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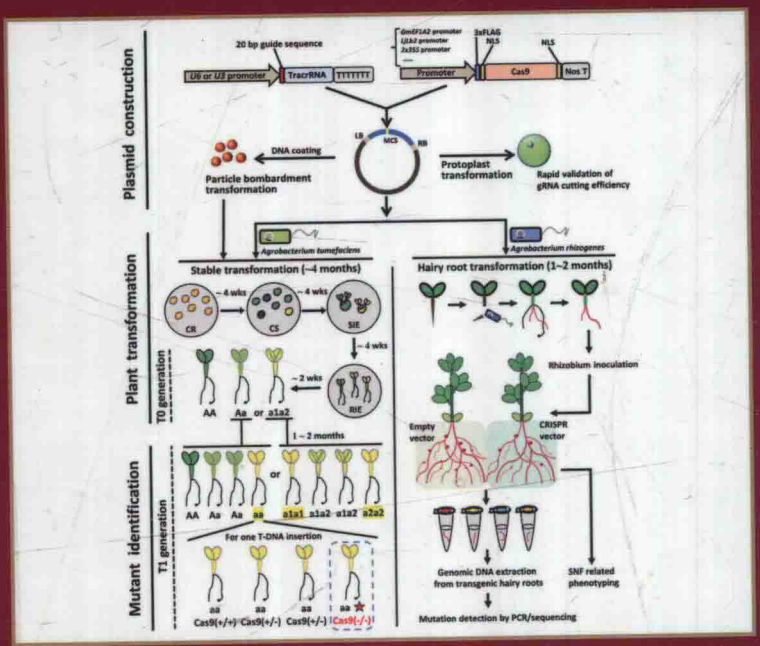
GENE EDITING IN PLANTS

EDITED BY
DONALD P. WEEKS AND BING YANG



VOLUME ONE HUNDRED AND FORTY NINE

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Gene Editing in Plants

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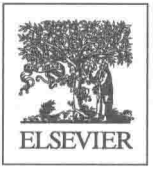

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PREFACE

Rapid development of new techniques for gene and genome editing is catalyzing phenomenal progress in basic biological research with animal, plant, and microbial systems as well as fostering practical applications in medicine, industry, and agriculture. For laboratory scientists using animals and plants for their research, there had been until recently a great envy of investigators who were using organisms (e.g., bacterial and yeast) whose genes could be precisely modified with relative ease in the laboratory. Such genetic modifications allowed a multitude of fundamental discoveries at a remarkable pace during the second half of the 20th century. These discoveries greatly expanded the knowledge of basic cell and molecular biology and also gave rise to incredible advances in many applied fields of science—including birth of the biotechnology industry. The advent of facile, but expensive, methods for inactivating or modifying specific genes in the mouse paved the way for important new understandings of mammalian development, physiology, and diseases—and, with this new knowledge, the development of improvements in medical care for humans and other animals. With the invention of zinc finger nuclease technology (and, later meganuclease technology) came the first break for researchers who were using organisms heretofore recalcitrant to gene-editing procedures. With these new tools, scientists could embark on new avenues of investigations that formerly were confined to use with organisms that could easily insert exogenous DNA sequences into their genomes using homologous recombination. Subsequent development of TALEN and CRISPR/Cas9 technologies, with their greatly improved ease of use and targeting specificities, has made available the power of gene editing to a broad spectrum of life scientists around the world.

In this book, we focus on exciting new developments in gene-editing technologies for plant biology research and their applications to plant-based production in agriculture. Chapter 1 by Baltes, Gil-Humanes, and Voytas provides an overview of the development of genome editing for use in agriculture and the opportunities and challenges that lie ahead. Zhang, Ma, Xie, and Liu (Chapter 8) provide an overview of the various gene-editing techniques available for plant biologists and recommendations regarding their use, while Davies, Kumar, and Sastry-Dent (Chapter 3) provide an in-depth discussion of zinc finger nucleases and their uses in crop improvement. Other chapters describe improvements in gene-editing techniques such as multiplex gene editing using either tRNA-processing systems

(Chapter 7 by Minkenberg, Wheatley, and Yang) or ribozyme-processing systems (Chapter 9 by He, Wang, Dai, and Zhao) for sgRNA production during multiplex gene targeting. Application of gene editing to improving plant response to abiotic stresses is addressed by Osakabe and Osakabe (Chapter 6), while efforts to develop plants with improved immunity to plant viruses are outlined by Zaidi, Tashkandi, and Mahfouz (Chapter 10). Wang, Wang, Zhou, and Duanmu describe how the CRISPR/Cas9 system can be effectively used to explore the complex systems associated with nitrogen fixation in legumes (Chapter 11). A few of the chapters focus on specific crops including maize and soybeans (Chapter 2 by Chilcoat, Liu, and Sander), rice (Chapter 5 by Bi and Yang), and the broad spectrum of polyploid plants that account for a large portion of crop production worldwide (Chapter 4 by Weeks). Finally, Wolt (Chapter 12) reviews important issues surrounding the governance and regulation of gene-edited plants and how these issues will play a pivotal role in how the technology will (or will not) benefit consumers in countries around the world.

It has been our goal in assembling this book that it will serve as a valuable source of information (and potentially inspiration) to students, scientists, and others who wish to learn about recent advances in gene-editing technologies and/or are contemplating the use of these technologies in their professional lives. We believe these technologies will no doubt revolutionize research in the plant sciences and have the potential to contribute significantly to the challenging task of feeding the rapidly increasing (and growingly affluent) world population.

We wish to acknowledge Dr. P. Michael Conn for his initial invitation to us to prepare this book and his steadfast encouragement along the way. The *Progress in Molecular Biology and Translational Science* series will serve as a concrete and continuing testament to his significant contributions to science. We sincerely thank Senior Editorial Project Manager, Ms. Helene Kabes, for her highly professional assistance during the preparation of this book and for her dedication to making the project a success. We and other authors of chapters in this book are indebted to Project Manager, Mr. James Selvam, for his efficient and conscientious final assembly and editing of our contributions. Finally, we would like to thank the outstanding authors of chapters in this book who volunteered their time and strong efforts to produce well-written and well-documented contributions that reflect the state-of-the-art science in gene editing in plants.

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CONTENTS

CHAPTER ONE

Genome Engineering and Agriculture: Opportunities and Challenges

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Abstract

In recent years, plant biotechnology has witnessed unprecedented technological change. Advances in high-throughput sequencing technologies have provided insight into the location and structure of functional elements within plant DNA. At the same time, improvements in genome engineering tools have enabled unprecedented control over genetic material. These technologies, combined with a growing understanding of plant systems biology, will irrevocably alter the way we create new crop varieties. As the first wave of genome-edited products emerge, we are just getting a glimpse of the immense opportunities the technology provides. We are also seeing its challenges and limitations. It is clear that genome editing will play an increased role in crop improvement and will help us to achieve food security in the coming decades; however, certain challenges and limitations must be overcome to realize the technology's full potential.

1. INTRODUCTION

1.1 The Role of Genome Engineering in Agriculture

As world population climbs from the current 7.3 billion to a projected 9.7 billion by 2050, there will be an increasing demand to efficiently produce and distribute food. It is predicted that food demand will increase 59%–98% by 2050,[1] which will likely necessitate rethinking current agricultural practices. This challenge—along with higher temperatures, drought, flooding, pests, and diseases—places food security at the top of the international political agenda. Alongside challenges in production, there is an increasing awareness and interest in functional foods—those that have healthier characteristics beyond basic nutrition.[2] Whereas a solution to these challenges is unlikely to come from a single technological advance, it is important to critically evaluate new technologies to determine their role in a solution.

One potential solution to improve food security and enhance food quality relates to the use of genome engineering to create new crop varieties. Genome engineering (or genome editing) can generally be defined as the targeted modification of DNA within living organisms. Due to the wide-ranging utility of modifying an organism's genome, the breadth of applications that fall under the genome engineering umbrella is enormous. Examples of such applications for agricultural purposes can range from basic biology (e.g., understanding gene function) to applied biology (e.g., altering

plant structure or characteristics to produce a useful product). In general, the common ground for most genome engineering projects is their reliance on tools that are capable of recognizing and altering a user-selected DNA sequence. This user-selected DNA sequence can include coding regions within genes to noncoding intergenic sequences; the modifications can range from single-nucleotide substitutions to large deletions or insertions. Being able to introduce a wide range of targeted DNA changes, in turn, results in a wide range of potential products, including those that could help address concerns related to food security or quality.

To apply genome engineering to produce useful agricultural products, three major questions need to be addressed: (i) what new, useful traits are to be introduced; (ii) what DNA modifications are required to generate the traits; (iii) how are these modifications physically introduced into a desired crop's genome? Unfortunately, answering these questions can be challenging, particularly for questions (ii) and (iii). For example, limited knowledge of the biology underlying certain complex plant traits (e.g., drought tolerance), and the inability to transform certain crop varieties can create significant bottlenecks when trying to generate new products. Nonetheless, significant progress has been made in applying genome engineering in agriculture. Within the last 5 years, numerous products have emerged from genome-editing platforms, including those with higher yield, drought tolerance, and improved oil characteristics. Here, we review the different types of genome modifications that can be introduced in plants and their potential cellular consequences (Section 2), the successes of applying genome editing in agriculture (Sections 3 and 4), and the current limitations and challenges within this field (Section 5).

2. GENOME EDITING IN PLANTS: POTENTIAL DNA MODIFICATIONS

2.1 Single-Nucleotide Polymorphisms

Perhaps the most subtle targeted genome edit is one that results in a single-nucleotide polymorphism. Here, the total size and organization of the crop genome remains unchanged; however, one nucleotide (out of the millions or billions within a plant's genome) is changed to a different nucleotide. Surprisingly, this subtle change can have profound impacts on cellular function. For example, if positioned appropriately within a gene, a single-nucleotide polymorphism can result in the complete inactivation of gene activity or

protein function. This so-called gene knockout can occur when a single-nucleotide polymorphism transforms a codon that normally codes for an amino acid into an early stop codon (Fig. 1B). Having an early stop codon within a gene's coding sequence results in the premature termination of protein synthesis, thereby producing a truncated protein with potentially reduced or no activity.[3–5] Notably, if there are introns downstream of an early stop codon, the mRNA can be subjected to nonsense-mediated decay—a cellular response to aberrantly processed mRNA, which results in degradation of the message.[6] Alternatively, a single-nucleotide polymorphism can destroy protein function if a nonsynonymous substitution is

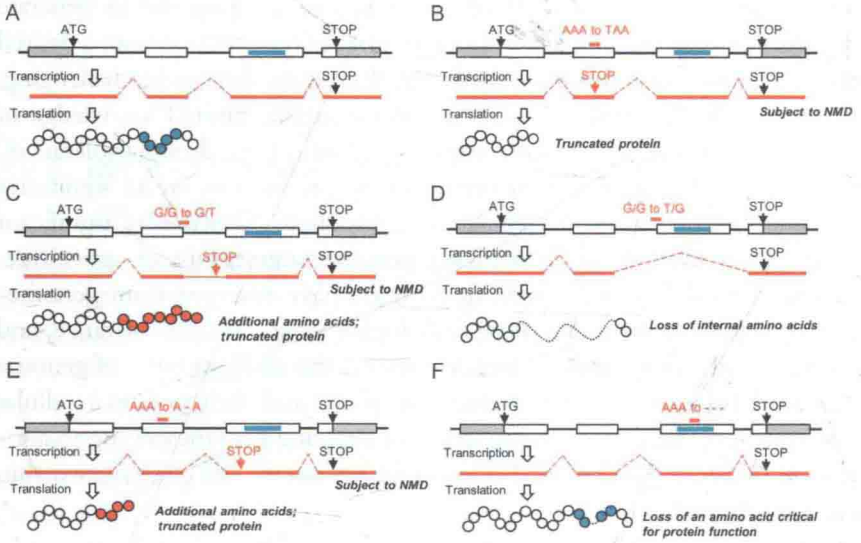

Fig. 1 Examples of genome edits that can alter gene or protein activity. (A) Illustration of transcription and translation of a "normal" gene. (B) Illustration of a single-nucleotide polymorphism leading to an early stop codon. (C) Illustration of a single-nucleotide polymorphism within the conserved 5′ splice site that can result in missplicing and inclusion of an intron. Within this example, the intron is shown to comprise an early stop codon. (D) Illustration of a single-nucleotide polymorphism within the conserved 3′ splice site that can result in missplicing and exclusion of a downstream exon. (E) Illustration of a single-nucleotide deletion that can result in a frameshift and early stop codon. (F) Illustration an inframe deletion within a site that encodes an amino acid critical for protein function. *Gray boxes*, 5′ and 3′ untranslated regions; *white boxes*, exons; *blue rectangle*, DNA region encoding amino acids critical for protein function; *red lines*, mRNA; *white circles*, amino acids; *blue circles*, amino acids critical for protein function; *red circles*, amino acids not normally encoded by the wild-type gene; *NMD*, nonsense-mediated decay; *forward slash*, intron–exon or exon–intron junction; *dash*, deleted nucleotide.