Identifying Novel Genes Potentially Involved in Maize Ear Meristem Development through Single-Cell Transcriptomics

Lillian Spade[1], Ravi Bika[2], Tengfei Liu[2], Sanzhen Liu[2]

[1]Luther College [2]Kansas State University, Department of Plant Pathology

Background

- Maize (*Zea mays*) is one of the most widely planted food crops globally
- Because of how widely planted it is, farmers are always looking to breed maize plants for better yield and improved resilience to both biotic and abiotic stresses
- The development of the maize plant is driven by undifferentiated cells called **meristem cells**
- Figuring out how meristem cells stay undifferentiated could let us rapidly speed up gene editing, which in turn would let us adapt maize to our needs faster
- Single cell RNA sequencing (**scRNA-seq**) is a tool that can quantify transcriptional levels of genes in individual cells
- This technique could help identify genes that are only expressed in meristem cells (and are therefore overlooked by bulk RNA sequencing techniques)

Research Hypothesis

Novel genes involved in maize ear meristem development can be identified using scRNA-seq, where genes sharing similar expression patterns and co-expressing with known regulatory genes are likely to participate in the same developmental processes.

Experimental Design

- Previous research has generated scRNA-seq data from developing maize ears (Xu et. al 2021)

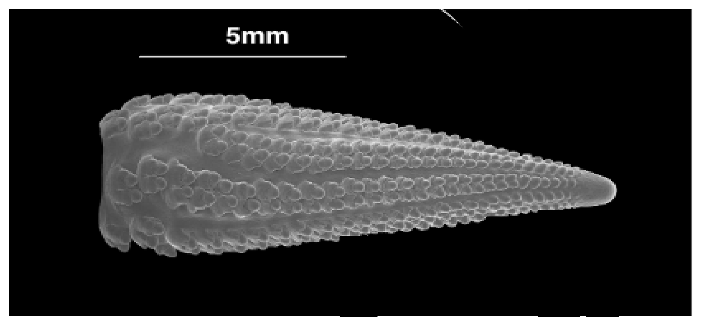

Diagram of a developing maize ear (Xu et al 2021)

- This dataset includes meristem cells as well as other types of cells; novel genes will be identified by comparing the expression of meristem vs other cells

scRNA-seq generates trimmed fastq file

↓ FastQC

STARsolo alignment

↓ Seurat in R

Clustering

↓ Known genes

Identifying clusters

↓ Cluster comparison

Novel Genes

Results

Clustering

Figure 1. Cell clustering visualization using Uniform Manifold Approximation and Projection (UMAP). Each point represents one cell. Cells were plotted based on their gene expression and 26 clusters were identified. Each cluster is indicated with a number and a unique color.

Identifying clusters of interest

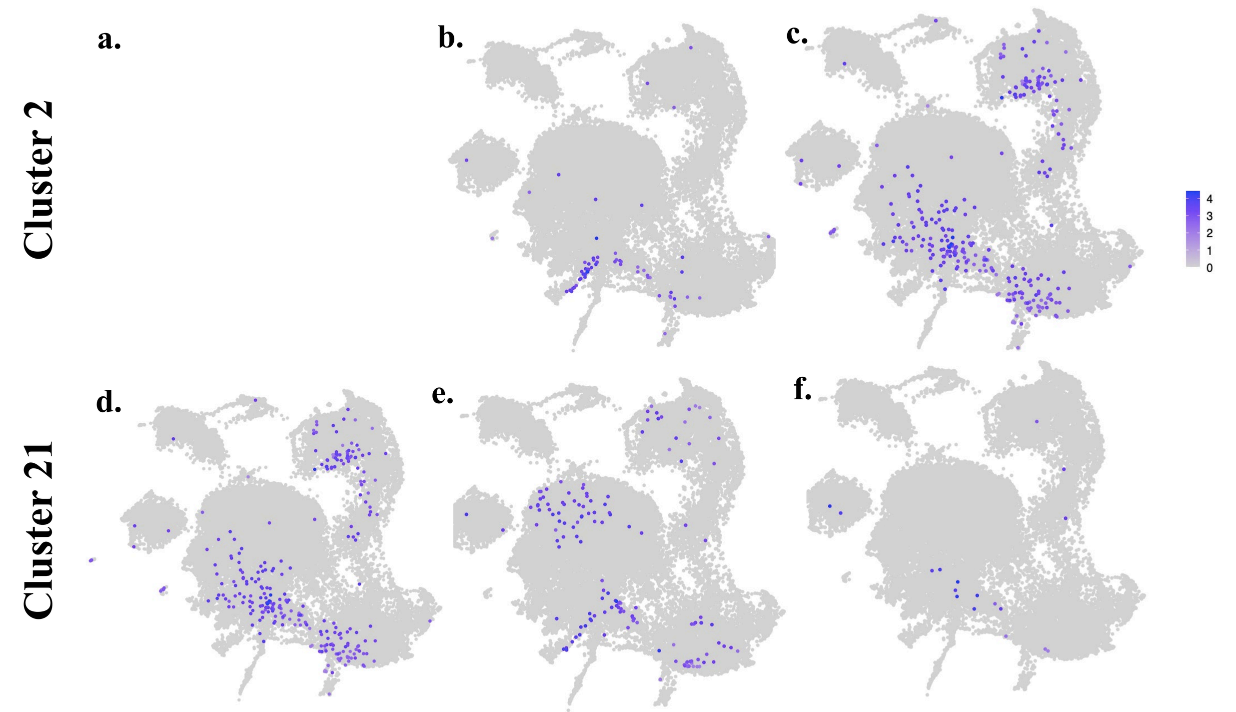

Figure 2. A sample of heatmaps showing the expression patterns of genes thought to be important for meristem cell development. The plots show significant upregulation of a gene in **a,b,c.** cluster 2, and **d,e,f.** cluster 21, respectively. In total, cluster 2 had 6 known genes upregulated, and cluster 21 had 5.

Table 1. List of new genes potentially associated with stem cells

Gene Name	Log2 Fold-Change	Known Function
Zm00001eb408900	6.5	sugar transmembrane transporter activity
Zm00001eb384670	6.1	N/A
Zm00001eb185580	5.9	N/A
Zm00001eb355030	5.6	auxin-activated signaling pathway
Zm00001eb097070	5.3	GTPase activity
Zm00001eb191600	5.3	carbohydrate binding
Zm00001eb022860	5.2	nucleic acid binding
Zm00001eb042190	4.8	integral component of membrane
Zm00001eb144010	4.8	auxin-activated signaling pathway
Zm00001eb287920	4.6	hydrolase activity

The top 10 genes showing upregulation in cluster 2. Log2 fold-change is a comparison between the gene's expression in cluster 2 vs the aggregate of all other clusters.

Discussion

- Cluster 2 showing a high amount of expression of these known genes means it may be of interest to investigate
  - These genes would still need testing to evaluate if they are marker genes
- We identified the top 10 genes that were highly expressed in cluster 2 compared to the rest of the clusters
  - These genes would still need functional testing
- If we find genes important for cell undifferentiation, we could utilize those genes to convert any cell type to a meristem cell
  - Future applications could use the gene identified from this study in genome editing, to convert any types of edited cells to meristem cells for whole-plant development.

Future Directions

- Confirming functions of the identified genes through functional validation
- Understanding and identifying marker genes that correspond to meristem cells
  - Further studies should confirm which genes can be considered marker genes
- Using a different scRNA-seq dataset to inspect if other datasets produce similar or different results
  - Potentially sample other types of tissues (not developing maize ear)

References

Xu X, et al. Single-cell RNA sequencing of developing maize ears facilitates functional analysis and trait candidate gene discovery. Dev Cell. 2021 Feb 22;56(4):557-568.e6.

Acknowledgements

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