Cell Wall Histochemistry of Maize Anthers

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Abstract

Relatively little is known about the cell wall composition of the anther, the male reproductive organ in flowering plants. Through a histochemical approach we characterized a variety of cell wall components across a timeline of anther development in *Zea mays* using specific cell wall stains. Lignin, pectin, and cellulose were all found to be enriched in specific somatic niche cell layers and to vary in concentration across the anther development timeline.

Introduction

Maize Reproduction

Maize pollen develops and matures in the anther. The maize tassel is composed of spikelets where the anthers reside in upper and lower florets (Figure 1). The anthers nurture the male germline through meiosis, pollen development, and shedding.[1]

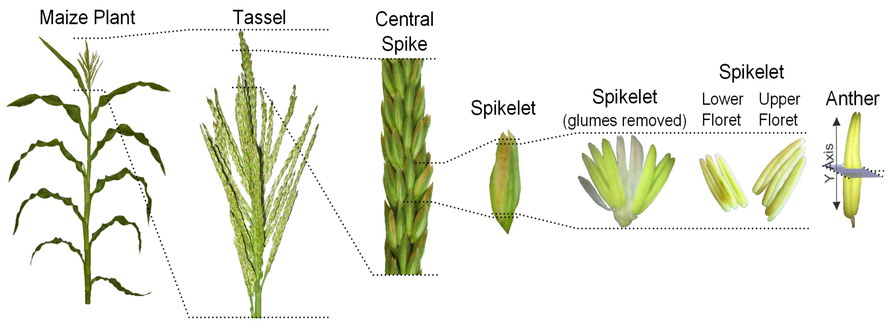


Figure 1: Maize anatomy.[2]

Anther Structure & Development

An anther is composed of four lobes surrounding a central vascular column. At its final stage prior to meiosis the lobe cell types are arranged as follows, from the center outwards: archesporial (AR), tapetum (TAP), middle layer (ML), endothecium (EN), and epidermis (EPI) (Figure 2).[1,3,4] Initially each anther lobe consists of a single layer of epidermis and a small number of equivalent subepidermal cells. Cells gain fate as they differentiate from the outside of the lobe inwards. Pre-meiotic development lasts approximately 8 days and anther length has been shown to correspond to developmental stage, within an inbred background.

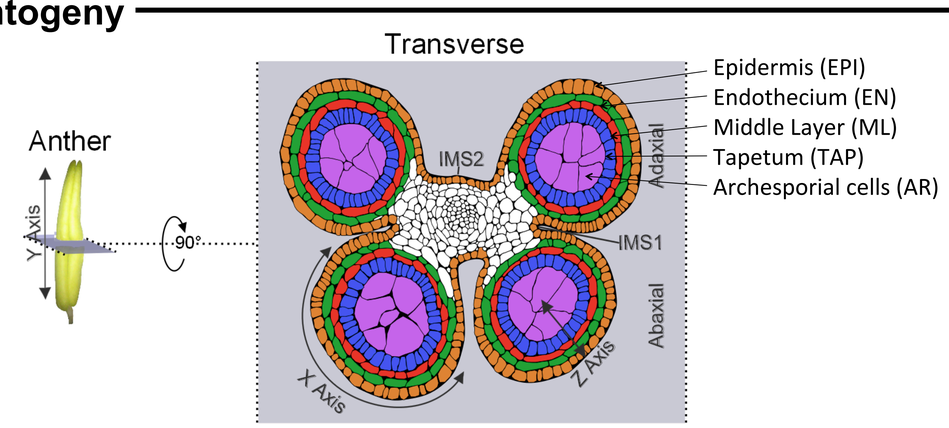
Cell Wall Composition

Surprisingly little is known about the cell walls of anthers. As the development of the anther involves highly coordinated cell division and differentiation into specialized cell types, understanding the composition of the newly built walls provides insight into the process of development and the roles of the different cell types. Previous experiments using solid-state NMR also indicate unexpected variations in the amounts of carbohydrates in the cell walls of select maize male sterile mutants (data not shown), indicating cell walls may play an important role in the development of viable pollen.

Histochemistry provides a simple, selective, visual targeting of cell wall components. We propose that this approach in anthers will allow us to identify differences in cell wall components between cell types across development.

Anther Ontogeny

Figure 2: Maize anther cell layers prior to meiosis.[2]



Methods

All tissues were fixed in FAA (50% ethanol, 3.7% formaldehyde, 5% glacial acetic acid, 1% DMSO, 0.5% Triton) and embedded in paraffin after an ethanol dilution and clearing process.

References

[1] Esau, K., 1965. *Plant Anatomy*. Second Edition ed. New York: John Wiley & Sons, Inc. [2] Zhang, H., et al. 2014. Transcriptomes and Proteomes Define Gene Expression Progression in Pre-meiotic Maize Anthers. *Genes, Genetics, Genomics*. (In Press) [3] Kelliher, T. & Walbot, V., 2011. Emergence and Patterning of the five cell types of the Zea Mays anther locule. *Developmental Biology*, (350), pp.32-49. [4] D'Arcy, W.G. & Keating, R.C., eds., 1996. *The Anther: Form, Function, Phylogeny*. Cambridge: Cambridge University Press.

Lignin

Lignin is a common cell wall component known to provide wall strength. Toludine Blue O is a metachromatic stain that colors lignin blue and carboxylic acids purple. Undifferentiated cells appear to have equal amounts of lignin (Figure 3A). During division of the SPL, only the ML and TAP are enriched in lignin (Figure 3B), and after division when all four somatic layers are present only the TAP is enhanced in lignin (Figure 3C). As the cell layers differentiate they appear to remodel and lose lignin enrichment.

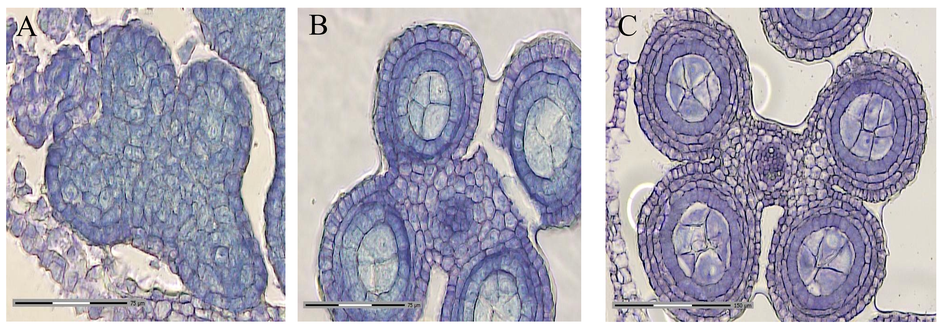


Figure 3: Anther undifferentiated (A), during SPL division (B), and after SPL division with all somatic layers present (C), all stained with Toludine Blue O. Darker blue indicates more lignin and darker purple indicates more carboxylic acids.

Pectin

Ruthenium Red was used to selectively stain for pectin, a component of primary cell walls. Like lignin, prior to any cell division all anther cells are enriched in pectin (Figure 4A). During SPL division, the ML, TAP, and AR are enriched (Figure 4B) and after division only the TAP and AR are enriched in pectin (Figure 4C).

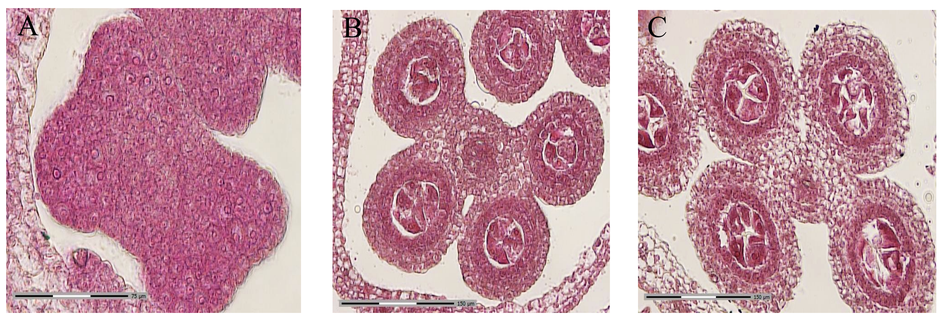


Figure 4: Anther stained with Ruthenium Red before division (A), during SPL division (B) and after SPL division with all four somatic layers present (C). Darker red indicates pectin enrichment.

Cellulose

Methylene Blue was used to selectively stain for cellulose. Prior to differentiation all cells are enriched (Figure 5A), during division of the SPL, the ML, TAP, and AR are enriched in cellulose while the EN and EPI are no longer (Figure 5B). After division, only the TAP is enhanced in cellulose (Figure 5C).

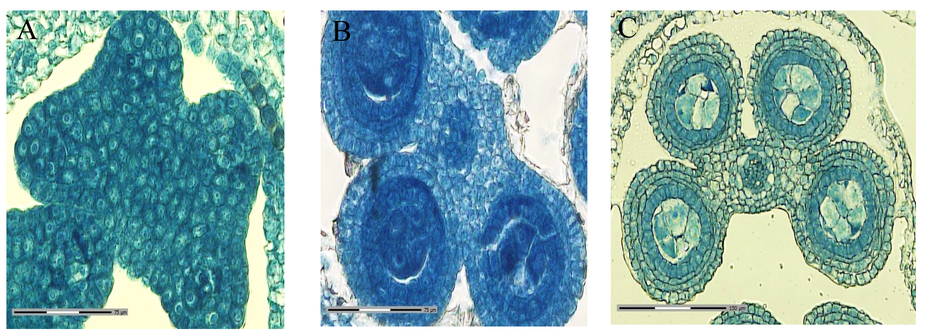


Figure 5: Methylene Blue stained anthers before division (A), during SPL division (B) and after SPL division with all four somatic layers present (C). Darker blue indicates cellulose enrichment.

Discussion

The walls of the different cell layers in the maize anther were found to be distinct in their composition and to change over developmental time. Before cell differentiation, all cells are enriched in lignin, pectin, and cellulose. During SPL division, the ML and TAP are enriched in these cell wall components, while the EN and EPI are enhanced in carboxylic acids and no longer in the other components. The similarity between the ML and TAP is likely due to their common precursor, the SPL. After SPL division is completed, only the TAP is enriched in lignin, pectin, and cellulose.

Each cell layer plays a distinct role in anther and pollen development. It also appears that as each cell gains its specific fate, so does its cell wall. Future work will utilize histochemistry on a variety of male sterile mutants that all have defects in anther somatic cell division and differentiation. This will provide further insight into the role of the cell walls in the development of viable pollen.

Acknowledgements

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