STRUCTURAL STUDIES ON CELL WALLS OF *PINUS RADIATA* WITH PARTICULAR REFERENCE TO CALLUS CULTURED CELLS

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ABSTRACT

A study of the composition of the primary cell wall of Pinus radiata has been undertaken.

1. Preliminary work with hypocotyl tissue showed that hemicelluloses of hypocotyl consisted of a xylan (probably (4-O-methylglucurono)xylan) and a xyloglucan. Acidic sugars examined, showed that galacturonic acid was the main component, and that 4-O-MeGlcA was present.

2. Cell walls were prepared from callus tissue either by wet sieving in 80% ethanol (Batch 1) or by disruption in a French Pressure cell and washing with aqueous potassium phosphate buffer; (Batch 2).

   Each batch was submitted to a series of extractions with different reagents in order to investigate the mode of bonding of polymers within the walls. The polysaccharide and protein components of each fraction were studied by monosaccharide and amino acid analysis.

   Fractions of Batch 1 were assayed for lignin and selected fractions from both batches were studied by methylation analysis.

3. The results of investigations led to the following major conclusions. The non-cellulosic components recognised in the wall preparations were: -

   a) A(1→3)-linked galactan and an arabino-3,6-galactan which were largely extractable from the cell walls by hot water and may be only loosely bound in the cell wall.

   b) The pectic components consisting of;
      i) pectin, a galacturonate polymer containing a linear (1→4)-galacturonan back bone interspersed with branched rhamnose residues,
      ii) branched (1→5)-arabinan and
      iii) linear (1→4)-galactan,

which occurred together in cell wall fractions and were not all extracted by classical extractants (such as hot aqueous EDTA), some being tightly bound in the cellulosic residue after alkali extraction.

c) A fucogalactoxyloglucan some of which was extracted by water or EDTA, but the majority was extracted by subsequent treatment with either alkali or in part by a strong chaotropic reagent (6M GTC). Thus the fucogalactoxyloglucan was probably bound in the cell wall by strong hydrogen bonding. Some other bonding may be involved in the GTC-resistant fraction.

d) A branched xylan which was removed by GTC and alkali, the larger level being removed by GTC.

e) A galactoglucomannan, identified only by 4-linked mannose residues in hot water extracts and strong alkali fractions.

f) Hydroxyproline-containing protein which was extracted from the cell wall by a variety of reagents but hydroxyproline-rich protein remains tighly bound after alkali extraction.

g) Lignin which was tentatively identified in the cell wall. It appeared likely that cross-linking with lignin would be responsible for the non-extractability of some polysaccharides and protein from the walls. A mild acid chlorite treatment followed by alkali extraction removed most of the residual pectic components, xylan and protein from the walls.

A basis has been laid for the further investigation of the wall structure and isolation of polysaccharides.

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ABBREVIATIONS

COMMON ABBREVIATIONS USED:

EDTA	Ethylene diaminetetra-acetic acid
TFA	Trifluoroacetic acid
TFA-enz	Trifluoroacetic acid -*Sclerotium rolfsii* enzyme treatment
Dimsyl	Dimethyl sulphinyl anion (CH_3-S-CH_2^-)
PEG	Polyethylene glycol
Amino Acids	Standard abbreviations are used
g.c.	Gas chromatography
g.c.-m.s. (G.C.-M.S.)	Gas chromatography-mass spectrometry
f.i.d./F.I.D.	Flame ionisation detection
m/e	Mass/charge ratio of ion fragments in mass spectrometry
mass spec.	Mass spectrometry
R_{xyl}, R_{xylose}	Coefficient of diffusion relative to xylose in paper chromatography
Inos	Inositol
2dglc - 2 deoxyglucose	2-Deoxy-D-glucose
Monocots	Monocotyledons
Dicots	Dicotyledons
Polysacch	Polysaccharide (only seldom used)
F.R.I.	Forest Research Institute (New Zealand)

SUGARS IN PLANT CELL WALLS:

Rha, L-Rha, L-Rha p	L-rhamnose,	L-rhamnopyranose
Fuc, L-Fuc, L-Fuc p	L-fucose,	L-fucopyranose
Ara, L-Ara, L-Ara f	L-arabinose,	L-arabinofuranose
Xyl, D-Xyl, D-Xyl p	D-xylose,	D-xylopyranose
Man, D-Man, D-Man p	D-mannose,	D-mannopyranose
Gal, D-Gal, D-Gal p	D-galactose,	D-galactopyranose
Glc, D-Glc, D-Glc p	D-glucose,	D-glucopyranose

OLIGOSACCHARIDES

GalA, D-GalA D-GalA p	D-galacturonic acid D-galactopyranosyl uronic acid
GlcA, D-GlcA D-GlcA p	D-glucuronic acid D-glucopyranosyl uronic acid
4-O-MeGlcA	4-O-methylglucuronic acid
4-O-MeGlcA-Xyl 4-O-MeGlcA-(1→2)-Xyl	4-O-methylglucuronosyl-(1→2)-xylose
GalA-GalA GalA-(1→4)-GalA	Galacturonosyl-(1→4)-galacturonic acid
GlcA-GalA GlcA-(1→4)-Gal	Glucuronosyl-(1→4)-galactose
GalA-Rha GalA-(1→2)-Rha	Galacturonosyl-(1→2)-rhamnose
GlcA-Xyl GlcA-(1→4)-Xyl	Glucuronosyl-(1→4)-xylose
MeGlcA-Xyl-Xyl	Methylglucuronosyl-(1→2)-xylose-(1→2)-xylose

POLYSACCHARIDES:

Named in text according to backbone structure. The general descriptions of polysaccharides are given in Chapter 1.

e.g.

β-(1→4)-mannan:	mannose (1→4)-linked in β configuration
Arabino-3,6-galactan:	polymer of (1→3) and (1→6)-linked β-D-galactopyranose units, to which are attached L-arabinofuranose units.
Arabino-(4-O-methylglucurono)xylan:	(1→4)-linked xylose backbone carrying arabinose, and 4-O-MeGlcA substituents.

METHYLATION DATA:

a) Sugar residues, with position of methyl groups,

e.g. 2,3,6-Tri-O-methyl-D-glucose

= 2,3,6-Me_3-glucose , or 2,3,6-Me_3Glc

b) Sugar residues indicating linkage positions, e.g. 4,6-linked glucose, are sometimes abbreviated to, e.g. 4,6-Glc.