Plant Physiol. (1977) 59, 337-340

Wall Analyses of *Lophocolea* Seta Cells (Bryophyta) Before and After Elongation[1]

Received for publication June 10, 1976 and in revised form October 12, 1976

ROBERT J. THOMAS
Department of Biology, Bates College, Lewiston, Maine 04240

ABSTRACT

***Lophocolea heterophylla* (Schrad.) Dum. (a leafy liverwort) produces sporophytes with seta cells that elongate 50-fold in 3 to 4 days. Wall components of these cells have been characterized by microscopic histochemistry, colorimetry, and gas chromatography of neutral sugars. Seta cell walls are qualitatively similar to primary cell walls of higher plants. The pectic fraction, however, responds differently to standard histochemical staining and extraction. Quantitatively, mannose, fucose, and rhamnose are in higher percentage, and arabinose and xylose are lower than typically found in vascular plants. Hexuronic acids increase on a percentage basis during elongation; pentoses decrease slightly, while hexose levels remain about the same. Increase in total wall carbohydrate after 2,400% elongation of setae was 1.8-fold.**

Changes in the molecular structure of plant cell walls are thought to play a central role in cell elongation (14, 19). There is no study, however, that quantitatively correlates bryophyte cell wall chemistry with growth. Wall analyses of these nonvascular land plants are few in number and restricted largely to either chemical characterization of mature gametophyte tissues (2, 3, 5, 6, 8, 9, 24), or to histochemical observations of conducting tissues (20, 23) and spores (7). The classic study by Overbeck (18) on liverwort setae is the only instance where a specific attempt has been made to correlate cell elongation with histochemical observations and physical properties of bryophyte cell walls.

The setae of Jungermannialean and Metzgerialean sporophytes are well suited for study on cell elongation. They undergo 50-fold extension in 3 to 4 days. Elongation is due solely to vacuolation of individual cells with no cell division and no accompanying tissue differentiation (18, 25). Soil water tension and low temperature inhibit seta growth (22). Exogenously supplied gibberellin and auxin are stimulatory (1, 26). Some irreversible stretching of seta walls can be obtained by artificial means (18), but despite considerable wall thinning, increase in wall carbohydrate accompanies elongation (25). The present study extends this latter finding with histochemical, colorimetric, and gas chromatographic analyses of cell wall carbohydrates before and after seta elongation.

MATERIALS AND METHODS

Plant Material. *Lophocolea heterophylla* (Schrad.) Dum., which regularly produces sporophytes in culture (26), was maintained axenically on a modified White's agar medium (10) supplemented with 1% (w/v) sucrose. The plants were grown at 16 C under 3,000 lux fluorescent light with 12-hr light and dark periods. Sporophytes are borne at the apex of leafy gametophyte stems. They consist of a spherical spore capsule, a cylindrical seta, and an anchor-shaped foot (Fig. 1). Mature sporophytes with unelongated (1 mm) and elongated setae (25 mm, average length) were selected for subsequent analysis. Sporophytes were carefully dissected from surrounding or adherent gametophyte tissue, and the capsule and foot of each sporophyte were removed with a razor blade.

Wall Histochemistry. Paraffin or fresh free-hand sections of setae were tested for the presence of cellulose, pectin, callose, lignin, sphagnol (the so-called "moss lignin" of the early literature), tannin, protein, and cuticular material. Standard histochemical methods were employed (11, 17). Observations for birefringence under polarized light were made using a Zeiss polarizing microscope with a 1/30 wavelength compensator. For colorimetric quantification of wall sugars, fresh setae were placed on a glass slide under a dissecting microscope and cut with a razor blade into lengths of 50 to 100 μm. The segments were transferred in 95% (v/v) ethanol to test tubes (10 × 75 mm) and soluble sugars were extracted according to procedures outlined by Jermyn and Isherwood (13). Alcohol-insoluble residues were either analyzed directly at this point or underwent further sequential extraction with water at 25 C for 12 hr, three 1-hr rinses of 0.5% (w/v) $(NH_4)_2C_2O_4$ at 90 C, one 6-hr rinse of 1.0 N NaOH at 25 C, and one 6-hr rinse of 4.4 N NaOH at 25 C (11). Extracts and residues were measured for amount of hexoses or pentoses by the cysteine reaction, or for hexuronic acids using carbazol as the color-producing agent; total carbohydrate content was determined using the orcinol method (12).

Gas Chromatography. Approximately 80 to 100 setae were homogenized at 4 C with 0.1 M (pH 7) phosphate buffer in a 2-ml glass microhomogenizer. Homogenized setae were washed and extracted for starch with α-amylase (16), dried *in vacuo* over P_2O_5 for at least 2 days, and weighed. The wall preparations were then acid-hydrolyzed and converted to alditol-acetates (4). *Myo*-inositol (0.5 μg) was added to the sulfuric acid hydrolysis reagent to serve as an internal standard. One-μl samples of the dichloromethane-eluted alditol-acetates were injected into the 200 C port of a Perkin-Elmer 880 gas-liquid chromatograph. The chromatograph was fitted with FID (190–200 C) and an aluminum column (7.5 m × 3.2 mm o.d.) packed with fluidized dried 4% (w/w) OV-225 on 100/120 mesh Gas-chrom Q. The carrier gas was nitrogen (30 ml/min). The column temperature was programmed for a 5-min postinjection hold at 120 C, a linear 4 C/min temperature rise to 180 C with a subsequent hold at 180 C until elution was complete. Electrometer output was recorded on a 2-mv strip chart recorder. Component peaks were identified by their retention times relative to the retention time of the internal standard. Quantification was based on triangulation of peak area relative to the *myo*-inositol peak, with correction against standards taken through the same procedure. Stan-

[1] This work is based in part on a dissertation prepared in the laboratory of Professor William T. Doyle and presented in partial fulfillment of Ph.D. requirements at the University of California, Santa Cruz.

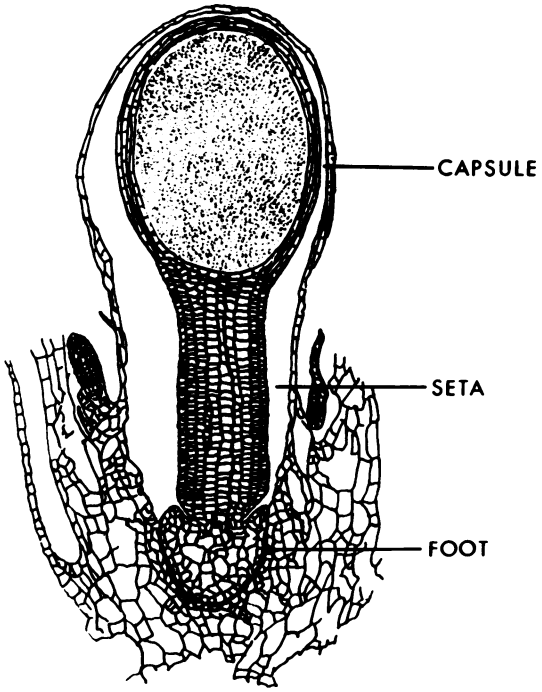

FIG. 1. Longitudinal section of an unelongate *L. heterophylla* sporophyte. The capsule has not yet broken through the thin cell layer of the enveloping calyptra of the gametophyte. Aborted archegonia and perianth tissue (the bulk of which has been removed in this section) are also depicted. The sporophyte remains attached to the gametophyte at the juncture of the foot. × 23.

dards were obtained as follows: D-galactose and D-mannose, Calbiochem Corp.; L-fucose and L-rhamnose, General Biochem; D-glucose, Mallinckrodt Chemical Works; D-xylose, Mann Research Labs; and *myo*-inositol, Sigma Chemical Co. All sugars were stored 72 hr in a vacuum dessicator at 50 C to remove residual moisture before weighing.

RESULTS

Positive staining reactions of seta wall components were obtained for cellulose (using zinc-chlor-iodine and IKI-H_2SO_4) and for an external wall cuticle (using Sudan IV). Cellulose molecules under polarized light displayed negative birefringence. Hydroxylamine-ferric chloride and ruthenium red staining for pectin was not effective. The presence of pectic compounds in the wall could only be inferred by comparison of periodic acid-Schiff-stained ammonium oxalate-extracted tissue and controls. Extraction with ammonium oxalate did not remove wall carbohydrates from the middle lamella, however; interlamellar spaces only appear after subsequent extraction with dilute base (Fig. 2). Extraction of the middle lamella with dilute acid or pectinase was also ineffective. Additional histochemical tests for wall callose (aniline blue), lignin (phloroglucinol and Schiff's reagent), sphagnol (Millon's reagent), tannin (ferric sulfate), and protein (mercuric bromphenol blue) were all negative.

Quantitative differences in wall carbohydrates during elongation of setae are elaborated by the colorimetric analyses (Fig. 3). Hexoses, pentoses, and hexuronic acids of the sequentially extracted wall fractions are depicted in unelongated and elongated setae. There is little change in the amount of water- and ammonium oxalate-soluble carbohydrates after 25-fold elongation of seta cells. Carbohydrates of the other wall fractions undergo quantitative increase with the largest change in fraction C. Overall increases in total hexoses, total pentoses, and total hexuronic acids of seta walls were statistically significant. Hexoses represent 54.1% of the wall carbohydrate before elongation, and are very close to that (52.0%) afterward. The hexose levels reported here may be somewhat high since some starch is present in this tissue (25) and was not removed prior to analysis. There is a small drop in the percentage of pentose after 25-fold elongation of setae (from 28.7 to 23.0%), while hexuronic acids increase from 17.1 to 24.9%. The orcinol-determined values for total carbohydrates of alcohol-insoluble residues were 16.4 ± 1.9 μg (SE)/seta for 1 mm setae, and 29.1 ± 3.0 after 25-fold elongation. These values vary by 8 and 12%, respectively, from the

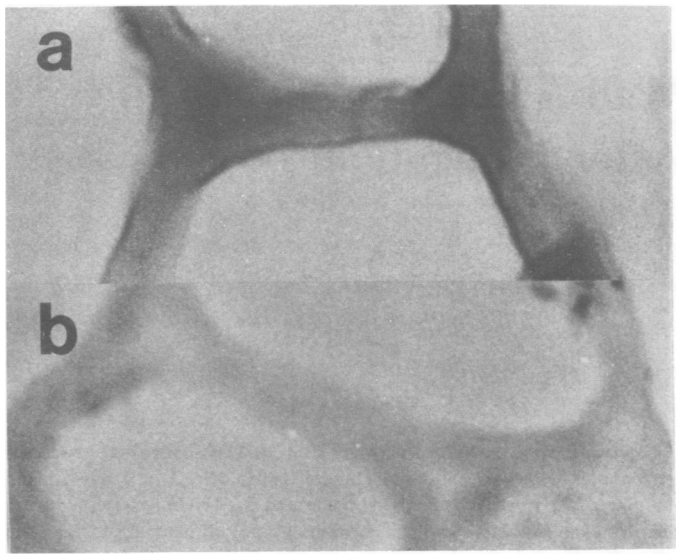

FIG. 2. Extraction of the middle lamella of seta cell walls. a: Ammonium oxalate-extracted, periodic acid-Schiff-stained unelongate seta cross-section (10 μm). b: ammonium oxalate and 1.0 N NaOH extracted, periodic acid-Schiff-stained serial section. Note separation of adjacent cells in the middle lamella region. × 1,930.

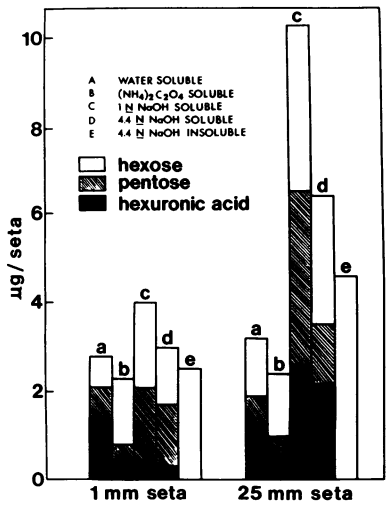

FIG. 3. Colorimetrically determined carbohydrate composition of seta cell walls. Wall components were sequentially extracted and quantified spectrophotometrically using the cysteine and carbazol color reactions (12). Means of at least three determinations are expressed in μg/seta in preference to wet or dry weight (since the number of cells in any given seta is constant at about 4,000 and no cell division takes place between the stages under study), with SE between 2 and 12% of the depicted values. Student's *t*-test at the 5% confidence level was used to determine the differences between means.

sum of three carbohydrate groups determined separately in unelongated and elongated setae.

Glucose, galactose, mannose, xylose, arabinose, fucose, and rhamnose are the neutral wall sugars of setae identified by GLC. Glucose is shown to be the predominant sugar present (Table I). Galactose and mannose are the next most prominent, whereas arabinose and xylose levels are rather low. Percentages of mannose, fucose, and rhamnose are high compared to the percentage of these three sugars usually found in the primary walls of higher plants (16); results with setae are comparable to those obtained using gametophyte tissue of *Lophocolea* (Table I; 24).

Changes in per cent of pentoses and hexoses determined by GLC substantiate the changes observed colorimetrically. GLC-determined hexoses comprise 27.3% of the wall dry weight before, and 28.5% after elongation. Pentoses decrease slightly from 6.4 to 3.7%. Discrepancy between GLC and colorimetric values can be attributed to differences between the analytical procedures. Acid hydrolysis of the wall for GLC solubilizes only 32 to 34% of the purified preparations. Similar treatment with angiosperm preparations solubilizes approximately 50% (16). This difference in response to standard hydrolysis is not atypical of liverwort cell walls (24).

DISCUSSION

With glucose, galactose, mannose, xylose, arabinose, fucose, rhamnose, and uronic acids as component wall constituents, the chemical nature of *L. heterophylla* seta cell walls appears to be qualitatively similar to primary cell walls of higher plants. Specific uronic acids of seta walls remain to be identified. In addition to the galacturonic and glucuronic acids of higher plants, they may contain mannuronic acid, a wall component detected by Das and Rao (6) in gametophytes of *Riccardia levieri*.

The absence of lignin in *Lophocolea* setae is consistent with observations on liverworts as a group. Other types of wall phenolics are known to occur in bryophytes (8) but were not revealed histochemically in *Lophocolea* setae. Protein in wall preparations of *L. heterophylla* gametophytes accounts for 0.33% of the wall dry weight (24). A similarly low protein content in seta walls probably accounts for the negative reaction to mercuric bromphenol blue. Although no further analysis of the protein content of seta walls was undertaken, such an analysis would be of interest. Hydroxyproline-rich glycoproteins have been identified in the walls of liverwort tissue (15). These proteins are comparable to the "extensin" of vascular plants. As possible sites for cross-linkage between wall polysaccharides, such proteins may be involved in cell wall expansion.

Negative birefringence of *Lophocolea* seta walls indicates preferred orientation of cellulose microfibrils in transverse direction around cells. This orientation (or tube texture) is maintained during elongation of seta cell walls despite enormous increase in cell length. Considering the helical pattern of seta growth (18), it seems likely that the arrangement of cellulose microfibrils may be slightly helical rather than tubular. Under these circumstances, the axis of maximum stress would not coincide with the axis of maximum strain so that one end of the seta cylinder would rotate with respect to the other. Helical growth is more commonly observed in isolated cell systems such as *Phycomyces* sporangiophores and internodal cells of *Nitella* (19); in the multicellular tissue of setae, it results in damage of internal cells as elongation nears completion.

Comparison with angiosperms focuses attention on the differences in solubility behavior and quantitative composition of seta cell walls. Hexuronic acids, for example, were detected in both dilute and concentrated base extracts of seta walls. This result compares favorably with the findings of Bricker and Doyle (5) who examined *Sphaerocarpos donnellii* wall preparations. It differs from observations on onion root tip (12) where the concentrated base extract lacks hexuronic acid. Another difference in response of setae is reflected in the pectic wall fraction. As in some other bryophyte tissue (5, 21), the relative amount of pectic material removed is less than that which would be removed from a higher plant cell wall taken through the same extraction procedure. Extraction for pectic substances, moreover, fails to remove carbohydrates from the middle lamellar region of seta walls (Fig. 2). These and other differences in wall solubility could be attributed to differences in chemical linkage between wall polymers. Perhaps the higher GLC-determined percentages of mannose, fucose, and rhamnose are related to the degree of chemical bonding within the wall.

The colorimetric and GLC-determined changes in wall composition of seta cells indicate that wall synthesis accompanies 25-fold elongation despite 4-fold thinning of the wall (25). Changes in carbohydrate composition need not be attributed to the unrelated event of vascular tissue differentiation in this system, nor does the utilization of low starch reserves in *Lophocolea* setae (25) interfere significantly with wall analysis. Wall analyses of setae were made only at the beginning and end of elongation; to establish a causal relationship between modifications in wall composition and growth, it would be desirable to investigate intermediate stages of elongation. Interrelationships between wall composition and molecular structure also need elucidation. It is not clear, therefore, what bearing the observed changes in composition have on the mechanism of elongation. Perhaps such changes are responsible for the increased wall plasticity required for seta growth.

Table I. Sugar Composition of Lophocolea heterophylla Cells Walls.

Percent contribution to the wall dry weight was determined after 72% sulfuric acid hydrolysis of wall preparations, conversion to alditolacetates, and separation by GLC on 4% OV-225.

Constituent	Gametophyte[1]	Unelongate Setae[2]	Elongate Setae[2]
Glu	17.0	16.7	17.6
Gal	7.4	4.9	5.1
Mann	3.5	4.4	4.9
Ara	3.1	2.4	1.1
Xyl	2.1	4.0	2.6
Fuc	0.8	0.8	0.5
Rhamnose	0.3	0.5	0.4
Total	34.2	33.4	32.2

[1] Average of three determinations

[2] Average of two determinations

Acknowledgments—The author expresses his appreciation to the following individuals: P. Green, Stanford University, for use of the polarizing microscope; M. Ashton and W. Jensen, University of California, Berkeley, for advice on microtechnique; L. Taiz, University of California, Santa Cruz, for advice on GLC; and to V. Curtis of Bates College for critical reading of the manuscript.

LITERATURE CITED

1. Asprey, G. F., K. Benson-Evans, and A. G. Lyon. 1958. Effects of gibberellin and indoleacetic acid on seta elongation in *Pellia epiphylla*. Nature 181: 1351.
2. Baillie, G. S. 1967. An investigation of the cell wall chemistry of the mosses *Mnium rugicum* and *Mnium blytii* and the hornwort *Phaeoceros laevis*. Master thesis. Colorado State University, Fort Collins.
3. Black, W. A. P., W. J. Cornhill, and F. N. Woodward. 1955. A preliminary investigation of the chemical composition of *Sphagnum* moss and peat. J. Appl. Chem. 5: 484-492.
4. Borchardt, L. G. and C. V. Piper. 1970. A gas chromatographic method for carbohydrates as alditol-acetates. Tappi 53: 257-260.
5. Bricker, C. and W. T. Doyle. 1964. Cell-wall carbohydrates in the liverwort, *Sphaerocarpos donnellii*. Am. J. Bot. 51: 666.
6. Das, V. S. R. and M. P. Rao. 1967. Cell wall sugars of the liverwort, *Riccardia*. Indian J. Exp. Biol. 5: 193-194.
7. Doyle, W. T. 1962. The morphology and affinities of the liverwort, *Geothallus*. Univ. Calif. Pub. Bot. 33: 185-268.
8. Erickson, M. and G. E. Miksche. 1974. On the occurrence of lignin or polyphenols in some mosses and liverworts. Phytochemistry 13: 2295-2299.
9. Geddes, D. S. and K. C. B. Wilkie. 1971. Hemicelluloses from the stem tissues of the aquatic moss *Fontinalis antipyretica*. Carbohydr. Res. 18: 333-335.
10. Hatcher, R. E. 1975. Towards the establishment of a pure culture collection of Hepaticae. Bryologist 68: 227-231.
11. Jensen, W. A. 1962. Botanical Histochemistry. W. H. Freeman & Co., San Francisco.
12. Jensen, W. A. and M. Ashton. 1960. Composition of developing primary wall in onion root tip cells. I. Quantitative analyses. Plant Physiol. 35: 313-323.
13. Jermyn, M. A. and F. A. Isherwood. 1956. Changes in the cell wall of the pear during ripening. Biochem. J. 64: 123-132.

14. LABAVITCH, J. M. AND P. M. RAY. 1974. Turnover of cell wall polysaccharides in elongating pea stem segments. Plant Physiol. 53: 669-673.
15. LAMPORT, D. T. A. AND D. H. MILLER. 1971. Hydroxyproline arabinosides in the plant kingdom. Plant Physiol. 48: 454-456.
16. LOESCHER, W. AND D. J. NEVINS. 1972. Auxin-induced changes in *Avena* coleoptile cell wall composition. Plant Physiol. 50: 556-563.
17. MOLISCH, H. 1921. Mikrochemie der Pflanze, Ed. 2. Gustav Fischer, Jena.
18. OVERBECK, F. 1934. Beiträge zur Kenntnis der Zellstreckung (Untersuchungen am Sporogonsteil von *Pellia epiphylla*). Z. Bot. 27: 129-170.
19. PRESTON, R. D. 1974. The Physical Biology of Plant Cell Walls. Chapman and Hall, London.
20. SCHEIRER, D. C. 1973. Hydrolysed walls in the water-conducting cells of *Dendroligotrichum* (Bryophyta): histochemistry and ultrastructure. Planta 115: 37-46.
21. SCHULZ, D. AND H. LEHMANN. 1970. Wachstum and Aufbau der Zellwand bei Moosen. Cytobiologie 1: 343-356.
22. SLADE, J. S. 1965. Physical factors influencing seta elongation in *Pellia epiphylla*. Bryologist 68: 440-445.
23. SMITH, J. L. 1966. The liverworts *Pallavicinia* and *Symphyogyna* and their conducting systems. Univ. Calif. Pub. Bot. 39: 1-83.
24. TAYLOR, J. AND P. B. KAUFMANN. 1976. Neutral sugar, uronic acid, cellulose, and protein content of cell walls isolated from gametophytes of the liverworts *Lophocolea heterophylla* and *Conocephalum conicum*. J. Hattori Bot. Lab. 40: 115-120.
25. THOMAS, R. J. AND W. T. DOYLE. 1976. Changes in the carbohydrate constituents of elongating *Lophocolea heterophylla* setae (Hepaticae). Am. J. Bot. 63: 1054-1059.
26. THOMAS, R. J., J. TAYLOR, AND M. J. MONTAGUE. 1970. The growth response of *Lophocolea heterophylla* seta segments to indole-3-acetic acid. Bryologist 73: 713-715.