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COMMENT

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Single-cell RNA sequencing opens a new era for cotton genomic research and gene functional analysis

PAN Xiaoping[1], PENG Renhai[2] and ZHANG Baohong[1*]

Abstract

Single-cell RNA sequencing (scRNA-seq) is one of the most advanced sequencing technologies for studying transcriptome landscape at the single-cell revolution. It provides numerous advantages over traditional RNA-seq. Since it was first used to profile single-cell transcriptome in plants in 2019, it has been extensively employed to perform different research in plants. Recently, scRNA-seq was also quickly adopted by the cotton research community to solve lots of scientific questions which have been never solved. In this comment, we highlighted the significant progress in employing scRNA-seq to cotton genetic and genomic study and its future potential applications.

Keywords Cotton, Single-cell RNA sequencing, Transcriptome

Introduction

With the quick development of next generation sequencing technology, including RNA sequencing (RNA-seq), in the past two decades, great progress has been made in the cotton genome and transcriptome landscape (Peng et al., 2021; Wen et al., 2023). The expression profiles have been sequenced in different tissue, organs, and cells under different conditions, which enhance our understanding of individual genes, including both protein-coding and non-coding genes (such as microRNAs), in cotton growth and development as well as response to different environmental stresses, which has fueled lots of discovery and innovation in cotton gene functional study in recent years (Peng et al., 2021; Wen et al., 2023). However, as we know, traditional RNA-seq generally performes gene expression on samples with mixed cell populations that contents at least thousands of different cells, this limited and even hindered us from understanding the functions of an individual gene in cell-type heterogeneity of biological samples at a single cell revolution level. The quick development of single-cell RNA sequencing technology revolutionized gene expression profiling analysis. Since it was first employed to profile the transcriptome in different *Arabidopsis* root cells and others in 2019 (Denyer et al., 2019; Horvath et al., 2019; Jean-Baptiste et al., 2019; Nelms et al., 2019; Ryu et al., 2019; Shulse et al., 2019; Zhang et al., 2019), scRNA-seq has been attracting more and more attention from both scientific and industrial communities, and the number of publications on scRNA-seq has been dramatically increased. Right now, the studied plant species not only include model plant species, such as *Arabidopsis* and rice, but also many agriculturally important crops, such as corn (Satterlee et al., 2020; Xu et al., 2021), peanut (Liu et al., 2021; Deng et al., 2024), and tobacco (Feng et al., 2023). The investigated field includes plant tissue and organ development and response to various environmental factors, including plant nutrient deficiency.

*Correspondence:
Zhang Baohong
zhangb@ecu.edu
[1] Department of Biology, East Carolina University, Greenville, NC 27858, USA
[2] College of Biology and Food Engineering, Anyang Institute of Technology, Anyang, Henan 455000, China

Table 1 Cotton research using single-cell RNA sequencing

Year	Cotton species	Targeted tissues/organs/cells	Goal of research	References
2022	*G. hirsutum*	Cotton fiber	Fate determination control of an individual fiber cell initiation	(Qin et al., 2022)
2023	*G. hirsutum*	Cotton fiber	Cell-specific clock-controlled gene expression program regulates rhythmic fiber cell growth	(Wang et al., 2023)
2023	*G. bickii*	Cotyledon	Molecular mechanisms underlying gland morphogenesis	(Sun et al., 2023)
2023	*G. hirsutum*	Cotyledon	Molecular mechanisms underlying gland morphogenesis	(Long et al., 2023)
2023	*G. hirsutum*	Leaf	Study terpenoid biosynthesis	(Lin et al., 2023)
2023	*G. hirsutum*	Hypocotyl and callus	Mechanisms underlying somatic embryogenesis and plant regeneration	(Zhu et al., 2023)
2024	*G. hirsutum*	Nonembryogenic calli (NEC) and primary embryogenic calli (PEC) tissues	Mechanisms underlying somatic embryogenesis and plant regeneration	(Guo et al., 2024)
2024	*G. hirsutum*	Anther	Spatial expression atlas and regulatory dynamics during anther development, and anther response to high temperature	(Li et al., 2024b)
2024	*G. arboreum*	Root	Transcriptional landscape of cotton roots in response to salinity stress	(Li et al., 2024a)

scRNA-seq is also revolutionizing cotton transcriptome and gene functional analysis

Due to its great potential to distinguish transcriptomes in different types of cells even adjacent cells, scRNA-seq has also quickly attracted lots of attention from cotton research communities (Table 1). In 2022, Qin et al. (2022) reported, for the first time, scRNA-seq was employed to study gene expression in cotton. They identified a total of 14 535 cells from cotton ovule cells, and these cells were divided into three major cell types, including fiber cells. Furthermore, they found that several core transcription factors, such as MYB25-like and HOX3, play a key role in regulating cotton fiber differentiation and tip-biased diffuse growth (Qin et al., 2022). Since then, in only about one year, at least 8 papers have been published utilizing scRNA-seq to study different biological processes in cotton (Table 1). Due to the fiber is the major product of cotton, there are always interest in investigating the molecular mechanisms underlying cotton fiber initiation and development. Following the research performed by Qin et al. (2022), Wang et al. (2023) also employed scRNA-seq to investigate the cell-specific clock-controlled gene expression landscape regulating rhythmic fiber cell growth. Their study used a well known cotton fibreless (fuzzless/lintless) mutant Xuzhou142fl and its wiltype Xue 142. Through scRNA-seq analysis, they identified five cell populations and successfully constructed the development trajectory for cotton finer lineage cells (Wang et al., 2023). Based on their study, they also found the circadian rhythmic process may regulate the primary growth of fiber cells, in which both small peptide RALF1 and certain *cis*-regulatory elements (such as CRE and TCPs) may play an important role (Wang et al., 2023).

scRNA-seq was also employed to study gland development and metabolite biosynthesis. Gossypol and its associated pigment gland are important for cotton evolution, which plays an important role for cotton plants against certain insects and diseases. Sun et al. (2023) employed scRNA-seq to study molecular mechanisms underlying gland morphogenesis using a unique wild cotton species, *G. bickii*, whose seeds are glandless but there are glands in other tissues. They found that both light and plant hormone gibberellin promoted the formation of pigment glands and three new genes, including ETHYLENE RESPONSE FACTOR 114 (ERF114), ZINC FINGER OF ARABIDOPSIS THALIANA 11 (ZAT11), and NAC TRANSCRIPTION FACTOR-LIKE 9 (NTL9) affected gland formation (Sun et al., 2023). Long et al. (2023) also employed scRNA-seq to study the pigment gland morphogenesis, in which the authors used two near-isogenic lines (NIL) (Gland cotton cultivar CCRI 12 and glandless cotton cultivar CCRI12gl); based on their results, several transcription factors, including PGF, ERF12, MYB14, and JUB1, serve as regulators for pigment gland morphogenesis in cotton. Recently, Lin et al. (2023) identified a hierarchical transcriptional regulatory network for terpenoid biosynthesis in cotton secretory glandular cells; they also found that two transcription factors, HSF4a and NAC42, directly affected terpenoid biosynthesis by targeting the expression of terpenoid biosynthetic genes.

Both transgenic and genome editing have become powerful tools for studying gene function and crop improvement (Zhang et al., 2021). However, the advanced techniques are majorly based on plant tissue culture and regeneration. Currently, compared with other plant species, it is hard to obtain transgenic or genome-edited regenerated plants because of the long period of culture

from callus induction to plant regeneration and the genotype recalcitrance, only a few genotypes can be used for plant regeneration in cotton. Thus, studying the potential mechanisms underlying plant regeneration is extremely important. Recently, Zhu et al. (2023) employed scRNA-seq to compare the transcriptome profile between an easily regenerable cotton genotype Jin668, and the recalcitrant genotype, also cotton genetic standard, TM-1. Based on the single-cell levels of mRNA profiling, they identified nine putative cell clusters and 23 cluster-specific marker genes for these two different cotton genotypes with different regeneration capacities. Combining with transgenic and genome editing, they also found that certain genes, such as *PLT3*, *LOX3*, and *LAX1/2*, are involved in reprogramming the fate of cotton cells and further control plant regeneration in cotton (Zhu et al., 2023). Guo et al. (2024) also employed scRNA-seq to analyze the transcriptome atlas during somatic embryogenesis in cotton; based on their scRNA-seq results, cotton calli were partitioned into four broad populations with six distinct cell clusters.

scRNA-seq was also used to study the transcriptome atlas and specific genes during cotton anther development, and plant response to environmental stress (Li et al., 2024a; 2024b). scRNA-seq shows that both high temperature and salinity stress induced certain gene expression aberrance in specific cell types in anther (Li et al., 2024b) and root (Li et al., 2024a), respectively (Table 1).

Summary and future prospects

Since scRNA-seq can be used to directly compare the gene expression profiling of an individual cell, it can assess transcriptional similarities and differences within a population of cells. Thus, it is possible for us for the first time to elucidate the cell heterogeneity and regulatory mechanism of each individual cell. Due to these unique features, scRNA-seq has been quickly adopted by the cotton research community to elucidate the molecular mechanism underlying many biological processes in cotton, such as fiber initiation and development, somatic embryogenesis, and response to different environmental stresses.

In scRNA-seq studies across various plant species, the initial and critical step of scRNA-seq is to isolate viable single cells from the targeted tissues. Because all plant cells have cell wall that not only provide support and protection to the cells but also allow the cells link together (Zhang et al., 2020), thus, for plant scRNA-seq, the first step generally is to isolate plant protoplast by using different cell wall breakdown methods and then followed by cell sorting using different methods, such as microfluidics. To achieve the high quality of cotton cell protoplast, there are a couple of explored methods for isolating protoplasts for cotton scRNA-seq (Liu et al., 2022; Zhang et al., 2023). Currently, most scRNA-seq studies in cotton employ protoplast isolation for harvesting single cell. However, enzymatic digestion-based protoplast preparation for scRNA-seq suffers one major weakness: no matter how good to isolate the protoplast, it needs a process to remove the cell walls which the process of protoplast preparation may be considered as cellular stress that will affect the gene expression levels. This suggests that some scRNA-seq results may pose false positives. Thus, better cell isolation methods may be considered to avoid these negative effects.

With the mature of scRNA-seq technology, more and more researchers will adopt this technique to explore new applications in cotton genomic research and crop improvement. Particularly as newly emerging technologies are created, such as the more advanced scRNA-seq, single cell stereo-sequencing technology (Stereo-seq) (Bawa et al., 2024), this technology will become more powerful for constructing spatially resolved developmental trajectories and the molecular regulatory mechanisms underlying cotton growth and development as well as yield and quality under different biotic and abiotic stresses. Combining scRNA-seq with the advanced clustered regularly interspaced short palindromic repeats (CRISPR) genome editing technology, the key genes controlling cotton fiber yield and quality as well as tolerance to stress will be identified. These findings will further be used for precision cotton molecular breed for creating new cultivars for sustainable cotton development.

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