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Integrative Omics Approaches for Wheat Improvement: Advances in Genomics, Transcriptomics, Proteomics, and Metabolomics

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ABSTRACT: Wheat (Triticum aestivum L.) is a staple crop that provides a major source of calories and protein for over one-third of the global population. However, its productivity is increasingly challenged by a wide range of abiotic and biotic stresses, exacerbated by the growing impacts of climate change. Traditional breeding methods, while effective to some extent, are insufficient to meet the rising food demand projected for 2050. In this context, omics technologies, genomics, transcriptomics, proteomics, and metabolomics, have emerged as transformative tools in wheat research and improvement. Genomics facilitates the identification of key loci and alleles associated with important agronomic traits through genome-wide association studies (GWAS), quantitative trait locus (QTL) mapping, genomic selection, and CRISPR-based genome editing. Transcriptomics provides insight into gene expression profiles under stress and during developmental stages, helping to uncover regulatory networks and transcription factors. Proteomics advances our understanding of functional protein networks and post-translational modifications, while metabolomics identifies key biochemical pathways and adaptive metabolites associated with stress tolerance and grain quality. More importantly, integrative multi-omics approaches enable a holistic understanding of the complex genotype-to-phenotype relationships that govern yield, resilience, and nutritional traits. This review comprehensively explores recent breakthroughs in wheat omics research, the integration of multi-layered datasets, and the application of computational biology and machine learning to accelerate precision breeding. It also discusses current challenges and future directions for translating omics-based discoveries into field-ready solutions, ultimately aiming to develop climate-resilient, high-yielding wheat cultivars to ensure global food and nutritional security. Keywords: Wheat genomics, Wheat transcriptomics, Wheat proteomics, Wheat metabolomics, multi-omics integration.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple crops globally, serving as a primary source of calories and protein for more than 35% of the world's population. It contributes approximately 20% of the total dietary energy and protein intake globally (FAO, 2021). Its adaptability to a wide range of agroclimatic conditions and diverse growing environments has made wheat a cornerstone of food and nutritional security, particularly in developing countries. As the global population is expected to reach nearly 10 billion by 2050, there is an urgent need to enhance wheat productivity to meet the escalating food demand (Ray *et al.*, 2013).

However, global wheat production is increasingly threatened by a range of abiotic and biotic stresses. Abiotic stresses such as drought, salinity, nutrient deficiency, and heat stress significantly reduce wheat productivity, especially under rainfed and marginal environments (Gupta *et al.*, 2008). Terminal heat stress, in particular, poses a critical threat in South Asia and Sub-Saharan Africa, leading to yield reductions of 20–40% depending on the intensity and duration of the

stress (Parvathi *et al.*, 2020). In parallel, biotic stresses such as rusts (leaf, stem, and stripe), Fusarium head blight, powdery mildew, and various insect pests continuously evolve and overcome host resistance, leading to significant yield losses and quality deterioration (Singh *et al.*, 2011). The unpredictable nature of climate change exacerbates the situation, affecting wheat phenology, stress interactions, and pathogen dynamics, making traditional breeding approaches insufficient.

In response to these complex challenges, advanced molecular tools and omics technologies have become essential to modern wheat improvement programs. The sequencing and public release of the fully annotated wheat reference genome (IWGSC RefSeq v1.0) marked a significant milestone in wheat research, opening avenues for genome-wide association studies (GWAS), QTL mapping, marker-assisted selection, genomic selection, and genome editing (Hernández *et al.*, 2018). Genomics allows for precise identification of loci associated with important traits, while transcriptomics (e.g., RNA sequencing) helps unravel gene expression profiles under stress or during developmental stages,

leading to the identification of key regulatory genes and transcription factors (Ramirez-Gonzalez *et al.*, 2018).

Similarly, proteomics and metabolomics have expanded our understanding of post-transcriptional and metabolic responses to stress. Proteomics enables the study of post-translational stress-responsive proteins, modifications, and signalling networks, while metabolomics allows the identification of stressinducible metabolites that serve as biomarkers or adaptive molecules (Weckwerth, 2011). These tools not only deepen our understanding of plant responses but also offer new targets for breeding climate-resilient, high-yielding, and nutritionally superior wheat varieties. However, understanding complex traits requires more than single-layer omics approaches. The integration of multi-omics data, combining genomics, transcriptomics, proteomics, and metabolomics, offers a holistic understanding of the genotype-to-phenotype continuum. These integrative approaches facilitate the discovery of key pathways, causal genes, and molecular interactions that govern stress tolerance, yield, and quality traits (Rane et al., 2021). Moreover, the advent of computational biology, systems biology, and machine learning has strengthened the predictive power of omics-based breeding, ensuring more efficient and targeted varietal development.

In this review, we discuss recent advances in the application of genomics, transcriptomics, proteomics, and metabolomics in wheat improvement, and explore how integrative omics strategies are transforming our approach to genetic gain under both optimal and stress conditions.

Genomics in Wheat Improvement. Recent advances in genomics have revolutionized wheat breeding by providing powerful tools to understand and manipulate the complex hexaploid genome of bread wheat (*Triticum aestivum* L.). The completion of the IWGSC RefSeq v1.0 genome assembly marked a major breakthrough, offering the first comprehensive reference for this vital crop. Subsequent improvements in RefSeq v2.0 (Zhu *et al.*, 2021) enhanced the assembly quality, particularly in challenging repetitive regions, enabling more accurate gene annotation and functional studies.

Pangenome initiatives have significantly expanded our understanding of wheat genetic diversity by sequencing multiple cultivars and wild relatives (Montenegro et al., 2022). These studies revealed extensive structural variations, including presence-absence variations (PAVs) and copy number variations (CNVs), that contribute to important agronomic traits (Bayer et al., 2020). Wild wheat species such as Triticum dicoccoides (Avni et al., 2022) and Aegilops tauschii have proven particularly valuable as sources of novel alleles for stress tolerance and disease resistance. Quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) have identified numerous genetic loci controlling key traits. Notable examples include disease resistance genes Lr34/Yr18 (Krattinger et al., 2019) and Fhb1 (Rawat et al., 2022), as well as grain quality determinants like the Glu-1 loci (He et al., 2021). Genomic selection (GS) has transformed wheat breeding by enabling prediction of breeding values Kumar et al., Biological Forum – An International Journal 15(11): 663-668(2023)

using genome-wide markers (Crossa *et al.*, 2017), with successful applications in yield improvement, drought tolerance, and heat stress adaptation.

Molecular markers developed through genomic studies have facilitated marker-assisted selection (MAS) for traits such as disease resistance (*Sr22, Yr15*) and abiotic stress tolerance (TaDREB2, TaHsfA6f). The advent of CRISPR-Cas9 genome editing has opened new possibilities for precise genetic modifications, including knockout of TaMLO for powdery mildew resistance (Wang *et al.*, 2021), editing of TaGW2 to increase grain size (Zhang *et al.*, 2022), and modification of TaFT1 to adjust flowering time.

Looking forward, the integration of multi-omics data with machine learning approaches promises to further enhance predictive breeding. However, challenges remain in data integration, phenotyping automation, and regulatory frameworks for gene-edited wheat. As these technologies continue to advance, genomics will play an increasingly central role in developing wheat varieties with improved yield, resilience, and nutritional quality to meet global food security challenges.

Transcriptomics in Wheat Research. Transcriptomics has emerged as a powerful approach for understanding gene expression dynamics in wheat under various developmental and environmental conditions. The application of RNA sequencing (RNA-seq) technologies has enabled comprehensive identification of stress-responsive genes, providing valuable insights into molecular mechanisms of stress adaptation (Ramírez-González *et al.*, 2018).

Temporal and spatial expression profiling has revealed distinct transcriptional responses to abiotic stresses including drought (Liu et al., 2021), heat, and salinity (Zhou et al., 2020). These studies have identified key stress-responsive pathways, such as those involving heat shock proteins (HSPs) and late embryogenesis abundant (LEA) proteins, which play crucial roles in stress tolerance. Differential gene expression analysis during grain development has uncovered stage-specific transcriptional programs regulating starch biosynthesis (Zhang et al., 2021), storage protein accumulation (Guo et al., 2020), and grain size determination (Dong et al., 2022). Similarly, transcriptome profiling during pathogen attacks has elucidated defence mechanisms against major wheat diseases like rusts (Dobon et al., 2019) and Fusarium head blight (Kugler et al., 2021).

Non-coding RNAs, particularly long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), have been shown to play important regulatory roles in stress responses (Ma *et al.*, 2022). Transcription factor families such as WRKY, NAC, and MYB have been identified as key regulators of stress-responsive gene networks (Shumayla *et al.*, 2021).

Advanced bioinformatics tools including HISAT2 (Kim et al., 2019) for read alignment, StringTie (Pertea et al., 2016) for transcript assembly, and DESeq2/edgeR (Love et al., 2014; Robinson et al., 2010) for differential expression analysis have become standard in wheat transcriptomics research. These tools enable comprehensive analysis of complex polyploid transcriptomes and identification of homoeolog-specific expression patterns. The integration of transcriptomic rnal 15(11): 663-668(2023) 664 data with other omics approaches is providing systemslevel understanding of wheat biology and facilitating identification of candidate genes for breeding programs (Borrill et al., 2019). Future directions include singlecell transcriptomics to resolve cell-type-specific responses and spatial transcriptomics to understand tissue-level gene expression patterns.

Proteomics in Wheat Biology. Proteomics has become an indispensable tool for understanding the functional dynamics of wheat proteins under various physiological and stress conditions. Advanced protein profiling techniques, including two-dimensional polyacrylamide electrophoresis (2D-PAGE) and gel liquid chromatography-tandem mass spectrometry (LC-MS/MS), have enabled comprehensive identification and quantification of wheat proteomes (Wang et al., 2021). These approaches have revealed extensive protein networks involved in stress responses and developmental processes.

Studies have identified numerous stress-responsive proteins, including heat shock proteins (HSPs) that function as molecular chaperones during heat stress (Hu et al., 2020), antioxidant enzymes like superoxide dismutase and catalase that mitigate oxidative damage (Li et al., 2021), and pathogenesis-related (PR) proteins that mediate defence against pathogens (Zhang et al., 2022). These protein markers provide valuable targets for breeding stress-resilient wheat varieties. Posttranslational modifications (PTMs), particularly phosphorylation, glycosylation, and ubiquitination, play crucial regulatory roles in wheat stress responses (Liu et al., 2022). Phosphoproteomic analyses have identified key signalling cascades activated during drought and pathogen challenges (Chen et al., 2021). Protein-protein interaction studies using techniques like yeast twohybrid screening and co-immunoprecipitation have mapped critical networks involved in grain development and stress adaptation.

However, wheat proteomics faces unique challenges due to the crop's complex hexaploid genome, which often results in multiple protein isoforms with high sequence similarity (Borrill et al., 2020). The dynamic nature of protein expression and modification under environmental conditions fluctuating further complicates data interpretation (Venske et al., 2022). Sample preparation difficulties, including efficient protein extraction from starch-rich tissues, also present technical hurdles (Tanner et al., 2021). Several specialized databases and resources support wheat proteomics research. UniProt provides comprehensive protein sequence and functional annotation. While Peptide Atlas offers a repository of experimentally observed peptides (Deutsch et al., 2022). The PRIDE database archives spectrometry-derived mass proteomics data (Perez-Riverol et al., 2022), facilitating data sharing and reanalysis. Wheat-specific resources like the Wheat Omics database integrate proteomic data with other omics layers for systems biology approaches (Ma et al., 2021).

Emerging techniques such as single-cell proteomics and spatial proteomics promise to revolutionize our understanding of protein dynamics at cellular and tissue levels. The integration of proteomic data with genomic Kumar et al.,

datasets and metabolomic is enabling more comprehensive models of wheat biology and accelerating marker-assisted breeding efforts (Afzal et al., 2022).

Metabolomics for Trait Dissection in Wheat. Metabolomics has emerged as a powerful tool for dissecting complex agronomic traits in wheat by characterizing the dynamic biochemical changes underlying stress responses and grain quality development. Advanced analytical platforms including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy enable comprehensive profiling of primary and secondary metabolites (Riedelsheimer et al., 2022). These technologies have identified key metabolic signatures associated with drought tolerance (e.g., accumulation of proline and polyamines), heat stress adaptation (e.g., increased flavonoids and tocopherols), and pathogen defense (e.g., phenylpropanoid pathway activation; Kage et al., 2022).

Secondary metabolites play pivotal roles in wheat stress physiology and nutritional quality. Flavonoids (e.g., tricin and its derivatives) contribute to both abiotic stress protection and human health benefits. Polyamines (spermidine, putrescine) regulate oxidative stress mitigation during drought, while sugars (raffinose, trehalose) function as osmoprotectants and energy reserves (Dumschott et al., 2022). Metabolite-GWAS studies have linked these compounds to genomic loci, enabling marker-assisted selection for stress-resilient varieties.

Recent advances in integrative omics approaches are transforming wheat systems biology by enabling comprehensive analysis of complex trait mechanisms. By combining genomics, transcriptomics, proteomics and metabolomics data, researchers can now reconstruct complete biological pathways from genotype to phenotype (Borrill *et al.*, 2020). This multilayered approach has proven particularly powerful for understanding stress responses in wheat, where sophisticated network analyses of gene co-expression patterns, protein-protein interactions and metabolic fluxes have revealed key regulatory hubs. Weighted gene co-expression network analysis (WGCNA) has emerged as a particularly valuable tool, identifying modules of co-regulated genes associated with drought and heat responses (Zhang et al., 2022). These networks frequently highlight transcription factors like TaNAC69 and TaDREB2 as central regulators coordinating molecular responses to abiotic stress.

Integrative Omics for Systems Biology in Wheat. Several landmark studies demonstrate the power of integrative omics in wheat research. A comprehensive investigation of drought tolerance combined GWAS data identifying chromosome 4A QTLs with transcriptomics showing upregulated TaNAC69 expression and metabolomics revealing proline and raffinose accumulation (Varshney et al., 2021). This systems-level analysis established TaNAC69 as a master regulator of osmotic adjustment. Similarly, studies of heat stress response integrated proteomic data showing HSP70 induction with metabolic profiling of Biological Forum – An International Journal 15(11): 663-668(2023) 665

flavonoid glycosides, pinpointing *TaCHS* and *TaFLS* genes as targets for thermotolerance breeding. For biotic stress resistance, multi-omics approaches have successfully linked the *Fhb1* locus to pathogenesis-related proteins and deoxynivalenol-detoxifying metabolites in Fusarium head blight resistance (Kage *et al.*, 2022).

The field continues to evolve with new computational and experimental approaches. Machine learning algorithms are being applied to predict trait performance from integrated omics datasets (Crossa et al., 2017), while emerging single-cell technologies promise to resolve tissue-specific responses to environmental stresses. However, significant challenges remain in data standardization and integration across different omics platforms. Initiatives like Wheat Omics 2.0 are addressing these challenges by developing unified databases and analysis pipelines (Ma et al., 2022). Visualization tools such as Cytoscape enable researchers to interpret complex multi-omics networks while pathway analysis platforms like Map Man facilitate functional annotation of omics data. As these integrative approaches mature, they are accelerating the development of climate-resilient, high-yielding wheat varieties through more precise identification of molecular targets for breeding and genetic engineering.

Challenges and Future Prospects in Wheat Omics Research. Despite remarkable progress, wheat omics research faces several key challenges that must be addressed to fully realize its potential. A major bottleneck lies in data integration and computational analysis, as the massive datasets generated by multiomics approaches require sophisticated bioinformatics pipelines and substantial computing power. The hexaploid nature of wheat's genome adds complexity, with homeolog-specific analyses necessitating specialized algorithms.

The field urgently requires high-throughput phenotyping platforms to bridge the gap between molecular data and observable traits. Automated phenotyping systems using drones, hyperspectral imaging, and IoT sensors are being developed to capture field performance data at scale. These must be integrated with omics databases through big data platforms that can handle the volume and variety of agricultural data. Translational genomics remains a critical challenge, as many laboratory discoveries fail to translate to field conditions. The "lab-to-field gap" can be addressed through multi-environment trials and the development of predictive models that account for genotype \times environment interactions. Collaborative networks between researchers, breeders, and farmers are essential to ensure omics innovations reach practical applications.

CONCLUSIONS

Omics technologies have revolutionized wheat research, providing unprecedented insights into the molecular basis of important agronomic traits. From the foundational IWGSC reference genome to cutting-edge single-cell omics, these approaches have transformed our ability to understand and manipulate wheat biology. The integration of genomics, transcriptomics, proteomics, and metabolomics has been particularly powerful, revealing complex networks underlying stress responses and quality traits.

Moving forward, maximizing the impact of omics research will require multidisciplinary collaboration across biology, computer science, engineering, and agronomy. By combining omics data with advanced phenotyping, environmental monitoring, and predictive modelling, we can accelerate genetic gains to meet the challenges of climate change and global food security. The future of wheat improvement lies in harnessing these integrated approaches to develop resilient, highyielding varieties through both conventional breeding and precision genetic strategies.

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