PscB: A Browser to Explore Plant Single Cell RNA-Sequencing Datasets

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One Sentence Summary:
The Plant Single Cell RNA-Sequencing Browser, with its comprehensive visualization tools, provides a critical resource to easily explore the intricate expression information inherent in scRNA-Seq data.

Dear Editor,

High-throughput single cell RNA-Sequencing (scRNA-Seq) provides unprecedented power for understanding gene expression within complex tissues. The development of droplet-based technologies (Macosko et al., 2015; Klein et al., 2015; Zheng et al., 2017), and the incomparable depth of data it affords, means scRNA-Seq has become a fundamental method for transcriptomic analysis in metazoan studies. With the first works applying this technology to plant tissues recently published (Denyer et al., 2019; Jean-Baptiste et al., 2019; Ryu et al., 2019; Shulse et al., 2019; Zhang et al., 2019), this is also, rapidly, becoming the case in plants. In anticipation of the proliferation of plant-cell datasets, a central browser with tailored visualization and analysis tools is required for biologists to easily
investigate scRNA-Seq data at the exceptional resolution it offers. The few current available
browsers present scRNA-Seq data in formats that describe expression in broad terms of cell
identity and cursory developmental stage but fail to fully embrace the unique and complex
spatiotemporal expression information inherent (Supplemental Table 1).

To address this need, we here present a web-based graphical user interface for easy but
comprehensive interrogation of plant scRNA-Seq data (https://www.zmbp-resources.uni-
tuebingen.de/timmermans/plant-single-cell-browser). The browser was built using the shiny
framework (Chang et al., 2018), which translates user-driven events, such as gene entries
and button clicks, into R reactive objects to explore scRNA-Seq data using the Seurat and
Monocle packages (Satija et al., 2015; Trapnell et al., 2014), and displays results as dynamic
web content. Detailed expression data for individual genes or gene lists can be visualised
and downloaded as (i) t-SNE plots, showing expression profiles across the cluster cloud (Fig.
1A-F), (ii) violin plots describing the distribution of expression values of cells within a given
cluster (Fig. 1G), and (iii) tables summarizing quantitative information on gene expression
across clusters (Fig. 1H). In addition, the highly resolved patterns of gene expression across
pseudotime-derived developmental trajectories can be visualized as scatterplot graphs and
heatmaps (Fig. 2). The browser also provides links to archival databases and relevant
publications, and includes a detailed customized tutorial to aid data interpretation beyond
the cluster level.

With our recent scRNA-Seq dataset as a starting point (Denyer et al., 2019), the browser
facilitates the intuitive assessment of gene expression across all cell types and
developmental stages of the Arabidopsis root. The nearly five thousand cells profiled
manifest in a cluster arrangement that captures the developmental dynamic of the root
both with respect to developmental time and the spatial relationships between cell and
tissue types. The cluster map shows that cells of the niche and meristem form a central core
from which cells of different identities radiate in contrasting directions (Fig. 1F; Denyer et
al., 2019). This map provides an immediate detailed view of a gene’s pattern of expression
displayed in t-SNE and violin plots (Fig. 1D, G). Further, downloadable tables summarize
mean expression per cell both as raw and Seurat-normalized expression values (Satija et al.,
2015), as well as the percentage of cells expressing a given gene in each cluster, offering
quantitative information for gene expression comparisons between clusters (Fig. 1H).
ScRNA-Seq data, however, captures spatiotemporal expression information at a resolution far beyond the clusters. Accordingly, many genes show expression in just a subset of cells within a cluster, often reflecting a more intricate distinction in the fate, state, or differentiation of cells (Fig. 1D). For instance, genes expressed in meristematic cells of a given tissue type mark a specific region in the meristem clusters positioned adjacent to their mature counterpart (Fig. 1D; AT3G22120). Likewise, cells at a given phase of the cell cycle co-localize to define subdomains within the meristem clusters. Accompanying the Browser’s visualization tools is a comprehensive tutorial that provides guidance in understanding the intricate distinctions of expression profiles localized to subdomains within cell clusters.

ScRNA-Seq data are particularly powerful in resolving progressions in gene expression connected to developmental time. Pseudotime analysis on our root dataset, using the Monocle2 software (Trapnell et al., 2014), reveals cells of progressive maturity spanning out from the central niche and meristem core. A “temporal” reference map aids in discerning differences in expression connected to differentiation (Fig. 2A, 1D; AT4G13390). Moreover, the browser provides a searchable format to interrogate gene expression across individual developmental trajectories. For example, the stem cell-to-mature trichoblast, atrichoblast, or cortex cell trajectories reveal waves of gene expression guiding development of these cell types that, based on evidence from reporter lines, are highly predictive of relative expression along the developing cell files (Denyer et al., 2019). Upon a query, heatmaps will display the expression dynamics of relevant genes along a trajectory (Fig. 2B). These are accompanied by a heatmap displaying all genes dynamically expressed across pseudotime, for simple comparison. Alongside these heatmaps, scatterplot graphs are produced depicting expression dynamics for genes of interest along pseudotime at individual-cell resolution (Fig. 2C). Together, this information can be used to assess the precise temporal gene expression changes cells undergo during the transition from stem cell through differentiation.

Several challenges, such as cell size and osmotic pressure sensitivities, are concerns for scRNA-Seq applications to plant tissues. However, perhaps the most prominent concern for plant biologists approaching scRNA-Seq is the process of producing viable protoplasts, and the downstream transcriptional effects of this ‘protoplasting’ procedure. The scRNA-seq browser uniquely presents the data in two formats: one where genes induced during the
protoplasting procedure are removed, and one where they are not. While inclusion of genes induced by the procedure appears to have little effect on clustering, applications such as marker selection, pseudotime analysis, and gene-network development from developmental trajectories, appear heavily influenced.

In summary, to address the obvious need for data accessibility tools that can navigate the numerous, complex scRNA-Seq datasets ahead, we developed a curated Plant Single Cell RNA-Sequencing Browser that allows biologists to quickly and easily explore gene expression at a variety of desired depths. Besides the immediate view of a gene’s pattern of expression obtained from t-SNE and violin plots, the Browser offers the unique opportunity to (i) gain quantitative information on gene expression across clusters, (ii) visualize highly resolved patterns of expression along pseudotime-derived trajectories, and (iii) discern the intricacies of spatiotemporal gene expression information captured within clusters. Additional distinguishing features of this Browser are listed in Supplemental Table 1.

To best serve the community, we invite colleagues to utilize this platform to make their scRNA-Seq data publicly available in this highly interpretable format. To do so, please contact the corresponding author. As each scRNA-Seq atlas, particularly those from tissues other than the root, will contain new intricacies in their cluster arrangement, customized detailed tutorials, such as the one accompanying the current root dataset, are a necessity for comprehensive appreciation of the full data inherent. The browser can be modified and updated to incorporate future analysis and visualization tools. The Plant Single Cell RNA-Sequencing Browser will thus provide an up-to-date and rich centralized resource for plant research, with the interpretative guidance required for properly deciphering this rich data format.

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SUPPLEMENTAL MATERIALS

Supplemental Table 1. Plant single cell RNA-Seq Browsers

FIGURE LEGENDS

Figure 1. The scRNA-Seq Browser interface. (A) Individual genes (≤ 4 genes) can be queried. (B) Alternately, a gene list (≤ 20 genes) can be uploaded. (C) Datasets that include or exclude genes induced by the protoplasting procedure can be queried. (D) For genes detected in a dataset, t-SNE plots are displayed showing expression across the clusters of cells. This can be localized to a defined subset of cells within a cluster. (E) t-SNEs are downloadable as a .png file. (F) Basic cluster maps aid with quick initial interpretation. (G) Gene expression values across clusters can be visualized as violin plots depicting the distribution of cells with a given expression value in each cluster. y-axis - gene expression level; x-axis - proportion of cells showing a given expression value. (H) In addition, histograms showing mean expression per cell for a given gene across clusters, either as raw UMI counts or as Seurat-Normalized-data (not shown here), and percentages of cells expressing, are displayed. Violin plots are downloadable as .png files, expression data as comma-separated files. C - cluster.

Figure 2. ScRNA-Seq resolves gene expression connected to developmental time. (A) Temporal reference map derived from pseudotime analysis of all cells aids in linking expression to stages of differentiation. (B,C) Gene expression along individual developmental trajectories provides additional information on temporal expression dynamics associated with differentiation. (B) An expression heatmap of selected genes dynamically expressed across a pseudotime trajectory is produced. The heatmap is oriented with expression in meristematic cells to the left and in mature cells to the right. (C) Scatterplots depicting expression dynamics for genes at individual cell resolution along pseudotime are simultaneously produced. Heatmaps and scatterplot graphs are downloadable as .png files.

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