# Applications and Prospects of Molecular Module Design Breeding Technology in Maize Breeding (Postprint)

**Authors:** Hao Huaiqing, Liu Lili, Yao Yuan, Feng Xue, Li Zhigang, Chao Qing, Xia Ran, Liu Hongtao, Wang Bochen, Qin Feng, Xie Qi, Jing Haichun

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## Abstract

Maize is a major food crop in China that plays a pivotal role in people's lives and the national economy. Ensuring its stable and sustained production is of great strategic significance for safeguarding national food security and serving the supply-side structural reform. Although traditional hybrid breeding technology has achieved a series of important accomplishments in maize genetic improvement, it still cannot meet the people's ever-growing needs for a better life. With the rapid development of biotechnology, molecular breeding has become an important direction and inevitable choice for maize breeding, among which molecular module design breeding integrates multiple disciplines to achieve optimized multi-module assembly at the whole-genome level, which will exert tremendous promoting effects on future maize breeding endeavors. This article reviews the developmental history of maize breeding technologies and the achievements of molecular module design breeding technology in maize breeding, and proposes prospects for future research.

## Full Text

## Topic: Molecular Module-based Crop Design

### Designer Breeding by Molecular Modules

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## Application and Prospect of Molecular Module-based Crop Design Technology in Maize Breeding

**HAO Huaiqing**[1], **LIU Lili**[1], **YAO Yuan**[2], **FENG Xue**[1], **LI Zhigang**[1], **CHAO Qing**[1], **XIA Ran**[3], **LIU Hongtao**[4], **WANG Baichen**[1], **QIN**

**Feng**[5]**, XIE Qi**[3]**, JING Haichun**[1]*

[1]Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
[2]Bureau of Major R&D Programs, Chinese Academy of Sciences, Beijing 100864, China
[3]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China
[4]Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China
[5]College of Biological Sciences, China Agricultural University, Beijing 100083, China

## Abstract

Maize is a major food crop in China that plays a decisive role in both people's livelihood and the national economy. Ensuring its sustained and stable production is of great strategic significance for safeguarding national food security and supporting supply-side structural reforms. Although traditional hybrid breeding technology has made important contributions to maize genetic improvement, it can no longer satisfy the growing demands of contemporary society for a better life. With the rapid development of biotechnology, molecular breeding has become an important direction and inevitable choice for maize breeding. Among these approaches, molecular module-based crop design technology, which integrates multiple disciplines to achieve multi-module optimization and assembly at the whole-genome level, will greatly promote the future of maize breeding in China. This article reviews the development history of maize breeding technologies, summarizes the achievements of molecular module-based breeding in maize, and discusses future research directions.

**Keywords:** maize, breeding technology, design breeding, molecular modules

## Current Status of Maize Breeding Industry

Maize is a high-yielding C4 crop that serves as a major food crop worldwide, the "king of feed," and an important raw material for industry and energy. With social progress, rising living standards, and the continuous expansion of animal husbandry, the practical value of maize in food, feed, energy, and medical applications is being continuously explored. However, given the growing world population, decreasing per capita arable land and water resources, and increasingly frequent extreme weather events, current maize production can no longer meet human demands. Simultaneously, as living standards improve, people's demand for nutritional quality in food grains has intensified, prompting the Food and Agriculture Organization of the United Nations to shift its strategic slogan from "food security" to "food and nutrition security." Consequently, ensuring improvements in both maize yield and quality has become a critical issue in current maize breeding.

China is one of the world's major maize producers, with total and per-unit-area yields second only to rice, playing a pivotal role in the nation's economy and people's livelihood. Through continuous efforts by scientists in the early period, China achieved remarkable success in maize breeding, which greatly contributed to national economic development. However, as foreign seed companies have increasingly entered the Chinese market, the competitiveness of domestic maize has declined significantly, and problems such as narrow genetic resources in original germplasm and backward breeding methods have become increasingly prominent. In recent years, various biotic and abiotic stresses, including drought, low temperature, soil infertility, and pests and diseases, have frequently occurred in some major maize-producing regions, causing large-scale yield losses. In 2016, the former Ministry of Agriculture implemented structural adjustments to maize planting areas, substantially reducing cultivation in non-advantageous regions such as the "Sickle Bend" area, with plans to further reduce planting by 50 million mu by 2020 to stabilize the total maize area at 500 million mu. With these national adjustments to crop planting structure, there is very limited room for expanding maize acreage in China. How to ensure national food security with limited resources represents a tremendous challenge for future maize breeding research in China. To continuously improve maize yield and quality, ensure national food security, satisfy people's demands for higher quality, and support new energy development, it is essential to select and breed new maize varieties with high yield, strong resistance, and superior quality through genetic improvement. This will be key to solving these problems and represents the primary direction for future maize breeding.

## Main Development History of Maize Breeding Technology

### Hybrid Breeding Technology: The Cornerstone of Maize Breeding

Hybrid breeding technology is based on sexual hybridization and utilizes heterosis for crop improvement through initial phenotypic selection and subsequent genetic recombination. This method cannot directly detect the genotype of breeding materials but instead infers genotype primarily through phenotypic traits. Although early researchers used hybrid breeding to develop a series of high-yield, high-quality, and stress-resistant varieties, the limitations of traditional breeding technology have become increasingly apparent with societal development: the breeding cycle is too long—typically requiring 7–8 years from selecting target phenotypic traits for hybridization to developing a new variety; breeding efficiency is low, often necessitating thousands of hybrid combinations to select one new variety; and phenotypic prediction of hybrid offspring is difficult. Traditional conventional breeding relies heavily on the experience of breeders, with limited understanding of the genetic basis, combining ability, and environmental influences of breeding materials, making it difficult to predict the performance of hybrid offspring. Additionally, the narrow genetic basis of maize inbred lines and the existence of reproductive isolation between species have restricted the improvement of specific materials to some extent [1-3].

### Molecular Breeding Technology: An Accelerator for Maize Breeding

Molecular breeding technology integrates biological genetics theory with conventional breeding to improve traits in target materials by utilizing genotype information, thereby cultivating new varieties that meet human needs. Early molecular breeding technologies mainly included marker-assisted breeding and transgenic breeding [4-8]. Marker-assisted breeding is based primarily on DNA polymorphism and improves traits by tagging target genes, enabling direct selection based on genotype. Transgenic technology introduces target genes into recipient materials through molecular biology methods to obtain desired biological traits in a short time. Currently, transgenic technology has played a very important role in herbicide resistance and pest resistance, with herbicide-resistance genes including phosphinothricin acetyltransferase (BAR) and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) genes, and insect-resistance genes primarily being Bt genes. Since its inception in 1987, transgenic technology has achieved great success, with some insect-resistant and herbicide-resistant varieties entering commercial promotion or production [9]. Compared with traditional hybrid breeding technology, marker-assisted breeding and transgenic breeding are considered efficient and precise methods for directional selection and improvement of target traits, capable of shortening breeding time and enhancing China's maize breeding level to some extent. However, their limitation lies in that they typically involve only single or a few genes and can only address simple agronomic traits.

### Molecular Module-based Crop Design: A New Breakthrough in Maize Breeding

With the continuous development of biotechnology, molecular design breeding has gradually become a major approach in maize breeding. This breeding concept was first proposed by Peleman [10] in 2003, and Chinese scientists have subsequently launched major projects to promote molecular design of important agronomic traits and the cultivation of new crop varieties. However, since most important agronomic traits in crops are complex traits controlled by multiple genes, and more importantly, the gene regulatory networks controlling these complex traits typically exhibit a "modular" characteristic [2,11], existing molecular breeding technologies cannot solve the challenge of genetically improving complex agronomic traits. Therefore, the novel concept of molecular module-based crop design was proposed. Molecular module-based breeding is actually a new type of molecular breeding that primarily integrates developments in genomics, molecular biology, systems biology, synthetic biology, and computational biology with maize breeding to cultivate new varieties with target traits. Molecular module-based breeding mainly includes three aspects: (1) discovery and functional characterization of molecular modules, conducting functional studies on important genes or QTLs (quantitative trait loci) that control complex traits; (2) organic coupling of discovered modules, performing theoretical simulation and functional prediction to propose optimal matching strategies; and (3) multi-module optimization and assembly at the whole-genome level to achieve directional improvement of complex traits [12]. Both theoretically and technically, molecular module-based breeding represents a new breakthrough in China's maize breeding enterprise, forming the foundation for molecular improvement of complex traits in maize and becoming the main force for new variety creation in future practice.

## Practical Application and Important Achievements of Molecular Module-based Breeding in Maize

Currently, molecular design breeding technology has become a major international trend in maize breeding. Global maize seed industry giants such as Bayer-Monsanto, DowDuPont, and Limagrain are all applying molecular design breeding to maize breeding through biotechnology means, effectively accelerating the breeding process, with R&D investments accounting for approximately 15% of their revenue. As early as 2007, Pioneer Hi-Bred International, Inc. (hereinafter referred to as "Pioneer") used maize whole-genome sequencing technology to sequence 10,000 genomes from over 600 elite maize inbred lines. Currently, both Pioneer and Monsanto possess tens of thousands of SNP markers [13]. Meanwhile, these seed companies are actively developing advanced phenotyping equipment to accurately collect and analyze field phenotypic data in maize, mining practical and usable molecular modules to provide information support for molecular design breeding. Concurrently, Chinese scientists have conducted a series of in-depth explorations in maize molecular design breeding, achieving important progress in whole-genome sequencing and SNP mining of elite inbred lines [14], functional characterization of key molecular modules controlling kernel oil content [15] and pest resistance [16], and the creation of new varieties.

In recent years, water scarcity has become a practical problem in agricultural production regions worldwide. In 2012, drought caused a 20% reduction in maize yield in the Midwestern United States. As a dryland crop, maize requires substantial water throughout its growth period, yet drought resistance in maize is a quantitative trait controlled by multiple genes with very complex genetic mechanisms. China is one of the most water-scarce countries in the world, and insufficient water availability in major maize-producing regions seriously affects maize growth and yield. Therefore, mining and characterizing molecular modules regulating water stress responses in maize and cultivating varieties with high water use efficiency are of great significance for increasing and stabilizing maize production in China. To address this goal, in 2013 the Chinese Academy of Sciences Strategic Priority Research Program (Category A) "Molecular Module-based Crop Design Innovation System" (hereinafter referred to as the "Molecular Module Pilot Program") specifically launched the project "Molecular Module Dissection for Efficient Water Use in Maize." Multiple CAS institutes conducted in-depth research from various perspectives, including seedling stage, flowering stage, root systems, leaf angle, and drought-salinity interactions, mining a batch of molecular modules with important production application value and successfully introducing some key modules into chassis varieties. The main progress is summarized below.

**Molecular Modules for Efficient Water Use at the Seedling Stage Have Been Mined and Characterized** The Qin Feng research group statistically analyzed seedling drought tolerance in 368 maize inbred lines from around the world and identified 83 genetic variation loci (resolved to 42 candidate genes) significantly associated with seedling drought resistance through genome-wide association analysis. They conducted in-depth characterization of functional variation sites and genetic effects of two mined molecular modules. The study revealed that in drought-sensitive materials, the promoter region of the ZmNAC111 gene carries an 82 bp miniature transposon insertion, which suppresses ZmNAC111 expression through small RNA-mediated DNA and histone methylation (Figure 1 [Figure 1: see original paper]). Increasing ZmNAC111 expression promotes stomatal closure under drought stress, significantly reducing stomatal conductance and transpiration rate and inducing upregulation of important water stress-responsive genes, thereby improving water use efficiency and enhancing maize drought tolerance [17]. The ZmVPP1 gene encodes a pyrophosphate hydrolase localized to the tonoplast membrane. In drought-resistant maize inbred lines, a 366 bp DNA fragment (InDel-379) is inserted in front of the ZmVPP1 gene promoter. Introducing the ZmVPP1 gene from drought-resistant materials into drought-sensitive materials can effectively improve seedling drought resistance [18]. Currently, the research group is continuing analysis of other candidate genes, and these research findings will provide extremely important genetic resources for the genetic improvement of maize drought tolerance and the cultivation of new drought-resistant maize varieties.

**Mining of Molecular Modules for Root Drought Tolerance** As the primary organ for plants to sense and absorb water, the root system is closely related to drought tolerance. Generally, a well-developed root system is more conducive to absorbing deep soil moisture under drought conditions and enhances resistance to drought stress. To deeply understand the mechanism by which root architecture changes at the adult stage contribute to maize drought tolerance and to identify molecular modules closely related to efficient water use in maize, the Xie Qi research group selected 23 inbred lines with large differences in root architecture (12 lines with few roots and 11 lines with many roots) from over 150 inbred lines for multi-year, multi-location drought experiments. They ultimately determined that an interval of approximately 6.66 Mb on chromosome 2 makes the greatest contribution to root phenotypes. Meanwhile, they have constructed backcross populations and are currently identifying backcross individuals containing the target interval and mining potential candidate molecular modules.

**Characterization and Preliminary Application of Leaf Angle Molecular Modules** Leaf angle is a major factor determining maize plant architecture and is closely related to yield and drought tolerance. The Liu Hongtao research group discovered that maize leaf angle mutants WF01 and WF02 are both caused by ectopic expression of the shoot apical meristem-specific gene RS1. Through promoter sequence analysis, they found that a 1089 bp long terminal repeat of the retrotransposon Flourf is inserted at the -758 bp position of the RS1 gene in both WF01 and WF02. Furthermore, these two molecular modules were introduced into ‘Chang 7-2’ to assemble improved versions of ‘Zhengdan 958.’ Preliminary yield testing showed that WF01 backcross-improved materials had comparable yield to ‘Zhengdan 958.’ The next step will involve continued backcrossing to purify the genetic background and increase planting density, followed by comparative analysis of the performance of improved lines with ‘Zhengdan 958’ under both normal and water stress conditions.

**Mining and Application of Molecular Modules for Drought Tolerance at the Flowering Stage** Research has shown that maize is most sensitive to drought stress before and after flowering, and even slight reductions in field water-holding capacity can seriously affect various physiological characteristics, ultimately causing yield losses [20]. To explore the molecular mechanisms of drought tolerance at the maize flowering stage and characterize molecular modules regulating water stress during reproductive growth, we conducted multi-year, multi-location drought treatment experiments on 1,100 maize inbred lines collected previously in Gansu, Xinjiang, and Inner Mongolia. We performed in-depth investigations of leaf stay-green characteristics, anthesis-silking interval, and yield-related traits, and conducted chip-based detection and GWAS analysis on 243 core germplasm resources, mining 44 genetic loci significantly associated with drought tolerance at the flowering stage. Simultaneously, combined with transcriptome sequencing analysis, we identified one core molecular module. After introducing this module into the Northeast China chassis variety ‘Jidan 27,’ leaf photosynthetic rate was significantly improved, and stay-green drought tolerance and yield stability were markedly enhanced.

The Xie Qi research group conducted comparative transcriptome analysis on leaves from extreme drought-tolerant line RIL70 and extreme sensitive line RIL93 selected from 181 recombinant inbred lines (RILs) constructed from PH4CV (drought-tolerant variety) and F9721 (drought-sensitive variety). Using the SIMM (simultaneous identification of multiple mutations) method to calculate line-specific introgression intervals, they ultimately identified two drought tolerance molecular modules [19]. Currently, drought-related molecular modules have been introduced into elite inbred lines ‘Zheng 58’ and ‘Chang 7-2.’ Using seedling saline-alkaline tolerance identification standards, the Wang Baichen research group evaluated seedling saline-alkaline tolerance in 69 maize inbred lines and identified a saline-alkaline tolerance molecular module, ZmTLP, which can explain 15% of phenotypic variation. Meanwhile, recombinant inbred lines of ‘Zheng 58’ and ‘Chang 7-2’ have been assembled for saline-alkaline tolerance screening, and related research work is ongoing.

Through the implementation of the Molecular Module Pilot Program, Chinese researchers have made progress in maize molecular module-based breeding, mining multiple molecular modules for efficient water use in maize with important application value and analyzing the mechanisms, regulatory networks, and functions of some modules. The next step requires continued in-depth exploration of the genetic networks regulating water stress responses in maize, identification of key molecular modules, mining of superior allelic variations, and establishment of associations between "molecular modules and water stress-regulated traits." Simultaneously, through multi-module computational simulation and directional design breeding technology, these key molecular modules should be optimized, coupled, and assembled to directionally improve chassis varieties, shorten breeding cycles, and achieve creation of new maize lines with efficient water use.

## Prospects for Molecular Module-based Crop Design in China

Maize is one of the most important cereal crops in China. With decreasing agricultural resources and increasing demand for high-quality maize, relying on technological innovation to vigorously develop maize breeding is imperative. Since important agronomic traits in maize, including stress resistance, yield, and quality, are all complex traits controlled by multiple genes, marker-assisted breeding and transgenic technology—which are limited to genetic improvement of single or a few genes—can no longer meet current demands for superior new varieties in maize production. The new breeding paradigm of molecular module-based crop design, which combines modern information technology with breeding practice, can aggregate multiple effective modules controlling complex agronomic traits in maize, improving breeding efficiency and providing strong theoretical and technical support for ensuring national food security. It is foreseeable that with the development of sequencing technology and big data, molecular module-based breeding will continue to play a leading role in future maize genetics and breeding. The urgent problems to be solved mainly include three aspects.

**1. Mining of Molecular Modules and Analysis of Regulatory Networks** Over the past few decades, numerous functional genes and QTL loci closely related to important agronomic traits in maize have been mined and identified, including stay-green drought tolerance, kernel quality, plant architecture, and disease resistance [21-27]. However, the molecular module networks for these important agronomic traits are not fully understood, which greatly hinders maize genetic improvement based on molecular module regulation. With the continuous development and updating of whole-genome sequencing technology, lower sequencing costs and high-throughput sequencing data have made large-scale mining of gene information controlling complex traits in maize possible—many elite maize inbred lines have been sequenced, and data in maize bioinformatics databases have increased substantially [28-30]. Meanwhile, the construction of functional genomics research platforms such as transcriptome sequencing, proteome sequencing, and gene chips, as well as maize mutant libraries, has laid a solid foundation for in-depth study of the regulatory networks of maize functional genes [31-35]. In the future, it will be necessary to utilize molecular biology, genomics, and epigenetic modification technologies to deeply explore the genetic basis and regulatory networks of complex agronomic traits in maize.

**2. Precise Detection and Utilization of High-Throughput Phenotypes** Phenotype is the result of interaction between genotype and environment. Efficient and accurate phenotypic detection and analysis are critical steps in maize production breeding and prerequisite conditions for superior variety selection and precision breeding. With improvements in sequencing technology, maize genomics, proteomics, and metabolomics have developed rapidly. However, due to the high complexity of maize phenotypes and limitations of traditional phenotyping techniques, maize phenomics research has lagged behind, preventing full utilization of a considerable portion of genomic information. Therefore, developing advanced phenotyping technologies and building high-throughput phenotyping platforms has become essential. Large enterprises and research institutions at home and abroad have successively established phenotyping platforms for various important agronomic traits under different environmental conditions, including artificial climate and field conditions, enabling automated collection of phenotypic data [36-38]. In future agricultural production, how to use advanced high-throughput phenotyping technologies and platforms to accurately collect phenotypic data information and combine it with multi-disciplinary technologies such as genomics to deeply mine molecular modules closely related to important agronomic traits in maize will be a focus of attention. This is also the theoretical foundation for achieving improvement of complex agronomic traits in maize.

**3. Interdisciplinary Integration and Fusion** As a multidisciplinary integration and intersection, molecular module-based crop design in maize is a highly comprehensive systematic project that requires combining genomics, phenomics, computational biology, systems biology, and synthetic biology to dissect, assemble, and couple molecular modules controlling complex agronomic traits and conduct molecular design and cultivation of new varieties. Notably, China's current maize breeding teams are relatively dispersed, and research lacks continuity and systematic organization. Therefore, future breeding work requires high-level resource integration, strengthened interdisciplinary cross-fertilization, and integration of common technologies to enhance the construction of maize molecular breeding platforms and improve and promote the maize molecular module-based crop design system. Utilizing molecular module-based breeding technology will undoubtedly enable comprehensive analysis of important molecular modules controlling complex agronomic traits in maize, achieve directional improvement of complex traits through multi-module coupling and assembly simulation, and cultivate new high-yield, high-quality maize lines.

China is a major agricultural country, and the sustained and stable production of maize is of great practical and strategic significance for ensuring agricultural sustainable development and national food security in China. Over the past few decades, China's maize breeding has achieved remarkable results with continuously increasing total production, but relatively low per-unit yield. Fully applying molecular module-based crop design to maize breeding represents a close integration of cutting-edge molecular biology knowledge with production breeding practice, playing an important leading role in the development of maize breeding, particularly in the genetic improvement of complex traits. Establishing an innovative system for maize molecular module-based crop design will help elevate the technical level of crop breeding in China, achieve the transformation from "experience-based breeding" to "precision breeding," and accelerate the selection process for new maize varieties in China.

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**HAO Huaiqing** is an Associate Researcher at the Institute of Botany, Chinese Academy of Sciences. Her research focuses on plant cell wall biosynthesis, membrane protein dynamics, and molecular mechanisms of crop stress resistance. She has published original research in *Plant Cell*, *Development*, *Plant Biology*, and *New Phytologist*.

**JING Haichun** is a Principal Investigator, Assistant Director, and member of the Academic Committee at the Institute of Botany, Chinese Academy of Sciences. He earned his Ph.D. from the University of Groningen in 2005 and was selected for the CAS Hundred Talents Program in 2010. His research focuses on the molecular genetics of biomass energy traits in sweet sorghum and maize.

*Corresponding author*

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