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Current Status and Prospects of Wheat Genome Research (Postprint)

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Abstract

China is the world's largest wheat producer and consumer, and its production holds major strategic significance for ensuring food security. Constrained by the large and complex genome of common wheat, the development of gene cloning and molecular design breeding technologies for its important agronomic traits lags far behind those of rice and maize. Supported by the 'Molecular Module Design Breeding Innovation System', Chinese scientists have made significant contributions to deciphering the wheat genome. Additionally, through years of dedicated efforts, they have successfully developed new wheat varieties using the theory of 'module coupling breeding'. This article focuses on elaborating these major achievements and future research directions.

Full Text

Introduction

China is the world's largest wheat producer and consumer, and wheat production holds major strategic significance for national food security. Constrained by the vast and complex genome of common wheat, gene cloning for important agronomic traits and the development of molecular design breeding technologies have lagged far behind those of rice and maize. Supported by the "Innovative System of Designer Breeding by Molecular Modules" program, Chinese scientists have made significant contributions to deciphering the wheat genome. Moreover, through years of dedicated effort, new wheat varieties have been successfully cultivated using the theory of "multi-module assembly breeding." This article focuses on elaborating these major achievements and future research directions.

As the world's second most important grain crop by total production, wheat is widely cultivated globally and feeds nearly 40% of the world's population. With

the global population projected to reach 9.6 billion by 2050, wheat productivity must increase by 1.6% annually to meet future demands, which can only be achieved through crop and trait improvement. However, since common wheat is an allohexaploid with a massive and complex genome (40 times larger than rice and 5.5 times larger than the human genome), its functional genomics research has fallen significantly behind that of rice and maize. This complex genetic background has long been a bottleneck restricting gene cloning for important agronomic traits and the development of molecular design breeding technologies. In 2005, scientists from the United States, France, and other countries initiated the International Wheat Genome Sequencing Consortium (IWGSC), organizing scientists from more than 20 major wheat-producing countries to collaborate on wheat genome sequencing. The Chinese Academy of Sciences Strategic Priority Program (Category A) “Innovative System of Designer Breeding by Molecular Modules” (hereinafter referred to as the “Molecular Design Breeding Priority Program”) proposed and established a modern biotechnology breeding innovation system from “molecular modules” to “variety design,” thereby rapidly achieving multi-module optimization and assembly at the whole-genome level to cultivate new super-varieties [1]. This program has also provided new development opportunities for wheat genetics research and molecular improvement breeding in China. This article reviews the wheat-related research achievements during the program implementation period and prospects for future research directions.

Major Breakthroughs in Wheat Genome Research

Common wheat is an allohexaploid with an AABBDD genome, whose formation involved three ancestral species and two natural hybridization events. Approximately 500,000 years ago, hybridization and chromosome doubling between the ancestral species *Triticum urartu* and a related *Aegilops* species formed the allotetraploid AABB. Then, about 8,000–10,000 years ago, this allotetraploid hybridized with wild *Aegilops tauschii*, producing the allohexaploid AABBDD and resulting in the massive and complex genome of common wheat. Since the relatively simple genomes of rice and maize were deciphered earlier, their molecular design breeding theories and technologies have become increasingly sophisticated. Consequently, a high-quality reference genome sequence map is key to achieving breakthroughs in wheat molecular design breeding research.

China has made outstanding contributions to cereal crop genome research, including the construction of fine maps for the AA and DD genomes and participation in the partial construction of the AABBDD hexaploid wheat fine map for “Chinese Spring.” The A genome is the foundational genome in wheat evolution and plays a central role in polyploid wheat evolution. The wheat research team at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (hereinafter referred to as “IGDB”) performed whole-genome sequencing of *Triticum urartu* using second-generation sequencing technology and completed a draft map of the wheat A genome in 2013. This effort annotated

34,879 protein-coding genes and predicted more than 16,000 simple sequence repeats (SSRs), over 739,000 insertion site-based polymorphisms (ISBPs), and more than 3.4 million single nucleotide polymorphisms (SNPs) as molecular markers, with the results published in *Nature* [2]. Subsequently, team members constructed a BAC library and physical map of the diploid *Triticum urartu* A genome. Through BAC-by-BAC sequencing combined with third-generation PacBio sequencing and the latest genome physical map construction technologies (10× Genomics, BioNano), they completed a fine map of the wheat A genome. The genome size was 4.94 Gb, with assembled contig (N-free) sequences totaling 4.79 Gb (97% of the genome) and a contig N50 of 344 kb; scaffold (N-containing) sequences totaled 4.86 Gb (98.4% of the genome) with a scaffold N50 of 3.67 Mb. The study annotated 41,507 protein-coding genes, with 81.42% of the genome sequence identified as repetitive sequences. Through comparative genomics research, structural variations in the wheat A genome during evolution were identified, and an evolutionary model for the seven chromosomes of the wheat A genome was deduced, providing a high-quality reference genome for wheat evolutionary analysis and gene cloning. The related research results were published in *Nature* in 2018 [3].

Additionally, a U.S. research team used the classic BAC-by-BAC sequencing approach combined with Bionano and third-generation sequencing technologies, while the team of Jia Jizeng from the Chinese Academy of Agricultural Sciences employed second- and third-generation sequencing technologies combined with NRGene assembly technology to respectively complete fine reference genome maps of *Aegilops tauschii*, the D genome donor of wheat. The results were published in *Nature* and *Nature Plants* in 2017 [4,5]. Meanwhile, the genome sequence analysis results for the AABB genome of wild emmer wheat (*Triticum dicoccoides*), the tetraploid ancestor of wheat, were published in *Science* [6]. Most importantly, the IWGSC used flow cytometry to isolate chromosomes from common wheat “Chinese Spring” (CS) and employed second-generation sequencing and NRGene assembly technology to annotate the CS reference genome sequence chromosome by chromosome, which has been publicly released (RefSeq-v1.0) [7]. This should currently be the best chromosome-level assembly for wheat.

As of August 2018, fine genome sequence maps for hexaploid wheat and its relatives, including AA, DD, AABB, and AABBDD, have all been completed, laying the foundation for wheat functional genomics, comparative genomics, and evolutionary genomics research. These achievements are particularly significant for understanding the origin, domestication, artificial selection, and genetic and epigenetic regulatory mechanisms underlying important agronomic traits in wheat at the whole-genome level, as well as for mining and utilizing superior alleles in breeding, which is crucial for ensuring China’s food security and sustainable agricultural development.

Successful Practice of “Module Coupling Breeding” Theory

The proposal of the “molecular module coupling breeding” theory represents a theoretical innovation of the Molecular Design Breeding Priority Program. After years of dedicated effort, this theory has been validated in practice in wheat breeding with remarkable achievements.

Southwest China is one of the country’s major wheat-producing regions and the primary source of wheat stripe rust. Breeding new wheat varieties resistant to stripe rust is particularly important for preventing and controlling wheat diseases at their source. Guided by the “multi-module coupling breeding” theory, the wheat research team at the Chengdu Institute of Biology, Chinese Academy of Sciences, successfully developed the new wheat variety “Chuanyu 25” by coupling stripe rust resistance molecular modules Yr7 and Yr17, awnlessness module Xgwm291, and dwarfing module Rht-D1b, resulting in a lodging-resistant, disease-resistant, high-quality, awnless variety suitable for mechanized harvesting. By coupling the large-grain molecular module QTgw.saas-5B and stripe rust resistance module YrCH42, they developed the high-yield, disease-resistant variety “Kemai 138,” the only breakthrough new variety in Sichuan Province in nearly a decade to achieve yield increases exceeding 10% in both regional trials and production tests, and was listed as a leading wheat variety in Sichuan Province in 2016. By introducing waxy molecular modules (Wx-A1b, Wx-B1b, and Wx-D1b) and low polyphenol oxidase (PPO) molecular modules Ppo2A1b/Ppo2D1a, they developed the full-waxy specialty high-quality wheat variety “Zhongke Nuomai 1,” which achieves organic integration of multiple superior traits including high quality, high yield, and disease resistance, with broad application prospects in food processing and brewing. By 2018, these three module-based new varieties had been promoted on a cumulative area of 1.58 million mu, leading the upgrading of wheat varieties in Southwest China.

Analysis of Salt and Drought Tolerance Molecular Modules

The Bohai Rim region of China has more than 40 million mu of medium- and low-yield fields and over 10 million mu of saline-alkali wasteland, suffering from long-term drought, waterlogging, and salinity disasters. Breeding new wheat varieties with drought and saline-alkali tolerance is particularly important for increasing local agricultural production and income. In 2017, “Xiaoyan 60,” developed by IGDB, passed the examination of the Hebei Provincial Crop Variety Committee (Approval No. Jianshenmai 2016030). The Yundong region of Cangzhou (east of the canal) is an area with severe soil salinity. By 2018, the cumulative demonstration and promotion area of “Xiaoyan 60” in this region had reached 21,000 mu. By constructing a recombinant inbred line population from “Zhongmai 175” and “Xiaoyan 60,” using the wheat 55K SNP chip to construct a genetic linkage map, and combining seedling and field adult-stage salt tolerance phenotyping data, dozens of quantitative trait loci (QTLs) related to salt tolerance have been mapped. Transcriptomic analysis revealed that “Xiaoyan 60” may enhance its salt and drought tolerance by regulating photosynthesis

and jasmonic acid signaling pathways.

Whole-Genome Sequencing and Association Analysis

Existing sequencing data have shown that the hexaploid wheat genome is very similar to those of diploid and tetraploid wheat, indicating that gene loss after polyploid formation is limited. However, a characteristic of the wheat genome is its large content of repetitive sequences that are highly similar but not identical. Consequently, the precise localization and isolation of wheat genes and transcripts remain quite challenging. Currently, long-read third-generation sequencing technology is becoming increasingly common, which undoubtedly facilitates wheat genome and transcriptome sequencing. It is hoped that subsequent technological transformations and analytical method improvements can further supplement and refine the existing fine map of the common wheat genome or complete genome assembly and annotation for more wheat varieties.

In terms of wheat reference genome sequence map construction, Chinese scientists have taken the lead and built a solid platform for wheat functional genomics research in China. Moreover, with continuously decreasing sequencing costs, whole-genome resequencing and association analysis studies are expected to become important research directions in the coming years. For instance, using materials such as wheat relatives, landraces, major cultivars, and translocation lines, addition lines, substitution lines constructed through distant hybridization, as well as saturated mutant libraries for whole-genome resequencing, research can focus on the genomics, epigenomics, comparative genomics, and evolutionary genomics of complex traits in wheat and its relatives. Such studies will reveal the history of wheat origin and domestication at the whole-genome level, as well as the genetic and epigenetic mechanisms of polyploidy and diploidization, dissect the genetic regulatory networks underlying important agronomic traits, and mine and utilize superior alleles. Furthermore, research on wild germplasm resources has shifted from collection and preservation to in-depth study and utilization. Through genetic population construction or deep resequencing of multiple germplasm resources, the full utilization and mining of wild genetic resources will facilitate the improvement of existing crop varieties.

Phenotyping Platform Construction and Technological Innovation

China possesses abundant wheat germplasm resources and has constructed numerous genetic populations, with incalculable human and material resources spent on phenotyping. How to rapidly and accurately obtain phenotypic data for individual wheat plants or lines has long been a dilemma for breeders and researchers. Phenotyping platforms are emerging large-scale scientific facilities that enable germplasm resource phenotyping research and precise identification. The IGDB research team has established phenotyping platforms for rice and wheat plant architecture and panicle types using high-resolution laser/soft

X-ray imaging systems, high-precision image analysis and reconstruction, and other methods and technologies targeting three-dimensional configurations. Additionally, the use of drones equipped with high-definition cameras or infrared instruments for large-scale field crop phenotyping collection through remote sensing technology is being implemented. The promotion and application of these new technologies and methods will further advance germplasm resource utilization and variety breeding processes.

Wheat Functional Genomics Research

Currently, functional genomics has become a competitive hotspot and key development direction in life sciences. Following the completion of human, model animal, and plant genome sequencing, life sciences as a whole have entered the post-sequencing era centered on functional genomics research, with countries worldwide attaching great importance to various post-sequencing genome projects. For example, developed countries and regions in Europe and America have successively launched ENCODE projects and four-dimensional nucleome projects for multiple species. However, China remains in a tracking and catching-up stage in the field of complex polyploid genomes and their functional genomics. The advent of the polyploid wheat reference genome provides an excellent opportunity. How to draw on the successful experiences of functional genomics research in model plants *Arabidopsis* and rice, combined with the characteristics of the wheat genome itself, to develop a set of rapid map-based cloning techniques suitable for wheat mutants should be a common challenge facing wheat researchers.

Mut-Map is a mature technology for rapid gene functional localization using sequencing of segregation populations constructed from wild-type and mutant plants. Given the huge wheat genome and expensive sequencing costs, researchers can simultaneously employ multiple approaches such as transcriptome sequencing, capture sequencing, RNA-Seq, and resequencing to help achieve rapid localization of wheat functional genes. BSR-Seq technology, which integrates bulked segregant analysis (BSA) and RNA-Seq analysis, is one such method enabling rapid wheat gene localization [8].

Gene Editing Technology

Gene editing technology is a genetic engineering technique that uses artificial nucleases for targeted genome modification and represents a research hotspot in today's life sciences. The team of Gao Caixia at IGDB has been dedicated to the research and application of crop genome editing methods. In 2014, this team first used TALEN technology to knock out the wheat *MLO* gene, conferring broad-spectrum resistance to powdery mildew [9]. The CRISPR/Cas9 gene editing system has become the most widely used gene editing technology due to its convenient design and high efficiency. Subsequently, this team was the first to successfully implement single-base editing technology in the three major

crops—wheat, rice, and maize—and apply it to trait improvement [10]. Furthermore, by assembling CRISPR/Cas9 protein and gRNA into ribonucleoprotein complexes (RNPs) *in vitro* and using biolistic transformation for targeted editing, the team has successfully established a complete DNA-free genome editing system in wheat [11]. This DNA-free gene editing technology offers advantages of precision, specificity, simplicity, and low cost, and helps minimize regulatory oversight, establishing a precise, biosafe new-generation breeding technology system that accelerates the industrialization of crop genome editing breeding.

Chip Development and Assisted Breeding

For a long time, molecular-assisted breeding technology has been difficult to promote due to the limited number and annotation information of wheat functional genes. With the development of genome sequencing and gene cloning technologies, an increasing number of wheat functional genes are expected to be cloned and applied in molecular breeding practice. In recent years, the development and application of wheat SNP chips have become more widespread, with new chips developed through collaboration between multiple domestic institutions and companies. These SNP detection technologies will provide important technical support for wheat genome-wide association analysis, important gene/QTL linkage mapping, and molecular detection of breeding parents and progeny materials. It has been reported that the Illumina Infinium iSelect 90K SNP chip technology combined with BSA can be used for large-scale localization of powdery mildew resistance genes in new wheat lines or varieties [12].

Moreover, the development and application of breeding chips have greatly improved the efficiency of high-throughput screening and identification of progeny populations. Traditional conventional wheat breeding generally relies on inter-varietal hybridization, leading to loss of genetic diversity. Under the leadership of Academician Li Zhensheng, the State Key Laboratory of Plant Cell and Chromosome Engineering has long been committed to wheat distant hybridization and chromosome engineering breeding research, successfully introducing chromosome sets, chromosomes, and chromosome segments from *Thinopyrum* into wheat to develop new hybrid types such as octoploid *Triticum-Thinopyrum*, alien addition lines, alien substitution lines, and translocation lines, as well as high-yield, high-quality, broad-spectrum disease-resistant wheat varieties such as “Xiaoyan 6.” However, wheat distant hybridization breeding research has long been limited to the cytogenetics level. With the aid of genome sequencing technology and breeding chip development, it is believed that the breeding of wheat distant hybridization varieties will be accelerated.

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