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Mini-review

Deciphering plant cell–cell communications using single-cell omics data

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ABSTRACT

Plants have various cell types that respond to different environmental factors, and cell–cell communication is the fundamental process that controls these plant responses. The emergence of single-cell techniques provides opportunities to explore features unique to each cell type and construct a comprehensive cell–cell communication (CCC) network. Although the most current successes of CCC inference were achieved in animal research, computational methods can also be directly applied to plants. This review describes the current major models for cell–cell communication inference and summarizes the computational tools based on single-cell omics datasets. In addition, we discuss the limitations of plant cell–cell communication research and propose new directions to expand the field in meaningful ways.

1. Introduction

Plants consist of various cell types that form a complex cell–cell communication (CCC, also known as cell–cell interaction) network, which is crucial for responding to a dynamic environment [1]. To better understand biological processes in plants, it is necessary to study the mechanisms by which CCCs control environmental responses and explore the unique features and roles of each cell type. Previous studies have investigated plant CCCs using experimental methods, such as fluorescence microscopy and laser ablation, and microdevice-based methods, such as microwells, single-cell traps, and droplet microfluidics [2,3]. Many molecules, including small RNAs, reactive oxygen species, and novel peptides, act as mobile signals in plant CCCs [4–6]. However, the low throughput of these technologies, which only focus on two cell types and a few signal candidates, has hindered their broad application in plant CCC research. Recent advances in high-throughput single-cell sequencing, including single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq), have enabled the characterization of cellular composition and function at the single-cell level. Several studies have explored cell activities, differential trajectories in various plant tissues (e.g., root [7–15], leaf [16–23], stem [24], shoot apical meristem [25–28], ear [29], seedling [30], seed [31], xylem [32] and flower [33–35]), and plant responses to different environmental stresses (e.g., low-nitrogen/high-salinity/iron-deficiency [36], heat and sucrose deficiency [9,37]). The emergence of spatial transcriptome (ST) technologies, such as Slide-seq [38], DBiT-seq (deterministic barcoding in tissue for spatial omics sequencing) [39], the 10X Genomics Visium platform [40], and scStereo-seq (single-cell Stereo-seq) [41], has facilitated the understanding of spatial cell and gene expression features. Gene expression information provided by single-cell omics data has facilitated the exploration of large-scale intercellular communication in plants. For instance, using a public scRNA-seq dataset from *Arabidopsis* heat-shocked roots, Xu et al. found that the AT1G28290–AT2G14890 ligand-receptor pair may play important roles in atrichoblast–cortex cell communication [42]. In addition, they found that genes downstream of the AT1G28290–AT2G14890 pair were enriched in the ribosome pathway, which provided new clues about the mechanism of plant response to heat stress. However, the majority of the currently published plant single-cell studies did not include CCC analysis.

Based on single-cell omics data, it is possible to infer plant CCCs using various bioinformatics and computational methods [42]. There are two widely accepted strategies in animal research [43]: ligand-receptor (LR) signal based mode and physical location based mode (Fig. 1). In this review, we outline various computational methods and tools for CCC inference and discuss the limitations and future perspectives of single-cell omics techniques in deciphering plant CCCs.

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2. LR signal-based mode to infer CCCs

In the LR signal-based mode, individual cells are first clustered based on their gene expression patterns, and cell types are assigned to different clusters based on known marker genes or golden reference datasets [44]. The intercellular interactions between the source and target clusters are explored. These interactions are normally achieved by a "sender" protein from the source cluster and a "receiver" protein from the target cluster, which are referred to as "ligand" and "receptor", respectively [45]. If the ligand and receptor are from the same cell/cluster, the interaction is autocrine. In contrast, if the ligand and receptor belong to different clusters, the interaction is paracrine [45]. Therefore, to accurately identify CCCs, a precise LR database must be created. LR information is typically extracted from various sources (Table 1). Most of the current resources for LRs are based on human or other animal models, which may be the reason for the lack of CCC analysis in plant research. To date, ScTensor [46] and PlantPhoneDB [42] are the only two databases to contain plant LR information (Table 1). ScTensor only collects *Arabidopsis* LR information extracted from protein-protein interaction in the STRING database with a combined confidence score over 400 for usage. The ligand and receptor candidates are retrieved from the SWISSPROT database and TrEMBL database. A total of 8697 and 94 *Arabidopsis* LR pairs were obtained by SWISSPROT and TrEMBL annotation in ScTensor. The current PlantPhoneDB contains 3514 unique LR pairs for *Arabidopsis*, which are curated from seven resources, including STRING, text-mining from the literature [42]. Compared with *Arabidopsis* in ScTensor, PlantPhoneDB contains 2727 unique LR pairs. Moreover, it also stores LR information for the other four plant species (e.g., maize, rice, poplar, and tomato), which are retrieved by orthologs with *Arabidopsis* using InParanoid [47].

Once sufficient LR information is available, a suitable method to measure CCCs should be determined. This process is usually performed in three major steps: score each LR pair based on expression patterns, aggregate LR scores from different cell types, and compute the significance of the CCC score. By systematically comparing 16 CCC inference resources and seven scoring methods, Dimitrov et al. found that both resources and methods have a considerable impact on CCC predictions [48]. However, Xu et al. attempted to use four different scoring methods to infer CCCs from a scRNA-seq dataset and found that the four scoring methods identified almost the same top-communicating CCCs [42]. Two methods, SingleCellSignalR and CellPhoneDB, were both evaluated by Dimitrov et al. and Xu et al., but different conclusions were achieved. Therefore, users should carefully consider different scoring functions and choose the most suitable methods for their dataset. Numerous computational tools based on the LR mode have been developed to infer CCCs for both individual cells and cell clusters, but they have mainly focused on humans and animals (Table 1). Erick et al. reviewed and grouped most tools into four categories according to the mathematical models used: differential combination-, network-, expression permutation-, and tensor-based tools [49]. We compared the different methods based on these criteria and their unique features. Most methods have tried to predict CCCs between different cell clusters, while SoptSC was able to infer individual cell interactions [50]. iTALK [51] and PyMINEr [52] first attempted to identify the differentially expressed genes between cell clusters and used them as candidates for final LR pair interactions. CellPhoneDB [53], CellChat [54], ICELLNET [55], CellTalkDB [56], Celllinker [57], CellCall [58], NATMI [59] and SingleCellSignalR [60] are expression permutation-based tools that calculate the interaction score for each LR pair and evaluate their significance via cluster label permutation, nonparametric tests, or empirical methods. Notably, CellPhoneDB, CellChat, and ICELLNET consider multisubunit complexes for ligands and receptors. In addition, CellChat integrates other important signaling cofactors, including soluble agonists and antagonists. Other methods, including NicheNet [61], SoptSC [50], CCCExplorer [62], and SpaOTsc [63], have been used to investigate the features of the connections between genes as a network. Intracellular gene–gene interactions were considered in the receiver cells of NicheNet and SoptSC. CCCExplorer considers crosstalk signaling pathways as a directed and connected network from LR interactions to transcription factors (TFs) and their target genes. Optimal transport is used in SpaOTsc to hypothesize intercellular communication. In contrast to these methods, which focus on pairwise analysis between different cell clusters, scTensor explicitly models LR interactions using a tensor decomposition involving multiple cell clusters. Two recent open-source tools, LIANA [48] and PlantPhoneDB [42], were developed to facilitate the incorporation of different methods and resources. In addition, some downstream analyses based on cell–cell interactions, including pathway or Gene Ontology enrichment and TF or target gene enrichment analyses, are conducted using multiple tools to determine the significant LR pairs (Table 1). Although these methods have been developed primarily

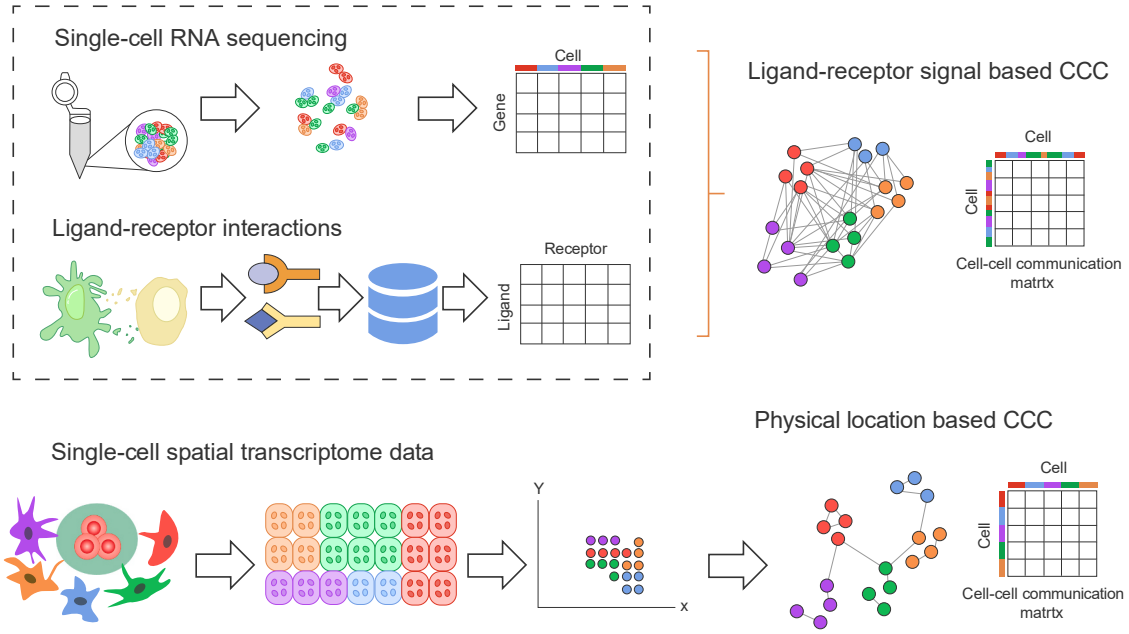

Fig. 1. Two modes used to infer plant cell-cell communications.

Table 1
Comparison between methods developed to infer cell-cell interaction using single-cell omics data.

Mode	Tool [Ref]	Species for LR	Detail of LR Resource	Visualization	Downstream Analysis	Method Overview
Ligand-Receptor Signal based	**Expression Permutation**					
	CellChat [54]	Human, Mouse	1939 and 2021 LRs for human and mouse, supporting multi-subunit complexes and cofactors	Circle plot, Bubble plot, Sankeyl plot, heatmap	Pathway enrichment	Score probabilities were calculated using law of mass action
	CellPhoneDB [53]	Human	1396 LRs for human, supporting multi-subunit complexes	Circle plot, Bubble plot, heatmap	NA	Randomly permute cluster labels to generate null distribution of LR scores to identify significant interactions
	SingleCellSignalR [60]	Human	3251 LRs for human	Circle plot, Bubble plot, heatmap	Pathway enrichment	Score probabilities were calculated using a nonlinear function of the product of LR expressions
	ICELLNET [55]	Human	380 LRs for human, supporting multi-subunit complexes	Circle plot, Bubble plot	NA	Interaction scores were calculated by multiplying the geometric means of ligand and receptor expressions
	CellTalkDB [56]	Human, Mouse	3398 and 2033 LRs for human and mouse, supporting multi-subunit complexes	Circle plot	NA	Use score functions from SingleCellSignalR
	Celllinker [57]	Human, Mouse	3700 and 3200 LRs for human and mouse	Bubble plot	NA	Significant interactions were calculated by permuting cell labels
	CellCall [58]	Human, Mouse	19,144 and 12,069 LRs-TFs for human and mouse, supporting multi-subunit complexes	Circle plot, Bubble plot, Sankeyl plot, heatmap	Pathway enrichment; TF enrichment	CCC scores are calculated by integrating the norm of LR interaction and score of downstream TFs
	NATMI [59]	Human	2293 LRs for human	Circle plot, Bubble plot, heatmap	NA	Interaction scores were calculated by the product of normalized LR expressions
	Network based					
	NicheNet [61]	Human	12,019 LRs for human, supporting cofactors	Circle plot, Sankeyl plot	TF and target gene analysis	LR links were predicted by combining their expression data with prior knowledge on signaling and gene regulatory networks
	CCCExplorer [62]	Human	1433 LRs for human	NA	TF and target gene analysis	Develop a computational model for crosstalk signaling discovery based on ligand-receptor interactions and downstream signaling networks
	SoptSC [50]	Human	1288 LRs for human	Circle plot	NA	Integrate downstream signals into LR score function
	SpaOTsc [63]	NA	LRs from Ramilowski et al., [75]	Circle plot, heatmap	NA	An optimal transport was used to infer cell interactions between different clusters. Also support physical location based inference
	Differential Combination					
	iTALK [51]	Human	2648 LRs for human	Circle plot	NA	Scores are calculated by differentially expressed LRs
	PyMINEr [52]	Human	52,612 LRs for human	Circle plot	Pathway enrichment	Enriched interactions are calculated by a Gaussian null distribution between cell clusters
	Tensor based					
	ScTensor [46]	*Arabidopsis*, 11 animals	12 species (21,882 [SWISSPROT]/472[TrEMBL] LRs for human, 8697/94 LRs for *Arabidopsis*)	NA	Pathway/GO enrichment	Tucker decomposition on a tensor of order three to identify key LRs in certain cell types
	Combination					
	LIANA [48]	NA	NA	NA	NA	7 methods from other tools
	PlantPhoneDB [42]	*Arabidopsis*, rice, tomato, maize, poplar	3514, 3762, 1751, 2823, 3110 LRs for *Arabidopsis*, rice, tomato, maize, poplar	Circle plot, heatmap	NA	Provide four scoring approaches to calculate interaction scores
Physical Location based	Cell2Cell [66]	Human	2005 LRs for human, supporting multi-subunit complexes	Circle plot	Pathway enrichment	Infer communication distance using Gaussian mixture model
	Giotto [67]	NA	NA	Circle plot, Bubble plot, heatmap	NA	ST data was used to filter interactions between cells
	stLearn [69]	NA	NA	Circle plot, heatmap	GO enrichment	Significant LR pairs were determined using CellPhoneDB based on normalized gene expression across spatial location
	SVCA [70]	NA	NA	NA	NA	Model gene expression as a function of intrinsic cell state effects, environmental effects and cell-cell interactions
	MISTy [68]	NA	NA	NA	NA	Interactions are calculated by weighting the gene expressions of local cell neighborhood
	DeepLinc [71]	NA	NA	NA	NA	Use a variational graph autoencoder with an adversarial network for regularization to infer cell interactions

for humans and other animal models, they can also be directly applied to plant datasets. For instance, PlantPhoneDB provides four different scoring methods and LIANA allows users to select any combination of resources and methods.

3. Physical location-based mode to infer CCCs

Generally, cells can only interact with each other in a limited space, which is missed in scRNA-seq or snRNA-seq data [49]. Some studies have attempted to de novo map scRNA-seq transcriptomes for a computational spatial representation of the studied organ [34,64,65]. The studies utilizing these methods tried to place single cells in space based on different assumptions. In some studies, it was assumed that cells with similar expression patterns were regarded as nearby [65], and other studies considered colocalized cells should have coexpressed ligands and receptors [64]. To reduce the false-negatives of CCC inference, it is crucial to incorporate the spatial location of mediators within the cells. This limitation is improved by the use of single-cell ST technologies. Most recent methods, such as Cell2Cell [66], Giotto [67], MISTY [68], SpaOTsc [63], stLearn [69] and SVCA [70], integrate scRNA-seq and additional intercellular distance information provided by ST. Commonly, these methods attempt to estimate similarity between single cells based on overlapping genes, and the similarity will be improved using ST information. Although CCC prediction is empowered by integrating scRNA-seq and ST, many methods have tried to use ST data directly to analyze CCC. All the above six tools could achieve this goal. Another tool, DeepLinc, attempts to reconstruct cell interaction networks de novo from ST data alone on the basis of a deep generative model of variational graph autoencoder (VGAE) [71]. Unfortunately, CCC analysis is still not prioritized in current ST studies in plants [41,72,73].

ScRNA-seq offers a means of precisely quantifying the state and trajectory/pseudotime of individual cells and thus may enable the construction of explicit, genome-scale dynamic cellular models [74]. Similarly, CCCs are also a temporal process during the life cycle of cells. However, there is still no method that considers this dynamic spatiotemporal aspect using ST data. With the improvement of the resolution of ST techniques, it will be possible to explore spatiotemporal CCC based on trajectories constructed by ST information.

4. Limitations and future perspectives

Despite the fact that CCC analyses are widely conducted in animals and humans, their application in plants is still rare. A major reason for this may be the rarity of single-cell-related studies in plants because of the difficulties in protoplast isolation and preparation due to the presence of cell walls [1]. With the improvement and increase in scRNA-seq and ST datasets in plants, it will be interesting to explore the activities of CCCs in different plants. Most of the current methods and tools can be directly applied to plant single-cell omics datasets. However, there are still several limitations to consider when investigating plant CCCs. Until now, ScTensor and PlantPhoneDB have only collected or curated LR pairs for *Arabidopsis*, and LR information for other plant species has been retrieved computationally by the InParanoid algorithm in PlantPhoneDB. Therefore, curated and precise LR information is required for other model plant species. Similar to CellChat, multisubunit complexes and other important signaling cofactors should be considered for plant LR pairs. Moreover, benchmarks or golden datasets must be established in plants so that researchers can compare different methods and choose the most appropriate one. The preparation of a real golden CCC network is challenging. Since plant cells are confined to their relative positions by cell walls, spatially adjacent cell types may have strong CCC. Compared with human and animal analysis, ST data is a good choice for parallel validation in plants. Most current CCC studies only focus on individual species/tissues; therefore, it will be interesting to compare the networks between tissues and species in the future.

As considerable efforts have been made to develop various CCC methods and tools, we can expect more novel insights into plant CCCs at the single-cell level in the future. Due to the increase in the number of single-cell atlases for different plants, scRNA-seq, ST, and single-cell proteomics and metabolomics can be used to expand the field of plant CCC research.

CRediT authorship contribution statement

Jingjing JIN: Resources, Methodology, Writing- Original draft preparation. **Shizhou Yu**: Methodology, Writing- Original draft preparation. **Peng Lu**: Resources, Methodology. **Peijian Cao**: Resources, Writing- Reviewing and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

JJJ and SHY did literature research. All authors contributed to discussions of the content, reviewed and/or edited the manuscript.

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