Rice Genomics and Genetics 2025, Vol.16, No.4, 199-210
http://cropscipublisher.com/index.php/rgg

Research Insight Open Access

Cell-type Specific Gene Regulatory Networks during Rice Grain Filling Revealed by scRNA-seq

Yanfu Wang, Danyan Ding
Institute of Life Sciences, Jiyang College of Zhejiang A&F University, Zhuji, 311800, Zhejiang, China
Corresponding email: danyan.ding@jicat.org

Rice Genomics and Genetics, 2025, Vol.16, No.4 doi: 10.5376/rgg.2025.16.0017
Received: 20 May, 2025
Accepted: 02 Jul., 2025
Published: 20 Jul., 2025

Preferred citation for this article:
Wang Y.F., and Ding D.Y., 2025, Cell-type specific gene regulatory networks during rice grain filling revealed by scRNA-seq, Rice Genomics and Genetics, 16(4): 199-210 (doi: 10.5376/rgg.2025.16.0017)

Abstract Rice grain filling is a critical determinant of yield and quality, relying on tightly coordinated gene regulation across diverse cell types. In this study, we applied single-cell RNA sequencing (scRNA-seq) to dissect the transcriptomic landscape of individual cell populations within developing rice grains, enabling the construction of cell-type specific gene regulatory networks (GRNs). We identified and classified distinct cell types involved in grain filling, characterized their unique gene expression patterns, and traced dynamic transcriptional changes across developmental stages. Through GRN inference, we uncovered key transcription factors and regulatory hubs governing starch biosynthesis, nutrient transport, protein accumulation, and stress responses, as well as their integration with hormonal and metabolic pathways. A focused case study on endosperm-specific starch biosynthesis revealed candidate regulators validated by transgenic and CRISPR-based approaches. Integration with spatial transcriptomics, proteomics, and metabolomics further reinforced the functional significance of these networks. These findings provide a high-resolution view of cell-type specific transcriptional regulation during rice grain filling, offering novel targets and strategies for genetic improvement of grain yield and quality.

Keywords Rice grain filling; Single-cell RNA sequencing; Gene regulatory networks; Cell-type specificity; Transcriptional regulation

1 Introduction

Grain filling in rice is a very crucial stage, directly affecting yield and quality. Rice is the staple food for more than half of the world's population. The efficiency of grain filling can affect the weight, size and nutritional components of grains, and thus has become an important goal in crop improvement and food security (Wang et al., 2008). The accumulation of starch accounts for the majority of the dry weight of rice, which is closely related to the grain-filling process. Therefore, to increase the yield and quality of rice, it is very important to optimize the grain-filling process (Xiao et al., 2025).

The process of grouting is rather complex, involving the transportation of carbohydrates and the synthesis of starch during the development of endosperm. The speed and completeness of grout filling will directly affect the final weight and quality of the grains. Poor grouting will lead to reduced yield and decreased quality (Peng et al., 2013; Wei et al., 2017). Environmental conditions, such as temperature and light, as well as gene regulation, all play important roles in this process (Chen et al., 2022).

The grains of rice have a variety of different cells, and each cell plays a different role in filling and development. Each cell type has its own gene regulatory network, which regulates processes such as cell division, cell expansion and nutrient accumulation, and ultimately determines the size and composition of the grain (Liu et al., 2022). Current research has found that transcription factors, micrornas and hormone signaling pathways can fine-tune gene expression in specific cells, which indicates that it is necessary to study regulatory mechanisms at the single-cell level (Panigrahi et al., 2021; Zhao et al., 2023).

The traditional RNA sequencing method involves testing many cells together, which can mask the differences among various cell types. Single-cell RNA sequencing (scRNA-seq) technology can analyze gene expression at

the level of each cell, helping us to clearly understand the transcriptional characteristics and regulatory networks of each type of cell. This technology can help us identify new grouting regulatory factors and cell-specific pathways, enabling us to better understand how gene regulation changes in time and space during grain development (Kim et al., 2017).

The main objective of this study is to investigate the gene regulatory networks of different cell types in rice grain filling using scRNA-seq technology. We plan to map the transcriptional profiles of each cell type, hoping to identify the important regulatory factors, signaling pathways and gene modules that affect grouting and quality formation. These results will provide useful references for improving rice yield and quality in the future, and may also help us find new breeding ideas in terms of stress resistance and grain trait improvement.

2 Overview of Rice Grain Filling Process: Stages, Cell Types, and Regulation

2.1 Stages of grain filling and key physiological changes

Grouting is not accomplished in an instant; rather, it starts from fertilization and progresses bit by bit. The process can roughly be divided into three stages: the early stage involves rapid cell division and endosperm expansion; the middle stage mainly involves the accumulation of a large amount of nutrients such as starch; and finally, it enters the stage of maturation and water reduction (Durbha et al., 2024). Of course, there are no clear boundaries between these stages, and they often overlap. The most obvious change in the early stage was the rapid proliferation of endosperm cells, which then began to convert sucrose into starch - the main storage substance in the grains. Meanwhile, the carbon in the leaves and stems is also re-mobilized to become the raw material needed for grout filling. At this time, the enzymes related to starch synthesis become active, and the metabolic direction shifts from maintaining life to reserve accumulation (Wang et al., 2021). However, these rhythms are not fixed. Changes in environmental conditions such as temperature and light can directly affect the speed and duration of grout filling, thereby influencing yield and rice quality (Shimoyanagi et al., 2021).

2.2 Major cell types involved in grain filling and their functions

There is not just one type of cell in a rice grain. The endosperm, embryo, seed coat and pericarp each have their own functions (Liu et al., 2025). The endosperm is the most important storage organ, occupying the majority of the space in the grain, where starch and protein accumulate. The path for transporting sucrose from the maternal tissue to the endosperm is not simple either, as it has to pass through multiple "channels" such as the parenchyma cells of the vascular vessels, the tubercle process, and the tubercle epidermis. Among them, sugar transporters like OsSWEET11 and OsSWEET14 play a key role (Fei et al., 2021). In addition, the peel and aleurone layer are also involved in material transfer and are not merely "shells". They also play a role in signal regulation during development (Ren et al., 2021).

2.3 Hormonal and metabolic regulation during grain filling

The role of hormones in the grouting process is hard to ignore. Cytokinin, auxin, abscisic acid (ABA), gibberellin and ethylene were all major participants (Figure 1) (Panda et al., 2018). They regulate not only cell division, but also the expression of genes related to starch synthesis and the "reservoir strength" of grains. For instance, cytokinin can promote the proliferation of endosperm cells and also facilitate the "filling" of grains. ABA accelerates grouting under adverse conditions and is a typical coping hormone (Yang et al., 2001). The concentration and duration of action of these hormones must be properly coordinated so that there will be no problems with grouting. There are also key links in metabolism. For instance, the process of converting sucrose into starch relies on several important enzymes, such as ADP-glucose pyrophosphorylase and starch synthase. The activities of these enzymes are influenced by both genes and the environment (Zhang et al., 2019), so the entire process is actually very subtle. It is easy to have problems, but it is very difficult to achieve coordination.

3 Principles and Advantages of scRNA-seq in Plant Research

3.1 Overview of scRNA-seq workflow and technical considerations

In plant research, the scRNA-seq technology has indeed changed the way people view differences in cell expression. It can perform transcriptional analysis at the single-cell level. Cell differences that were previously "averaged out" in mixed samples can now be seen separately (Bawa et al., 2022).

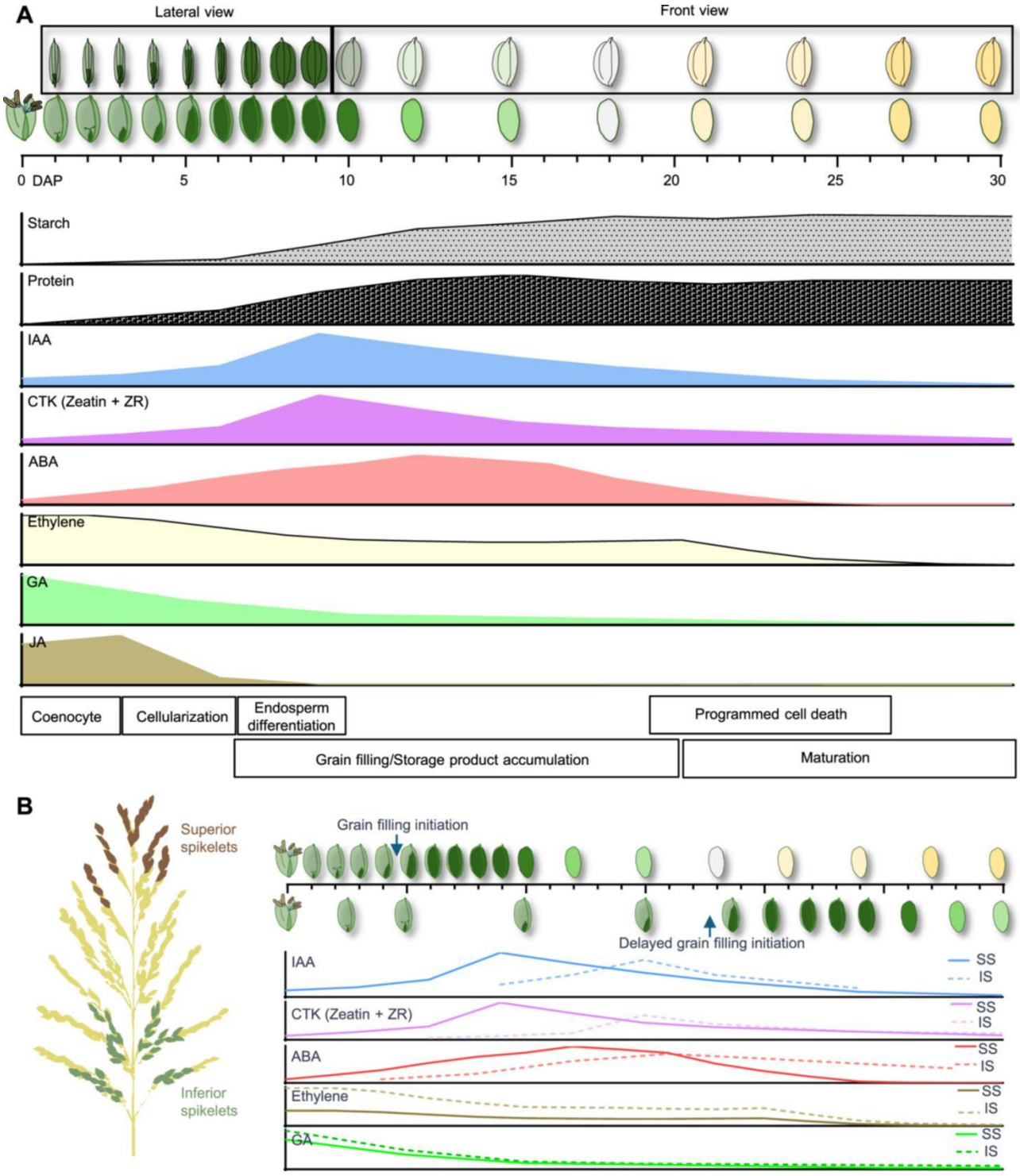

Figure 1 Schematic overview of grain development in rice (Adopted from Liu et al., 2025)

Image caption: (A) The relative volume of caryopsis within the grain and morphological event is presented throughout the developmental process of rice grain. Total starch and protein content in grain (relative to maximum volume) is shown in parallel with the time course (days after pollination (DAP)). (B) Timeline diagram of developmental differences and hormone level changes between superior and inferior grains. The solid lines represent superior spikelet grains (SS), and the dashed lines represent inferior spikelet grains (IS), illustrating the developmental differences and changes in hormone levels over time (Adopted from Liu et al., 2025)

However, the initial steps of doing scRNA-seq on plants are actually not simple. For example, to isolate living single cells or cell nuclei from tissues, this step often requires enzymes for processing because the cell walls of

plants are too hard, or they are directly made into protoplasts. However, this may cause stress and even affect the accuracy of the experiment (Sun et al., 2024). The subsequent process includes RNA extraction, reverse transcription, amplification, library construction, and then high-throughput sequencing. The final step is to classify the cells using computational methods, identify their respective marker genes, and attempt to restore the regulatory networks among them. Sometimes, if the samples are difficult to handle, researchers will also choose single-core RNA sequencing (snRNA-seq) as an alternative. It can avoid some deviations caused by cell disruption and can also handle frozen tissues (Wang et al., 2022). Of course, no matter which method is used, the quality of RNA and the control of experimental details will directly affect the final analysis result.

3.2 Advantages over bulk RNA-seq in resolving cell-type specific expression

Traditional bulk RNA-seq often mixes a bunch of cells together for measurement, and the result is an average value. In this way, rare cells or the subtle differences between cells can be easily "smoothed out". Unlike scRNA-seq, it can observe the expression of each cell separately (Denyer et al., 2019). This enables us to identify some newly discovered cell types or observe the transformation trajectories of certain cells during their development. For example, when studying plant development or environmental response, the spatial distribution and regulatory patterns of cell expression are crucial (Shaw et al., 2020). Nowadays, many plants, especially model plants and crops, have established their own single-cell atlases.

3.3 Challenges in applying scRNA-seq to plant tissues

However, when scRNA-seq is actually applied to plants, it is not without difficulties. The most common trouble is cell separation. Plant cells have thick cell walls, and some tissues are particularly complex. It is not easy to "disassemble" them into individual cells. Even if the isolation is successful, the RNA content of individual cells is very low and they are easily affected by noise. In addition, the possible stress response during the digestion of cells will also affect the data quality (Jovic et al., 2022). Furthermore, not all plants have complete reference maps, which also limits the depth of interpretation. Some people may have overlooked another issue: Batch effects and RNA degradation may cause bias, and at the same time, the requirements for analytical tools are also very high (Islam et al., 2024). However, over the years, the separation methods, database construction processes and data analysis techniques have all been continuously optimized. Although there are many challenges, these improvements are gradually expanding the application scope of this technology in plant research.

4 Cell-type Specific Transcriptomic Profiles during Rice Grain Filling

4.1 Identification and classification of distinct cell populations in the grain

It is now quite clear that there are actually many types of cells in rice grains, and their functions vary greatly. Thanks to techniques such as laser capture microdissection (LCM) and RNA sequencing, researchers were able to analyze several major tissues in grains separately, such as endosperm, alomalone layer, transverse cells, pericarp epidermis, and ovule vascular bundles (OVT) (Ram et al., 2020). The tasks of each cell group in these tissues are not exactly the same: endosperm mainly stores nutrients, the aleurone layer participates in nutrient metabolism, transverse cells assist in transportation, the epidermis of the globule is related to the development of endosperm, and OVT is more like a bridge, responsible for bringing in nutrients from the mother. I didn't know much about this area in the past, but now it's gradually becoming clear.

4.2 Gene expression patterns unique to specific cell types

There is a pattern to which genes are expressed by different tissues. By means of spatial transcriptomics, researchers have found that each type of cell has its own "preferred" set of genes. For example, the genes expressed in this part of OVT are closely related to hormone transducers and transporters, which also indicates that it is quite crucial in nutrient delivery (Wu et al., 2020). For instance, endosperm, where the genes are concentrated on starch and protein storage. The gene expression in the aleurone layer is rather complex and involves many metabolic regulations. In addition, the research also identified many cis-acting elements through promoter analysis, some of which had never been seen before. It is precisely these elements that enable different tissues to perform their respective duties during grouting, with some genes on and others off, clearly distinguishable.

4.3 Dynamic changes in cell-type specific gene expression during filling stages

During the grouting period at different stages, gene expression will also constantly change. In the early stage, genes related to energy, such as those associated with photosynthesis and oxidative phosphorylation, will increase significantly; However, as grouting enters its peak period, the expression of genes controlling glucose metabolism and starch synthesis gradually becomes stronger (Katara et al., 2020). However, the situation is not completely static. For example, micrornas are also involved in regulation. They selectively switch on and off certain genes according to different stages and tissues, such as adjusting hormone balance or controlling starch accumulation (Peng et al., 2013; Yi et al., 2013). Although these changes seem chaotic, it is precisely this "rhythmic chaos" that has driven the grains to develop and mature smoothly in the end.

5 Construction of Gene Regulatory Networks (GRNs) from scRNA-seq Data

5.1 Methods for inferring GRNs from single-cell data

Not all single-cell RNA sequencing analyses require sophisticated algorithms, but some commonly used ones nowadays are indeed quite complex, such as graph neural networks (GNNS), graph attention models (like AttentionGRN, GRLGRN), and some deep generative models (like DeepSEM). Their goal is actually quite clear: to figure out which transcription factors control which genes, and also to know that this control is directional and functional (Shu et al., 2021; Gao et al., 2025). However, in the face of such thorny problems as data sparsity, background noise, and inter-cell differences, attention mechanisms, graph convolution, and causal reasoning have to be relied upon to deal with them (Lin and Le, 2022). If it is scRNA-seq data with time sequence, then cyclic autoencoders or causal frameworks can also be used to track the time changes, such as the regulatory changes during the grain filling stage of rice (Chen et al., 2025).

5.2 Key transcription factors and regulatory hubs identified in rice grain filling

Although these studies seem to focus more on methods, in fact, they have already been able to help us identify some key regulatory factors and "hub" genes. In other biological systems, systems like TFAP2A and TEAD4 have been identified in this way. This type of method may also reveal the important regulatory nodes during the grain filling period of rice (Wang et al., 2025). Most of these nodes regulate the gene modules related to starch synthesis, hormone signaling, and nutrient transport - these modules play a crucial role in the smooth development of grains.

5.3 Cross-talk between cell-type specific GRNs and hormonal/metabolic pathways

The GRN derived from RNA-SEq is not merely about transcription factors and target genes. It also reveals some deep connections - the interaction between the regulatory network and hormones and metabolic signals is actually very frequent. Plant hormones such as auxin, cytokinin and abscisic acid often "disrupt" along with metabolic pathway signals. They jointly regulate gene expression, help grains fill smoothly and improve quality (Mao et al., 2023). This regulatory approach is not static; it adjusts according to changes in the developmental stage or the external environment to ensure that the grains can develop healthily and respond to stress.

6 Functional Insights from Cell-type Specific GRNs in Rice Grain Filling

6.1 GRNs governing starch biosynthesis and storage protein accumulation

During the grain filling period, the regulatory networks related to starch synthesis in the endosperm are particularly crucial, and many genes come into play at this stage. For instance, the *GIF2* gene encodes the large subunit of ADP-glucose phosphorylase (AGP) and is an important node in the regulatory network. Once *GIF2* loses its function, the activity of AGP will decrease, resulting in problems in starch synthesis, and the expression of genes such as starch synthase, branching enzyme, and debranching enzyme will also be affected (Wei et al., 2017). These changes indicate that during the development of grains, the accumulation of starch and stored proteins is actually closely regulated. There is another type of small molecule RNA, such as miR1861 and miR397, which are also involved in the regulatory process. They "fine-tune" starch synthesis by influencing the expression of certain negative regulatory factors, especially when the external environment changes (Teng et al., 2021).

6.2 GRNs controlling nutrient transport and assimilation

Not only the endosperm but also the pericarp tissue plays an important role. In the peel, the expression levels of

the two transcription factors ONAC127 and ONAC129 are very high. They control the expression of sugar transporters (such as OsMST6 and OsSWEET4) and some calmodulin-related genes (Ren et al., 2021). These genes affect the efficiency of sugar transport from the mother to the endosperm. Meanwhile, mitochondrial pyruvate kinase complexes like OsPK3-OsPK1 and OsPK4 also play a role in the transport of sucrose. The expression of these genes is phased and usually occurs in the tissues responsible for sucrose transport, thereby helping grains obtain nutrients efficiently during the filling period (Hu et al., 2020). Overall, these regulatory networks act like a "coordination center", integrating the signals between the parent and the grains to make the distribution of carbohydrates more reasonable.

6.3 GRNs involved in stress response and grain quality traits

When environmental conditions change rapidly, especially at high temperatures, it is very easy to interfere with grain filling. At this point, some cell type-specific regulatory networks begin to come into play. Heterodimers like ONAC127 and ONAC129 not only regulate nutrient transport but also participate in responses to stresses such as high temperatures. If they are knocked out or overexpressed, the grain filling will deteriorate and the tolerance to high temperatures will also decrease. Meanwhile, plant hormones such as ABA (abscisic acid), IAA (growth hormone), and polyamines are also involved in these regulatory processes. Interestingly, some spikelets often have poor filling and low grain weight due to relatively low hormone content (Zhang et al., 2016). Micrornas are no outsiders either. They help plants better adapt to adverse conditions by regulating hormone synthesis and signal transduction, and also influence the quality of the final grains.

7 Case Study

7.1 Identification of endosperm-specific transcription factors regulating starch genes

It has been repeatedly mentioned in the research that some transcription factors are expressed only in the endosperm or at particularly high levels there, and these factors can directly regulate genes related to starch synthesis. For example, OsbZIP58 has a particularly high expression level when endosperm starch synthesis is most active, and it can directly bind to the promoters of six key genes, namely *OsAGPL3*, *Wx*, *OsSSIIa*, *SBE1*, *OsBEIIb* and *ISA2*, to control their expression (Wang et al., 2013). In addition, there are two proteins, OsNAC24 and OsNAP. The complex composed of them can regulate OsGBSSI and OsSBEI. The regulatory process is more like "fine-tuning" (Jin et al., 2023). NF-YC12 is also an example. It specifically regulates FLO6 and OsGS1;3 in the endosperm. Affecting the accumulation of starch and stored protein (Xiong et al., 2019). As for OsbZIP60, also known as OPAQUE3, it is involved not only in the synthesis of starch and protein, but also in maintaining the stability of the endoplasmic reticulum (Figure 2) (Cao et al., 2022).

7.2 Construction and analysis of starch-related GRNs from scRNA-seq data

By combining laser microdissection technology with transcriptome analysis, it is now possible to map out the gene regulatory network (GRN) within the endosperm of rice, down to specific spatial positions. Studies have found that in the early stage of grouting, most of the genes related to starch synthesis are first expressed in the central starch endosperm (CSE), and then extend to the lateral and dorsal regions (Ishimaru et al., 2021). Network analysis shows that there is synergy between transcription factors and metabolic genes at different times and in different Spaces, and hormone signals and regulatory factors related to cell death have also been integrated into these networks. Recent large-scale GRN analyses have also identified hundreds of transcription factors that can regulate starch synthase. One enzyme may even be regulated by multiple factors, which also indicates that the entire system is regulated very complexly and there is redundancy (Huang et al., 2025).

7.3 Functional validation through transgenic and CRISPR approaches

To verify the effects of these regulatory factors, researchers employed methods such as transgenic overexpression, knockout, and CRISPR/Cas9 (Chen and Zhang, 2024). For instance, knocking out OsbZIP58 or OsNAC24 will directly alter the starch content, the ratio of amylose to amylopectin in the grains, as well as the grain quality. These results also confirm their regulatory roles. For instance, knocking out Waxy/GBSSI with CRISPR/Cas9 reduces amylose and simultaneously triggers compensatory responses in the expression of other related genes,

indicating that there may be a feedback mechanism regulating this network (Perez et al., 2019). Similarly, if the factors regulating OsGBSSI are knocked out, the content of amylose can also be reduced, and the edible quality of rice will be improved. These results provide a clear direction for the improvement of rice quality.

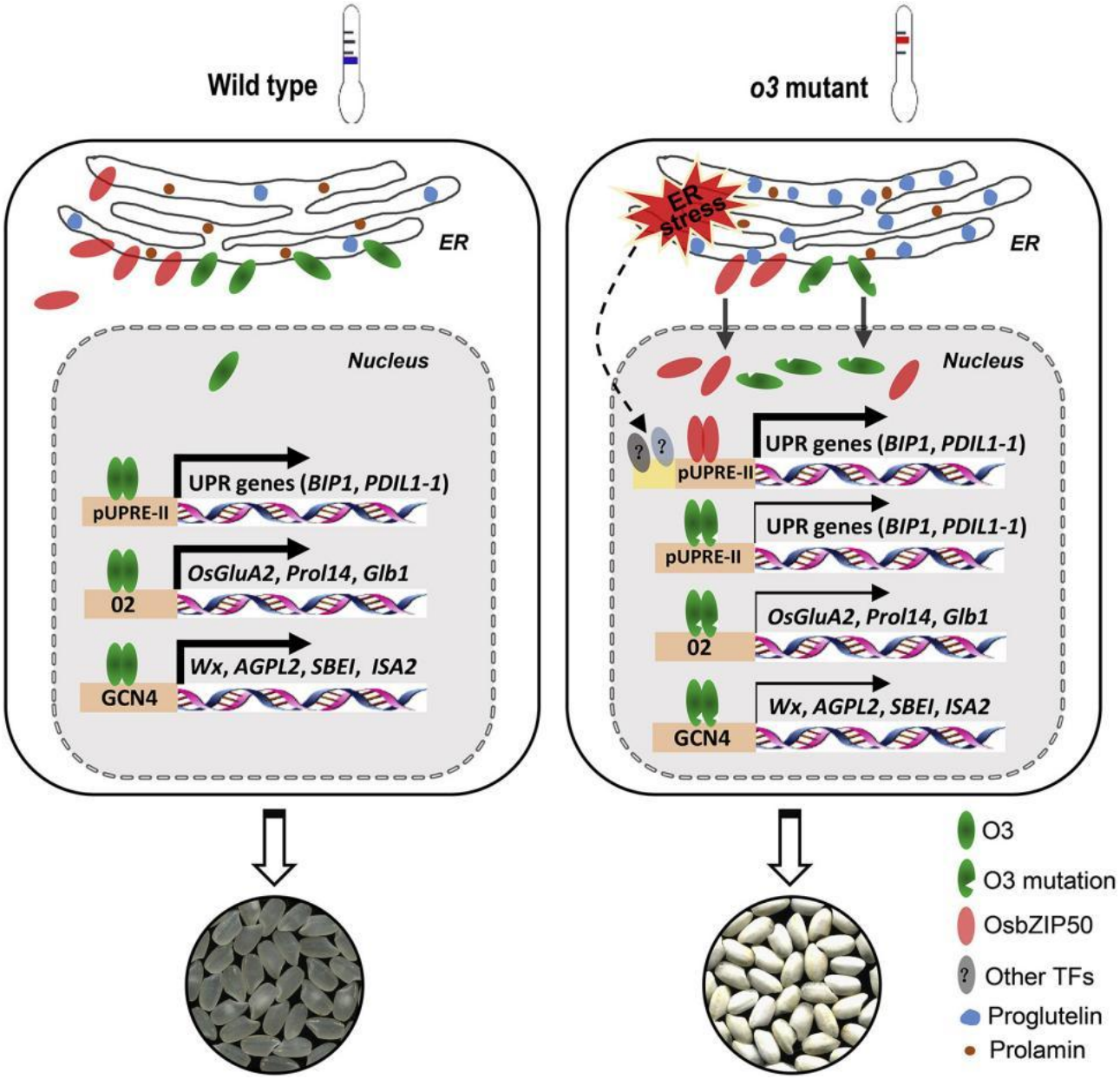

Figure 2 Proposed model of the role of O3 in maintaining ER homeostasis and regulating endosperm storage protein and starch biosynthesis in rice (Adopted from Cao et al., 2022)

Under normal conditions in the WT, O3 is located in the ER and the nucleus. It simultaneously regulates ER protein processes and secretion as well as storage protein and starch biosynthesis in endosperm cells by binding to specific motifs, such as pUPRE-II, O2, and the GCN4 box, to activate transcription of UPR genes and storage protein and starch biosynthesis genes, ultimately ensuring normal development of rice grains. However, mutation of O3 leads to downregulated expression of ER stress-related genes, such as *OsBIP1* and *PDIL1-1*, as well as genes related to storage protein and starch biosynthesis, resulting in ER stress and an impaired protein folding process in the ER. This leads to excessive accumulation of the 57-kDa glutelin precursor and reduces the contents of starch and storage proteins. High temperature can aggravate ER stress and lead to more abnormal grain development in the *o3* mutant. As physiological feedback, more O3 (mutated) is transferred to the nucleus from the ER, together with OsbZIP50 and other unknown TFs, to activate expression of key UPR genes to maintain ER homeostasis, especially under high-temperature conditions (Adopted from Cao et al., 2022)

8 Integration with Other Omics and Validation Approaches

8.1 Combining scRNA-seq with spatial transcriptomics for localization of GRNs

Single-cell RNA sequencing (scRNA-seq) enables us to see which genes each cell expresses, but it has a drawback - it "cannot reveal" the original location of these cells in the tissue. The spatial transcriptome (SRT) technology precisely fills this gap, as it can map gene expression to specific locations. Nowadays, many studies combine the use of the two methods. In this way, researchers can not only identify the regulatory networks (GRNS) in specific cell types, but also know the spatial layout of these networks in tissues. Some analytical tools, such as Seurat, SpaOTsc, Tangram and gimVI, have been able to match RNA-SEq data with spatial location information, estimate undetected gene expressions, and even draw possible spatial regulatory maps (Yan et al., 2024). These tools are very helpful for studying how cells interact and how the surrounding environment affects regulatory mechanisms (Williams et al., 2022).

8.2 Integration with proteomics and metabolomics for functional confirmation

Just looking at the transcriptome data is not enough. After all, gene expression does not necessarily mean protein production. So, some researchers began to analyze scRNA-seq together with proteomic and metabolomic data. This multi-omics integration can provide a clearer view of exactly what is happening at the functional level of cells. Now there are some deep learning and graph models, such as MCNET and DEMOC, that can integrate transcriptome, proteome and epigenetic data, thereby more accurately identifying the key modules in the regulatory network (Zou et al., 2022; Tiwari and Trankatwar, 2023). More importantly, this approach can also verify whether the changes in mRNA are truly reflected in proteins and metabolites. This step is crucial for confirming the biological significance of the regulatory network (Fan et al., 2025).

8.3 Validation using gene editing and mutant analysis

Although various analytical methods are powerful, paper inferences are not evidence after all. To confirm the true function of a certain gene or regulatory element, one still needs to conduct "hands-on experiments" for verification. Gene editing technologies like CRISPR/Cas9 and mutant analysis are all commonly used methods (Huang, 2024). By knocking out a gene, overexpressing it, or interfering with its regulatory region, we can observe how gene expression changes, whether proteins change, and whether the phenotypes of plants or cells also change (Rostom et al., 2017). These intervention experiments are like "practical tests" of previous computational predictions and are also an important part of transforming results from data into biological understanding (Bridges and Miller-Jensen, 2022).

9 Concluding Remarks

Our understanding of the grain filling process of rice has actually undergone considerable changes in recent years. Especially after the emergence of single-cell RNA sequencing (scRNA-seq), it has enabled people to observe from a more detailed perspective how different each type of cell is in terms of gene regulation. This technology is not a solo effort. Together with other transcriptomics methods, it gradually unifies the regulatory relationships among genes, micrornas and targets during grain development. Take the miR1432-OsACOT pathway as an example. It was found to be related to fatty acid metabolism and hormone synthesis, and surprisingly, it affected the grouting speed and even the yield. The more such research there is, the clearer we can see the complex molecular mechanisms.

In fact, under high-throughput sequencing, many new discoveries have been made: hundreds of mirnas have been found to be highly expressed only at a certain stage, and most of these mirnas are related to hormone balance, nutrient transport or starch accumulation. From this perspective, research at the single-cell level not only clarifies the regulatory map but also clarifies when and in which cells it takes effect, which is of great help to genetic improvement.

These studies are not confined to the laboratory only. For instance, some research has been verified in the field - by suppressing miR1432 or modifying OsACOT, both the grain weight and total yield of rice were indeed increased. It is evident that to breed breeds that are more capable of filling, more nutritious, and more resistant to

environmental stress, experience alone is not enough. The more we understand miRNA and the target genes they regulate, the more precisely we can apply our efforts.

However, on the other hand, these advancements are also inseparable from a broader context: single-cell technology is gradually integrating with other omics methods (such as spatial transcriptomics, proteomics, and metabolomics) into a complete system. Although data analysis is still not an easy task, especially when there are significant cell differences and complex regulation, the current tools are far more powerful than ever before. In the future, if these methods can be widely applied to crop breeding, the process of studying in the fields should become faster and more accurate.

Acknowledgments

We are grateful to Mr. Xu for critically reading the manuscript and providing valuable feedback that improved the clarity of the text.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Bawa G., Liu Z., Yu X., Qin A., and Sun X., 2022, Single-cell RNA sequencing for plant research: insights and possible benefits, International Journal of Molecular Sciences, 23(9): 4497.
https://doi.org/10.3390/ijms23094497

Bridges K., and Miller-Jensen K., 2022, Mapping and validation of scRNA-Seq-Derived cell-cell communication networks in the tumor microenvironment, Frontiers in Immunology, 13: 885267.
https://doi.org/10.3389/fimmu.2022.885267

Cao R., Zhao S., Jiao G., Duan Y., Ma L., Dong N., Lu F., Zhu M., Shao G., Hu S., Sheng Z., Zhang J., Tang S., Wei X., and Hu P., 2022, *OPAQUE3*, encoding a transmembrane bZIP transcription factor, regulates endosperm storage protein and starch biosynthesis in rice, Plant Communications, 3(6): 100463.
https://doi.org/10.1016/j.xplc.2022.100463

Chen Z.F., and Zhang D.P., 2024, Genome editing and rice improvement: the role of CRISPR/Cas9 in developing superior yield traits, Genomics and Applied Biology, 15(4): 182-190.
https://doi.org/10.5376/gab.2024.15.0014

Chen H., Wang T., Gong Z., Lu H., Chen Y., Deng F., and Ren W., 2022, Low light conditions alter genome-wide profiles of circular RNAs in rice grains during grain filling, Plants, 11(9): 1272.
https://doi.org/10.3390/plants11091272

Chen L., Dautle M., Gao R., Zhang S., and Chen Y., 2025, Inferring gene regulatory networks from time-series scRNA-seq data via GRANGER causal recurrent autoencoders, Briefings in Bioinformatics, 26(2): bbaf089.
https://doi.org/10.1093/bib/bbaf089

Denyer T.X., Klesen S., Scacchi E., Nieselt K., and Timmermans M., 2019, Spatiotemporal developmental trajectories in the Arabidopsis root revealed using high-throughput single-cell RNA sequencing, Developmental Cell, 48(6): 840-852.
https://doi.org/10.1016/j.devcel.2019.02.022

Durbha S., Siromani N., Jaldhani V., Krishnakanth T., Thuraga V., Neeraja C., Subrahmanyam D., and Sundaram R., 2024, Dynamics of starch formation and gene expression during grain filling and its possible influence on grain quality, Scientific Reports, 14: 6743.
https://doi.org/10.1038/s41598-024-57010-4

Fan X., He Z., Guo J., Bu D., Han D., Qu X., Li Q., Cheng S., Han A., and Guo J., 2025, Leveraging TME features and multi-omics data with an advanced deep learning framework for improved cancer survival prediction, Scientific Reports, 15: 14282.
https://doi.org/10.1038/s41598-025-98565-0

Fei H., Yang Z., Lu Q., Wen X., Zhang Y., Zhang A., and Lu C., 2021, OsSWEET14 cooperates with OsSWEET11 to contribute to grain filling in rice, Plant Science, 306: 110851.
https://doi.org/10.1016/j.plantsci.2021.110851

Gao Z., Su Y., Tang J., Jin H., Ding Y., Cao R., Wei P., and Zheng C., 2025, AttentionGRN: a functional and directed graph transformer for gene regulatory network reconstruction from scRNA-seq data, Briefings in Bioinformatics, 26(2): bbaf118.
https://doi.org/10.1093/bib/bbaf118

Hu L., Tu B., Yang W., Yuan H., Li J., Guo L., Zheng L., Chen W., Zhu X., Wang Y., Qin P., Ma B., and Li S., 2020, Mitochondria-associated pyruvate kinase complexes regulate grain filling in rice, Plant Physiology, 183(3): 1073-1087.
https://doi.org/10.1104/pp.20.00279

Huang Y.M., 2024, Genomic insights into grain size and weight: the *GS2* gene's role in rice yield improvement, Plant Gene and Trait, 15(3): 141-151.
http://dx.doi.org/10.5376/pgt.2024.15.0015

Huang K., Lu F., Chen P., Jiao G., Lin H., Zhang J., Zhao S., Cao R., Shao G., Sheng Z., Hu S., Tang S., Hu P., and Wei X., 2025, A large-scale gene regulatory network for rice endosperm starch biosynthesis and its application in genetic improvement of rice quality, Plant Biotechnology Journal, 23(7): 2583-2594.
https://doi.org/10.1111/pbi.70079

Ishimaru T., Parween S., Saito Y., Masumura T., Kondo M., and Sreenivasulu N., 2021, Laser microdissection transcriptome data derived gene regulatory networks of developing rice endosperm revealed tissue-and stage-specific regulators modulating starch metabolism, Plant Molecular Biology, 108: 443-467.
https://doi.org/10.1007/s11103-021-01225-w

Islam M., Liu Y., Hassan M., Abraham P., Merlet J., Townsend A., Jacobson D., Buell C., Tuskan G., and Yang X., 2024, Advances in the application of single-cell transcriptomics in plant systems and synthetic biology, Biodesign Research, 6: 29.
https://doi.org/10.34133/bdr.0029

Jin S., Xu L., Leng Y., Zhang M., Yang Q., Wang S., Jia S., Song T., Wang R., Tao T., Liu Q., Cai X., and Gao J., 2023, The OsNAC24 - OsNAP protein complex activates OsGBSSI and OsSBEI expression to fine - tune starch biosynthesis in rice endosperm, Plant Biotechnology Journal, 21: 2224-2240.
https://doi.org/10.1111/pbi.14124

Jovic D., Liang X., Zeng H., Lin L., Xu F., and Luo Y., 2022, Single - cell RNA sequencing technologies and applications: a brief overview, Clinical and Translational Medicine, 12(3): e694.
https://doi.org/10.1002/ctm2.694

Katara J., Verma R., Parida M., Ngangkham U., Molla K., Barbadikar K., Mukherjee M., Parameswaran C., Samantaray S., Ravi N., Singh O., and Mohapatra T., 2020, Differential expression of genes at panicle initiation and grain filling stages implied in heterosis of rice hybrids, International Journal of Molecular Sciences, 21(3): 1080.
https://doi.org/10.3390/ijms21031080

Kim J., Chae S., Jun K., Pahk Y., Lee T., Chung P., Kim Y., and Nahm B., 2017, Genome-wide identification of grain filling genes regulated by the OsSMF1 transcription factor in rice, Rice, 10: 16.
https://doi.org/10.1186/s12284-017-0155-4

Lin Z., and Le O., 2022, Inferring gene regulatory networks from single-cell gene expression data via deep multi-view contrastive learning, Briefings in Bioinformatics, 24(1): bbac586.
https://doi.org/10.1093/bib/bbac586

Liu B., Meng S., Yang J., Wu J., Peng Y., Zhang J., and Ye N., 2025, Carbohydrate flow during grain filling: Phytohormonal regulation and genetic control in rice (*Oryza sativa*), Journal of Integrative Plant Biology, 67: 1086-1104.
https://doi.org/10.1111/jipb.13904

Liu C., Ma T., Yuan D., Zhou Y., Long Y., Li Z., Dong Z., Duan M., Yu D., Jing Y., Bai X., Wang Y., Hou Q., Liu S., Zhang J., Chen S., Li D., Liu X., Li Z., Wang W., Li J., Wei X., Ma B., and Wan X., 2022, The OsEIL1 - OsERF115 - target gene regulatory module controls grain size and weight in rice, Plant Biotechnology Journal, 20(8): 1470-1486.
https://doi.org/10.1111/pbi.13825

Mao G., Pang Z., Zuo K., Wang Q., Pei X., Chen X., and Liu J., 2023, Predicting gene regulatory links from single-cell RNA-seq data using graph neural networks, Briefings in Bioinformatics, 24(6): bbad414.
https://doi.org/10.1093/bib/bbad414

Panda B., Sekhar S., Dash S., Behera L., and Shaw B., 2018, Biochemical and molecular characterisation of exogenous cytokinin application on grain filling in rice, BMC Plant Biology, 18: 89.
https://doi.org/10.1186/s12870-018-1279-4

Panigrahi S., Panigrahy M., Kariali E., Dash S., Sahu B., Sahu S., Mohapatra P., and Panigrahi K., 2021, MicroRNAs modulate ethylene induced retrograde signal for rice endosperm starch biosynthesis by default expression of transcriptome, Scientific Reports, 11: 5573.
https://doi.org/10.1038/s41598-021-84663-2

Peng T., Sun H., Du Y., Zhang J., Li J., Liu Y., Zhao Y., and Zhao Q., 2013, Characterization and expression patterns of microRNAs involved in rice grain filling, PLoS ONE, 8(1): e54148.
https://doi.org/10.1371/journal.pone.0054148

Pérez L., Soto E., Farré G., Juanos J., Villorbina G., Bassie L., Medina V., Serrato A., Sahrawy M., Rojas J., Romagosa I., Muñoz P., Zhu C., and Christou P., 2019, CRISPR/Cas9 mutations in the rice Waxy/GBSSI gene induce allele-specific and zygosity-dependent feedback effects on endosperm starch biosynthesis, Plant Cell Reports, 38: 417-433.
https://doi.org/10.1007/s00299-019-02388-z

Ram H., Singh A., Katoch M., Kaur R., Sardar S., Palia S., Satyam R., Sonah H., Deshmukh R., Pandey A., Gupta I., and Sharma T., 2020, Dissecting the nutrient partitioning mechanism in rice grain using spatially resolved gene expression profiling, Journal of Experimental Botany, 72(6): 2212-2230.
https://doi.org/10.1093/jxb/eraa536

Ren Y., Huang Z., Jiang H., Wang Z., Wu F., Xiong Y., and Yao J., 2021, A Heat stress responsive NAC transcription factor heterodimer plays key roles in rice grain filling, Journal of Experimental Botany, 72(8): 2947-2964.
https://doi.org/10.1093/jxb/erab027

Rostom R., Svensson V., Teichmann S., and Kar G., 2017, Computational approaches for interpreting scRNA - seq data, Febs Letters, 591: 2213-2225.
https://doi.org/10.1002/1873-3468.12684

Shaw R., Tian X., and Xu J., 2020, Single-cell transcriptome analysis in plants: advances and challenges, Molecular Plant, 14(1): 115-126.
https://doi.org/10.1016/j.molp.2020.10.012

Shimoyanagi R., Abo M., and Shiotsu F., 2021, Higher temperatures during grain filling affect grain chalkiness and rice nutrient contents, Agronomy, 11(7): 1360.
https://doi.org/10.3390/AGRONOMY11071360

Shu H., Zhou J., Lian Q., Li H., Zhao D., Zeng J., and Ma J., 2021, Modeling gene regulatory networks using neural network architectures, Nature Computational Science, 1: 491-501.
https://doi.org/10.1038/s43588-021-00099-8

Sun Y., Sun J., Lin C., Zhang J., Yan H., Guan Z., and Zhang C., 2024, Single-cell transcriptomics applied in plants, Cells, 13(18): 1561.
https://doi.org/10.3390/cells13181561

Teng Z., Chen Y., Yuan Y., Peng Y., Yi Y., Yu H., Yi Z., Yang J., Peng Y., Duan M., Zhang J., and Ye N., 2021, Identification of microRNAs regulating grain filling of rice inferior spikelets in response to moderate soil drying post-anthesis, The Crop Journal, 10(4): 962-971.
https://doi.org/10.1016/j.cj.2021.11.004

Tiwari A., and Trankatwar S., 2023, MCNET: multi-omics integration for gene regulatory network inference from scRNA-seq, medRxiv, 23290691: 1-16.
https://doi.org/10.1101/2023.05.29.23290691

Wang E., Wang J., Zhu X., Hao W., Wang L., Li Q., Zhang L., He W., Lu B., Lin H., Ma H., Zhang G., and He Z., 2008, Control of rice grain-filling and yield by a gene with a potential signature of domestication, Nature Genetics, 40: 1370-1374.
https://doi.org/10.1038/ng.220

Wang J., Xu H., Zhu Y., Liu Q., and Cai X., 2013, OsbZIP58, a basic leucine zipper transcription factor, regulates starch biosynthesis in rice endosperm, Journal of Experimental Botany, 64: 3453-3466.
https://doi.org/10.1093/jxb/ert187

Wang K., Li Y., Liu F., Luan X., Wang X., and Zhou J., 2025, GRLGRN: graph representation-based learning to infer gene regulatory networks from single-cell RNA-seq data, BMC Bioinformatics, 26: 108.
https://doi.org/10.1186/s12859-025-06116-1

Wang K., Zhao C., Xiang S., Duan K., Chen X., Guo X., and Sahu S., 2022, An optimized FACS-free single-nucleus RNA sequencing (snRNA-seq) method for plant science research, Plant Science, 326: 111535.
https://doi.org/10.1016/j.plantsci.2022.111535

Wang W., Cui W., Xu K., Gao H., Wei H., and Zhang H., 2021, Effects of early-and late-sowing on starch accumulation and associated enzyme activities during grain filling stage in rice, Rice Science, 28: 191-199.
https://doi.org/10.1016/J.RSCI.2021.01.008

Wei X., Jiao G., Lin H., Sheng Z., Shao G., Xie L., Tang S., Xu Q., and Hu P., 2017, GRAIN INCOMPLETE FILLING 2 regulates grain filling and starch synthesis during rice caryopsis development, Journal of Integrative Plant Biology, 59(2): 134-153.
https://doi.org/10.1111/jipb.12510

Williams C., Lee H., Asatsuma T., Vento-Tormo R., and Haque A., 2022, An introduction to spatial transcriptomics for biomedical research, Genome Medicine, 14: 68.
https://doi.org/10.1186/s13073-022-01075-1

Wu T., Müller M., Gruissem W., and Bhullar N., 2020, Genome wide analysis of the transcriptional profiles in different regions of the developing rice grains, Rice, 13: 62.
https://doi.org/10.1186/s12284-020-00421-4

Xiao Y., Dong Y., Zhou M., Wang Y., Liu X., Lu X., Zhang G., Wang F., Tang W., and Deng H., 2025, Regulation of rice grain quality by exogenous kinetin during grain-filling period, Plants, 14(3): 358.
https://doi.org/10.3390/plants14030358

Xiong Y., Ren Y., Li W., Wu F., Yang W., Huang X., and Yao J., 2019, NF-YC12 is a key multi-functional regulator of accumulation of seed storage substances in rice, Journal of Experimental Botany, 70: 3765-3780.
https://doi.org/10.1093/jxb/erz168

Yan C., Zhu Y., Chen M., Yang K., Cui F., Zou Q., and Zhang Z., 2024, Integration tools for scRNA-seq data and spatial transcriptomics sequencing data, Briefings in Functional Genomics, 23(4): 295-302.
https://doi.org/10.1093/bfgp/elae002

Yang J., Zhang J., Wang Z., Zhu Q., and Wang W., 2001, Hormonal changes in the grains of rice subjected to water stress during grain filling, Plant physiology, 127(1): 315-323.
https://doi.org/10.1104/PP.127.1.315

Yi R., Zhu Z., Hu J., Qian Q., Dai J., and Ding Y., 2013, Identification and expression analysis of microRNAs at the grain filling stage in rice (*Oryza sativa* L.) via deep sequencing, PLoS ONE, 8(3): e57863.
https://doi.org/10.1371/journal.pone.0057863

Zhang D., Zhang M., Zhou Y., Wang Y., Shen J., Chen H., Zhang L., Lü B., Liang G., and Liang J, 2019, The rice g protein γ subunit DEP1/qPE9-1 positively regulates grain-filling process by increasing auxin and cytokinin content in rice grains, Rice, 12: 91.
https://doi.org/10.1186/s12284-019-0344-4

Zhang W., Cao Z., Zhou Q., Chen J., Xu G., Gu J., Liu L., Wang Z., Yang J., and Zhang H., 2016, Grain filling characteristics and their relations with endogenous hormones in large-and small-grain mutants of rice, PLoS ONE, 11(10): e0165321.
https://doi.org/10.1371/journal.pone.0165321

Zhao Y., Zhang X., Cheng Y., Du X., Teotia S., Miao C., Sun H., Fan G., Tang G., Xue H., Zhao Q., and Peng T., 2023, The *miR167-OsARF12* module regulates rice grain filling and grain size downstream of *miR159*, Plant Communications, 4(5): 100604.
https://doi.org/10.1016/j.xplc.2023.100604

Zou G., Lin Y., Han T., and Le O., 2022, DEMOC: a deep embedded multi-omics learning approach for clustering single-cell CITE-seq data, Briefings in Bioinformatics, 23(5): bbac347.
https://doi.org/10.1093/bib/bbac347