On the cutting edge of development: Laser-assisted microdissection of the Arabidopsis gynoecium reveals tissue-specific gene expression patterns

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Multicellular organisms require extensive spatial and temporal control of transcription in order to generate cell-type specific gene expression patterns. These expression patterns make up the coordinated cellular networks that act in concert to perform functions essential for organogenesis, growth, and fitness. In plants, cell type-specific expression patterns take on another layer of complexity as plant cells are immobile and contain cell-cell communication pores (plasmodesmata). Consequently, cellular fate and function are both deeply informed by the context in which cells differentiate. Fruit development is a prime example of such a coordinated cellular response, as fruits form from mature gynoecia (female reproductive organs) and internally develop seeds and the chambers that house these seeds. Fruit development requires precise spatial coordination and is not induced until a specific temporal cue is given. In Arabidopsis and other dehiscent species, gynoecium development also requires the formation of marginal regions that undergo programmed cell death and separation, allowing for seed dispersal. All of these distinct developmental processes require intricately controlled gene expression patterns, coordinated across macroscopic cellular distances. Gynoecium development has huge agricultural relevance, as farmers seek to maximize their yield and prevent seed loss in their crops.

In this issue of *Plant Physiology*, Luna-García et al., 2023 examine gene expression profiles of multiple tissue types in Arabidopsis gynoecia at distinct timepoints, using laser-assisted microdissection (LAM) to isolate specific regions of the developing gynoecium for RNA-sequencing analysis. RNA sequencing can elucidate cell-type specific gene expression, but the techniques used to sequester cellular subpopulations, such as fluorescent activated cell sorting (FACS), causes these analyses to lose spatial information of the cell-cell contacts that are essential for development. These cell-sorting techniques also require reporter genes, so prior knowledge of gene expression patterns in these cells is necessary. In tissues with clear delineation between physical subdomains, such as the gynoecium, LAM allows for information on cell populations' physical location to remain intact and has no need for marker genes. Using LAM, the authors collected tissue from developing gynoecia from two distinct tissue types at two different time points to study temporal and spatial differences in gene expression (Figure 1A). In early-stage gynoecia, they dissected out the medial carpel margin meristem and the lateral carpel wall tissue, while in late-stage gynoecia, they dissected out the fully mature medial septum and lateral valve tissues (Figure 1B).

The authors investigated the differentially expressed genes (DEG) among these tissue types that may regulate their different developmental potentials. They found that previously characterized transcription factors that mediate the development of these distinct tissue types were specifically enriched in those tissues whose development they regulated. They also observed that many DEGs expressed in both the medial and lateral tissues were related to auxin signaling, though different auxin response proteins are expressed in these distinct spatial domains. In the lateral domain, ARF3 (ETTIN/AUXIN RESPONSE FACTOR 3), a known regulator of gynoecium development (Nemhauser et al., 2000), is expressed, whereas in the medial domain, ARF6 and ARF8 (AUXIN RESPONSE FACTORS 6 and 8) are expressed. It is known that different ARFs regulate oppositional or sequential developmental pathways in other plant organs, such as during leaf polarity establishment (Manuela and Xu, 2020) and lateral root development (Taylor-Teeples et al., 2016). It would be interesting to determine whether these different ARFs regulate distinct gene circuits in the gynoecium to differentiate these spatial domains.

This study also showed that auxin biosynthesis genes, such as *YUC1* (*YUCCA 1*) and auxin transport proteins, such as PIN3 (PIN215 FORMED 3) are specifically expressed in medial tissue types, suggesting that not only downstream responses to auxin but also auxin concentration gradients themselves are established in a spatially regulated manner in the gynoecium. Auxin source-sink dynamics are essential morphogenic signals in developmental processes that form along an axial gradient, such as the balance between cell division and cell

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elongation in the root (Guillotin and Birnbaum, 2020). The expression patterns of auxin synthesis, transport, and perception genes in medial versus lateral gynoecium tissue may indicate that auxin is synthesized in medial tissue and transported laterally and apically to regulate the balance between cell division and cell differentiation that promotes the elaboration of the mature silique into an elongated tube structure. The interplay between cell proliferation and cell elongation is in fact known to be a distinguishing feature between medial and lateral gynoecium tissue. To examine whether their DEGs reflected this distinction, the authors compared expression of cell cycle regulators in their four tissue types. They found that the carpel margin meristem, i.e. early-stage medial domain tissue, expressed more cell cycle-promoting genes, which could mediate the meristematic activity in this tissue at this timepoint. It would be interesting to investigate if there is a corollary increase in expression of cell elongation-promoting genes in the early lateral tissue, the carpel wall, given the necessity for substantial cell elongation in this tissue during silique growth (Ripoll et al., 2019).

The deep RNA sequencing of these four temporally and spatially distinct gynoecium tissue types in this article identified uncharacterized genes with differential expression patterns. These genes could potentially be unknown regulators of fate specification in these tissues. The authors characterized T-DNA insertional mutants of twenty-one of these genes, finding that mutants of seven of the genes tested had defects in gynoecium development. Many of the genes are known regulators of other developmental processes, such as FP2 (FOLDED PETALS 2) and PRS (PRESSED FLOWER), which respectively regulate cell elongation (Takeda et al., 2014) and cell division (Matsumoto and Okada, 2001) in floral organs. These known floral phenotypes may suggest that these genes play a similar role in the balance between cell division and elongation in the gynoecium. The authors also found DEGs for several WOX (WUSCHEL-RELATED HOMEOBOX) genes, key drivers of cellular differentiation in root and shoot meristems (Kitagawa and Jackson, 2019). Mutants of these WOX paralogs developed siliques that were shorter and had fewer seeds than wild type plants (Figure 1C). One mutant of a gene specifically expressed in septum tissue, TRN2 (TORNADO 2), showed further defects, including twisted gynoecium (Figure 1D), which resulted in an unfused septum (Figure 1E) and a silique lacking a transmitting track, in line with TRN2's expression pattern. Finally, disruption of *REM1* (*REPRODUCTIVE MERISTEM 1*), which is specifically expressed in the carpel margin meristem, led to a defect in pollen viability (Figure 1F) and shorter siliques. REM1's dual role in male and female reproduction could be an interesting avenue to further explore, to determine whether this gene regulates similar development circuitry or distinct downstream targets in pollen and gynoecium development.

Luna-García, et al. revealed the value in global transcriptomic profiling at a cell-type resolution, as many of the genes identified in this study as differentially expressed in the gynoecium play pleiotropic roles in earlier developmental processes, such as flower and root meristem development. Mutant phenotypes of these genes, particularly genes that are necessary for these earlier processes, could obscure their role in gynoecium development, as the mutants will fail to develop gynoecia in the first place. While the authors found several differentially expressed genes affected gynoecium development using global T-DNA mutants, use of an inducible gene repression system may allow for the identification of other genetic drivers of gynoecium development that play stronger pleiotropic roles in vegetative and flower development. Another intriguing line of research that this transcriptomic method could address is how seed dispersal is regulated in Arabidopsis, and specifically how valve margin cells undergo apoptosis to open up the silique and allow mature seeds to escape. The authors found that SHP2 (SHATTERPROOF 2), which regulates specification of valve margin cells (Liljegren et al., 2000), is expressed in medial tissue. If these valve margin cells could be specifically isolated by LAM, this could determine whether SHP2 expression is enriched in this population of cells, and reveal novel determinants of seed release. Similarly precise LAM in the medial tissue could isolate developing seeds versus the transmitting tract to determine gene expression enrichment in these heterogenous cell populations. Fertilization, gynoecium growth, and seed release are all developmental processes absolutely indispensable for fitness. Luna-García, et al. illustrate the complex regulation that underpins these pathways, and demonstrates the utility of physical methods to isolate tissue types in transcriptomic assays. Expanding this analysis to key crops that produce fruit and seed products could enrich our ability to predict and engineer the outcomes of these developmental events.

Figure 1: Laser-assisted microdissection of Arabidopsis gynoecium determines spatially and temporally distinct developmental transcriptomes. A. Visualization of gynoecium cross-sections at early and late developmental stages with the tissue types dissected in this study labeled. B. Cross-sections of Arabidopsis gynoecium at early developmental stage, where lateral carpel wall (purple) and medial carpel margin meristem (red) tissue was harvested, and at late developmental stage, where lateral valve tissue (purple) and medial

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septum tissue (red) was harvested. C. Comparison of silique growth in WT (Col-0) and several WOX transcription factor mutant lines (*wox3*, *wox12*, and *wox1*). Scale bars are 1 cm. D. Comparison of silique morphology in WT (Col-0) and *trn2* mutants. Scale bars are 0.5 cm. E. Cross-sections of WT (Col-0) and trn2 mutant siliques, showing loss of transmitting tract in *trn2* mutant. Scale bars are 10 μm. F. Peterson's staining analysis of pollen viability in WT (Col-0) and *rem1* mutants. Non-viable pollen is stained green. Scale bars are 10 μm. Figure adapted from Luna-García, et al.

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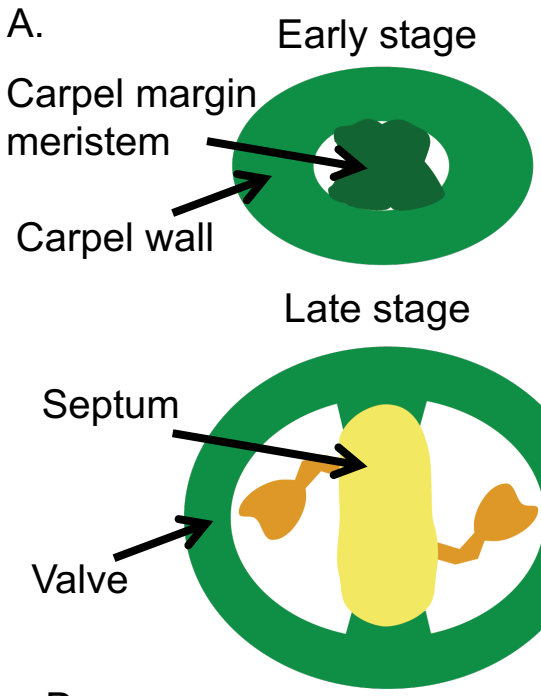

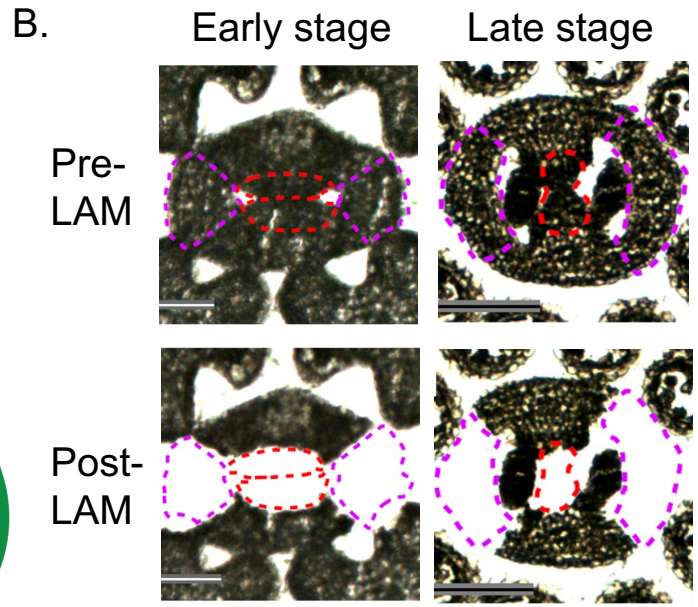

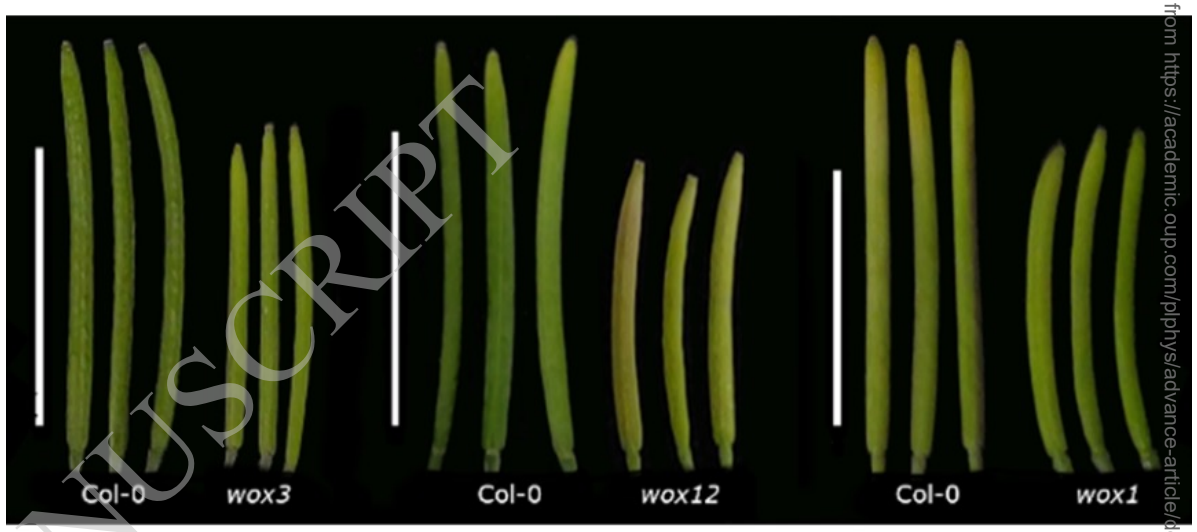

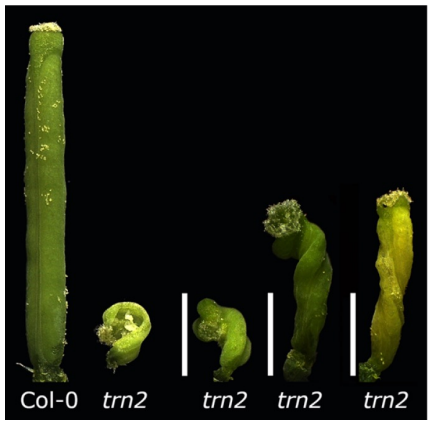

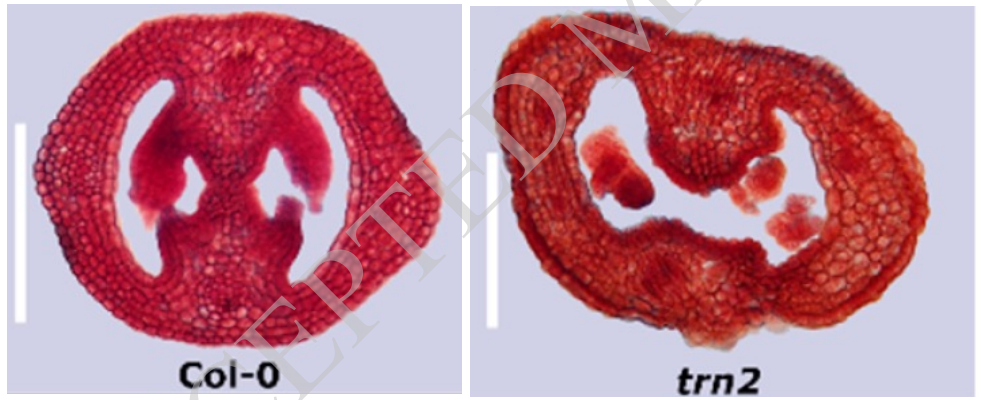

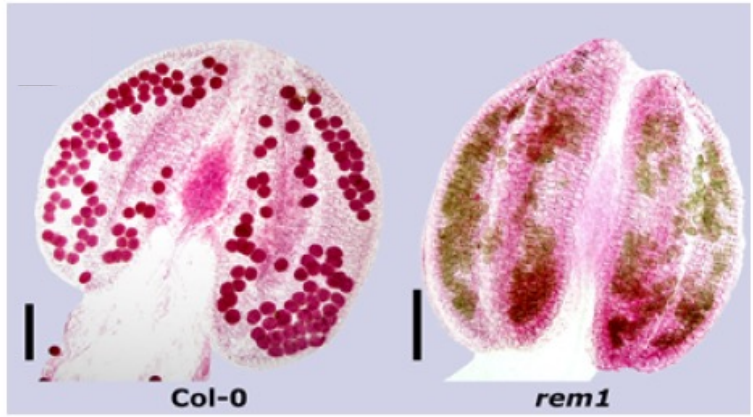

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