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Spatiotemporal Transcriptome Atlas of Wheat Grain Filling Stage

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Abstract The grain-filling period is a crucial stage that determines the yield and quality formation of wheat grains, involving a complex spatio-temporal regulation process. To gain a deeper understanding of the dynamic changes in gene expression and their molecular mechanisms during grain development, this study, based on multiple time points and multiple tissues, constructed a spatio-temporal transcriptome map of wheat grains covering the entire grain-filling period. Three key periods - early, middle, and late grain-filling - were selected, and the main tissues such as endosperm, embryo, and aleurone layer were collected respectively. High-quality transcriptome data were obtained by using high-throughput RNA sequencing technology. Through differential expression analysis, time series clustering, tissue-specific expression analysis and functional annotation, a large number of key genes and transcription factors related to carbon and nitrogen metabolism, starch synthesis, hormone regulation, stress resistance response, etc. were identified. This study also constructed multiple regulatory networks closely related to grain development and revealed the potential roles of non-coding Rnas and epigenetic factors in regulation, providing resource support for the analysis of the complex regulatory networks of grain development. This research will lay a solid foundation for the improvement of wheat quality, molecular breeding and the functional study of key regulatory genes during the grain-filling period.

Keywords Wheat; Grouting period; Transcriptome; Spatio-temporal map; Grain development

1 Introduction

Wheat (*Triticum aestivum* L.) feeds nearly one-third of the world's population, not only because it is grown in large quantities - with a global planting area of approximately 230 million hectares, but more importantly, it provides a crucial source of heat and nutrients. But when it comes to output and quality, it depends on the grouting period. During this period, whether the grains are ultimately large or small, whether they can be ground well and roasted fragrant all depend on this stage (Zhang et al., 2023). The structures such as the embryo, endosperm and seed coat are not independent of each other. Instead, they jointly regulate the accumulation process of nutrients through the coordinated expression of genes and also influence the formation of various functional areas within the grain (Li et al., 2025).

Of course, to understand these processes clearly is not about thinking. In recent years, technological advancements in transcriptomics, proteomics, metabolomics and other fields have indeed deepened our understanding of the molecular networks of wheat grain development, especially in grain filling (Rangan et al., 2017). After applying high-throughput RNA sequencing and spatial transcriptomics technologies, researchers can identify thousands of differentially expressed genes at one time and also clearly understand the respective roles played by different cell types in regulation. But then again, most of the early studies focused on the "whole", such as the entire grain or a certain organ. Although this approach has reference value, it obviously lacks "partitioning operations", especially being far from sufficient at the cell type and spatial levels (Zhang et al., 2021; 2023). This makes it very difficult for us to truly figure out exactly which cells, at what point in time and through what mechanism are promoting grain development and quality formation.

This study integrated high-resolution gene expression data from multiple cell types and developmental time points, constructed a spatiotemporal transcriptome map of wheat grains during the grain-filling period, classified wheat grains into different cell types, identified marker genes, and mapped the regulatory network driving grain development and quality formation. By providing a detailed molecular map, this map fills the key knowledge gap

in cell type-specific regulation and offers new genetic resources for breeding high-yield and high-quality wheat varieties. This study aims to construct a high-resolution spatiotemporal transcriptome map, clarify the regulatory mechanism of grain filling at the cellular level, and promote targeted genetic improvement for global food security.

2 Physiological and Developmental Characteristics of Wheat Grains during the Grain Filling Stage

2.1 Developmental stages and characteristics of grains during the grain filling stage

Once wheat enters the grain-filling stage, the development pace of the grains begins to accelerate significantly. However, this process is not accomplished all at once but is carried out in stages. Starting from flowering, the grains roughly go through three filling stages: early, middle and late. Each stage has its own "main activity" - in the early stage, it is cell division and differentiation; in the middle stage, it focuses on dry matter accumulation, such as a large amount of starch and protein piling up in the endosperm; in the late stage, it gradually turns to maturity and water loss (Guan et al., 2022). But this rhythm is not static. The environmental and genetic background can change at any time. When common stresses such as high temperature and shading occur, photosynthesis is affected, and the homoides decrease. As a result, the size of the grains, starch content, and final weight often have to be reduced (Chunduri et al., 2021; Mirosavljevic et al., 2021; Hou et al., 2024; Li et al., 2025).

2.2 Functional differences among various tissues

Ultimately, a wheat grain is not a "unified combat unit", but is composed of different functional organizations that "divide labor and cooperate". Endosperm is the main warehouse, which also contains multiple parts such as the aleurone layer, sub-aleurone layer and endosperm transfer cells. Its main task is to store starch and protein for the germination of seedlings or for the nutrition of human food. The aleurone layer acts as a "transport hub", rich in various enzymes, and it is the key to mobilizing nutrients at critical moments. The embryo should not be overlooked either. It is the starting point of future plants, with active metabolism and unique protein expression. Many proteomics and metabolomics studies have shown that each of these tissues has its own specific expression rhythm and metabolic pathways (Zhang et al., 2021; 2023). To put it bluntly, it's about who should do what and when they should do it, which is already planned within the body.

2.3 Dynamics of carbon and nitrogen metabolism during grain filling

To ensure that the grains are fully filled, water alone is not enough; the key lies in how carbon and nitrogen "flow" and "transform". The carbon source is mainly sucrose, and nitrogen enters the grains in the form of amino acids, which are eventually converted into starch and storage proteins (Wang et al., 2021). But this transformation is not achieved overnight; there is a series of enzymes "holding the stage" in between. Just as sucrose synthase and starch synthase are responsible for carbon metabolism, GOGAT and GDH play a key role in nitrogen assimilation. The activity and expression of these enzymes are closely related to the type of tissue and the time point of development. Different "switches" are turned on at different times. But then again, once there are problems with the environment, such as insufficient nitrogen or excessively high temperatures, these metabolic activities will also be disrupted. Eventually, it is often the case that starch rises but protein drops, and the composition of the grains changes accordingly. Throughout the entire filling period, endosperm is the most crucial "metabolic main battlefield", where the synergistic operation between carbon and nitrogen plays the greatest role, and has a particularly direct impact on yield and quality.

3 Gene Expression Dynamics during the Wheat Grain Filling Stage

3.1 Global spatiotemporal patterns of gene expression

During the grain filling process of wheat, the changes in gene expression are not merely a "start - enhance - end" process. In fact, there is a complex, cell type-specific regulatory system behind it, and this system is constantly changing over time and space. Research in spatial transcriptomics has found that gene activity is not evenly distributed throughout the grains. As development progresses, the expression activity around the embryo and endosperm is the highest, while the expression levels of peripheral structures such as the seed coat and fruit coat

gradually decline. The aleurone layer, starch endosperm, transferred cells and other regions each exhibit different expression profiles. Currently, 10 types of cells can be distinguished and 192 marker genes have been identified. Transcription factors like TaABI3-B1 are particularly active only in the embryo and its adjacent endosperm. They regulate not only the embryo size but also influence the final formation of the entire grain. These findings once again demonstrate the significance of spatial dimension expression regulation in terms of yield and quality (Figure 1) (Li et al., 2025).

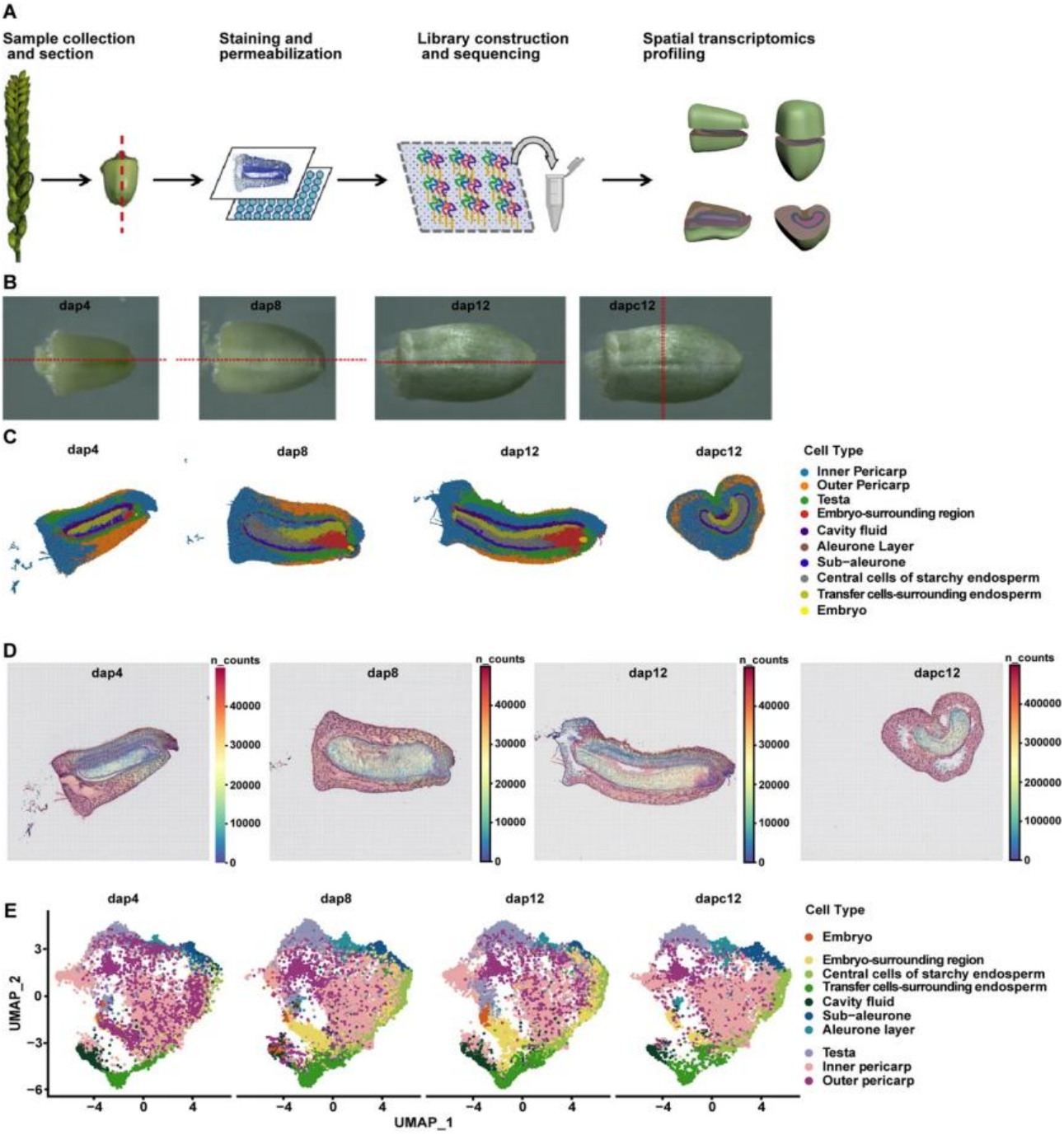

Figure 1 Spatially resolved transcriptome analysis of wheat grains. A A workflow for sampling and sequencing of wheat grains on a BMKMANU S1000. B Developing grains at dap 4, dap 8, and dap 12. C Spatial visualization of the unbiased spot clustering for dap 4, dap 8, and dap 12 sections. Merged bright field images and spatial clusters of the other three sections. The tissue/cell-type identity of each cluster was assigned based on the location of each cluster. D Heatmap and spatial distribution map of total expression counts in the spot of each sample. E UMAP of spatial spots from dap 4, dap 8, and dap 12 wheat sections. Dots correspond to individual spots on the BMKMANU S1000 chip; colors indicate cell type annotation for each spot (Adopted from Li et al., 2025)

3.2 Temporal clustering analysis of differentially expressed genes (DEGs)

Many people think that grain development is just a quantitative change, but in fact, the temporal expression changes are equally important. Transcriptome data analysis from the early to mid-stage of grouting revealed that there were tens of thousands of differentially expressed genes (DEGs), among which over 1 400 were transcription factors. These genes are not randomly "online"; they are automatically divided into different expression clusters according to their developmental stages. Between 0 and 6 days after pollination, the most active genes are those involved in cell division and hormone metabolism. However, by the 8th to 10th day, the situation changes, and genes related to starch synthesis and protein storage start to take the lead. Upon further examination through co-expression clustering analysis and WGCNA, several key modules and hub genes have emerged - such as those regulating processes like sucrose conversion to starch and hormone signaling, which control the "switch button" from the cellular activity stage to the storage and accumulation stage (Guan et al., 2022; Hou et al., 2024).

3.3 Functional prediction of tissue-specific expressed genes

If it is not detailed enough from an overall perspective, then it is necessary to delve into the organizational level. Transcriptome and proteome analyses of the endosperm and embryo have revealed many genes that only "speak" in specific tissues. The genes with high expression levels in the endosperm region are mostly related to the synthesis of starch and storage proteins, while the genes that are active in the embryo are more inclined towards developmental control and stress response. Interestingly, functional prediction has identified some "seed players" - such as those that can regulate sucrose transport and enhance starch synthase activity, as well as genes linked to hormone signaling pathways (like abolic acid and ethylene). The emergence of such genes not only explains the differences in tissue functions, but also provides direct candidate resources for subsequent breeding and directed genetic engineering (Liu et al., 2023; Shi et al., 2024).

4 Functional Pathways and Molecular Network Analysis

4.1 Enrichment analysis revealing metabolic and signaling pathways

During the crucial period of grain filling for wheat, it was actually quite lively inside. Whether it is starch and sucrose metabolism, or glycolysis, amino acid synthesis, photosynthesis and other pathways, they are almost all "online" - at different tissues and different time points, these metabolic pathways are not static but constantly changing (Rangan et al., 2017; Zhang et al., 2021; Li et al., 2025). However, things didn't go as smoothly as expected. Environmental stresses such as shading or high temperatures, once they occur, can easily disrupt these core pathways. For instance, the expression of photosynthetic antenna proteins, carbon fixation efficiency, and even the activity of genes related to starch synthesis will all be affected, and ultimately it may be reflected in grain size, weight, and quality (Chunduri et al., 2021; Hou et al., 2024). More systematic functional annotations and pathway analyses have revealed that there are over seventy identified metabolic and signaling pathways, including hormone synthesis (such as abscisic acid and ethylene), protein synthesis and degradation, as well as some specialized metabolic processes closely related to grout (Zhang et al., 2023).

4.2 Construction of transcription factor regulatory networks and identification of key nodes

When all the regulatory factors are considered together, network construction becomes a crucial link. Using analytical methods such as WGCNA and graphic LASSO, researchers gradually pieced together the full picture of the TF network behind grouting regulation. Of course, not all transcription factors have the same "weight". Some like Q, TaTPP-7A, and members of the OsNF-Y family are basically control hubs. They do not simply activate one gene, but can mobilize multiple functional regions, involving multiple directions such as carbon and nitrogen metabolism, starch synthesis, and hormone signaling (Fang et al., 2022). For instance, the Q gene binds to the gene motifs related to photosynthesis and nitrogen metabolism, driving up both production and protein levels. TaTPP-7A is involved in the T6P-SnRK1 pathway, integrating glucose signaling and ABA hormone response to regulate sucrose distribution and starch accumulation (Liu et al., 2023). Behind the entire network lies a complex regulatory mechanism that is clearly layered yet highly interwoven.

4.3 Roles of non-coding RNAs in spatiotemporal regulation

As for non-coding RNA, it may not have received much attention in the past, but now it can be seen that its "behind-the-scenes operations" should not be underestimated either. Especially small molecules like miRNA have been confirmed to be involved in the detailed regulation of the grain filling process. They usually do not directly express functional proteins but rather "target" core genes in key pathways, such as nutrient absorption, hormone response, and stress response, which have all been identified (Gupta et al., 2021). Of course, the specific role of non-coding RNA in wheat grout filling is still under continuous exploration. However, one point has basically become a consensus: they are playing an increasingly crucial role in regulating the spatiotemporal rhythm of gene expression, especially in ensuring the final development and quality stability of grains, and their status is rapidly rising.

5 Case Studies: Functional Validation of Key Genes and Pathways

5.1 Spatiotemporal expression profiles of starch synthesis-related genes

As soon as the grouting period begins, the synthesis of starch is never idle. However, it is not the result of a few genes working alone, but rather a complete regulatory network. TaTPP-7A is one of the ones that has been proven to have a very obvious effect. This trehalose 6-phosphatase gene is specifically expressed during the grain development period. Once overexpressed, it not only activates the genes related to starch synthesis but also directly increases the thousand-grain weight (TKW) and yield. How was it done? The core pathway is T6P-SnRK1, and together with the signal "dialogue" between sugar and ABA, they coordinate the decomposition, flow and utilization of sucrose (Figure 2) (Liu et al., 2023). These regulations are not "cast the net evenly". Spatial transcriptome data further reveal how transcription factors such as TaNAC019 and TaNF-Y regulate starch synthesis genes in specific cell types, thereby affecting endosperm structure and grain size (Li et al., 2025).

5.2 Protein accumulation-related genes and the molecular basis of quality formation

Not all proteins accumulate in large quantities simultaneously in the grains, nor does it all depend on the timing. The location of the tissue and the stage of development jointly determine which types of proteins are expressed in which area. Both proteomics and transcriptomics have shown that storage proteins, including gliadin and gliadin, each have their own distinct expression regions. For instance, low-molecular-weight glutenin is particularly active in the strong gluten genotype, especially in the later stage of filling, which directly contributes to bread quality (Araya-Flores et al., 2020). At the spatial distribution level, the accumulation sites of disulfide isomerase and glutamine under the endosperm and alar layers precisely illustrate how macropolymers are formed step by step - this is a key step directly related to the final quality (Zhang et al., 2021). In addition, cell type specific proteomics studies have also shown that the regulatory mechanisms of nitrogen metabolism and protein synthesis in different regions within endosperm are not the same, and these minor differences will eventually affect the nutritional level of grains (Zhang et al., 2023).

5.3 Case studies of stress-responsive genes during the grain filling stage

Not only is the regulation under normal conditions worthy of attention, but the genetic response in adverse circumstances is also a greater test of the system's resilience. Once stress such as high temperature and shading occurs, the gene expression pattern of wheat will immediately "switch". In some heat-tolerant varieties, transcription factors such as TaAP2/ERF are up-regulated, and the ethylene signaling pathway is also activated to maintain the stable development of seeds (Magar et al., 2024). For instance, "emergency components" such as heat shock proteins and catalase can also be induced to help regulate osmotic pressure, enhance resilience and grouting efficiency (Rangan et al., 2020; Sihag et al., 2023; Hou et al., 2024). Of course, not all coercion can be withstood. For instance, shading can inhibit the key pathways of starch synthesis and photosynthesis, resulting in small grains and low starch content. However, such research is not all about bad news. Some candidate genes (such as TaLFNR1-7A and TaFd-7A) have been discovered, and they can still maintain a relatively high photosynthetic efficiency under low light. The functional verification of such genes also provides more precise targets for breeding, especially in enhancing the stability and resilience of crops in adverse conditions.

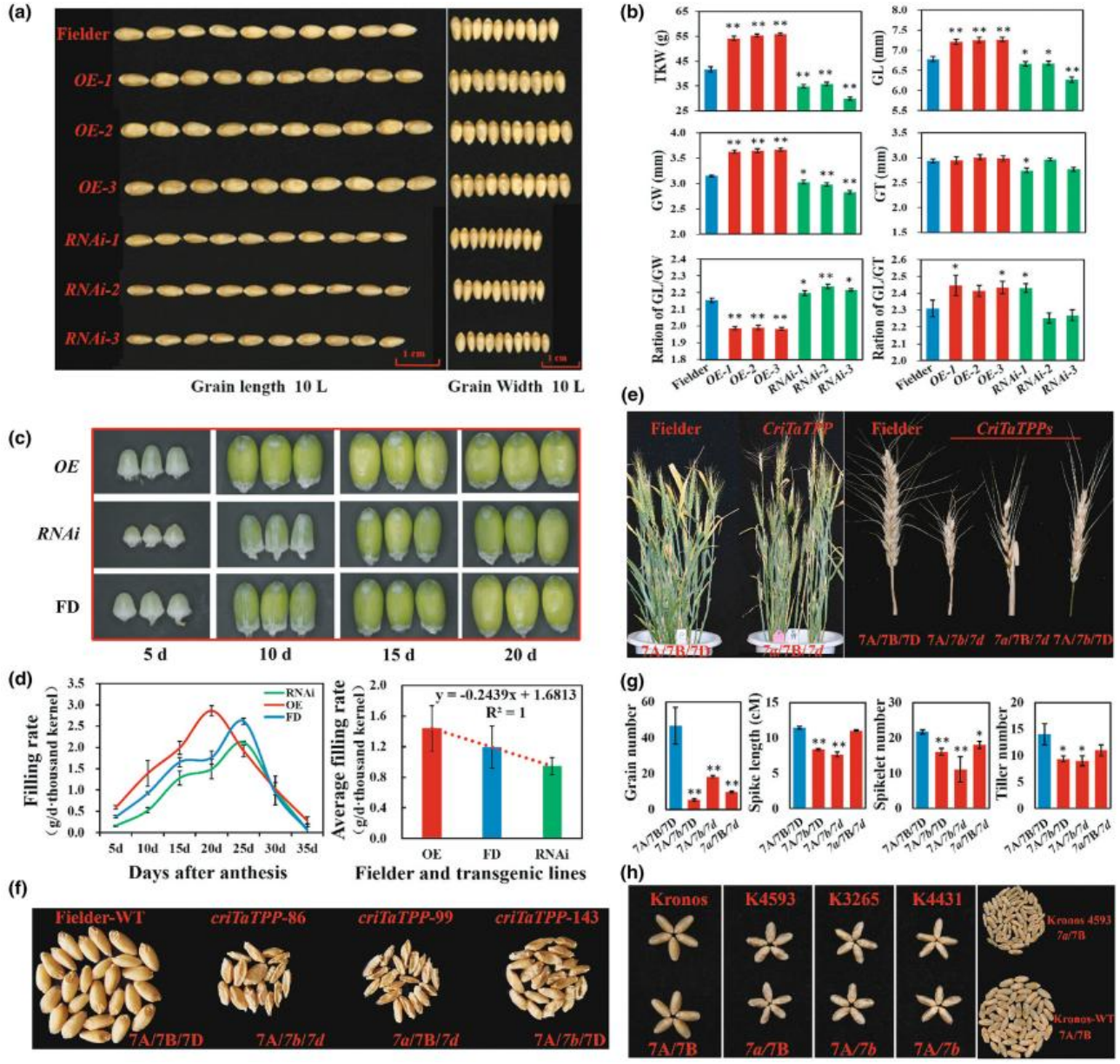

Figure 2 Grain phenotype and agronomic traits of transgenic *TaTPP-7A* lines (a) Mature grains of *TaTPP-7A* OE and RNAi wheat lines and the WT cv. Fielder. The grains show differences in length and width. Bar = 1 cm. (b) Grain traits of *TaTPP-7A* OE wheat lines and Fielder. TKW, thousand kernel weight; GL, grain length; GW, grain width; GT, grain thick; GL/GW, GL/GT, or GW/GT, the ratio of GL to GW, GL to GT, or GW to GT, respectively. (c) Developing grain phenotype. (d) Grain filling rate. R^2 and dashed red lines denote the correlation coefficient and the association between filling rate and transgenic lines, respectively. Bars show the standard error. (e, f, g) Phenotypes (whole plant, spike, and grain) and agronomic traits of *CriTaTPPs* mutants. *CriTaTPPs* denote the CRISPR/Cas9-edited lines of *TaTPPs*. Capital letters (7A/7B/7D) or italic lowercase letters (7a/7b/7d) denote the WT and mutant chromosomes. (h) Mature grains of the *K-TaTPP-7A/7B* mutants (Adopted from Liu et al., 2023)

6 Associations between Spatiotemporal Transcriptomes and Grain Quality Traits

6.1 Regulatory mechanisms of starch deposition and grain filling capacity

During the grain-filling process, how starch deposits and whether the grains can be filled up mainly depend on the "time point" and "location" of gene expression. Spatio-temporal transcriptome analysis tells us that core genes involved in carbohydrate metabolism, such as granule-binding starch synthase I and several soluble starch synthases, are not expressed in the same way everywhere-they have obvious enrichment patterns in different tissues during the grain development stage, especially in endosperm cells. If expressed early and in the right position, sufficient starch will accumulate. Behind this, it is not just a single gene at work; a complete set of

regulatory networks is also involved. For instance, transcription factors like TaABI3-B1 are a crucial part of it. They regulate downstream genes and ultimately affect not only the size of the grains but also the overall efficiency of grain-filling. As for whether these pathways are useful or not, genome-wide association analyses and QTL studies have long provided the answer: they have direct contributions to both yield and processing quality (Li et al., 2023; 2024; 2025).

6.2 Spatiotemporal expression basis of protein synthesis and processing quality

Whether the gluten in wheat is strong or not and whether the protein content is sufficient, in the final analysis, it is still a matter of the "timely and punctually" expression of genes. Especially for those high-molecular-weight glutenin subunits and storage protein genes, the expression time and spatial location are very carefully selected, and their activity is most obvious in the endosperm layer and aleurone layer. Meta-QTL and transcriptome integration analysis has helped us identify a number of candidate genes and regulatory elements that affect the accumulation rate and intensity of proteins, and some of these variations can explain the significant differences in bread quality (Gudi et al., 2022). The value of spatial transcriptome maps is not just for show; it can help breeders identify key marker genes and modules for subsequent quality improvement.

6.3 Formation of secondary metabolites and nutritional quality

It is not only starch and protein that determine the nutritional value of grains. Secondary metabolic pathways such as amino acid synthesis and arginine metabolism are equally important. The expression of related genes is actually quite "selective". At different tissues and developmental stages of the grain, they each have their own positions and time points. It is precisely these specific expression patterns that determine which regions can accumulate more nutritious or biologically active compounds. That is to say, whether the health value is high or not sometimes does not depend on the total amount but on the distribution. To identify these genes, traditional methods alone are insufficient. Spatial transcriptome combined with QTL mapping is the key breakthrough (Li et al., 2023; 2025). Such achievements provide practical paths and targets for improving the nutritional quality of wheat during the breeding process.

7 Application Value of Transcriptome Atlas in Wheat Genetic Improvement

7.1 Application potential of key regulatory genes during the grain filling stage in molecular breeding

Ultimately, whether the yield and quality of wheat can be improved during the grain-filling stage depends on whether there are precise control targets. And the spatio-temporal transcriptome atlas precisely provides quite a few clues for this matter. The expression patterns of genes involved in starch synthesis, protein accumulation, or coping with adversity at different tissue and developmental stages have long been depicted more and more clearly. Some key genes have passed functional verification and have become key targets in molecular breeding. Not every candidate gene is worth a try, but those that are highly expressed in specific tissues or time periods can indeed be given priority. Breeders can directly utilize these expression data for marker-assisted selection and even gene editing to rapidly cultivate wheat varieties with strong grain-filling capacity and stable quality (Dong et al., 2015; Cao et al., 2020).

7.2 Integration with quantitative trait loci (QTL) mapping and genomic selection

Of course, relying solely on transcriptome data is not enough. Only by combining it with QTL can the positioning accuracy be guaranteed. Especially when it comes to complex traits like grain weight and plant height, it is difficult to explain clearly from a single data source. The current approach is usually to integrate transcript abundance with consensus maps and SNP data to identify more stable and reliable QTLS and candidate genes in different planting environments and genetic backgrounds (Cao et al., 2020; Qu et al., 2021). On the other hand, incorporating transcriptome information into genomic selection models has indeed enhanced the accuracy of predicting the genetic value of certain traits, especially in controlled environments. Of course, this method is not "cheap". Large-scale analysis is costly and not easy to operate. Therefore, many teams are now beginning to explore another direction - integrating environmental variables and genomic information as a more realistic and practical alternative strategy (Liu et al., 2024).

7.3 Prospects of multi-omics integration (transcriptome, metabolome, epigenome)

At present, if the genetic improvement of wheat is to make further progress, the integration of multiple omics is probably an inevitable trend. It is difficult to comprehensively explain the complex processes of grain development and quality formation with a single data source. Looking at transcriptomics, metabolomics and epigenomics together not only expands our understanding of regulatory mechanisms, but also helps to discover truly useful biomarkers. In the past, due to cost or technical limitations, omics analysis was mostly confined to a small scale. But now, with the emergence of efficient and economical expression profile analysis methods, research on large groups is no longer out of reach. Meanwhile, spatial transcriptomics and single-molecule techniques are constantly enriching our understanding of gene annotation, and new regulatory elements are thus constantly being discovered (Dong et al., 2015; Wang et al., 2021). If integrated properly, multi-omics can enable breeders to identify more reliable targets, understand how genes and the environment interact, and ultimately breed wheat varieties that are both high-yielding and of high quality, as well as resilient to stress (Liu et al., 2024).

8 Conclusion and Outlook

To understand exactly what happens to wheat grains during the grain-filling period, the spatiotemporal transcriptome undoubtedly plays a significant role. This type of research not only sketches out a "timetable" and "spatial map" of gene expression, but also goes further by breaking down the grains by different cell types to identify hundreds of representative marker genes. Regulatory factors like TaABI3-B1 have been confirmed to be directly linked to the size of embryos and grains. And some co-expression modules and conserved motifs have gradually woven a net for regulating yield and quality. These data themselves are of great value. They not only bring us closer to the core of the developmental mechanism but also provide many targets for breeding.

But then again, the matter is far from being "concluded". At present, spatial transcriptome technology is still at the "coarse granularity" level. It is considered good if it can be classified into tissue or cell types, but there is still a considerable distance to go to achieve true single-cell resolution. Moreover, although omics integration sounds wonderful, it is actually quite troublesome to implement. Protein, metabolism, and phenotype data are inherently high-dimensional. If they are to be pieced together, it is basically impossible to rely solely on traditional analytical methods. There is another overlooked issue: the data coverage related to the late grouting stage and environmental stress is still scarce, and many conclusions are thus biased. In addition, the lack of unified analytical standards and reference maps makes it difficult to compare different experiments and limits their application to actual breeding.

Where should the next work go? At least several directions are very clear. One is to further enhance the precision of spatial expression - single-cell and even subcellular levels are all worth exploring. Another aspect is that the time dimension cannot be overlooked. Grouting must be managed from start to finish, in both favorable and adverse circumstances. Omics integration is not merely a concept. In the future, it is indeed necessary to analyze the transcriptome, proteome, metabolome, epigenome, etc. within the same network. Fortunately, technology is also advancing. For instance, more efficient sequencing methods, more accurate spatial barcode technology, and some new computing tools all make it possible to construct complex and high-resolution maps. Once these resources are established, it is only a matter of time before the key regulatory factors are identified, and precision breeding will truly have a "handle" - not only to produce more and of better quality, but also to better withstand the uncertainties of the future.

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Conflict of Interest Disclosure

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

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