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Building a Spatial and Transcriptomic Framework for Transcription Factor Regulation in Wolffia australiana

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Understanding how plants grow, develop, and respond to their environment requires not only identifying the genes involved, but functionally characterizing what each gene does. To understand how transcription factors regulate genes across tissues and organs, transcriptional data needs to be placed within a spatial, cellular, and organismal context. *Wolffia australiana* is an attractive system for addressing this fundamental question due to its small body plan, fast clonal growth rates, and compact non-redundant genome. In our lab, we use high-resolution, 3D imaging technologies and transcriptomics to pinpoint the localization of transcription factors and how they globally regulate genes in a whole plant. We used X-ray microscopy to capture the 3D architecture of *W. australiana*, resolving the structure of the meristematic region and laying the groundwork for future cell segmentation and quantification. We further employed expansion microscopy, which enables nanoscale imaging beyond the diffraction limit, achieving roughly 4x physical expansion of intact *W. australiana* and enhanced resolution of nuclei and chloroplasts. Together, these imaging approaches provide a spatial and cellular scaffold for mapping transcriptional activity across the whole plant. To begin applying this framework, we examined the transcriptomic response to early salt stress by generating a bulk RNA-sequencing atlas over a 48-hour timecourse, which identified specific transcription factors and functional pathways underlying the salt stress response. These imaging and transcriptomic approaches together lay the groundwork for localizing transcription factor activity within a defined spatial and cellular context in *W. australiana*.

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