The effects of desiccation on callose in food-conducting cells of the moss *Polytrichum commune*

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Abstract

Sieve elements and leptoids are the food-conducting cells of tracheophytes (seed plants and ferns) and mosses, respectively. In addition to performing a common function of sugar and nutrient transport, these two cell types show structural similarities that include an elongated shape with oblique end walls containing plasmodesmata. In sieve elements of tracheophytes, a collar of callose surrounds plasmodesmata and acts as a sphincter regulating molecule transport from cell to cell. Callose abundance also increases during stress responses in sieve elements. Aside from a few studies using aniline blue fluorescence that yielded confusing results, little is known about the occurrence and function of callose in moss leptoids. In this study, we investigated the location and development to callose, and changes in abundance of this polymer in leptoid cell walls during drying in the moss *Polytrichum commune*. Using aniline blue fluorescence and immunogold labeling in the transmission electron microscope, we document the localized occurrence of callose around plasmodesmata in end walls of leptoids in this moss. During differentiation and following 7 and 14 days of drying, callose increases in abundance around plasmodesmata. This work provides definitive evidence that callose is a key cell wall polymer around plasmodesmata in leptoids from their inception. Increases in callose during desiccation suggest a similar role of this cell wall polymer in regulating nutrient transport in mosses as has been documented in sieve elements of seed plants and ferns.

Introduction

Polytrichum commune is a moss with a complex anatomy that rivals that of tracheophytes, land plants with true xylem and phloem. The central vascular system in *Polytrichum* consists of hydroids, water conducting cells, and leptoids, food-conducting cells. Similarities between leptoids and sieve elements, food conducting cells of tracheophytes, include elongated cells with aggregates of plasmodesmata (PD) along lateral walls and oblique end walls with modified plasmodesmata (PD). Sieve elements not only transport nutrients throughout the plant, but they regulate transport by conglomerating callose and P-protein along PDs, thereby inhibiting flow of water and dissolved molecules across cells. The polymerization and de-polymerization of callose are the mechanisms for stress responses to pathogens, salts, drying and mechanical stress.

Callose is a well-studied polymer in tracheophytes, however, research on callose in bryophytes is limited. Callose localization in leptoids of bryophytes is restricted to aniline blue fluorescent with conflicting results. The present study uses immunogold labeling in the transmission electron microscopy, a highly specific detection method, to addresses the following questions related to callose in leptoid cell walls of *P. commune*. **1. Where is callose found in the cell wall of leptoids, specifically is this polymer associated with PDs? 2. How does callose labeling change during development of leptoids? 3. Does the intensity of callose labeling in leptoids change following dehydration stress?**

Acknowledgements

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Results

Figure 1-3 Aniline blue fluorescent showing callose localization. H, hydrome (water-conducting); L, leptome (food-conducting)

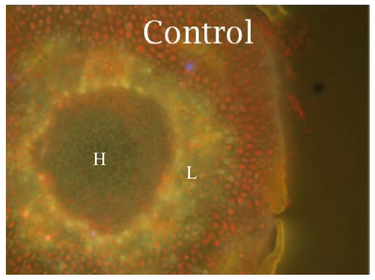

Figure 1. Control lacks aniline blue and shows autofluoresecence. Red=chlorophyll

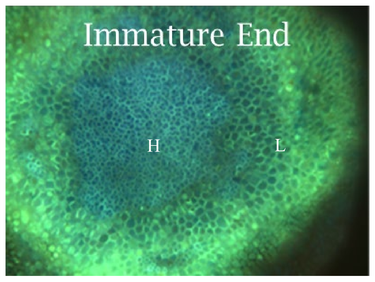

Figure 2. Immature region of stem with abundant callose (bright yellow green florescence).

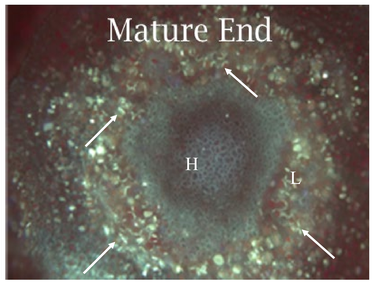

Figure 3. Mature region of stem showing florescence in end walls of leptoids (arrows).

Table 1. Anti-callose abundance table across dry times for *P. commune*. A higher density of labels are counted in day 7 and 14.

	Density Average	Label Sum	Score
Day 0	4.267	256	+
Day 7	6.833	410	++
Day 14	5.783	347	++

Figures 4-7. Immunogold labeling for callose in leptoid end walls. Dots identify callose in cell walls (Arrows). Bars = 50nm

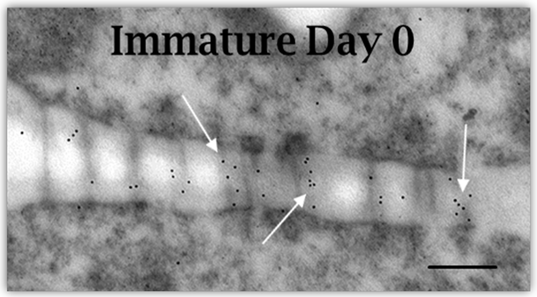

Figure 4. Undifferentiated cell with sparce callose associated with plasmodesmata.

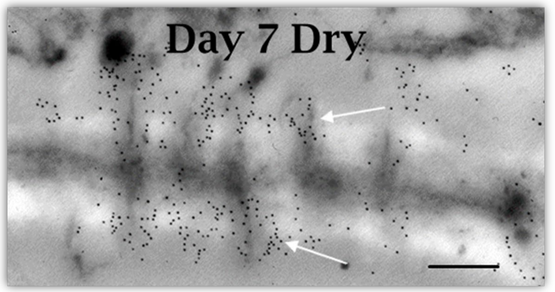

Figure 6: Abundant callose after 7-days of drying.

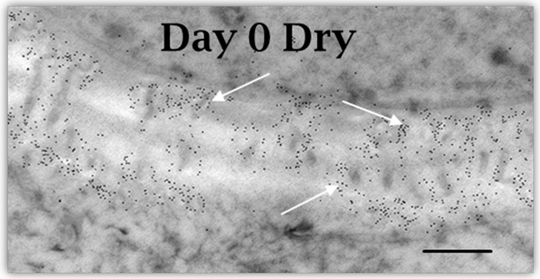

Figure 5. Mature end wall in hydrated plant with abundant labels around plasmodesmata.

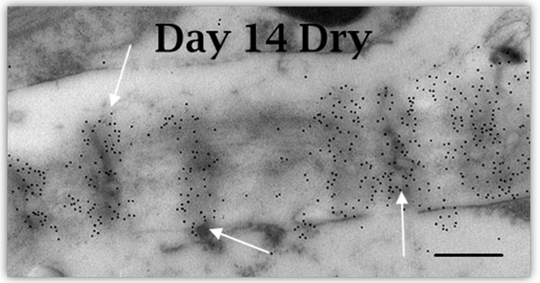

Figure 7: Labels are dense around plasmodesmata after 14-days of drying

Materials and Methods

Dehydration: Control samples were prepared upon collection. Treatment samples of mosses were dried in a shaded area outside for 7 and 14 days and then processed.

Specimen Preparation: Cut portions of stem, specifically the apex, were fixed overnight in glutaraldehyde and 0.5M phosphate buffer pH7.2, rinsed 3x in the same buffer for 15 min each. Plants were post-fixed in osmium tetroxide and rinsed with autoclaved water. Specimens were dehydrated in a graded ethanol series, embedded in LR white resin, cured at 65°C, and sectioned.

Immunogold Labeling: Sections were collected on 200 mesh Ni grids, blocked in BSA/PBS, and put into primary antibody, anti-callose, overnight. Following four washes in BSA/PBS, grids were placed in the gold-conjugated secondary antibody, washed in PBS 4x, rinsed and dried. Grids were observed and imaged on a Hitachi H7650 microscope at the SIU Image center.

Scoring Labels: Four 100x100 pixel frames were randomly placed on the images. The labels within frame were counted (15 images were used for each group). Counts were then averaged and scored. An average of 1 to 4 labels per frame were assign a single plus (+). If the average was 5 to 9 labels, two pluses (++) were given.

Histochemical Staining: To test for callose, hand-sectioned material was placed in 1% aniline blue in 0.067 M Na2HPO4 (pH 8.5) in the dark at 4°C for 24 hours, and rinsed in buffer. Controls were made using the buffer without stain. Specimens were viewed with a Leica DM500B fluorescence microscope (excitation filter ultraviolet fluorescence between 360-400nm). Images were collected digitally using a Q-Imaging Retiga 2000R digital camera.

Discussion

Question 1: Where is callose found in leptoid cell walls?

Aniline blue fluorescence indicates callose is present in *P. commune* walls from their inception. Immunogold labeling specifies that callose is localized around plasmodesmata that are numerous in immature cells.

Question 2: How does callose labeling change as cells mature?

Close to the apex, undifferentiated cells have abundance plasmodesmata in thinner walls. Aniline blue results show more florescence in the apical region than in the mature region due to the many PD. However, the labeling in these cells is visible less than mature PD. Mature cells have a pronounced collar of callose around plasmodesmata on leptoid end walls.

Question 3: Does the intensity of callose labeling in leptoids change following dehydration stress?

Statistical analysis indicates more callose labeling is present following drought stress. The abundant labeling in the 7- and 14-day dried plants can be interpreted as the cells way to plug transport across end walls.

Conclusions

This study finds that callose is present in PD of *P. commune* by histochemical staining and immunogold labeling techniques. Also, immature cells located at the apex have many connections between walls in the form of callose lined PD. The abundance table (Table 1) reveals plants that undergo desiccation put more callose into their walls. This suggests the moss *P. commune* may be acting as the water stress response described in tracheophytes. Finally, these results continue to demonstrate the striking similarities between leptoids and sieve elements that has been attested by past researchers.