Jichun Tian · Jiansheng Chen
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Genetic Analyses of Wheat and Molecular Marker-Assisted Breeding, Volume 2

Conditional QTL Analysis and MAS

小麦的遗传解析和分子标记辅助育种（第2卷）
——条件QTL分析和MAS

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Jichun Tian
State Key Laboratory of Crop Biology
Shandong Agricultural University
Tai'an
China

Jiansheng Chen
State Key Laboratory of Crop Biology
Shandong Agricultural University
Tai'an
China

Guangfeng Chen
College of Ecology and Garden Architecture
Dezhou University
Dezhou
China

Peng Wu
College of Food Science and Engineering
Shandong Agricultural University
Tai'an
China

Han Zhang
Institute of Crop Science
Shandong Academy of Agricultural Science
Jinan
China

Yong Zhao
College of Agronomy
Hebei Agricultural University
Baoding
China

ISBN: 978-7-03-045742-4
Published by Science Press
Jointly published with Springer Science+Business Media Dordrecht

Translation from the Chinese language edition: 小麦主要性状的遗传解析及分子标记辅助育种 by 田纪春, © Science Press, Beijing 2015. All rights reserved

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解析小麦农艺
性状推动分子
育种实施

甲午年 李振声

Translation: Dissecting Wheat Agronomic Traits and Promoting Molecular Breeding Implementation.

Author: Zhensheng Li is Academician of the Chinese Academy of Sciences and receiver of the National Supreme Scientific and Technological Award.

Foreword 1

Modern biotechnologies, prevalent in nearly all aspects of crop breeding programs, have been developing so rapidly that one could anticipate new breakthroughs on a regular basis. The invention of polymerase chain reaction (PCR) in 1985 by Kary Mullis (USA), for example, has allowed us to dissect various crops' quantitative trait loci (QTLs) at the level of a single gene. According to the statistics, more than 4200 genetic maps of various crops have been constructed by the end of 2012 using various molecular markers, many of which are SSR maps. These maps have made it possible to conduct the QTL mappings and efficacy analyses on traits associated with plant morphology, yield, quality, as well as stress resistance. Molecular markers developed by QTL mappings have been utilized in marker-assisted selections (MASs), resulting in enhanced tracking efficiency of major effect genes and QTLs, thereby accelerating germplasm development and the speed of variety development. With the advent of these breeding trends, Peleman and Vander Vort (Belgium Academy of Sciences) proposed a novel breeding concept in 2003, known as "breeding by design" which will undoubtedly become a mainstream technology in crop genetic improvement and enhance the in-depth crop breeding significantly in the near future. However, wheat "molecular breeding" is currently at the conceptual phase. As an allohexaploid, the wheat genome is much larger than that of rice, corn, and many other crops. Adding to the challenges, the genome sequencing is incomplete. The lack of progress in wheat molecular breeding is, in a similar fashion, due to the fact that most of the traits responsible for wheat yield, quality, and various others are controlled by multiple QTLs. Furthermore, some issues derived from MAS per se have yet to be resolved. This is evidenced by the fact that grain weight of a specific lineage containing large grain QTL gene is not necessarily high when selection of grain weight within the hybrid progenies is based on a single or a few QTL markers. This is especially true when selection is conducted within different selection populations with diverse genetic backgrounds, resulting in significant incertitude and perplexity for the application of MAS in wheat.

Fortunately, the author of this book has conducted MSA on the basis of his extensive experience in conventional breeding and he is also well versed in the advantages of molecular breeding. The author's unique background made it possible to create a synthesis of traditional breeding and MAS breeding. Noticeably, the focal points of this book, unlike many other molecular biology monographs (published domestically and abroad) majoring in introductions of basic concepts, research tools, and/or experimental techniques, are the results of an authentic summary of constructions of various genetic maps and applications of QTL analyses and molecular markers in wheat. The author, together with the rest of his team members, has contributed to identifications of a number of main QTLs and molecular markers that are associated with yield, quality, and stress resistance, which will provide a better foundation for the MAS breeding; creations of combinations of the breeding elements containing advantageous QTL genes; maintaining or eliminating any specific F1 hybrids based on the QTL gene gathering levels and their heterosis strengths; and applications of molecular markers to track QTL genes within the F2-F6 pedigree selections. Most importantly, these findings provide practical tools and techniques for MAS breeding of many other crops including wheat. It is for these reasons that I am honored to pen the preface for this book in order to express my support, recognition, and inspiration to the author and his team members.

Xu Liu
Academician of the Chinese Academy of Engineering
Beijing, China

Foreword 2

Tackling global food security depends on our ability to develop and deliver technologies that lead to increased food production. However, due to limitations in arable land, we must achieve this increase without expanding the area under production, and in future environments where the frequency and severity of climate shocks and extremes are expected to increase as a result of climate change. Over the past few decades, we have relied on access to water and energy to drive major yield increases through expansion of irrigation schemes and large-scale use of fertilizers, but this is not an option for the next phase of productivity gains. We will be increasingly dependent on the ability of our breeders to produce varieties that show improved yield under conditions where water and nutrients are likely to be limiting.

Agriculture has a long history of major production gains through the application of new technologies. The early farmers would have selected lines that supported their production systems and improved harvesting and storage. As a result, they developed lines that showed major changes in characteristics that suited them to farming compared to their wild relatives. A key change would have been selection for plants that grew well as a crop or community. Over time, farmers developed plants that suited their environment and this led to the generation of diverse landraces that supported human development.

A flow of genetic material over regions occurred as farmers exchanged grain with their neighburs and along trading routes. This communication, trade, and sharing would have slowly expanded the germplasm available to farmers and helped spread innovation. The process of practical germplasm exchange and selection underpinned modern agriculture, but it was not until the introduction of selective breeding that we saw a dramatic acceleration in the rates of genetic improvement. The discovery of the principles of genetics laid the foundations for the rapid improvements in crops that have occurred over the past century. As knowledge of genetics and genes expanded, the rates of genetic gain in crop improvement also accelerated.

Systematic breeding is based on the use of variation to develop new gene combinations. Breeders have selected the best performing plants from crosses to continually enhance the yield and quality of our crops. The opportunity for advances in breeding outcomes is closely tied to the diversity of the variation available, the size of the populations screened, and the intensity of selection. Therefore, selective breeding is essentially a numbers game since many important crop characteristics, such as yield and drought tolerance, controlled by a very large number of genes. Consequently, the chance of finding the best or improved combinations of genes can be difficult. However, new technologies have greatly assisted breeders; for example, mechanized sowing and harvesting has allowed breeders to grow and assess thousands of genetic combinations. In addition, improvements in computing power and sophisticated statistical methods have supported the accurate assessment of new plant lines. More recently, DNA markers have allowed breeders to follow individual genes, gene variants, and genomic regions as they are passed to the progeny of crosses. Based on the DNA fingerprint, the breeders can predict many of the key characteristics of the plants when grown as a crop, such as disease resistance, quality, and even yield. Marker technology has helped create new breeding strategies and reduced the costs of monitoring genes and controlling the frequency of useful alleles in breeding populations.

To tackle global food security and enhancing crop production, it is important to address factors limiting production for our major food crops. Wheat is the world's most widely grown crop and contributes around 20 % of calories and protein for the daily human diet. To meet the predicted food demands of a world population of over 9 billion people by 2050, wheat production will need to increase by 60 % relative to 2010. This means we need to increase the rates of yield improvement from the current level of 1 % per year to at least 1.6 %. The effective application of new technologies and increased investment in wheat research and breeding will be critical if these targets are to be met. This challenge was recognized in 2011 when the G20 group of countries agreed to establish a global Wheat Initiative with the "aims to encourage and support the development of a vibrant global public–private research community sharing resources, capabilities, data and game changing ideas, and technologies to improve wheat productivity, quality, and sustainable production around the world." The Wheat Initiative currently brings together 16 countries, nine private companies, and two international research centers and continues to grow. The Wheat Initiative recently launched a Strategic Research Agenda that identified key targets and priorities for global research. These included a major focus on developing our knowledge and understanding of the genetic control of major traits affecting wheat production, stress tolerance, disease resistance, and quality. The agenda also highlighted the importance of cooperation and exchange of information and knowledge about wheat.

Most work on wheat genetics over the past few decades has focussed on germplasm and traits of relevance to the European and North America production systems. These environments have been only poorly representative of the conditions facing breeders and farmers in the world's largest wheat producing countries, such as China and India. Of these two major wheat producing countries, India has been well connected to the mainstream of germplasm development through close links with the international wheat improvement program led by CIMMYT in

Mexico. The flow of germplasm has been both to and from India with a long history of use Indian germplasm in international breeding programs. In contrast, the Chinese programs have for a long period been overlooked by the international wheat breeding community. However, there have been several important examples where germplasm sourced from China has had a major impact on the international breeding community.

These two volumes represent a new milestone in international wheat research by providing an overview of modern wheat genetic research from the perspective of the Chinese wheat research community. Importantly, the work covers trait dissection based on Chinese germplasm and covering traits of relevance to wheat production in the world's biggest wheat producing country. Through this work, we gain an insight into both the success and challenges faced by Chinese researchers and breeders. The complexity of the challenges faced in China to deal with demands for improvements in the sustainability of production under pressures to reduce inputs and in the face of climate change represent a model for strategies to tackle the global challenges. These volumes will help develop a framework for wheat genetic research and open the Chinese experience to wheat researchers from around the world.

Peter Langridge
University of Adelaide

Foreword 3

It is projected that world population will reach 9.7 billion by 2050, and the global food need will increase 40 % by 2030 and 70 % by 2050 approximately (FAO). The challenge of meeting this demand is made still more difficult by climate change, global temperature warming, and more frequent extreme weather events. To increase cereal production is one of the most important ways to keep food security.

Total cereal yields are a function of land area devoted to cereals multiplied by the yield per unit of area. This means that if cereal production is to increase, either the agricultural land area or yield per unit area will need to increase, or a combination of both. In reality, the agricultural land area is not expected to increase in the future and may even decrease with currently productive land being lost to rising sea levels, desertification, etc. Therefore, if the challenge of increasing production is to be achieved, it will come from increasing yield per unit area. However, this achievement must be not adversely affecting the environment, just as John Beddington said "The challenge for global agriculture is to grow more food on not much more land, using less water, fertilizer and pesticides than we have historically done." Therefore, breeding new crop varieties to increase yield per acre is the best way of economic efficient and environment sustainable.

Wheat (*Triticumaestivum* L.) is one of the most important food crops around the world, which provides a fifth of human calories. High stable yield and good quality are the key objectives of breeding programs, but since 1980, the rate of increase in wheat yield has slowed. According to the statistics in China, the rate of increase in wheat yield per unit area was over 7 % in the 1980s and 1990s of the last century, but has slowed to less than 3 % in this millennium. However, higher yield cultivars are still being developed in China (e.g., Shannong 20 released 2010, 11.9 t/ha^{-1}; Lankao 198 released in 2012, 12.2 t/ha^{-1}; and Yannong 999 released 2011, 12.3 t/ha^{-1}), which have contributed greatly to increase total wheat production and emphasize the importance of continued genetic improvement.

In order to meet the fast-growing demand for wheat, researchers have presented different strategies to dramatically increase wheat productivity. In the UK, the Rothamsted research team developed the "20:20 Wheat" strategic program that seeks to provide the underpinning knowledge and tools to increase the yield potential of wheat in the UK to 20 t.ha^{-1} in 20 years. This project identifies "maximizing yield potential and protecting yield potential" as central approaches. The identification of the genetic basis for relevant traits and the dissection of their interdependent relationships are critical to the realization of this goal. The rapid developing of genome sequencing, combined traditional breeding program, and molecular marker-assisted selection (MAS) increase the probability of successfully increasing yields.

However, the development of efficient molecular markers is important for molecular MAS or molecular design breeding. Therefore, quantitative trait loci (QTL) mapping for important wheat traits to capture major and stable QTLs is a key step. This book cohesively describes the developments in genetic mapping, QTL analysis, and molecular marker-assisted breeding that occurred over the past decades. In the first volume, this book introduces the core concepts and research methods of QTL; then, the authors illustrate six molecular genetics maps constructed by their group and QTL mapping for more than twenty important wheat traits, including quality, physiology, and various stress resistances. The second volume is mainly about conditional QTL mapping analyses and their applications to wheat breeding and cultivation. The authors introduced the concept and advantage of conditional QTL and illuminated their research results using the method for dissecting the temporal and spatial expressions and interrelations of some QTL. Molecular marker exploration methods and practical examples are also described in this part, which provided a good perspective on wheat breeding.

The book provides a great deal of novel information, in-depth knowledge of wheat genetics and molecular breeding, which will be extremely valuable to academics and to wheat breeders.

Prof. Martin Parry
Associate Director
Rothamsted Research, Harpenden,
Herts, AL5 2JQ, UK

Preface

Publishing a scientific research monograph not only requires extraordinary accumulation of data derived from technical endeavors that often spans a decade, but also requires the authors to invest many months or even years of writing. Writing and publishing a book offers the authors neither benefit nor satisfaction when taking into consideration the myriad of factors such as contemporary fast-paced research rhythm combined with professional title and salary promotions, research grant proposals and project evaluations, the high cost of book publications, limited number of readers, and the relatively small market. Nevertheless, there are multitudes of factors that motivated us in striving to compose and publish this book. First and foremost, there is a need for achieving breakthrough research in order to develop competitive wheat varieties. According to Li and Wan (2012), the demand for wheat production in China is projected to increase by at least 28 % by 2020. With the continuous depletion of arable land in China, the only way to meet this demand is to develop innovative varieties with high yield. It is true that the conventional breeding has made great contributions to the increased wheat production in China since 1949, and the techniques for field selections are still irreplaceable at present. However, this traditional breeding method has a number of disadvantages including selections being based only on phenotype, which inherently results in low efficiency and less superior varieties. For example, several major commercial varieties in China, such as Jimai 22, Aikang 58, Zhoumai 18, and Shanon 20, perform well within the boundaries of their plant habits and stress tolerances, but to reach another breakthrough presents entirely new sets of significant challenges. The development of super varieties with multiple beneficial traits controlled by collective elite alleles requires molecular markers to identify, track, and accumulate these superb genes, which needs the multidisciplinary knowledge (Peleman and Vander Vort 2003). Secondly, there is a need for combining molecular breeding and conventional breeding. Since the advent of modern molecular biology techniques represented by PCR, rapid developments in plant genetic diversity analysis and identification and cloning of elite genes have been made over the last three decades. The wealth of data in regard to genomics, proteomics, metabolomics, and phenotypes and numerous patents are too many to mention. It is my belief that "molecular breeding" and "molecular design breeding" are still at the stages of concept development and project applications. This is largely due to the poor combination of molecular breeding with traditional breeding. The current scientific research system is the cause of the "mismatch"—researchers on molecular breeding are mainly scattered in the confines of academic institutions and/or universities within which they do not fully understand or consider the needs of conventional breeding, while the conventional breeders who often work at local breeding stations and agricultural corporations have less interest in the "molecular design breeding" (it is "computer breeding" according to them). Furthermore, because wheat genome is characterized by its immense size and enormous complexity of QTLs, trait selections based on only one or a few molecular markers from populations with diverse backgrounds and environments are often not ideal. For example, genes with large grain gene/QTL and grain weight may not be necessarily high. Similarly, lines with disease resistant gene/QTL may be susceptible to diseases in the field. Having worked at Shandong Agricultural University for several decades, the author takes advantage of the unique situations experienced in both traditional breeding and molecular breeding and implemented the synthesis of the two breeding approaches with good results. This book publishes the summaries of my team research results and my past 16 years' research experience. Thirdly, we wish to express our gratitude for the monumental support from the national science and technology policy for many of our wheat breeding projects. Over the last decade, we have received research funds for a number of national research projects, including the State "973" program (No. 2009CB118301) for molecular improvement of high-yield wheat and development of molecular breeding elements aiming for creating super wheat high yield (supported by the Ministry of National Science and Technology); four projects (No. 30471082, 30671270, 30971764, and 31171554) supported by the Natural Science Foundation of China; two projects on wheat transformation supported by the National Development and Reform Commission; and the Mega Project on "Development and commercialization of super wheat varieties in Shandong Province." The success of these milestone projects and the wealth of research data presented in this volume are the results of the continuous support we received over the past ten years from the state and province, which allowed me and all of my team members (including all the graduated students) to focus and conduct these studies. By publishing this book, it is my intention to express my sincere thanks to the state and provincial leadership as well as all of the counterparts in China for their support and inspiration during this painstaking period of research.

Based on the foundation of the molecular biology and bioinformatics, Belgian scientist Peleman et al. (2003) recently proposed a novel breeding concept known as "breeding by design." This idea consists of three core concepts: mapping QTL-associated agronomic traits; evaluating the allelic variations at these loci; and implementing molecular design breeding. The premise of the research conducted by my laboratory over the past ten years was based on the concepts of molecular breeding and molecular design breeding. Constructive data (e.g., creations of molecular elements and molecular markers) derived from the research have been successfully applied to traditional breeding programs, enabling us to make the right cross combinations followed by good pedigree selections. This book compiles wheat molecular genetics map construction and genetic diagnosis of major wheat traits (QTL analysis). The book is divided into seven chapters. Chapters 1 and 2 mainly introduce "research progress of crop quantitative traits" and "the core concept and research methods of quantitative traits," which establish the necessary backgrounds for the contents of the subsequent chapters. Chapter 3 presents "six wheat genetic molecular maps" established by us with the details of map characteristics and their merits of applications. Chapters 4–7 discuss the following subjects: genetic analyses of QTLs associated primarily with wheat yield, quality, physiology, and stress resistance, respectively, have obtained more than 120 major QTLs of dozens of major traits and their molecular markers as well. In order to give readers a comprehensive understanding of the latest research progress, the volume presents not only the results of QTL mapping and efficacy analysis of each major QTL primarily based on our own research projects, but also, in addition, the summaries of similar projects at both home and abroad.

Introduction of the concepts and methods consists of only about 10 % of this volume, and the bulk of the content—more than 90 %—contains the summary of our research data, thereby indicating that this is not a biotechnological book with emphasis on the foundations of methodology and techniques. Rather, this book begins with establishment of molecular genetic maps, QTL analyses, followed by molecular marker-assisted breeding, thereby resulting in a science monograph with a comprehensive and in-depth research system. Ultimately, this publication is not only the collection of the findings of the emerging and ever-evolving wheat molecular marker breeding, but also the prerequisite for the implementations of the newly proposed "molecular design breeding."

The contents of this book are contributed by the members of my Wheat Quality Breeding Team stationed at the State Key Laboratory of Crop Biology, Shandong Agricultural University. Data presented in this volume are the results of several generations of wheat breeding efforts evidenced by development of a novel wheat variety (PH82-2-2) with high protein content and other superior qualities in the 1980s (awarded a 2nd Prize by the National Technology Invention); creations of seven new wheat varieties with high yield and superior quality over the past ten years, including Shannong Youmain #2 (evaluated at the provincial level in 2001 and at the state level in 2009), #3, Shannong #11 and #12 (evaluated at the provincial level in 2003, 2004, and 2005, respectively), #19 and #20 (evaluated at the state level in 2010 and 2011, respectively), and #26 (evaluated at the state level in 2014); and the comprehensive understanding of advantages and disadvantages of the conventional wheat breeding programs. The author has 36 years of career endeavors divided equally between teaching and research, with primary focus on plant physiology and biochemistry in addition to plant genetics and breeding. The fundamental knowledge of these two disciplines enabled me to successfully combine the traditional breeding with the modern molecular biology. For instance, the establishment of various genetic populations (RIL, DH, CIL, ad NL) began as early as 1998, which laid the foundation for the subsequent QTL mapping and molecular marker-assisted breeding. The rate of selected variety combinations versus cross combinations has increased from 1/1000 by traditional breeding to 1/500 by this strategy, whereas the selected lines for potential varieties from traditional breeding are only 1/1,000,000 compared to 1/10,000 using our selection system. Furthermore, land requirement for breeding studies is about 50 % less than that of a decade ago, and the cost of breeding has decreased significantly, while breeding efficiency has experienced remarkable improvement.

During my nearly 40 years of breeding experience, I have presided over a number of programs on molecular breeding and molecular design breeding at the state level. Participating in writing this book includes young faculty members, graduate students who have left the author's laboratory and are currently working across the country, and those who are currently still in their graduate programs at both Ph.D. and master levels, as well as the field technicians. Each of them provided his/her utmost effort to contribute to this publication. However, due to the rapid development of molecular biology and marker-assisted breeding technology, over time it is inevitable to identify insufficient information in this book. We hope that this volume would provide service and impart knowledge to the readers, but at the same time, we also welcome the readers to submit comments, feedbacks, or concerns.

Tai'an, China
January 2015

Jichun Tian