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Is *Chara corallina* a suitable model plant for studying cell-failure mechanisms in fruit skins?

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Summary

The freshwater alga, *Chara corallina*, is often used as a model system in studies of cell-wall mechanics. *Chara* has a unique and very simple architecture, in which a number of node cells form the interface between two giant internode cells. Given this architecture, when investigating a specimen comprising a single internode cell, a tensile test stresses just its cell walls. However, when investigating a specimen comprising two internode cells and their intervening node, the stress is imposed on both the cellulosic cell wall components but also on the (in-series) pectin middle lamellae between the cells comprising the node. This setup should allow comparative study of the properties of the cellulose of the cell walls in the absence of middle lamellae and also of the pectin layers of the middle lamellae in the node. Such information would be of particular interest in the case of fruit skins that in tension typically fail along the middle lamellae causing separation of adjacent cells. The objectives were to establish whether the mechanical properties of the pectin middle lamellae between the *Chara* node cells may be inferred by comparing tensile properties of an internode cell and of a node.

Chara cell walls are composed of cellulose and de-esterified pectins as indexed by staining with calcofluorwhite and LM19. Staining with 2F4 indicates the presence of homogalacturonans crosslinked by Ca. Compared with internodes, nodes had lower stiffness, lower maximum force at failure and lower maximum strain. For the internode+node+internode samples, failure in the internode was more frequent than failure in the node. Lowering the medium pH had no effect on stiffness, maximum force at fracture or maximum strain of either internodes or nodes but it did increase the frequency of node vs. internode failure. Incubation in EGTA extracted Ca and decreased stiffness, maximum force at fracture and maximum strain of both internode and node samples. There was little effect of EGTA on the frequency of node vs. internode failure. Incubating internodes and nodes in pectinase decreased stiffness, maximum force at failure and maximum strain.

The pectin components of *Chara* cells are almost identical to those of fruit skin cells. The tensile properties of both internodes and nodes are affected by pectins. This makes it difficult to infer those of the interfacing middle lamellae.

Keywords: Cell wall, Middle lamella, Pectin, Calcium, Tensile test

Introduction

Skin failure is an initial event in a number of commercially important fruit disorders such as rain cracking and russeting (KNOCHE and LANG, 2017). From a mechanical point of view, the epidermal and hypodermal cell layers form the structural 'backbone' of most fruit skins, the cuticle's contribution to skin mechanics is at most low, but more often it is negligible (BRÜGGENWIRTH et al., 2014; KHANAL and KNOCHE, 2014). Tomato is a notable exception (MATAS et al., 2004). Here, the cuticle becomes increasingly important as a structural component as maturation and ripening proceed (BARGEL and NEINHUIS, 2005).

In principle, failure of a stressed fruit skin may occur either as a fracturing of the cell walls (failure mode 'across') or by separation of the walls of neighbouring cells along the interfacial middle lamella (failure mode 'along'). Only a few studies have investigated the failure mode of fruit skins. In these, fracture occurs most often by separation of cell walls 'along' the middle lamellae, thereby exposing pectins on the fracture surface (SCHUMANN et al., 2019). Less frequent is fracture 'across' cell walls where the cellulosic cell walls rupture. In sweet cherry, cell wall swelling is an important factor affecting the failure mode (BRÜGGENWIRTH and KNOCHE, 2017). Non-swollen cell walls fail across cell walls, whereas swollen cell walls fail along. In the latter case, the water absorption by cell-wall constituents leads to swelling that, in turn, weakens cell-to-cell adhesion, thereby permitting separation of adjacent cells along the middle lamella. Because of the enormous economic importance of fruit skin failure to the horticultural industry there is significant merit in investigating and manipulating cell to cell adhesion and cell separation.

For a long time, Charophytes (a group of freshwater green algae) have been used as model plants to investigate aspects of the basic physiology of terrestrial plants (for review see DOMOZYCH et al., 2016). Of special interest are the origins and composition of its cell walls (for review see DOMOZYCH and DOMOZYCH, 2014). *Chara corallina* is a representative of the Charophytes. Its thallus consists of nodes and internodes (Fig. 1a). Every internode represents a single giant cell. There are no cortical cells surrounding the internode cell. Commonly, the nodes are simple but multicellular (Fig. 1b). The primary constituents of *Chara* cell walls are cellulose and pectins. Epitopes of cell wall constituents were identified in nodes, internodes, lateral branches, antheridia and oogonia of *Chara* using monoclonal antibodies (mABs; DOMOZYCH et al., 2010). The main pectins in nodes and internodes were homogalacturonans methylesterified to different degrees and complexed with Ca.

The similarity of the primary cell wall constituents of *Chara corallina* to those of higher plants and the simple geometry of its thallus make it an interesting and potentially useful model system for studying factors affecting the mechanical properties of the cell walls of higher-plants – in our case the epi- and hypodermal cells of fruit skins. In particular by tension testing a *Chara* internode sample, we expect the mechanical properties to reflect primarily those of the cellulose composite. In contrast, when tension testing a node sample that comprises a small complex of cells intervening between two internode cells, not only are the cellulose composites stressed and strained, but also the pectin middle lamellae holding the node cells together and attaching them to the two adjacent internode cells. Thus, comparisons of the mechanical properties of internode and node samples should allow inferences to be made regarding the mechanical properties of the pectin middle lamellae.

The objectives were (1) to establish the tensile properties of internode and node samples of *Chara corallina* and (2) to identify the effects on these properties of manipulating the pectins.

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Methods

Plant material

Thalli of *Chara corallina* KLEIN ex C.L. WILLDENOW were obtained from a stock culture at Kiel University (Centre for Biochemistry and Molecular Biology, Kiel University, Kiel, Germany). Thalli were cultivated in a tank held at 19 ± 1 °C. A compost of *Fagus sylvatica* L. leaves served as the substrate. The tank was filled with deionised water. The height of the water level was 18 cm. Light was provided (15 W LED Pflanzenleuchte Wachstumslampe, Top Hengda, Ningbo, China) during a 14 h photoperiod. Explants of the stem comprising the top three nodes and internodes (Fig. 1a) were harvest at weekly intervals, transferred to the laboratory, and held at 23 °C in the dark for no longer than 5 d.

Immunolabeling of cell walls

Samples of the internodes and of the nodes were excised from the thallus using a razor blade. Longitudinal sections and cross sections were cut under a binocular microscope (MZ10F, Leica, Wetzlar, Germany). Cell wall strips were prepared by cutting internodes longitudinally. Cell contents were extracted by incubating samples in acetone (10 min), subsequent washing for 1 to 5 min in 80% aqueous ethanol, followed by acetone (10 min). Samples were then transferred to phosphate buffered saline (PBS buffer, 292 mOsmol kg^{-1}, pH 7). Any cell contents that still remained were carefully removed using a soft, camel-hair brush with subsequent rinsing in PBS buffer. The procedure was carried out under a microscope.

The cell-wall constituents at the surface were identified using immunolabelling of the exposed epitopes and the procedure described by AVCI et al. (2012). The procedure comprised the following steps: (1) Blocking of non-specific protein binding sites using 3% (w/v) non-fat dry milk powder dissolved in PBS buffer for 30 min. (2) Removal of the milk powder by 3 × 5 min washing in PBS buffer. (3) Incubating samples for 1 h in solutions containing primary monoclonal antibodies (mAbs; PlantProbes, Leeds, UK). All mAbs selected react with homogalacturonan: LM7 (WILLATS et al., 2001), LM19 (VERHERTBRUGGEN et al., 2009), LM20 (VERHERTBRUGGEN et al., 2009). These mAbs (all anti rat) were diluted 1:10 in PBS buffer. The mAb 2F4 (anti mouse) (LINERS et al., 1989) was also diluted 1:10 and prepared in Tris(hydroxymethyl)methylamin buffer (TRIS-HCl) at pH 8. (4) Following a 1 h incubation to allow binding, the mAbs were removed and the samples washed 3 × 5 min in PBS buffer solution without mAbs. (5) The primary antibodies that had bound to the epitopes were labelled using a secondary antibody carrying a fluorescence marker (Alexa Fluor 488 anti-rat for LM mAbs and Alexa Fluor 488 anti-mouse for 2F4). The secondary antibodies were diluted 1:100 and prepared in PBS. (6) The next step comprised 2 × 5 min rinsing with PBS buffer, followed by a 20 min incubation in 0.1% calcofluor white (fluorescent brightener 28; Sigma-Aldrich Chemie, Munich, Germany) prepared in water. (7) Finally, samples were washed twice for 5 min in PBS buffer and a PBS-based antifadent solution (Citifluor AF3; Science services, Munich, Germany) was applied. For controls, samples were treated using the same procedure except for the absence of mAbs labelling and calcofluor white staining. (8) Samples were then transferred to the stage of a binocular fluorescent microscope (MZ10F equipped with filters GFP-plus: 480-440 nm excitation, ≥510 nm emission wavelength and UV: 360-440 mm excitation, ≥420 emission; Leica, Wetzlar, Germany). Calibrated images were taken using a DP73 camera (Olympus, Hamburg, Germany) and the images processed using cellSens Dimension 1.7.1 (Olympus).

Uniaxial tensile tests

Samples of the internode (25 mm long) and of the node (25 mm long) were excised using a razor blade and the positions of the samples along the axis of the thalli recorded (Fig. 1a). Lateral stems were cut to 1-2 mm length (Fig. 1b). The internode samples comprised the central portion of an individual internode, those of a node comprised the ends of the subtending proximal and distal internodes and the intervening node. To facilitate handling during preparation and mounting, the samples were mounted in a light frame made of paper and masking tape (Tesa Krepp; Tesa, Hamburg, Germany). Unless otherwise specified, the internodal and nodal samples were equilibrated in deionised water (controls) or in a treatment solution for 24 h in the dark at 23°C. For measurement, a sample was mounted (Fig. 1c) in a universal material testing machine (Z0.5; Zwick/Roell, Ulm, Germany) equipped with a 5 N force transducer (XForce HP 5N, Zwick/Roell, Ulm Germany). The distance between the clamps was 15 mm, the speed of the crosshead was set at 0.2 mm min^{-1}. Immediately before measurement, the paper frame was cut open. During a tensile measurement, the specimen was held in air, saturated with water vapour using a humidifier (Pari-Boy, PARI, Starnberg, Germany). The tensile force (F; Newton) and the displacement (Δl; mm) of the crosshead were recorded. Uniaxial strains (ε; mm mm^{-1}) were calculated by dividing Δl by the initial distance between the clamps (l_0; mm):

$$\varepsilon = \frac{\Delta l}{l_0}$$

The stiffness (S; Newton) was obtained as the maximum slope of a linear regression fitted through a plot of force vs. strain (Fig. 1d). Tensile measurements were continued to the point of fracture. Maximum force (F_{max}) and strain at maximum force (ε_{max}) were determined. The F_{max} is closely related to the force at fracture ($F_{fracture}$; $F_{fracture}$ = - 0.02 (± 0.01) + 1.02 (± 0.01) × F_{max}; r^2 = 0.99, n = 40). The site of fracture either in the internode or in the node was recorded (Fig. 1e, f) and digital images were taken (MZ10F, Leica; DP73, Olympus). Occasionally, a sample would fracture within, or very close to, a clamp. The records from such runs were discarded because

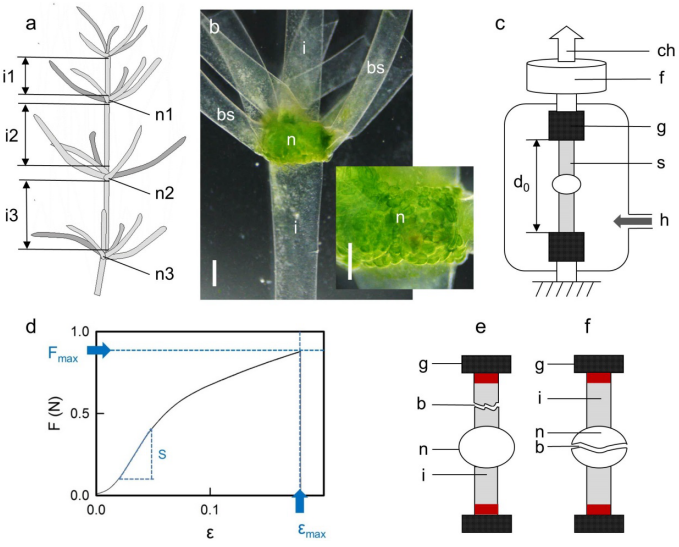

Fig. 1: Tensile test for stem samples of *Chara corallina*. a: Sketch of thallus with internodes (i) and nodes (n); b: Microscopic view of thallus with node (n) with internodes (i) and branching stems (bs) as prepared for immunolabelling, detail = multicellular node, bars = 0.5 mm; c: Setup of tensile test, ch = cross head, f = force transducer, g = grip, s = specimen, d_0 = grip distance, h = humidifier for control of relative humidity; d: Representative plot of force (F) versus strain (ε). The maximum force (F_{max}) corresponded to the maximum force recorded, the maximum strain (ε_{max}) to the strain at F_{max}. The stiffness (S) corresponded to the maximum slope. e, f: Failure of the internode or node using node samples, b = break.

damage to the sample during handling, mounting or clamping could not be excluded.
For each internodal sample, diameter was measured at three positions along the internode (cellSens Dimension 1.7.1., Olympus). For each nodal sample the diameters of the internode stubs, just proximal and just distal to the node, were measured.

Experiments
The effect of internodal position along the plant (i1, i2, i3) (measured from the apex) and nodal position (n1, n2, n3) on mechanical properties was investigated (Fig. 1 a). The number of replicates was 20-21.

The effect of pH was studied following incubation of samples in 70 mM K_2HPO_4 buffer for 24 h. The pH was adjusted to pH 3, pH 4, pH 5, pH 6, or pH 7 using HCl or NaOH. The number of replicates was 9-10.

The role of Ca in cell wall mechanics was studied following a 24 h incubation in ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) at concentrations ranging from 0.1 to 2.5 mM. The pH was adjusted to pH 7 using NaOH. Following incubation, the Ca content of the supernatant of the incubation solution was quantified by atomic absorption spectrometry (AAS) (AAnalyst 300; Perkin Elmer, Waltham, MA, USA). The AAS was equipped with a Ca lumina hollow cathode lamp (wavelength 422.7 nm, slit 0.7 nm) and an air-acetylene flame. The Ca analysis and the tensile tests were carried out with 10 replicates each.

The role of pectins in determining the mechanical properties of internodal and nodal cell walls was established by incubating samples in a solution containing pectinase (Fluka BioChemika, Sigma-Aldrich, Steinheim, Germany). This pectinase represented an endo-pectinase extracted from *Aspergillus niger*. The activity of the pectinase solution was adjusted to 20 U ml^{-1}, the pH of the solution to pH 5 using NaOH. The number of replicates was 10.

Data processing, statistics
Data are presented as box plots where the upper and lower border of the box represented the 25th and the 75th percentiles, the whiskers the 10th and 90th percentiles, and the filled circles the outliers. The line within the box represents the mean. Analysis of variance and regression analyses were carried out using SAS (SAS Institute, Cary, NC, USA). Mean comparisons were carried out using Tukey's Studentised range test ($P < 0.05$).

Results

The *Chara* cell wall was found to be composed of cellulose as indexed by staining with calcofluor white (Fig. 2, 3). In addition, pectins occurred in the internodes and in the nodes. The pectins are primarily de-esterified as indexed by intensive staining with LM19 (Fig. 2 n, p, r). There was essentially no staining of the internodes with LM7 (specific for partially esterified homogalacturonans) and only limited staining of the nodes (Fig. 2 h, j, l). Results were similar

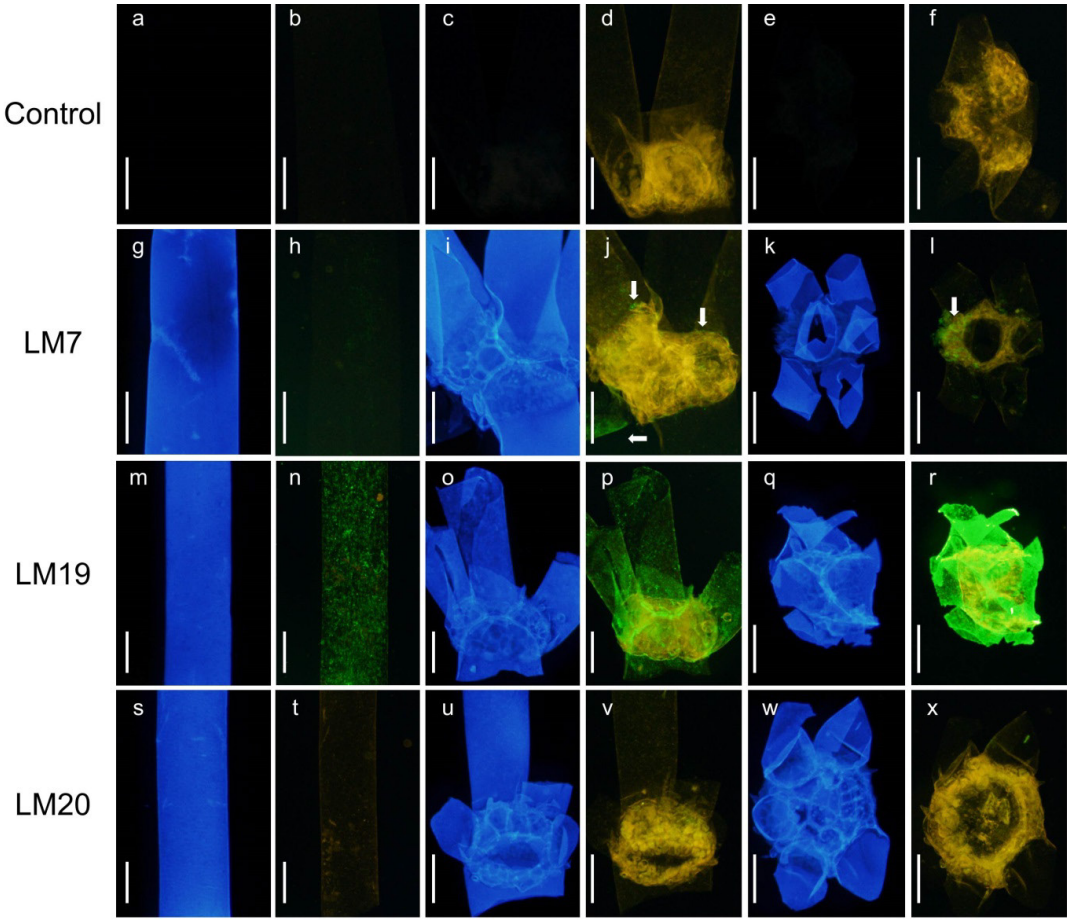

Fig. 2: Fluorescent micrographs of *Chara corallina* explants: internodes (a, b, g, h, m, n, s, t), longitudinal sections of nodes (c, d, i, j, o, p, u, v) and cross sections of nodes (e, f, k, l, q, r, w, x); stained with calcofluor white (g, i, k, m, o, q, s, u, w) and immunolabelled with LM7 (h, j, l), LM19 (n, p, r), LM20 (t, v, x); for control (a-f) the incubation was without calcofluor white or primary and secondary antibodies; bars = 0.5 mm.

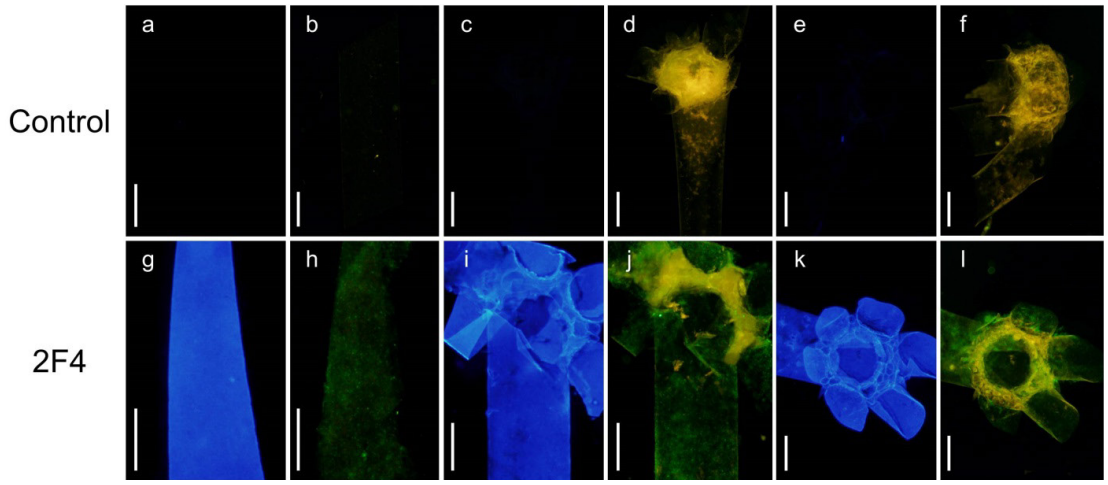

Fig. 3: Fluorescent micrographs of *Chara corallina* explants: internodes (a, b, g, h), longitudinal sections of nodes (c, d, i, j) and and cross sections of nodes (e, f, k, l); stained with calcofluor white (g, i, k) and immunolabelled with 2F4 (h, j, l); for control (a-f) the incubation without calcofluor white or primary and secondary antibodies; bars = 0.5 mm.

with LM20 (specific for highly esterified homogalacturonans) but a lot less intense (Fig. 2 t, v, x). Staining with 2F4 indicates the presence of homogalacturonans crosslinked by Ca, particularly in the nodes (Fig. 3 h, j, l).

The position of the internodes and the nodes along the axis of the thalli affected the stiffness of the specimens but the effects on the maximum force or maximum strain were too small to be significant (Fig. 4). The stiffness of the first internode and of the first node were lower than those of the second or third internodes or nodes. Most of the node specimens (> 80%) fractured in the internode across the cell walls, the others (< 20%) fractured in the node along the middle lamellae (Tab. 1).

Normal probability plots were generally linear indicating that stiffness, maximum force and maximum strain followed a normal distribution. Compared with the internodes, the nodes had lower stiffness, lower maximum force and lower maximum strain (Fig. 5, Tab. 2).

There was no effect of pH on stiffness, maximum force or maximum strain of internodes or nodes (Fig. 6). The percentage of specimens that fractured in the node increased with decreasing acidity (Tab. 1).

Increasing the EGTA concentration in the incubation solution increased the amount of Ca extracted from internode and node samples (Fig. 7). It also decreased their stiffness, their maximum force and maximum strain to similar extents (Fig. 8). There was little effect of EGTA on the relative frequency of fracture in the internode vs. in the node (Tab. 1).

Incubating internodes and node samples in pectinase decreased their stiffness, maximum force and maximum strain (Fig. 9). The frequency of fracture in the node increased markedly compared with the control without pectinase (Tab. 1).

Discussion

The mechanical properties of *Chara* cell walls are affected by pectins

The pectin components have marked effects on the mechanical properties of the cell walls of both nodes and internodes. First, incubating internodal and nodal cell walls in pectinase decreased stiffness, maximum force and maximum strain. Second, treatment of the cell walls with EGTA decreased stiffness, maximum force and maximum strain. The effect is consistent with the reduced stiffness and strength reported on treatment with CDTA (Toole et al., 2002). These chelators extract significant amounts of Ca from the cell wall. This decreases cross linking of homogalacturonan by Ca and solubilises pectins in the cell walls of both internode and node. It is worth noting that the magnitudes of these reductions in mechanical properties are comparable to those achieved by enzymatic degradation of pectins using pectinases.

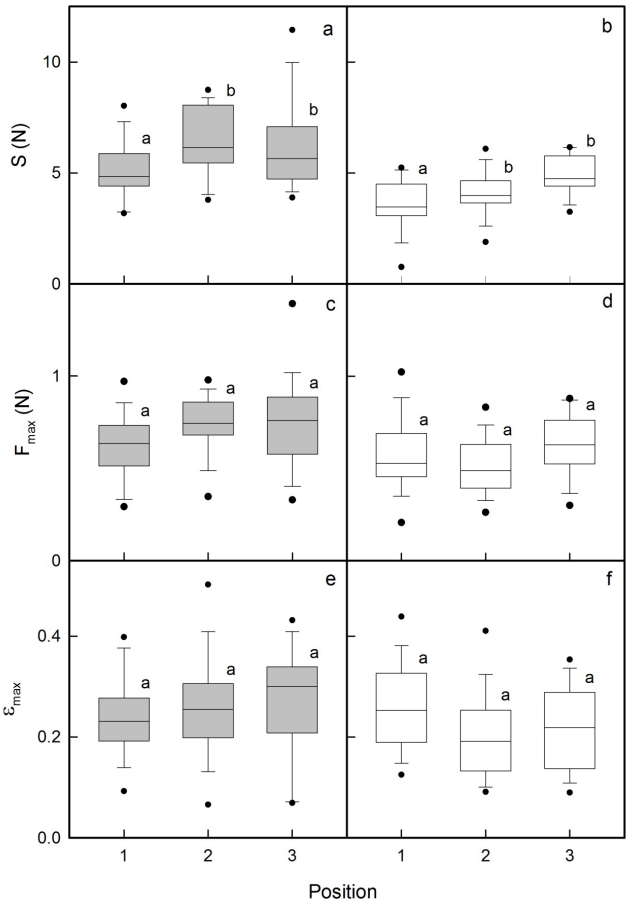

Fig. 4: Stiffness (S) (a, b), maximum force (F_{max}) (c, d) and maximum strain (ε_{max}) (e, f) of internodes (a, c, e) and nodes (b, d, f) of *Chara corallina* as affected by the position along the stem (counted from top, see Fig. 1 a). Means followed by the same letter do not differ significantly, $P < 0.05$.

Tab. 1: Frequency of failure of node samples of *Chara corallina* at the node or the internode when subjected to uniaxial tensile tests (Position 1, 2, and 3 refer to the position of the specimen along the plant axis). Unless otherwise specified, internodes and nodes were taken from position 2 and 3.

Experiment/Treatment	Frequency of failure (%)	
	Internode	Node
Position 1	80	20
Position 2	90	10
Position 3	90	10
pH 3	80	20
pH 4	90	10
pH 5	60	40
pH 6	67	33
pH 7	67	33
0.0 mM EGTA	100	0
0.5 mM EGTA	100	0
1.0 mM EGTA	100	0
2.5 mM EGTA	70	30
Control (- Pectinase)	100	0
Pectinase	50	50

Pectins also affect the fracture mode of the node samples. Both the extraction of Ca using EGTA and treatment with pectinases increased the frequency of schizogenous fracture i.e. along the middle lamellae between individual node cells and at either end of the internode cells where these adjoin the node cells.

There is little difference in the mechanical properties of the nodes and internodes of *Chara*

Failure of fruit skins occurs by failure of the pectin middle lamella and not by rupture of cellulose or hemicelluloses. Hence, if *Chara* was to be used as a model system, this model must reproduce the failure mode of fruit skins. Therefore, our experiments focussed on manipulating of pectins in the middle lamella between the two internode cells layer and the interfacing nodal cells. Clearly, the statistical analysis revealed significant differences. However, the magnitude of these differences was remarkably small. This similarity of the mechanical properties of internodes and nodes and also of their responses to EGTA and pectinase treatments were unexpected. However, the similarity is consistent with the lack of major compositional differences between their cell walls as indexed by staining using mAB. EGTA and pectinase treatments interact primarily with pectins and, therefore, we expected the node samples to be more affected, and the internode samples less affected. It is only in the nodes that the pectin middle lamellae form load-bearing links in the force transduction chain between the cells. These links were stressed in the uniaxial tensile test. In contrast, a middle lamella was not involved in our tensile tests of the internode specimens – not even in shear because the *Chara* internode is a single giant cell. Nevertheless, there were also marked effects of the EGTA and pectinase treatments on the tensile properties of the internode. This is consistent with the presence of pectins in the cell walls of both the internodes and nodes.

Cell walls and their mechanical properties in Chara and in fleshy fruit: a comparison

The cell wall of *Chara* is similar to that of a fleshy fruit, such as sweet cherry. This conclusion is based on the following observations. In *Chara,* the cell walls contain a combination of cellulose and pectins in both internode and node with only minor differences be-

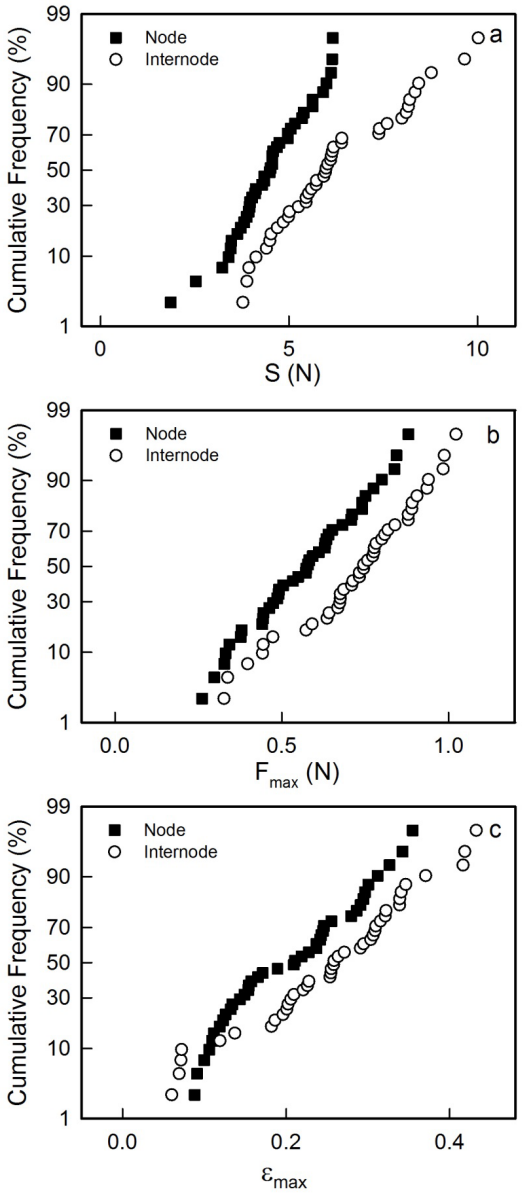

Fig. 5: Normal probability plot of the cumulative frequency distribution of stiffness (S) (a), maximum force (F_{max}) (b) and maximum strain (ε_{max}) (c) of internodes and nodes of *Chara coralline*. Pooled data for positions 2 and 3.

Tab. 2: Mean, median, minimum, maximum and coefficient of variation of stiffness (S), maximum force (F_{max}) and maximum strains (ε_{max}) of internodes and nodes from position 2 and 3 of *Chara corallina*. Means of internodes and nodes are significantly different, $P < 0.05$.

Parameter	Specimen	n	Mean	Median	Range		
					Minimum	Maximum	cv
S (N)	Internode	41	6.35 a	5.99	3.77	11.52	28.40
	Node	41	4.52 b	4.50	1.86	6.16	22.12
F_{max} (N)	Internode	41	0.74 a	0.75	0.33	1.41	27.41
	Node	41	0.58 b	0.58	0.26	0.88	28.77
ε_{max}	Internode	41	0.26 a	0.26	0.06	0.51	39.16
(mm mm^{-1})	Node	41	0.21 b	0.21	0.09	0.41	39.99

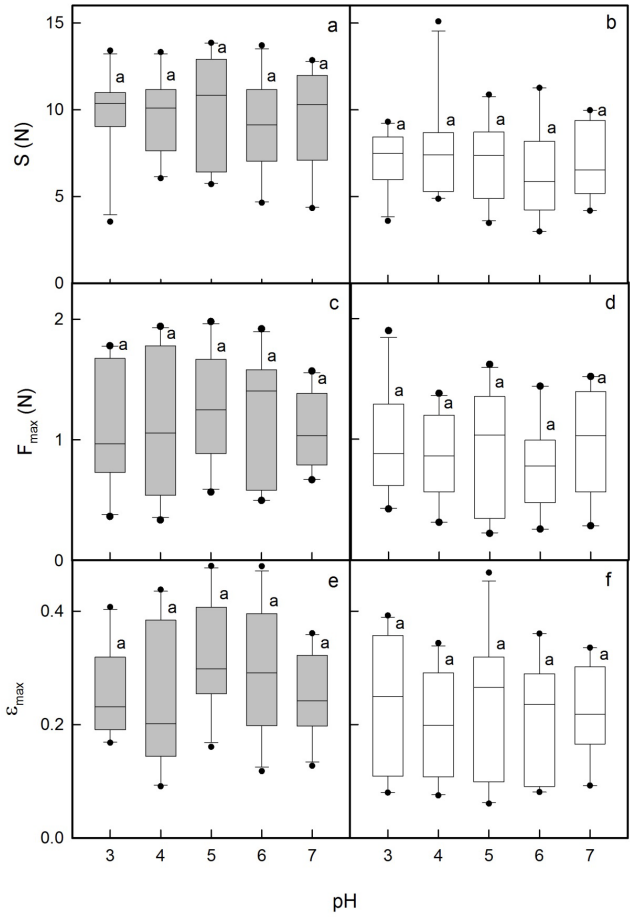

Fig. 6: Effect of pH on stiffness (S) (a, b), maximum force (F_{max}) (c, d) and maximum strain (ε_{max}) (e, f) of internodes (a, c, e) and nodes (b, d, f) of *Chara corallina*. Pooled data for positions 2 and 3. The effect of pH was not significant ($P < 0.05$).

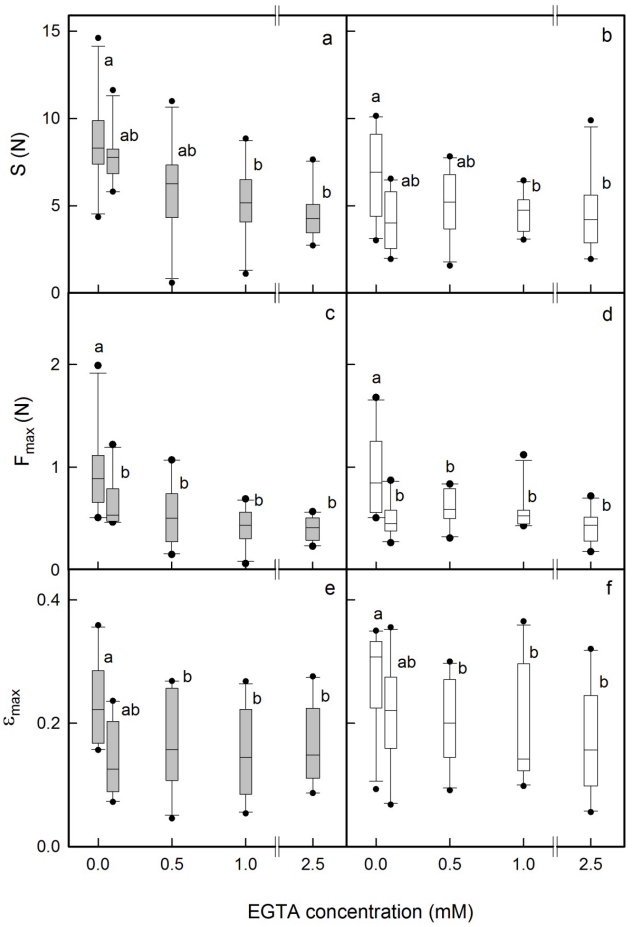

Fig. 8: Effect of EGTA concentration on stiffness (S) (a, b), maximum force (F_{max}) (c, d) and maximum strain (ε_{max}) (e, f) of internodes (a, c, e) and nodes (b, d, f) of *Chara corallina taken* from positions 2 and 3 along the stem. Means followed by the same letter do not differ significantly, $P < 0.05$.

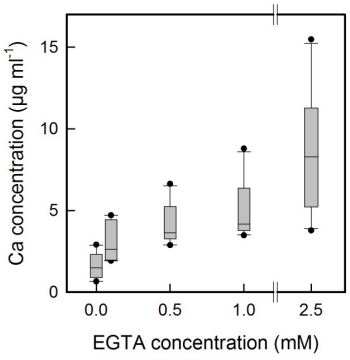

Fig. 7: Effect of the EGTA concentration during incubation of a single node and internode taken from positions 2 and 3 on the amount of Ca released.

tween the two (Fig. 2, 3; DOMOZYCH et al., 2010). This is the case also in the cell walls of sweet cherry fruit skin (SCHUMANN et al., 2019). The pectins present show low or no esterification of homogalacturonans as indexed by the binding of LM19 (SCHUMANN et al., 2019). A lack of highly esterified homogalacturonans (as indexed by a lack of binding of LM20) was evident in both *Chara* and sweet cherry (SCHUMANN et al., 2019). Slight differences between the cell walls of *Chara* and sweet cherry were observed in the binding of 2F4. This mAb identifies Ca bridges that cross link pectins. There was significant binding of 2F4 in *Chara* internode and node cell walls, but very little binding in sweet cherry (SCHUMANN et al., 2019). *Chara* is enveloped by a coating of Ca deposits (DOMOZYCH et al., 2010). In contrast, there are no Ca deposits in sweet cherry. Instead, the Ca content of sweet cherries is low and decreases during development (WINKLER and KNOCHE, 2019).

Pectins play a decisive role in the mechanical properties of the cells both of *Chara* and of fruit skins. Treatment with pectinases or EGTA or organic acids markedly increased failure of fruit skins as indexed by the incidence of fruit cracking (GLENN and POOVAIAH, 1989; WINKLER et al., 2015) or a decrease in fracture force of sweet cherry fruit skins (BRÜGGENWIRTH and KNOCHE, 2017). Conversely, additions of Ca significantly strengthened the cell walls of sweet cherry fruit skin with increases in both fracture force and modulus, but decreases in fracture strain (BRÜGGENWIRTH and KNOCHE, 2017). Thus, fruit skins and *Chara* node and internode samples respond in identical ways to the manipulation of cell wall pectins.

However, the failure modes of *Chara* and of sweet cherry fruit skins were consistently different. In *Chara*, failure occurred most frequently across cell walls (rhexigenous), whereas in fruit skins failure occurred most frequently along the middle lamellae (schizogenous) as demonstrated by the binding of LM19 (SCHUMANN et al., 2019). This observation indicates that in *Chara* the fracture force of the cellulose microfibrills was similar to that of the viscoelastic, gel-type pectin middle lamella. In contrast, in the sweet cherry fruit skin, the pectins of the middle lamellae represent the weak link in a strained sample of tissue.

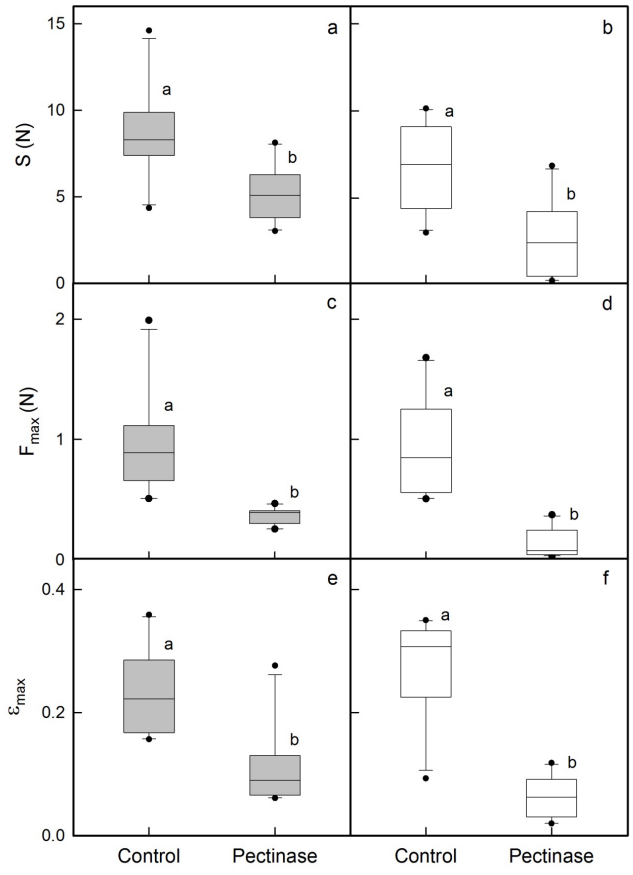

Fig. 9: Effect of pectinase treatment on stiffness (S) (a, b), maximum force (F_{max}) (c, d) and maximum strain (ε_{max}) (e, f) of internodes (a, c, e) and nodes (b, d, f) of *Chara corallina*. Pooled data for positions 2 and 3. Means of controls and treatments are significantly different, $P < 0.05$.

Thus, notwithstanding the obvious attraction of working with the highly simplified 'tissue' system of this alga, studies of the mechanical properties and failure modes of *Chara* are considered unlikely to offer useful insights into these properties for higher-plant fruit skin.

Conclusion

Our results demonstrate that the effects of manipulation of the pectins on the mechanical properties of the *Chara* cell walls are similar to those reported for the excised skins of fleshy fruit. The properties of both are strongly affected by pectins. However, the tensile properties of internode and node are too similar to allow conclusive inferences to be made of the rheological properties of the middle lamella from intercomparison of the tensile properties of the *Chara* internode vs. its node. Based on these findings, *Chara* quite fails to serve as a useful model for research into the failure characteristics of fruit skins.

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Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

AR, EG carried out the experiments, AR, EG, MK analysed data, EG, MK, AL wrote the manuscript. The data supporting the findings of this study are available from the corresponding author upon request.

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