CFW FEATURE

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Cell Walls of Cereal Grains

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The mature cereal grain is composed of the maternal tissues of the pericarp-seed coat and the nucellar remnants. These tissues overlie the tissues of the progeny, comprising the embryo or primordial plant, and are separated from the starchy endosperm-aleurone layer by the scutellum. Each of these tissues is composed of cells whose protoplasts are enclosed by a cell wall. In the case of the pericarp-seed coat tissues, at maturity the contents of the cells have disappeared, and only the thick walls remain. The cells of the starchy endosperm, which are also dead, are packed with starch granules and storage protein and usually have quite thin walls. In contrast, the cells of the aleurone, which are still alive, have thick bilayered walls enclosing the protein and lipid-rich cell contents (Fig. 1).

Impact of Cell Walls and Their Components on End Uses of Grains

The cell walls of the various grain tissues and their components have important impacts on the end uses of grains. In milling, water uptake during the conditioning step and the breakage pattern are influenced by cell wall organization and the composition of the pericarp-seed coat tissues. In the development of doughs for breads and pastas, the wall components directly affect water absorption, mixing behavior, dough rheology, and the final properties of the baked product. In malting (germination), water uptake and endosperm dissolution rates are critically affected by the physical structure and composition of the cell walls. In brewing, the mashing, wort, and beer filtration steps and the character and stability of the final beer product are affected by wall structure and composition. Dietary fibers from grains, which have important implications in human nutrition and disease prevention, are found primarily in the cell walls. The robust walls of aleurone cells (Fig. 1) are not broken during roller milling, and because they are not susceptible to digestion in the upper alimentary tract, nutrients in the cell (e.g., high-quality proteins, lipids, and B vitamins) remain unavailable until the aleurone particles reach the large intestine. In monogastric animals (pigs and poultry), the integrity of the grain cell walls is implicated in depression of feed quality and in compromised carcass and egg hygiene.

Composition and Structure of Cell Walls in Relation to Biological Functions of Grain Tissues

The compositions of endosperm, aleurone, outer pericarp (beeswing bran), and bran (pericarp, seed coat, aleurone) cell walls are compared in Table I. The walls of the starchy endosperm and the overlying aleurone cells are rich in polymers, including polysaccharides as the most abundant components and smaller amounts of proteins. The walls of the pericarp-seed coat cells are qualitatively similar in terms of polysaccharide composition, but some cell types also contain significant amounts of lignin, a hydrophobic, polyphenolic polymer, and the walls of the epidermal cells have an outer layer of cutin, a water-repellant polyester.

These cell wall compositions reflect the different functions of the tissues to which the cells belong. The starchy endosperm is a transient food reserve, and during germination, the simple, thin walls are readily degraded by hydrolases synthesized in, and secreted from, the aleurone, allowing access to their starch and protein substrates. The bilayered walls of aleurone cells are themselves a barrier to the release of hydrolytic enzymes. During the early stages of germination, the thicker, outer aleurone wall layer is preferentially degraded. The inner layer persists, however, until the cells die. The pericarp tissues, which are in fact modified leaves, protect the developing and mature endosperm-embryo. The closely associated seed coat that overlies the outer surface of the aleurone has a layer of suberin (cork) that forms an impermeable barrier and prevents the penetration of water into the underlying endosperm.

The polysaccharide composition of the cell walls of both the pericarp-seed coat tissues and the tissues of the progeny (starchy endosperm, aleurone, scutellum, and embryo) characteristically contain cellulose and two noncellulosic polysaccharides, arabinoxylans and (1 3,1 4)-β-glucans, although their proportions depend on the cell type (Table II). These noncellulosic polysaccharides are characteristic of the grass family among the monocotyledons and set them apart from the remainder of

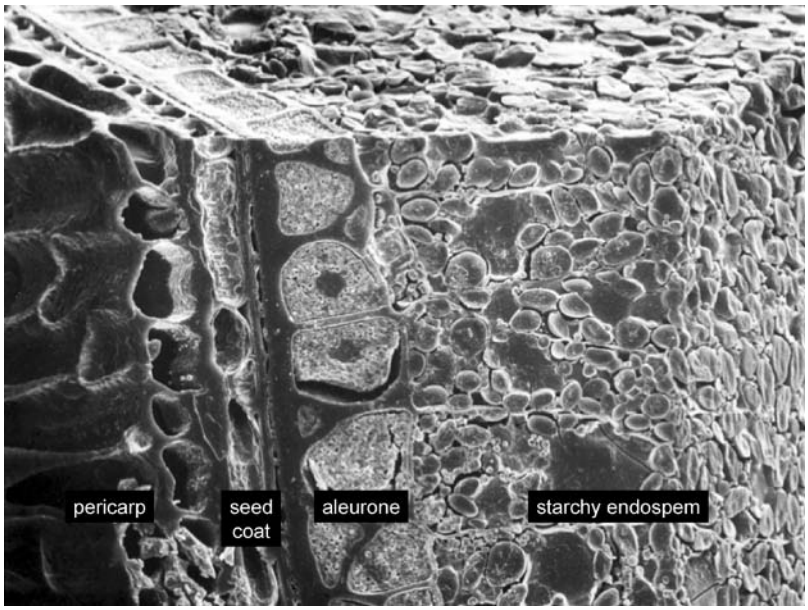

Fig. 1. Scanning electron micrograph of a portion of a wheat grain, showing the starchy endosperm, aleurone and overlying nucellar remnants, seed coat, and inner pericarp. The outer pericarp has been lost in preparation. (Reprinted from Joyner [12])

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the monocots and dicots, whose major noncellulosic wall polysaccharides are typically xyloglucans and pectins.

Organization of Wall Components

The model of a cell wall shown in Fig. 2 suggests the noncellulosic polysaccharides (in the case of grasses, arabinoxylans and (1→3,1→4)-β-glucans) and proteins constitute a gel-like matrix phase in which the microfibrillar cellulose and associated glucomannans are embedded.

The surfaces of unextracted walls of wheat endosperm viewed using transmission electron microscopy are without structural features (15), but after extraction of water-soluble polymers, chiefly arabinoxylans, profiles of cellulose microfibrils can be seen in an amorphous background. Further extraction of water-insoluble arabinoxylan and (1→3,1→4)-β-glucan with 1 *M* NaOH fully exposes a web of microfibrils, as expected based on the cell wall model (Fig. 2). The cellulose microfibrils themselves are associated with 4 *M* KOH-soluble glucomannans.

Significantly, there is little difference in the basic chemistry or molecular size distribution of water-extractable and -unextractable (1 *M* NaOH-soluble) arabinoxylans from endosperm walls (16). The differential extractability of the noncellulosic polysaccharides most likely depends on the nature and extent of noncovalent and/or covalent associations between wall polymers. The model (Fig. 2) shows physical associations between arabinoxylans and (1→3,1→4)-β-glucans themselves, with one another (11), and between these polymers and the surfaces of the cellulosic microfibrils.

The involvement of covalent cross-links between arabinoxylans through dehydrodiferulic ester bridges (Fig. 3A) has also been proposed to play a role in wall integrity. The observation that treatment with dilute alkali, specifically neutral hydroxamic acid, liberates a substantial fraction of the arabinoxylan from water-extracted endosperm walls (16) may support this proposal. However, dilute alkalis may also alter the physical form of noncellulosic polysaccharides, causing them to dissociate from the wall. A comprehensive analysis of these interactions remains to be done. A physicochemical assessment of polymer interactions in the native wall using currently available physical methods might provide the needed information.

The availability of isolated walls from wheat aleurone cells (2,22,26) has allowed possible covalent associations between polymeric components to be explored. As in the walls of starchy endosperm cells, the noncellulosic polysaccharides are chiefly (1→3, 1→4)-β-glucans and arabinoxylans, but the content of monomeric hydroxycinnamic acids esterified to the arabinoxylans is a much higher (1.8% compared with 0.05%) (Table I). Consequently, the aleurone walls are strongly autofluorescent (2,21). Only very small amounts of esterified dehydrodiferulic acid are present, as a result bridges between arabinoxylan chains involving these structures are not important in aleurone wall organization. Proteins comprise 1% of the aleurone wall and are readily detected throughout the wall using the fluorescent dye Ponceau Red. At least three protein types are present: glycine-rich (37–86%), proline-rich (11–39%), and serine-rich (up to 23%) (22). Removal of arabinoxylan and (1→3,1→4)-β-glucan using specific hydrolases leaves a residue rich in protein (4.5%)

Table I. Composition of different types of cell walls in wheat grain (%)

Type of Cell Wall	Cellulose	Gluco-mannan	(1→3,1→4)-β-D-Glucan	Hetero-xylan	Phenolic Acids	Lignin	Protein[a]
Aleurone[b]	2	2	29	65	1.85		1
Starchy endosperm[b]	4	7	20	70	0.05		0.5
Bran (pericarp, seed coat, aleurone)[c]	29		6	64	0.45	8.3	9.2*
Beeswing bran (outer pericarp)[d]	30			60	0.5	12	6*

[a] * indicates value may include cellular proteins.
[b] Bacic and Stone (1,2).
[c] Selvendran and coworkers (24).
[d] Ring and Selvendran (23).

Table II. Comparative compositions of cell walls of different cereal grains

Cereal Type of Cell Wall	Component (% wt)				
	Cellulose	Gluco-mannan[a]	(1→3,1→4)-β-D-Glucan	Heteroxylan	Protein[b]
Wheat (*Triticum aestivum* L.)[c]					
Aleurone	2	2	29	65	1
Starchy endosperm	4	7	20	70	0.5
Barley (*Hordeum vulgare* L.)[c]					
Aleurone	2	2	26	71	6*
Starchy endosperm	2	2	75	20	5*
Rice (*Oryza sativa* L.)[d]					
Starchy endosperm	28	15**	20	27	18*

[a] ** indicates genotype has a very low glucomannan content. Value given is for xyloglucan/mannan content.
[b] * indicates values may include cellular proteins.
[c] Bacic and Stone (1,2).
[d] Shibuya and coworkers (25). Rice starchy endosperm also contains 3% (wt) pectin.

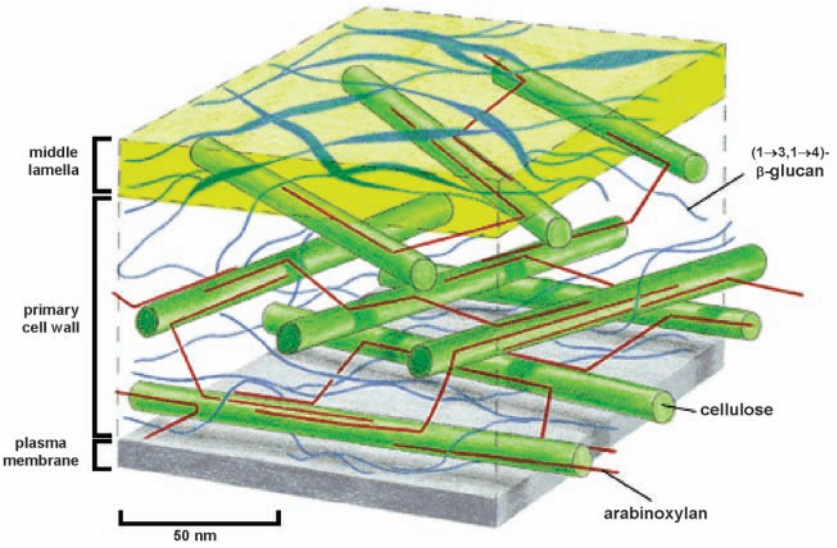

Fig. 2. A simplified schematic representation of the spatial arrangement of polymers in a primary cell wall of a cereal, e.g., the wall of a starchy endosperm cell. The cellulosic microfibrils are embedded in a network of noncellulosic matrix polysaccharides (arabinoxylans and (1→3,1→4)-β-glucans and proteins [not shown]) that are associated with the surfaces of several microfibrils. The immature primary wall may contain ≈60% water, but during development of walls of some cells in the pericarp-seed coat, the water is replaced by lignin, which encrusts the noncellulosic polysaccharides and proteins and may be covalently bound to them. (Based on McCann and Roberts [19])

and a highly branched arabinoxylan that retains its UV-induced autofluorescence even after alkali treatment. This has led to the suggestion (22) that the ferulic acid esterified to the arabinoxylan may be cross-linked to wall protein through ferulate-tyrosine bridges, as originally proposed by Geissmann and Neukom (8) (Fig. 3B).

Additionally, some of the wall proteins themselves may be cross-linked through tyrosine-tyrosine dimers (Fig. 3C). Small amounts of such dimers have been reported in endosperm proteins (9). Although proteins are well-known cell-wall components, their potential for interaction and perhaps reinforcement of the predominant polysaccharide components needs to be explored further. In contrast to the walls of the endosperm and aleurone cells, many of the pericarp-seed coat walls are reinforced with lignin, which replaces the water in the gel-like matrix (Fig. 2) and overlies the noncellulosic polysaccharides and proteins found there. As a consequence, the walls are physically strong and resistant to digestion by microorganisms in human and monogastric large intestines and in the rumen. Lignified cell walls in the stems (internodes) of grasses have compositions similar to those of the pericarp (Table I), and there is a clear relationship between the decrease in in vitro digestibility and the accumulation of wall-bound hydroxycinnamic acids during maturation (13).

Detailed examination of the forms of wall-bound hydroxycinnamic acids shows that some of the ferulic acid esterified to wall polysaccharides (arabinoxylan) forms dehydrodiferulic acid bridges between arabinoxylan chains (Fig. 3A). In addition, some of the ferulic acid (as well as dehydrodiferulic acid) esterified to the arabinoxylan is also etherified to lignin, forming ester-ether bridges between the two wall polymers (10). A correlation between ester-ether bridge content, but not lignin content, and in vitro digestibility has been shown for internodes of the pasture grass *Phalaris aquatica* (14). Proteins could also be involved in this cross-linking. The covalent cross-linking between the polymeric components in the lignified wall matrix would exclude polysaccharide hydrolases and so prevent access to their substrates. The poor digestion of lignified cell walls by ruminants is largely explained by this barrier.

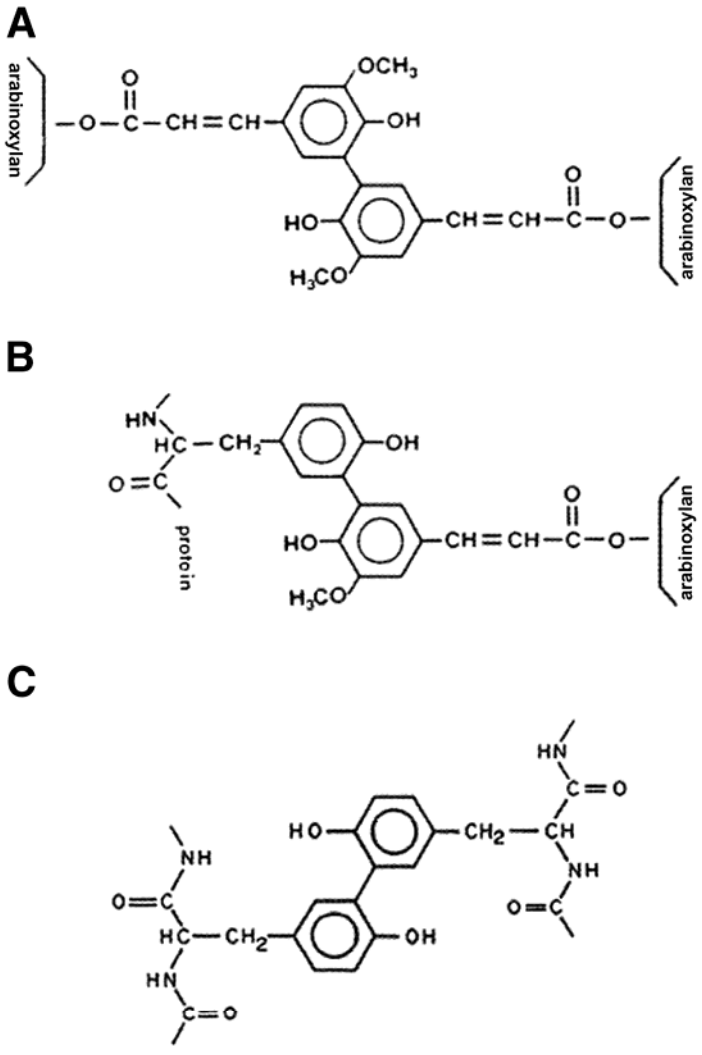

Fig. 3. Three modes of covalent cross-linking between cell wall polymers: **A,** a diferulate cross-link between two arabinoxylan chains; **B,** a tyrosyl-ferulate cross-link between a protein and a feruloylated arabinoxylan; and **C,** a dityrosyl cross-link between two protein chains. (Based on Geissmann and Neukom [8])

Development of Endosperm Cell Walls

Endosperm development in wheat begins with the cellularization of the multinucleate primary (3n) endosperm cell within five or six days after anthesis (15,18). The periclinal walls of first-formed cells are deposited between nuclei lining the periphery of the endosperm mother cell, without nuclear division or normal phragmoplast formation (18). This mode of wall initiation is quite unlike that in vegetative cells, in which the new wall is deposited centripetally at the cell plate situated in a phragmoplast between the daughter nuclei (20).

The endosperm cellularization process in cereals has been described in detail by Brown and coworkers (3). A (1→3)-β-glucan–specific monoclonal antibody has shown that the (1→3)-β-glucan callose is a major wall component in the developing periclinal walls in the syncytial endosperm of rice (4). Extension of these observations to cellularizing barley endosperm using four specific monoclonal antibodies has shown that the initially deposited callose in periclinal walls is replaced by (1→3,1→4)-β-glucan and that arabinoxylan and glucomannan are later added to the wall (27). Although the cytology of periclinal wall development in the nuclear endosperm is different than normal wall formation, a transient deposition of callose is common to both types (20).

Approaches to Manipulating Wall Structure and Composition

The critical effects of cell walls and their components on the end uses of cereal grains suggests that changing wall composition (e.g., increasing or decreasing (1→3,1→4)-β-glucan content or altering the degree of cross-linking of polysaccharide components) could be advantageous for specific applications. Already, selection based on natural variation in wall composition is possible, and marker-assisted selection for traits such as Ara/Xyl ratio and (1→3,1→4)-β-glucan, ferulic acid, ester-ether bridges, and wall protein contents is feasible. In some cases markers have already been identified. Changes in wall composition have also been observed following mutation. Generation of transgenic cereals with desirable cell-wall compositions using current technology is now a possibility with the recognition of genes for synthases for cellulose (5), heteromannans (7), and (1→3,1→4)-β-glucans (6). Postharvest chemical and enzymatic treatments have been applied to the walls of the pericarp-seed coat of grains to increase their fermentability by microorganisms.

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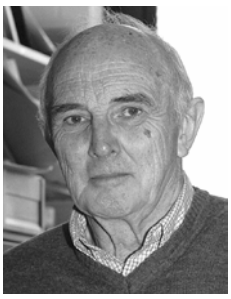

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