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An Insight into Plant Cell Wall Protein

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Introduction

The primary plant cell wall is mainly consisted of a network of polysaccharides viz., cellulose microfibrils, hemicellulose that wraps and entangles cellulose microfibrils, and pectin. After the cells have finished growing, secondary walls synthesize other compounds such as lignin, wax and cutin. Cell wall proteins (CWPs) differ among plants and their distribution within functional classes differs based on cell types, organs, developmental stages and/or environmental conditions. The plant cell wall is a dynamic structure that undergoes changes during its development. Cell wall proteins (CWPs) play important part in plant cell walls development and adjustment to the environment. There are various experimental techniques available to characterize cell wall proteomes.

Structure of cell wall

The plant cell wall is multi-layered and consists of outermost layer of the cell wall. They are identified as the middle lamella, primary cell wall, and secondary cell wall. All plant cells not necessarily have a secondary cell wall.

- **Middle lamella:** This layer is made up of Pectin-containing polysaccharides, the outer cell wall. The major function of pectins is to facilitate cell adhesion by assisting the adherence of the cell walls to the surrounding cells.
- **Primary cell wall:** In developing plant cells, the layer between the middle lamella and plasma membrane is called the primary cell wall. Hemicellulose fibres and pectin polysaccharides form a gel-like matrix that is predominantly made up of cellulose microfibrils. The basic cell wall offers the durability and adaptability required for cell development.
- **Secondary cell wall:** In some plant cells, this layer develops between the primary cell wall and plasma membrane. Once the primary cell wall stops dividing and expanding

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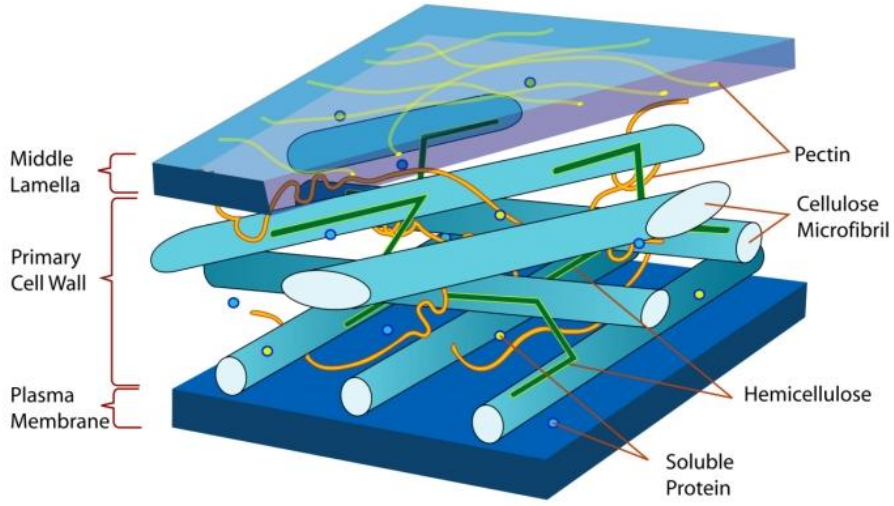
it may thicken to create a solid layer called secondary cell wall. Some secondary cell walls furthermore include lignin in addition to cellulose and hemicellulose. Lignin helps plant vascular tissue cells transmit water and reinforces the cell wall.



General role of CWP

CWP involved in the modification of cell wall components, signal transduction, interaction with apoplasts and plasma membrane and almost all cell wall-related processes. CWP is normally split functional categories such as carbohydrates, proteins that act on oxidoreductases, Proteins associated with the most common classes, lipid metabolism and proteases which are the abundant compounds in the cell wall.

Specific features of CWPs

(i) CWPs represent only 5–10% of the total cell wall mass. Depending on the type of cell wall, it is embedded in a complex matrix of carbohydrate polymers, aromatic compounds, waxes, or cutin.

(ii) CWP can interact with cell wall components via non-covalent bonds. They can also covalently bind to form insoluble networks such as proline-rich proteins (PRPs) or structural protein networks of extensins.

(iii) Unlike other intracellular compartments, the cell wall of a plant forms an open space that connects cells within the tissue. It is Located between the plasma membrane and the cuticle of the air organs or the Suberin layer of the root, it provides waterproofness and protection to the plant from stresses.

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(iv) Mostly CWPs are basic proteins directed towards the secretory pathway, such as structural proteins linked to the wall and those secreted into the apoplast and extracellularly.

(v) CWPs undergo post-translational modifications (PTMs), like hydroxylation of proline (Pro) residues converting them to hydroxyproline (Hyp), N-glycosylation, O-glycosylation or addition of a glycosylphosphatidylinositol (GPI) - anchor.

CWP and their interactions with cell wall components

The current model of the plant cell wall illustrates the placement of its components in two structurally independent interacting networks embedded in the pectin matrix. Cellulose microfibrils and hemicellulose form the first network. The second is formed by structural proteins. All proteins secreted into the extracellular space, as well as proteins located at the interface between the plasma membrane and the cell wall, are used as CWPs. The three types of CWP can be distinguished by their interaction with cell wall components. CWP interacts little or no with cell wall components and is therefore free to move in extracellular space. Such proteins can be found in cell suspensions, in liquid media, or extracted with low ionic strength buffers. This fraction is called an "unstable/labile protein". Most of them have an acidic pH in the range of 2-6. Alternatively, CWP can weakly bind to the matrix via van der Waals interactions, hydrogen bonds, hydrophobic or ionic interactions. Such proteins can be extracted by salts and most of them have a basic pH in the range of 8-11, so they are positively charged at the acidic pH of the cell wall. Most cell wall polysaccharides are neutral, but negatively charged pectins contain polygalacturonic acid, which negatively charges their interactions with high pH proteins. Such interactions are tuned by pH, pectin esterification, Ca2 + concentration, and the mobility and diffusivity of these macromolecules. Finally, CWP is resistant to salt extraction because it can bind strongly to cell wall components. For example, extensin is cross-linked via covalent bonds and peroxidases and can have a high affinity for Ca2 + pectate.

Functional classification of CWPs

The number of non-redundant proteins was collected from each species and sorted into functional classes. proteins acting on carbohydrates (PACs), oxidoreductases (ORs), proteases (Ps), proteins related to lipid metabolism (LMs), proteins possibly involved in

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signaling (Ss), proteins with predicted interaction domains (IDs), miscellaneous proteins (Ms), proteins of unknown function (UFs) and structural proteins (SPs).

Proteins acting on carbohydrates (PACs

Glycosyl Hydrolase (GH) families are proteins involved in cell wall carbohydrates remodeling and can be regulated during development. These families are used in enzymatic cocktails for biomass degradation in second-generation ethanol production. It is numerous in monocots and some are involved in endogenous metabolism. Starch is a plant carbohydrate often linked to storage organs, and its breakdown is mediated by amylases. In grains, there is an extracellular matrix enriched in starch, which is degraded by secreted enzymes, the alpha-amylases. Type II cell walls present as mixed glucans as the principal hemicellulose which is the substrate for GH that displays glucan-1,3-β-glucosidase activity. These enzymes are used in enzymatic cocktails for biomass deconstruction and are considered one of the most efficient enzymes in breaking glycosidic bonds in hemicelluloses. As grasses are used as raw materials for biofuels production, the identification of GH functions could be valuable in solving the difficulties related to biomass deconstruction. It regulates carbon partitioning by cleaving apoplastic sucrose and helping in the process of carbon import into the cell thus involved in the sugar transport.

GH families have more members in plant proteome and a possible explanation is that their substrates are xyloglucans and galactans, the last related to pectins. Galactan is involved in xyloglucan structure and mediates the interaction between xyloglucan and cellulose thus in cell elongation and in biomass deconstruction, as they are rich in arabinoxylans. They also involved in and hemicellulose hydrolysis along with the enzyme hemicellulases. Pectin is one of the main constituents of the primary cell wall and involved in wall porosity, charge density and microfibril spacing. Consistently with the fact that type II-wall plants have lower pectin content, Pectin Methyl Esterases (PMEs) and Pectate Lyase-like proteins. After the transport from the Golgi apparatus to the cell wall, pectin is partially deesterified by PMEs, exposing a carboxyl group on galacturonosyl residues and allowing the pectin to be stiffened by ionic crossbonding with calcium ions. The degree of methylation impacts on the wall stiffening and access to enzymes.

Oxidoreductases

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ORs mostly comprise several class III peroxidases (Prxs), multicopper oxidases, plastocyanins, berberine-bridge enzymes (BBEs) and blue copper-binding proteins. Prxs, part of large multigenic families, can either oxidize phenolic compounds, and consume hydrogen peroxide or generate reactive oxygen species. They have been involved in several functional roles, such as cell elongation, lignin metabolism, stress responses and germination. As Prxs are versatile proteins, they can both promote cell wall expansion or the crosslinking of its components, favoring cell wall strengthening; it is difficult to establish a correlation between their higher or lower proportion and their metabolic function. Polyploidy species are having higher amount of peroxidases and different Prxs were identified when using destructive and non-destructive CWPs extraction which was suggested to be due to a differential level of pectin-binding capacity. The proteins related to lignification, germination and the second to cell elongation inhibition and cell wall strengthening were also identified in plants. Some peroxidase was also linked to lignin content which controls the lignification of tissues and changes the cell wall properties, to higher production of reactive oxygen species that led to cold tolerance, evidencing the multiple roles of peroxidases in plant development. Some berberines (BBEs) can be identified as monolignol oxidoreductases, and are related to lignin formation.

Proteins Related to Lipid Metabolism

Lipid Transfer Proteins (LTPs) are encoded by large multigenic families, which are considered to be essential to land colonization by plants, and are among the most abundant secreted proteins, but their exact in vivo role is still unclear. It has been suggested that LTPs mediate the transference and adhesion of molecules required for the composition of lipid barriers that are water-resistant, such as cutin, suberin and wax. In the leaves of C4-metabolism plants, suberin surrounds the plasma membrane of bundle sheath cells, inhibiting CO_2 diffusion, which could be a possible explanation for increased LTPs in these species. Accordingly, in few plants some LTPs were only identified in leaves. LTPs were also associated with lipid deposition for cell expansion, as their transcripts were differentially expressed in maize elongating internodes in comparison to non-elongating ones. LTPs are sometimes negatively regulates plant defense mechanisms through the regulation of the antagonism between abscisic and salicylic acids, and some are disease-related marker show some level of redundancy in plant immunity. Sometimes LTP linked with lipid rafts in root-

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hair tip growth, suggesting that root hairs could be used as a model to study lipid rafts in plant development.

Proteases

Essentially, Ps break peptide bonds and control several relevant plant processes, such as protein transport, activity and half-lives, being generally divided into aspartyl (Asp), serine, cysteine, metallo and threonine proteases. The senescence-associated subtilisin is a serine protease identified in spme monocot species cell wall proteomes. Subtilisin was associated with the regulation of abscisic acid (ABA) signaling and drought tolerance, probably through a conserved mechanism between dicots and monocots, given the relevant role it plays. The cysteine protease papain-like protein has been associated with senescence and necrotic cell death as its expression increases along with leaf development pointing to its use as a senescence marker in monocots.

Proteins with Interacting Domains (IDs)

IDs encompass Pectin Methyl Esterase Inhibitors (PMEIs), proteins with leucine-rich repeat (LRR) and Lysm domains, protease inhibitors such as cystatins, Bowman-Birk inhibitors, lectins and jacalins. The LRR-containing domain is conserved throughout evolution in the plants, displaying activity in the innate immune system through the sensing of pathogen-associated molecular patterns. LRR-domain protein was associated with reduced damage caused by infection of a root nematode by inducing plant camalexin and indole-glucosinolate pathways. Perhaps both proteins could be part of the conserved defense mechanisms against nematodes in dicots and grasses.

Proteins Possibly Related to Signaling

This class of CWPs is composed by fasciclin-like arabinogalactans (FLAs), leucine-rich repeat receptor protein kinases (LRR-RKs) and COBRA-like proteins (COBLs). Among the CWP, FLAs seem to be more numerous and are related to cell-to-cell adhesion, mechanical strength for secondary cell walls and cellulose biosynthesis and in elasticity. These FLA is act on the lateral root and shoot formation in tissue culture and they could display organ-specific activities. COBL are specific plant proteins and associated with cell expansion and cellulose level of crystallinity, and specifically in elongating tissues. COBL protein is linked to cellulose biosynthesis in the secondary wall, affecting plant mechanical strength. LRR domain-containing proteins are conserved in both dicots and monocots.

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Components of structural proteins

Biological structures have one thing in common that is structural proteins. Some of these proteins have been studied in detail and have contributed significantly to the understanding of their structure. The amino acids Gly and Pro (or Hyp) are commonly found as the main components of these structural proteins and appear to be suitable components of structural proteins. The plant cell wall, which is a major structural part of plant cells, also contains structural proteins. Many primary amino acid sequences of these proteins have been deduced from cDNA and genomic sequences, but little is known about the exact localization and developmental expression patterns of these proteins. However, both synthesis and cross-linking of these wall proteins have been found to be under strict developmental control. In addition, their synthesis and cross-linking can be environmentally driven by several triggers. Despite increasing knowledge of these proteins, their biological function remains largely speculative. To date, three major classes of structural wall proteins have been recognized: extension, PRP, and GRP. Other wall proteins described may also play a structural role, but little is known about these proteins and this discussion will focus on well-characterized wall proteins.

Conclusions

Knowledge of dicotyledonous and monocotyledonous CWP needs to be expanded to gain new insights into Type II cell wall specializations and their adaptive benefits. This helps to genetically adapt plants to improve efficiency and biomass for the production of commercially important products such as second generation bio fuels. In addition to the benefits provided by the initial study of monocotyledonous CWP, determining the role of CWP requires data mining and integration using multiple omics and protein-protein interaction studies.