin and associated waxes deposition (Teixeira et al., 2018). Interestingly, ABA mediates spatio-temporal fine-tune changes in cell metabolism, transport processes, and wall composition in response to salt stress in roots, namely prompting localized regulation of suberin and lignin biosynthetic genes in root endodermal and exodermal cells (Barberon et al., 2016; Geng et al., 2013). In sugarcane, ethylene, in synergistic action with ABA, was implicated in leaf abscission and in internode ripening, and consequent regulation of sugar storage levels and sink strength of the internode (Cunha et al., 2017; Xu et al., 2018), physiological mechanisms dependent on tissue suberization, and lignification in order to cope with tissue disruption or the strong osmotic potential generated. Genes coding for transcription factors involved in ethylene perception and signal transduction, ETR2 (ethylene receptor 2), ERF1 (ethylene responsive transcription factor 1), and EIN4 (ethylene insensitive 4), were upregulated during suberin deposition associated with good quality cork layer formation in *Q. suber* (Teixeira et al., 2018). Moreover, sugarcane ERF3 was induced by suberin-linked stress conditions as ABA, salt stress, and wounding and when constitutively expressed in *Nicotiana tabacum* conferred elevated tolerance to drought and osmotic stress to the transgenic plants (Trujillo et al., 2008).

6 | SUGARCANE GENOMIC AND TRANSCRIPTOMIC RESOURCES

The genetically diverse germplasm of sugarcane constitutes a powerful source for improving sugarcane forage crops through biotechnological or breeding approaches. Important features such as biomass yield, fiber content, cell wall amount and composition, and sugar content are genotype-dependent in *Saccharum* genus that includes great genetic variation among varieties and also between different species. Curiously, though *Saccharum* spp. hybrids derive from a small genetic background, a relatively high genetic variability is observed due to sugarcane heterozygosity and high polyploidy. Also, the parental sugarcane species show higher variability comparing to cultivated *Saccharum* spp. hybrids. *S. spontaneum* has the larger anatomical diversity, from stalks yield, sucrose content, and fiber amount to biotic and abiotic stress tolerance (Paterson et al., 2013). Noteworthy, today's diversity among *Saccharum* spp. hybrids results largely from introgression from *S. spontaneum* (de Setta et al., 2014). Other sources of genetic diversity are found in the "*Saccharum* complex," namely the genus *Miscanthus* and *Erianthus* that can potentially be interbreed to sugarcane (Paterson et al., 2013). This way, the diversity in genome content and structure and especially the allelic variation between sugarcane parental species, mainly *S. officinarum* and *S. spontaneum,* and other close species constitute an important genetic source for sugarcane biomass improvement.

The allelic variation of sugarcane complex genome has been decoded through a number of resources such ESTs (expressed sequenced tags), full-length cDNA, transcriptomics and bacterial artificial chromosome (BAC) libraries. The largest collection of sugarcane ESTs was generated by the SUCEST project from cDNA libraries of different tissues from several sugarcane varieties (Vettore et al., 2003) and was important for sugarcane gene discovery, transcript profiling, genome exploration and transcriptome and proteome analysis. Recently, the transcriptome analysis by next-generation sequencing technology RNA-seq has furnished important data to identify molecular key intermediaries of cell wall biosynthetic mechanism and of biomass yield and recalcitrance (Mattiello et al., 2015; Singh et al., 2018; Vicentini et al., 2015; Xu et al., 2018). On the other hand, the genome of sugarcane due to its high polyploidy is extremely complex and has not been completely sequenced or a detailed physical map produced. A draft sugarcane genome based on whole-genome shotgun sequencing of the SP80-3280 cultivar has been released recently (Riaño-Pachón & Mattiello, 2017). The main genomic resources to date have been BAC libraries of cultivar R570, *S. officinarum* and *S. spontaneum* among other varieties genomes (de Setta et al., 2014; Hotta et al., 2010). Noteworthy, sorghum genome is fully sequenced and annotated and has been used as reference for sugarcane genomic comparative analysis, as sorghum phylogenetically is the most related species to sugarcane with high levels of microcollinearity between both genomes (de Setta et al., 2014).

7 | CONCLUSIONS AND FUTURE PROSPECTS

In grasses, a major constrain to forage digestibility is the total content in recalcitrant biopolymers, particularly lignin and suberin. It is important to note that the rumen microflora constitute a complex microenvironment that adjusts to large extent to grass forage digestion. Curiously, grass forage digestion is largely confined to the secondary cell wall due to poor digestibility of mature primary cell wall caused by heavy lignification and the presence of the suberin lamella. Ruminant diets based in grass forages are rich in cellulose and have intermediary levels of soluble sugars, activating cellulolytic and sucrolytic bacterial enzymes in favor of starch lytic bacteria. Grass-based diets release also high levels of *p*-coumaric acid that restrain microorganism multiplication and activity of the polysaccharide degrading bacteria; however, there are rumen microorganisms able to detoxify hydroxycinnamates (Arcuri, Lopes, & Carneiro, 2011).

A profuse and detailed knowledge about the transcription factors and regulation mechanisms of suberin and associated waxes biosynthesis will allow accurate biotechnological approaches to the production of more feasible crops (Table 2). The study of suberin-related transcription factors may prove useful to define the flux of key metabolic intermediaries toward suberin production in the broader network of regulatory factors that intervene also in phenylpropanoid metabolism and lignin biosynthesis. Another important aspect is to elucidate the responsive factors controlling suberin and associated waxes accumulation in sugarcane without unbalance the proportion of the different aliphatic and hydroxycinnamic monomer components and compromising the remarkable features of impermeability, flexibility, and anti-microbial resistance of suberin necessaries to plant survival. The cross-linking mediated by hydroxycinnamic acids between the suberin, lignin, and glucuronoarabinoxylans matrices may be an additional target into engineering sugarcane hybrids with improved digestibility.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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