Unleashing the power of single cell technologies: combining single-nuclei RNA-seq and Molecular Cartography ™ in soybean

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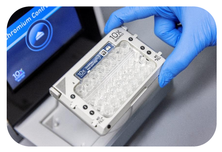
The sustainable production of crops necessary to meet demand requires the development of innovative new strategies. Genomic engineering represents an attractive solution to develop the next generation of high-performing crops. However, the implementation of such strategies requires a deep and high-resolution understanding of the role and activity of crop genes. Considering that plants are composed of various cells and cell types sharing the same genetic information, plant cells differentially use their genomic information to establish cell-type-specific transcriptional programs and to gain unique biological functions. To precisely characterize the activity of each gene in a cell-type, we and others reported the use of single cell/nucleus RNA sequencing (scRNA-seq/sNucRNA-seq) and single nucleus Assay for Transposase Accessible Chromatin sequencing (sNucATAC-seq) technologies; mostly on the model plant Arabidopsis thaliana [1-12]. However, even though these technologies prove useful in characterizing cell type-specific transcriptomes, the functional annotation of the identified clusters remains challenging; especially when considering non-model plant species. In the present study, we demonstrated the use of Molecular Cartography™ alongside sNucATAC-seq to add a spatiotemporal dimension to our Soybean root (A-E) and nodule single cell expression profiles (F-I). This technology allowed us to visualize the expression of 100 preselected genes at cellular resolution. Given our results, we expect single cell/nucleus technologies paired with high resolution spatial analysis to provide a new understanding of plant gene activity and function. Such detailed knowledge will be essential in establishing meaningful genomic engineering strategies to improve crop performance.

To characterize the activity and regulation of the soybean genes at the single-cell resolution, we applied Single Cell Multiome ATAC + Gene Expression technology and sNucRNA-seq technology on the soybean root and the soybean nodule, respectively (**A** and **F**). The transcriptomic/epigenomic profiles of each cell composing the root and nodule were analyzed to generate Uniform Manifold Approximation and Projections (UMAPs) (**B** and **G**). On the UMAPs, the relative location of a cell/nucleus is a reflection of its transcriptomic/epigenomic profile. Our data led to the identifications of 16 and 10 different cell types composing the soybean root and nodule, respectively.

Functional annotation of the clusters is usually achieved by using known molecular marker genes (e.g., **C.1**). However, soybean cell-type marker genes are poorly characterized. To support the annotation of other root and nodule clusters, we applied Molecular Cartography™ (**D.1, E.1, H.1, H.2, and I.1**). This new technology allowed us to question the expression of cluster-specific marker genes in the context of the morphology of the root and nodule. To validate the use of this technology, we first analyzed the activity of a set of root hair-specific genes (**D.2, red shape**). Molecular Cartography™ confirmed our functional genomic approach (**C.2, red shape**). We extended the use of Molecular Cartography™ to reveal other soybean root cells types including the epidermal, cortical, endodermal, pericycle, xylem, and phloem cells (**E.2, brown shape**). Applying the same molecular probes on nodule cross section, we identified the clusters composing the cells infected by *B. diazoefficiens*, the soybean nitrogen-fixing symbiont (**H.3, grey shape**), and those composed by cells from the vascular bundle (**I2., cyan shape**).

Soybean

Microfluidic System



A

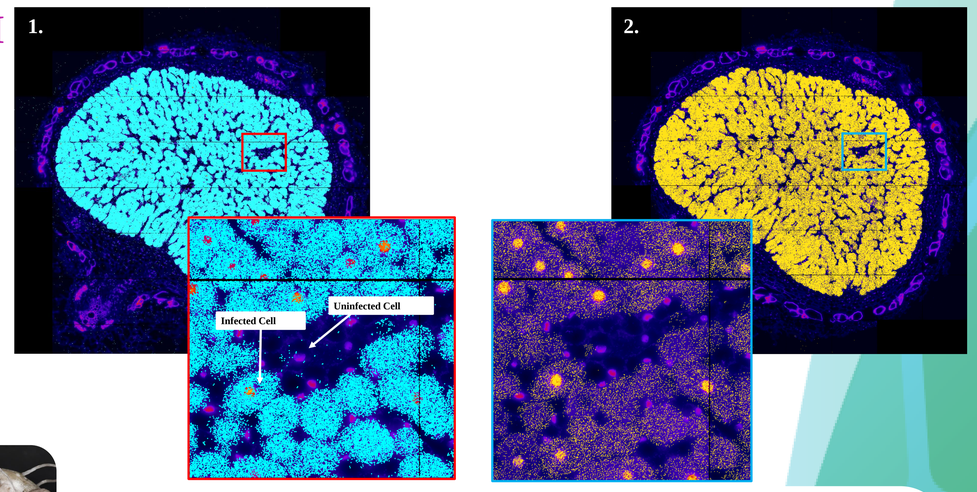
Root tip

F

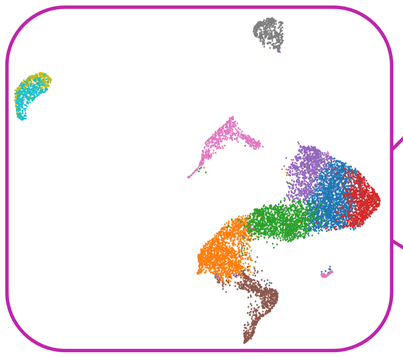
Nodules

H. Using a set of probes designed against *B. diazoefficiens* transcripts not only highlighted the set of infected cells of the soybean nodule at the center of the organ (**H.1**) but also served as quality control of our Molecular Cartography™ experiments. Notably, we noticed the very low amounts of noise generated by the *B. diazoefficiens* probes in uninfected nodule cells (**H.1**). The identification of a soybean gene-specific expressed in the infected cells of the nodule (**H.2**) allowed us to identify the *B. diazoefficiens*-infected cell cluster on the sNucRNA-seq UMAP (**H.3**, grey shape).

H



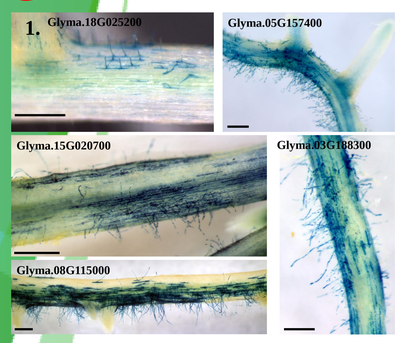
G **sNucRNA-seq UMAP of the soybean nodule**

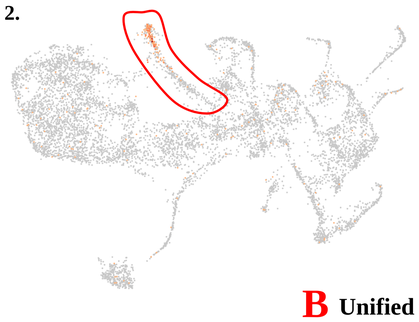


Functional annotation of the soybean "nodule *B. diazoefficiens*-infected cell" cluster

Functional annotation of the soybean "nodule vascular bundle" cluster

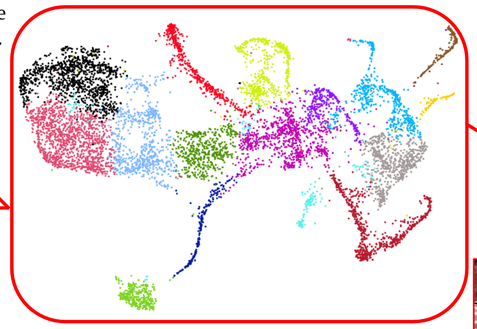
C





C. Several root hair-specific marker genes have been previously functionally characterized (**C.1**, [13]). The analysis of their transcriptional activity on the soybean root UMAP revealed the soybean root hair cell cluster (**C.2, red shape**).

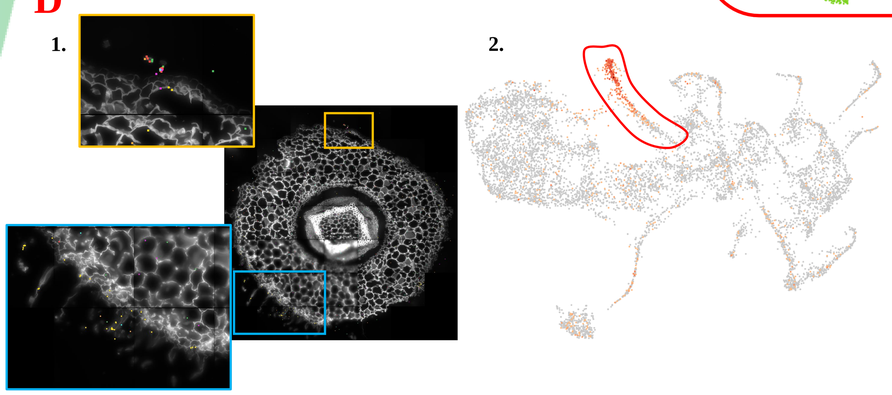
B **Unified transcriptomic/epigenomic UMAP of the soybean root**

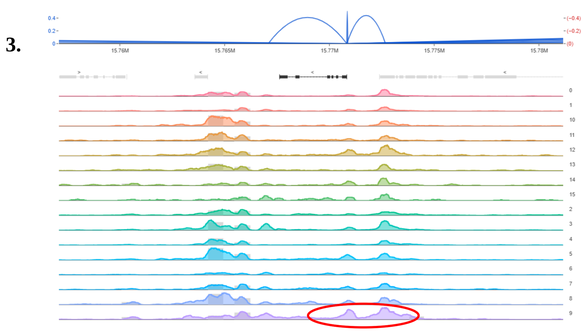


Functional annotation of the soybean "root hair" cluster

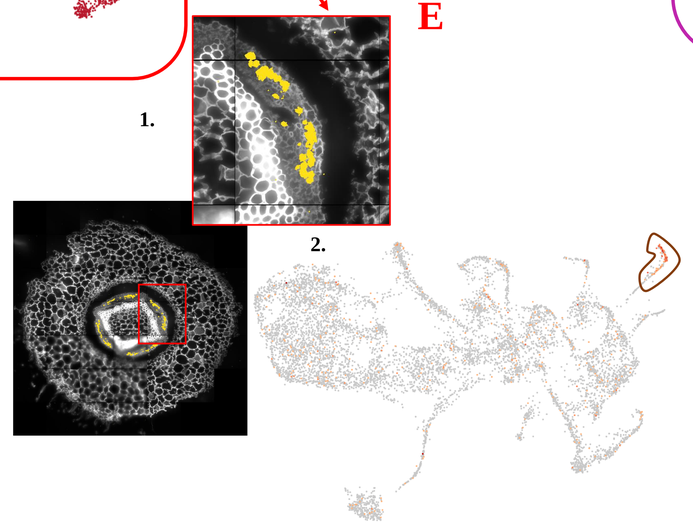
Functional annotation of the soybean "phloem" cluster

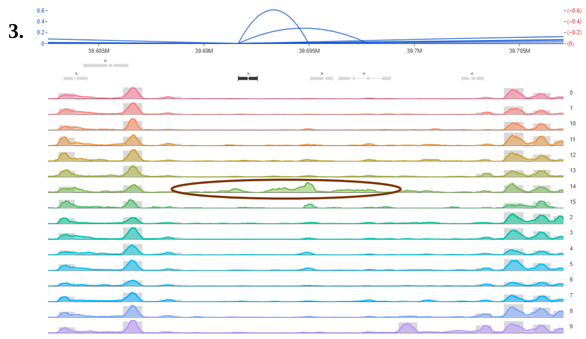
D





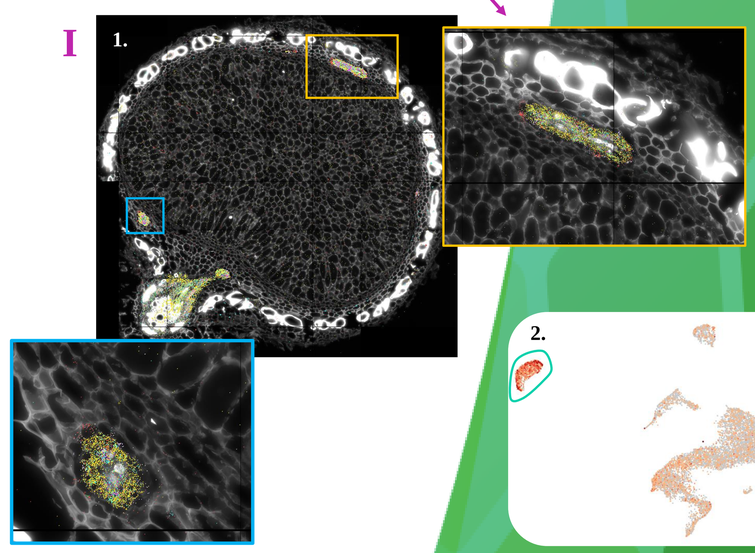
E





D and E. Molecular Cartography™ of soybean genes specifically expressed in the soybean root hair (**D.1**) and phloem cells (**E.1**). The use of Single Cell Multiome ATAC + Gene Expression technology on the soybean root enables the unification of gene expression and profiles of chromatin accessibility, and the characterization of regulatory elements that control gene activity. Significant correlations were identified between DNA fragments located upstream and downstream of the root hair (**D.3, blue curves**) and phloem-specific genes of interest (**E.3, blue curves**). These correlations support the discovery of accessible regulatory elements in the soybean root hair (**D.3, red circle**) and phloem cells (**E.3, brown circle**).

I



I. Molecular Cartography™ of soybean genes specifically expressed in the vascular bundles of the soybean nodule (**I.1**). The mining of their transcriptional activity in the soybean nodule sNucRNA-seq UMAP supports the functional annotation of one nodule cell cluster as the "vascular bundle" cluster (**I.2**, cyan shape).

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