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Review

Composition of plant cell walls

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Die Zusammensetzung pflanzlicher Zellwände

Zusammenfassung. Der Artikel faßt jüngere Forschungsergebnisse (ungefähr ab Beginn der achtziger Jahre) über die Zusammensetzung der Zellwände von Pflanzen zusammen mit einer Beschreibung der in den mikrofibrillären und amorphen Phasen enthaltenen Polysaccharide: Cellulose, Hemicellulosen und Pektinsubstanzen sowie der übrigen Komponenten: Lignin, Proteine und Enzyme. – Cellulose ist ein lineares Homopolymer aus Mikrofasern, deren parakristalline Struktur durch Wasserstoffbrückenbindungen stabilisiert wird. Die Hemicellulosen stellen eine wichtige Gruppe unter den Polysacchariden dar, die untereinander und mit den Cellulose- und/oder Pektin-Mikrofibrillen verbunden sind. Als wichtigste seien genannt: Xylane, Arabinoxylane, Mannane, Galactomannane, Glucomannane, Xyloglucane, Glucuronomannane, Arabinogalactan II, β-1,3- und β-1,4-Glucane. Die Pektinsubstanzen sind eine komplexe Mischung von kolloidalen Polysacchariden, die der Zellwand mit Wasser oder löslichen organischen Komponenten (chelating agents) entzogen werden können, wobei die bedeutendsten folgende sind: Rhamnogalacturonan I, Rhamnogalacturonan II, Arabinan, Galactan, Arabinogalactan I und D-Galacturonan.

Abstract. The present study reviews the most recent research published (starting approximately in the 1980s) on the composition of plant cell walls, with a description of the polysaccharides contained in the microfibrillar and amorphous phases: cellulose, hemicellulose and pectic substances, as well as the other components: lignin, proteins and enzymes. Cellulose is a linear homopolymer made up of microfibrils that form a para-crystalline structure stabilised by hydrogen bridges. The hemicelluloses constitute an important group of polysaccharides, which are inter-linked and also linked to microfibrils of cellulose and/or pectins, the most important being: xylans, arabinoxylans, mannans, galactomannans, glucomannans, arabinogalactan II, β-1,3-glucan and β-1,3-β-1,4-glucans. The pectic substances are a complex mixture of colloidal polysaccharides that can be extracted from the cell wall with water or chelating agents, the most significant being: rhamnogalacturonan I, rhamnogalacturonan II, arabinan, galactan, arabinogalactan I and D-galacturonan.

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Introduction

The cell wall is a complex, semi-rigid structure that surrounds the cytoplasmic membrane and performs a series of functions which are connected with its structure. It allows the cell to resist internal and/or external pressures, it provides a structural barrier to some molecules and it protects against insect invasion [1]. In addition, it constitutes a source of nutrients, fibre and energy and acts as a buffer between the environment and the protoplasmic content that plays various roles in the life of the tissues and organs. The cytoplasm obtains the substances necessary for its metabolism through the cell wall and excretes other substances across it. By constituting the armour which gives substance to the plant, it is important in determining its texture. Thus, changes in its composition and structure lead to changes in the texture of the plant. For this reason a knowledge of the structure of the cell wall is of great importance.

According to the description given by Showalter [2], the cell wall is made up of a complex amalgam of carbohydrates, proteins, lignin and water, as well as other substances embedded in it such as cutin, suberin and certain inorganic compounds that vary from one plant to another. Some models of cell walls have been described [3, 4] that give a very real idea of the polymers forming a primary cell wall and of the different kinds of bonds that can appear between them.

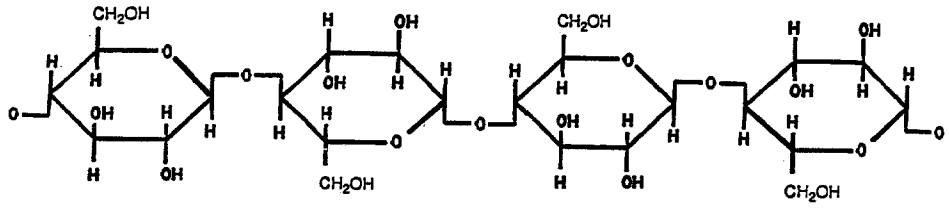

Fig. 1. The structure of cellulose

Morphologically three zones can be differentiated and these are perfectly distinguishable by photonic microscopy [5]: middle lamella, primary wall and secondary wall.

The *middle lamella* is the most external of all three and is shared by two contiguous cells between which it acts as a sort of separating panel. This is the first zone to be formed in the cell during cytoplasmic division. It is colloidal, optically inactive and consists almost exclusively of pectic substances.

Once the middle lamella has been formed, carbohydrates begin to be deposited on both sides of it giving rise to the *primary wall* which is the most external of the two wall zones. This continues to thicken for as long as cell growth lasts. The carbohydrates of this wall, fundamentally cellulose, are synthesised on the surface of the cytoplasmic membrane and may be secreted to the outside of the plasmalemma by exocytosis. The primary cell wall controls cell growth and forms the structural base of the skeleton of the plant.

Many cells have a cell wall formed only by these two layers. However, some specialised cells exist that, once cell expansion is complete, may continue synthesising polymers and give rise to the *secondary wall* [6]. The secondary wall is considered to be a supplementary wall with a predominantly mechanical function [7]. Its appearance gives rise to certain changes in the composition of the middle lamella and primary wall such as lignification [8, 9]. Generally, the secondary wall consists of three layers which are denominated, from outside to inside, S_1, S_2 and S_3. These are distinguishable ultrastructurally by differences in the orientation of their cellulose microfibrils [10]. Normally lignification begins in the middle lamella and primary wall when expansion ceases, and extends into the secondary wall. It is the latter which has the greatest quantity of lignin of the plant.

Fibrous components of the cell wall

In the primary and secondary cell walls, a fibrillar phase can be distinguished, which consists fundamentally of cellulose and another, amorphous, so-called matrix, with a very complex chemical composition.

The microfibrillar phase

This is formed by extremely long and narrow microfibrils which are circular or oval in section. Each of these contains 30–100 molecules of cellulose. It is distinguishable from the matrix phase by its high degree of crystallinity and its relatively homogeneous chemical composition; it is also readily visible by an electron microscope [11].

Cellulose is a polydispersed linear homopolymer of poly-β(1,4)-D-glucose [12] with a degree of polymerisation greater than 15,000 (Fig. 1). This is found in the microfibrils forming a para-crystalline structure, stabilised by intra- and intermolecular hydrogen bonds oriented in a parallel or antiparallel manner. X-Ray diffraction studies suggest that this crystalline structure is surrounded by another which is less organised, in which other polysaccharides, linked to the cellulose by covalent bonds, are found. These polysaccharides, for example mannans and xylans, have a structure similar to those of the amorphous phase. These findings have been confirmed by studies of the composition of α-cellulose (the fraction obtained after separating hemicelluloses, pectic substances and lignin), in which measurable amounts of sugars corresponding to these non-cellulose polysaccharides have been found [13–15]. This finding is supported by the fact that the bonds between cellulose and mannan or xylan are extremely resistant and cannot be broken even by treatment with acids [16].

The amorphous phase or matrix

When viewed by electron microscopy, the amorphous phase appears as a background partially transparent to electrons, which the microfibrillar phase can be distinguished. Its composition is heterogeneous, being made up of a large variety of polymers which are interlinked, as well as being linked to the microfibrils by a variety of different forces [11]. It is difficult to characterise this phase, because to do so it is necessary to isolate its constituent polymers which would involve breaking the bonds that keep them together. In some cases these bonds are covalent and can give a distorted image of the cell wall. Despite this fact, to date almost all of the polysaccharides have been studied and their structure known. Generally, they have been fractionated and sequenced by means of gas chromatography and mass spectrometry together with the use of certain specific enzymes. Due to its highly irregular three-dimensional structure, lignin is the most poorly understood component of the matrix.

Non-cellulose polysaccharides of the matrix

Two large groups of such polysaccharides, hemicelluloses and pectic substances, can be distinguished on the basis of the treatment necessary to extract them from the wall. This classification is widely used.

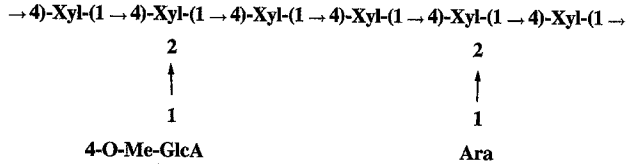

Fig. 2. Xylan

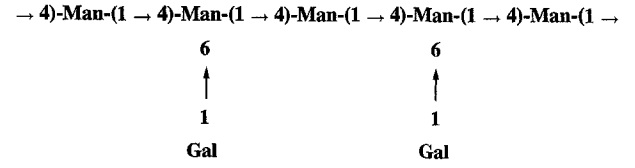

Fig. 3. The structure of galactomannans

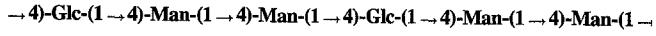

Fig. 4. The structure of glucomannans

Hemicelluloses

Hemicelluloses constitute a very important group of polysaccharides which form links between each other and also with other polysaccharides such as the microfibrils of cellulose and pectins. The definition of hemicelluloses is very generic, but is accepted at present. Under this heading are included those polysaccharides closely linked to the cellulose that are easily soluble in dilute alkali after elimination of the lignin. Furthermore, other polysaccharides which have a similar structure and are soluble in water are also included as are those that degrade upon extraction and, therefore are not isolated. From the alkaline solution these are separated by different precipitation systems. All of the above gives some idea of the enormous complexity of the group of hemicelluloses. Below, a description is given of the most representative hemicelluloses found within the cell wall.

The *xylans* contain a main chain formed by D-xylopyranose residues linked by β-1,4 linkages (Fig. 2). Only one pure xylan has been characterised from esparto-grass (*Stipa tenacissima*). This contains 95–98% xylose [17]; it is more usual to find branched xylans.

The C2 atom of some xylose residues is substituted by a unit of α-4-*O*-methylglucuronic acid. Substitution on C2 or C3 may also occur by α-arabinofuranose, or they may be esterified by acetyl groups. At the same time, xylans with longer side chains also exist. These contain arabinose and other sugars [11]. Below are some examples of the branches that have been characterised in some plant products, the majority of these being linked to the main chain through the C3 of a xylose residue [1].

Gal-(β-1,4)-Xyl-(β-1,2)-Ara$_f$-
Gal-(β-1,5)-Ara$_f$-
GluA-(β-1,4)-Xyl-(β-1,4)-Gal-
Xyl-(β-1,2)-Ara$_f$-,

where Gal denotes galactose, Xyl xylose, Ara arabinose and Glu glucose.

The distribution of the branches is not uniform in all the xylans. Thus, for example, highly substituted xylans can be found that are derived from cells of the mesophyll of rye, the walls of which are very thin. Xylans with a lower degree of substitution are found in the epidermis and vascular tissue [18]. Xylans also exist with ferulic acid substituents. The diferulic bridges could be a form of interaction with the rest of the cell wall components [11, 19]. The links with polyphenolic compounds can be so strong that a previous delignification stage is necessary in order to extract them [8]. This is true for the majority of the gymnosperms [20].

The xylans are very abundant in plants; in soft woods they are present at 7–12% and in hardwoods at 25% [11]. In wheat bran they are present at 5.4% and contain small amounts of arabinose, galactose and uronic acids [13]. In the tomato, an acid xylan has been found that contains residues of 1,2- and 1,2,4-rhamnopyranose, which indicates that they could be linked to some pectic polysaccharide [21]. In the olive (*Olea europaea*) a xylan has been described that has a ratio of xylose: 4-*O*-methylglucuronic acid of 24:1. This is formed by a main chain of β-1,4-xylopyranose and branches between the *O*-2 of the xylose residue [8, 22]. In the spruce (*Pseudotsuga menziesii*) there is a xylan with residues of 1,3-arabinose and 1,2-glucuronic acid [23] and in the pod of the bean (*Phaseolus coccineus*) there is a glucuronoxylan in which the ratio of glucuronic acid to its 4-methyl ester is 1:5.4 [24].

The *arabinoxylans* constitute the major xylan component of the cell wall of the gramineas; one has been isolated from wheat. The side chains of this component contain one single residue of arabinose united to the C3 and/or C6 atom of the main chain [25]. This polysaccharide has also been located in couch grass (*Cynodon dactylon*). In this species, the arabinose side chains contain polyphenolic residues that dimerise after various days of exposure to light, thus increasing the fibrousness of the cell wall [26].

The *mannans* are formed by a chain of β-1,4-mannose. If there is also a α-1,6-galactose chain present, they are called *galactomannans*. The pure mannans are very compact polysaccharides due to the presence of abundant intramolecular hydrogen bridges and can even come to occupy positions between the cellulose microfibrils [11], while the galactomannans are soluble in water and can be classified with the gums [27] and mucilages [28].

Two morphologically different mannans have been identified in the ivory nut (*Phytelephas macrocarpa*). One has a crystalline structure and the other an amorphous structure. The latter has a microfibrous appearance analogous to cellulose [17]. Mannans have also been isolated from coffee beans, these containing at least one residue of galactose for every hundred residues of mannose [29] and also from sunflower [14] and sugar beet [18].

Galactomannans with a low mannose:galactose ratio (of the order of 3:4) (Fig. 3), have been detected in the seeds of the three-spined acacia (*Gleditsia triacanthos*) [30] and in seeds of *Crotalaria medicaginea* [31].

The *glucomannans* are made up of a chain of β-1,4-glucose and β-1,4-mannose without any regularity in its sequence (Fig. 4). Those that contain a single galactose

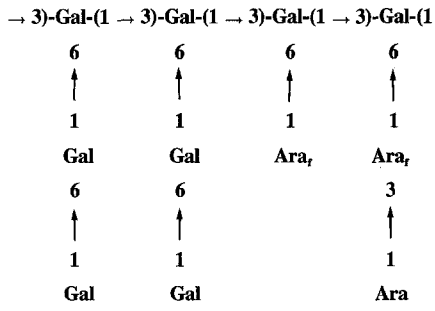

Fig. 5. The structure of arabinogalactan II

residue in the side chains in a proportion similar to that of glucose are called *galactoglucomannans* and have a lower degree of polymerisation than those described previously.

The glucomannans are difficult to isolate since, from the structural point of view, they lie between cellulose and mannans; in the case of the galactomannans isolation is easier since they are more soluble in water [20]. A partially acetylated glucomannan with a ratio of glucose : mannan of 2 : 3 has been isolated from the asparagus seed [32]. In the blackberry (*Rubus fructicosus*) a galactomannan with a galactose : glucose : mannose ratio of 17 : 24 : 20 has been isolated and characterised [33].

The *xyloglucans* are heteropolysaccharides with a repeated structure of different monopolysaccharides. These constitute the principal hemicellulose of the primary wall of dicotyledons. The units which have been identified at present are a heptosaccharide and a nonasaccharide, the first made up of four residues of β-1,4-glucose and three of terminal xylose, linked to the main glucose chain by α-1,6 links [34]. The nonasaccharide possesses, in addition, fucosylgalactose linked to one of the xylose residues. In monocotyledonous plants these are present in smaller proportions and have a different structure to those previously mentioned [1].

In the bean (*Phaseolus coccineus*) two xyloglucans with different degrees of branching have been identified [35] and one with almost 50% of terminal xylose residues has been identified in the cabbage (*Brassica oleracea*) [36]. The excretion of this polysaccharide to the cell wall is associated with the synthesis of cellulose. When the plasmalemma fuses with the membranes of the vesicles that transport xylogluans, these are liberated to the outside and they link up by means of hydrogen bridges to the β-1,4-glucans (cellulose) that are being synthesised on its surface. This fact possibly explains why the cellulose of the primary wall, as observed by X-ray diffraction studies, has a greater degree of crystallinity than that of the secondary wall, and also why its fibres are narrower [37].

The xyloglucans are involved in the growth of the cell wall that occurs in response to various stimuli, and in the maintenance of its rigidity [38]. The involvement in growth has been demonstrated by studying the internal and external zones of the hypocotyl of the pumpkin (*Cucurbita maxima*) germinated in the dark. The xyloglucan of the internal tissue has a lower molecular weight than that of the external tissue (210 kDa, compared to 455 kDa), which indicates that in the former it is expanding, while in the second growth is still inhibited, the molecular weight being greater [5]. It is found as the storage polysaccharide in some legumes [11, 28] and easily forms gels. This characteristic is exploited by the food industry which uses it as an additive [20].

The *glucuronomannans* are polysaccharides the main chain of which is made up of units of α-1,4-mannose and β-1,4-glucuronic acid with branches either of xylose or galactose linked to the mannose by β-1,6 links or of arabinose joined to the mannose by β-1,3 links. Small amounts have been located in the cell wall of tobacco and of gum exudates [39].

The *arabinogalactan II* is a polysaccharide with a highly branched structure with β-D-galactopyranose residues linked by 1,3 and 1,6 links to the main chain and branches, respectively (Fig. 5). It is common to find L-arabinofuranose and L-arabinopyranose in the side chains as well as D-glucuronic acid (or its 4-methyl ester) and rhamnose [30].

It is found joined to proteins rich in hydroxyproline (HRGP), which indicates that it could form a point of union between polysaccharides of the cell wall and proteins. Furthermore, the fact that it has rhamnose in its branches suggests that it is also linked to rhamnogalacturonans by covalent bonds [40].

β1,3-Glucan, also called *callose*, is a polysaccharide that the cell synthesises and that is easily degraded. The β-1,3 link gives it a helical conformation which allows it to form microfibres. It appears as deposits of different sizes and with a variable frequency throughout the whole zone of contact between the endosperm and the aleurone layer and it appears to be associated with the internal surface of the external cell walls of the endosperm.

β-1,3-β-1,3-Glucans are linear polysaccharides that occur preferentially in the monocotyledonous plants and usually have β-1,3 links separated by two, three or four residues united by β-1,4 links, although, with time, the proportion of β-1,4 links increases.

A glucan of this type has been obtained from wheat flour [41] and from barley [42]. It is synthesised at a stage prior to the appearance of the callose and is located throughout the endosperm in a more or less uniform manner.

Pectic substances

The pectic substances consist of a complex mixture of colloidal polysaccharides that can be extracted from the cell wall with water, or with solutions of chelating agents. Pectic material is heavily involved in the structure and texture of the cell walls of vegetable products, polymers with high degree of esterification (DE) have little capacity of interaction with other components of the cell wall and, therefore, are readily water soluble; those with medium and low DE are stabilised by ionic bonds with calcium forming gels ("egg-box structure") and can be solubilised by chelating agents such as oxalate. Others could be linked covalently to hemicelluloses, cellulose or even proteins, and they are released only by treatment with alkali or dilute acid [43].

→ 4)-GalA-(1 → 2)-Rha-(1 → 4)-GalA-(1 → 2)-Rha-(1 → 4)-GalA-(1 →

Fig. 6. The structure of rhamnogalacturonan I

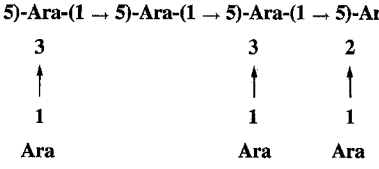

Fig. 7. The structure of arabinan

→ 4)-Gal-(1 → 4)-Gal-(1 → 4)-Gal-(1 → 4)-Gal-(1 → 4)-Gal-(1 →

Fig. 8. The structure of galactan

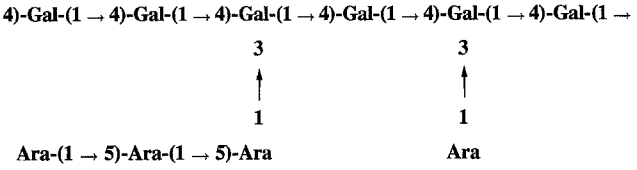

Fig. 9. The structure of arabinogalactan I

→ 4)-GalA-(1 → 4)-GalA-(1 → 4)-GalA-(1 → 4)-GalA-(1 → 4)-GalA-(1 →

Fig. 10. The structure of galacturonan

The most characteristic features of these polysaccharides are the residues of anhydrogalacturonic acid, although they also have residues of rhamnose, arabinose and galactose. Generally the rhamnose forms part of the main chain, while the arabinose and galactose are found on the side chains linked to the main chain by covalent bonds. Below, the most representative pectic polysaccharides of the cell wall are described.

Rhamnogalacturonan I (RG-I) has a zig-zag conformation, due to the fact that in the α-1,4-galacturonic acid chain, residues of 1,2-rhamnose appear (Fig. 6). When this is further branched at C4 a Y configuration is formed. In its structure highly branched zones alternate with other zones with no side chains, giving rise to high molecular weight compounds.

RG-I has been isolated from tissue of the sycamore (*Acer pseudoplatanus*) and has a rhamnose : galacturonic acid : arabinose : galactose ratio of 1 : 2 : 1.5 : 1.5. It is isolated from the cell wall using the enzyme α-1,4-endopolygalacturonase [44], or by sequential treatment with cyclohexane-*trans*-1,2-diamine-tetraacetate (CDTA) and sodium carbonate [21].

Rhamnogalacturonan II (RG-II) differs from RG-1 in two important respects: (1) it contains apiose residues, 2-*O*-methylfucose and 2-*O*-methylxylose, the fucose is very abundant and, as well as being found as terminal residue, appears as 1,3-fucose and 1,3,4-fucose [23]; (2) residues of 1,2-arabinopyranose appear in significant amounts, these, together with the terminal residues, being the only arabinose residues measurable. The rhamnose is found chiefly as a 1,3 residue, the bridging points being 1,2,4- and 1,3,4-.

Apart from the differences already mentioned, it is difficult to differentiate these two rhamnogalacturonans and, in fact, it is very common to find in the literature the generic name. At times, mention is made that to the fact that there are two types, but no distinction is made between them. However, the differences between the two have been demonstrated perfectly. For example, in spruce cell cultures, residues of 1,2-rhamnose and 1,2-arabinofuranose typical of RG-I are found together with others of 1,3,4-fucose and 3′-apiose, characteristic of RG-II [23]. Similarly, in maize and millet, the two groups are perfectly distinguishable, although they appear to be linked by covalent bonds [45].

Arabinan is made up of α-L-arabinofuranose residues connected by 1,5 links to the highly branched zones at C3 and, to a lesser extent, at C2 [46]. The branches, chiefly α-arabinofuranose, are found homogeneously distributed along the whole length of the molecule (Fig. 7). Arabinans are widely distributed in the plant kingdom, being found, among others, in seeds, vegetables and fruits, in red gram (*Cajanus cajan*) [47] and parsnip (*Pastinica sativa*) [48].

Although it is difficult to isolate, due to the extreme sensitivity of the α-L-arabinofuranose unit to acids, its structure has been studied in parsnip, and it has been found to contain 83 % arabinose. By statistical calculations it has been established that 1 unit composed of 40 residues has 20 of 1,5-arabinose, 7 of 1,3,5-, 2 of 1,2,3,5- and 11 non-reducing terminal residues.

Galactan is a homopolymer of β-1,4-galactose (Fig. 8), although at times it has branches at C6 of galacturonic and glucuronic acids. It has been characterised from the lupin (*Lupinus*) and has been shown to be a storage polysaccharide that is mobilised totally during germination. Furthermore, it functions as a structural polysaccharide in a wide variety of tissues and organs [20].

Arabinogalactan I is made up of a main chain of β-1,4-D-galactose with arabinose branches at C3, linked by α-1,5 links [20] (Fig. 9) and, at times, it appears linked to RG-I. An arabinogalactan I with a highly branched structure has been isolated from the cell wall of celery. This contains L-arabinose, D-galactose and D-glucose in a ratio of 2.53 : 1.00 : 0.012, together with a small proportion of 1,3,6-galactose typical of arabinogalactan II and double-branched L-arabinose characteristic of an arabinan.

D-*Galacturonan* is a polysaccharide consisting fundamentally of α-1,4-galacturonic acid (Fig. 10) with a variable degree of esterification. Significant amounts of this have been found in the walls of spruce cells in culture, as well as in the cell wall of onions where it appears as a rigid, scarcely esterified polymer [49].

As some authors have suggested, pectin molecules are composed of alternating "smooth regions" (homogalacturonans) and "hairy regions" (rhamnogalacturonans) [50, 51].

Proteins

The proteins making up the cell wall are classified into two large groups: structural proteins and enzymes.

Structural proteins

Generally, the structural proteins are glycosilades and are rich in hydroxyproline [52], and for this reason they are also called hydroxyproline-rich glycoproteins (HRGP). In addition, they contain alanine, serine and threonine in relatively high amounts. They may be glycosidated by chains of arabinose (extensins) [2] or of arabinogalactan (AGP). In the former, the hydroxyproline may be glycosidated by chains of from one to four arabinofuranose residues connected by 1,2 links or 1,3 links. In AGP, only 4 % of the total carbohydrate of the protein is present in the tetraarabinose chain.

Some of the glycoproteins isolated from the cell wall have *lectin* activity [53], these being proteins that are able to join specifically to certain polysaccharides. In these, 16 % of the total amino acids is hydroxyproline and 92 % of the carbohydrates is arabinose. Other types of protein rich in glycine have also been detected and these could substitute in the HGRP when it has a structural role [54, 55].

All of these structural proteins are synthesised by the plant tissue during the normal process of growth and also as a response to external lesions, a fact that has been confirmed in proteins of the cell wall of soya (*Glycine max*). In the normal development of its hypocotyl, a protein rich in proline and hydroxyproline has been isolated and, by causing a lesion to the same tissue, another similar protein has been detected. Selective antibodies against the first protein react intensely to the second [56].

They have a levo-rotatory helical structure, the stability of which is maintained by the side chains of carbohydrates and the chains are interlinked by residues of isodityrosine, which makes them relatively insoluble [57].

Studies carried out to elucidate the location of the structural proteins of different plant products, such as soya, tomato and potato etc, have shown the presence of proteins rich in glycine (GRP), proline (PRP) and hydroxyproline (HGRP) in xylem, phloem and the cambium zone [58, 59].

Enzymes

In the cell wall peroxidase and hydrolase enzymes have been detected, these being involved in different processes such as maturation, softening, as well as in response to exposure to pathogens. There are a large number of hydrolases, including cellulases, pectinases and a series of glycosidases such as α- and β-galactosidase, α- and β-mannosidase, etc [60].

The peroxidase catalyse the formation of links between the macromolecules of lignin, proteins, hemicelluloses and ferulic acid. The cellulases are involved in defence mechanisms and in the processes of fruit ripening, softening and abscision. The pectinases have a great effect on the softening of fruits. Among the glycosidases, α-ammosidase is associated with the synthesis and maintenance of glycoprotein, β-glycosidase may play a role in the lignification of the tissues and α-arabinosidase is activated during seed germination and fruit softening before the specific pectic enzymes have started to act.

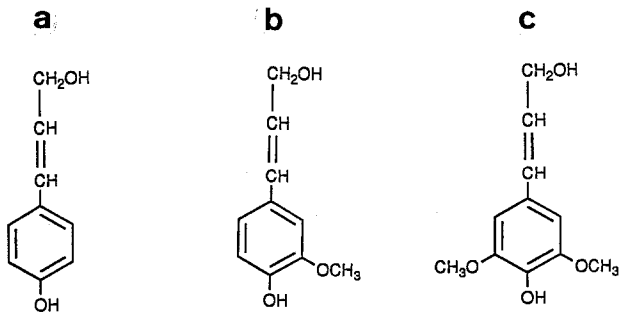

Fig. 11 a–c. The precursors of lignin **a** p-coumaryl alcohol; **b** coniferyl alcohol; **c** sinapyl alcohol

Lignin

When the plant tissue has finished its growth phase, some specific differentiated cells synthesise lignin, which can be described as a three-dimensional polysaccharide made up of units of phenylpropane [9]. Although the chemistry of lignin has not been completely elucidated, it is recognised to be a complex network that has, as precursors, the coniferyllic, coumaric and sinapyllic aromatic alcohols (Fig. 11), that are united by a large variety of bonds. Polymerisation does not end as long as there are primer spaces in the network and activated precursors. Thus, the molecule tends to expand throughout the cell wall, displacing water and producing, as a result, a strongly hydrophobic framework that interacts with the other components of the wall and keeps them united.

The negative correlation between the lignin content of the cell wall and the cell wall digestibility is well documented [61, 62]. The presence of lignin in the wall is the main factor responsible for the difficulty in degrading plant fibre, since it forms a type of screen which makes it almost impossible for chemical and/or enzymatic agents to penetrate through to the other components of the fibre. Its presence reduces the digestibility of the fibre and it is important to take this into account when considering animal feeding. Thus, the pectic substances, which constitute the most digestible fraction of the cell wall, the celluloses and hemicelluloses are protected by, and possibly are bonded directly to, a barrier of lignin [63]. The lignin also forms an effective barrier against pathogens and, therefore, gives good protection against possible infection.

In addition to the lignin, other phenolic compounds may be present, the most significant being *ferulic acid* that esterifies arabinose and galactose in the pectic substances. In the internodal regions of the wheat plant, ferulic acid forms esters and ethers in comparable proportions, but as maturation proceeds, the esterified forms diminish. In

contrast, coumaric acid is found in a predominantly esterified form and the concentration of this form increases with maturation [64].

In the cell wall of the red chickpea (*Cajanus cajan*) the following protocateic acids have been identified; *p*-hydrobenzoic acid, vanillic acid and caffeic acid. All of these phenolic acids are found both in the free forms and bonded to the wall by ester or glycosidic bonds [65].

Polymerisation of the lignin begins in the middle lamella, extends to the primary wall and, subsequently, to the secondary wall. The alcohols are oxidised in the wall by the action of peroxidases giving rise to mesomeric phenoxy radicals that interact spontaneously to form lignin. For example, dimers of *p*-coumaric, ferulic-coumaric and diferulic acids have been found. These are formed spontaneously by photochemical reaction in sunlight [66].

Oligosaccharides of xyloglucan and arabinoxylan have been isolated by enzymatic hydrolysis of bamboo shoots. These contain residues of ferulic and *p*-coumaric acids, which indicates that polyphenolic bridges link a variety of cell wall polysaccharides by means of covalent bonds.

The lignin is found in the sclerenchyma, tracheids and xylem vessels as well as in other cells in response to infections and other external stimuli. Its chemical composition differs in the different plant groups: the gymnosperms have a high proportion of coniferyllic alcohol, while the dicotyledonous angiosperms have equal proportions of coniferyllic and sinapyllic alcohols. The monocotyledons, on the other hand, have equal amounts of the three alcohols [67].

Types of bonds in the cell wall

The cell wall is a complex framework both in terms of its composition and its structure. The polymers can be united by means of covalent, ionic or hydrogen bonds, by hydrophobic forces, etc. As a result a structure is formed which gives unity and mechanical resistance to the cells.

There is no uniformity as far as the cell walls of plants is concerned, the monocotyledons and dicotyledons etc, showing differences. Differences also exist within the same organism (primary secondary wall). However, within any one particular group, for example the primary or secondary wall of dicotyledons, the final wall structures and polymer interrelationships are fairly consistent.

In the formation of the cross-linked structure, peroxidase and pectinesterase enzymes seem to be involved in the structure of the primary wall of dicotyledons. The former catalyse the formation of isodityrosine and diferulate bridges, while the latter produce acid zones within the pectin molecule that are capable of forming calcium bridges.

The arabinogalactans, arabinans and galactans are linked to RG-I by *covalent bonds* and, at the same time, RG-II is linked covalently with other acid polysaccharides of the wall [1]. The acid polysaccharides can also be interlinked by means of diferulic bridges. Ferulic acid esterifies arabinose and galactose and two residues of this acid can unite under the action of the peroxidase enzyme. Ester-type bonds can also be formed between acid polysaccharides and cellulose, or between glucuronoxylan polysaccharides and lignin [11].

The xyloglucans are united to the cellulose fibres by numerous *hydrogen bonds*, this type of bond being the most important in the cell wall, and prevents the cellulose fibres from adhering to form aggregates characteristic of the secondary wall. Other polymers, such as glucuronoarabinoxylans, arabinoxylans and xylans also exist and these link to the cellulose by means of hydrogen bonds. These types of bonds serve to form links between the hemicelluloses and also between the hemicelluloses and the cellulose fibres. The *ionic bonds* are formed between molecules with charged groups and, thus, probably exist between molecules of extensin and galacturonans [68]. The *hydrophobic forces* are formed between the highly methylated galacturonans, between protein molecules and between proteins and lignin.

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