Foreword

In the following pages, some of the world's most renowned researchers take a look at the state of the art and science of introducing novel genes into plant cells and plants. The various chapters deal with a wide range of products, from genetically modified seeds and plants to commodities made by such transgenic plants, including enzymes or vaccines. One important consideration is where and how the new genes are integrated into the host plant. The donor DNA may be inserted into the plant chromosome at random places or targeted to a specific location, by recombination or by employing site-specific nucleases. A future targeting technology may employ a minichromosome, an artificial vector assembled from parts of a normal chromosome (Chapter 13). A minichromosome is actually a megavector, which will be especially attractive for the introduction of a block of genes, for example those encoding an entire biochemical pathway for production of a valuable metabolite. At the other extreme of size, free replicons such as a (modified) plant DNA viral genome might be the most useful vector for some traits. Whatever the form and location of the vector, the DNA construct itself must mimic the plant's strategy for dictating quantity, timing, and location for the encoded protein to be made. In Chapter 2, Dr. Nuccio et al. provides a wellspring of information on plant trait gene design and approaches that have worked.

This book addresses many of these issues and will be useful to the plant genetic engineer, whether student or accomplished professional. I found new ideas and information in each chapter. I skipped around as my curiosity led me, and was excited to discover how many different types of challenges plant genetic engineering has posed, and how many creative solutions have been devised. I found the book quite readable for a technical work, with a refreshing honesty about the sometimes halting progress of scientific research.

While we are on the topic of honesty, I must confess to a motive underlying my writing of this foreword. I wanted to reach you, readers of this book, with one more message. Let me begin with a brief story: When my sons were quite young, we subscribed to a journal about the environment called Ranger Rick. One month it carried a story about insect galls, describing how the mother insect uses chemical signals to stimulate growth of the plant cells into a gall at the site where she deposits her eggs. When the insect larvae hatch, the gall serves her babies as a nice source of food. By coincidence, my colleagues and I at the University of Washington had recently begun a research project on crown gall tumors, induced by *Agrobacterium* in plants. The insect gall story, aimed at children, made me think. Crown galls were known to produce new metabolites—octopine or nopaline, depending on the *Agrobacterium* strain that incited the gall. Could octopine and nopaline be baby food for *Agrobacterium*? When it was my turn to talk at our weekly research group meeting, I reported on the Ranger Rick article, and proposed that *Agrobacterium*, like the mother insect, might be producing the crown gall as a means of feeding its progeny. I can well recall the laughter and ridicule that ensued. The concept was named the Ranger Rick Hypothesis, and I was teased mercilessly about it for many months, until our competitors in France, Australia, and Belgium announced this very same concept as the "rationale of the gall" (in three languages). It became a respectable idea, eventually supported by increasing amounts of evidence.

There are several potential morals to this story, and I invite you to consider any of them that interest you. For me, the moral is that *Agrobacterium* truly was a genetic engineer before my colleagues and I ever thought of the possibility. The process that we now use to make genetically modified plants, the topic of this volume, is a natural one at core, invented first by a microbe and only refined by *Homo sapiens*. *Agrobacterium* worked out a way to transfer its desirable genes to the host plant cells, genes that caused abundant growth (the gall) and delicious (we suppose) meals for future generations. I hope that you who take a serious interest in the contents of this book will take equally seriously the need to inform the public that gene transfer is a natural and normal process. The products made by genetic modification of plants are more precise and predictable than those made by plant breeding, especially plant breeders use of wide crosses for introduction of new traits from wild relatives of crop plants.

By the year 2050, the world's population is expected to grow from its current 7 billion to 9 billion, a 30 % increase in the number of people. A distressing number of our present population is already hungry, even starving. Biotechnology alone cannot solve this problem, but it certainly has the potential to be an important part of the solution. Unless people accept foods produced through biotechnology, progress in food security will be slow. I believe that the principal risk of genetically modified crops is public perception, not the safety of the products themselves, which are thoroughly tested. If you share my view, I hope that you will not keep it a secret. Seek opportunities to speak to school children, garden clubs, church groups, or anyone who will listen. Tell them that there is nothing unnatural about gene transfer to plants by *Agrobacterium*. I believe that the success of genetically modified plant products depends upon the efforts of scientists like you and me to communicate to the public the safety and sanity of biotech plants.

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Preface

When we decided to edit a book on gene expression in plants, we realized that the most valuable contribution would be to combine reports from the biotech industry, and academic and research institutes that would focus on gene expression studies with economically important crops and related enabling technologies. Such a volume should be useful for students and researchers at all levels. Tremendous progress has been made in introducing novel genes and traits into plant genomes since the first creation of transgenic plants 30 years ago, and the first commercialization of genetically modified maize in 1996. Consequently, cultivation of biotech crops with useful traits has increased more than 100-fold from 1.7 million ha in 1996 to over 175 million ha globally in 2013. This achievement has been made possible by continued advances in understanding the basic molecular biology of regulatory sequences to modulate gene expression, enhancement of protein synthesis, and new technologies for transformation of crop plants.

In this book, authors who are experts in their fields describe current advances on commercial crops and key enabling technologies that will underpin future advances in biotechnology. They discuss state-of-the-art discoveries as well as future challenges. This book has three parts that encompass knowledge on genetically modified (GM) food crops that are currently used by consumers, those that are anticipated to reach the market place in the near future and enabling technologies that will facilitate the development of next generation GM crops. Part I focuses only on genetically modified maize and soybean (three chapters each), while Part II discusses the GM food crops rice, wheat, sorghum, vegetables, and sugarcane. Part III covers exciting recent developments in several novel enabling technologies, including gene targeting, minichromosomes, and *in planta* transient expression systems.

In the first chapter, Lu et al. provide a detailed overview of fascinating aspects of maize protein expression. This chapter reviews current understanding and future perspectives on key aspects that affect recombinant protein expression in this crop. These authors have summarized various factors that control gene expression, including promoters, subcellular targeting, and different regulatory elements, including introns, 5′ and 3′ untranslated regions (UTRs), spacers and insulators. In Chapter 2, Nuccio et al. present a detailed understanding on transgene design with plant trait gene expression cassette design. The authors characterized several native maize promoters, and used the structure of these promoters to design constructs that deliver high-level gene expression/accumulation in maize. Chapter 3 is also devoted to maize. Howard and Hood review different strategies to maximize recombinant protein expression in kernels and discuss the characteristics that make maize a popular choice for recombinant protein production. These authors also assess various factors that contribute to high-level expression of heterologous proteins, together with examples of successful approaches.

In Chapter 4, Ramachandra et al. outline the breeding and biotech approaches to improve yield in soybean. The use of transgenes to complement traditional breeding through "gene stacking" will be important to further increase soybean yield and overcome biotic and abiotic stresses. One of the most successful innovations of biotech that had a major impact on farming is the introduction of herbicide tolerance in plants. Consequently, Huang et al. in Chapter 5 discuss the details of genes/traits, which have been exploited to make plants tolerant to herbicides. Tolerance to broad-spectrum herbicides makes weed control more efficient, which greatly assists the farming community. However, the increase of resistant weeds is creating new challenges for the biotech industry. In order to address this concern, authors discuss the use of trait stacking to manage hard-to-control and resistant weeds. They also describe the development of a new herbicide trait system for dicamba tolerance. Herman and Schmidt (Chapter 6) have focused on modification of soybean seeds for their use as protein bioreactors. Soybean seeds have high protein content and are used as a protein source in animal feed. These authors present the success and limitations of different approaches to produce heterologous proteins in seeds. They describe a protein rebalancing approach that increases expression of a model protein (green fluorescent protein) from 1.5 to 8 % of the total soy seed protein.

Significant progress has been made in cereal biotechnology. Many traits have been engineered into the rice genome to protect against biotic and abiotic stress or to improve grain and nutritional quality. In Chapter 7, Nandi and Khush review strategies to increase heterologous protein expression in rice grains. These authors summarize key factors responsible for controlling expression, including regulatory sequences, translational efficiency, posttranslational modifications, and compartmentalization of foreign proteins. They also discuss strategies to down-regulate endogenous protein expression in order to boost heterologous protein accumulation. In Chapter 8, Jones summarizes current advances in wheat biotechnology, particularly methods adopted for wheat transformation. He also summarizes progress in enhancing tolerance to biotic stress and to improve quality traits such as those for breadmaking. Biotechnology plays an important role in meeting the global demand for wheat, which is anticipated to increase more than 50 % by 2050. Recent advances in sorghum biotechnology are outlined by Do and Zhang (Chapter 9), with the challenges related to the tissue culture and transformation of this crop. The biotech approaches for insect pest management in vegetable crops are featured in Chapter 10 by Sreevathsa et al. The Bt protein was tested in vegetable crops to control insect pests, with discussion of different promoters used to achieve high-level expression, conferring greater resistance against target pests. The authors also discuss other strategies, including the use of inhibitors of insect digestive enzymes, or engineering secondary metabolism of volatile communication compounds to combat pests. In recent years, there has been more biotechnology research directed to sugarcane not only for sugar production, but also for its use as biofuels. In Chapter 11, Wu discusses techniques for boosting sugar content through genetic engineering, including the expression of novel sugars.

As the opportunities of biotechnology increase, more complex tools are needed to deliver desired targets. In addition, newly acquired plant genomes' sequences provide a wealth of data that can be exploited. A key to understanding the functions of specific genes is the ability to rapidly overexpress or turn them off. Part III explores these enabling technologies. In Chapter 12, Petolino et al. describe gene targeting in plants by using Zinc Finger Nucleases (ZFNs). These authors explain how ZFNs are exploited for target mutagenesis, gene deletion and site-specific transgene integration. They also discuss other nuclease technologies, such as TALENs, meganucleases, and CRISPRs, as well as the relative advantages and limitations of these procedures. Minichromosomes combine native chromosome structural elements, like centromeres, along with transgenes for introduction into crop plants. Birchler (Chapter 13) reviews the status of "Minichromosome" technology in plants. One of the key advantages of artificial chromosomes is that multiple genes of interest could be stacked into plant genomes as a single entity without linkage to other chromosomes. Birchler also discusses both the challenges and opportunities associated with this novel technology.

Studies on gene function(s) utilizing stable transformation is time consuming and expensive. However, *in planta* transient sytems, using viral vectors developed in recent years, make it possible to study gene function by knocking down target genes or overexpression of genes of interest, although this approach has been limited to small genes (< 1.5 kb) in crop plants. There are efforts to build viral vectors, which can accommodate larger inserts. In Chapter 14, Lee et al. review various *in planta* transient expression systems for both RNAi-mediated down-regulation and over expression of target genes in monocotyledonous plants. These authors discuss the increasing use of transient *in planta* expression systems, such as virus-induced gene silencing (VIGS), virus-mediated overexpression (VOX), and cell culture-based transient approaches, as well as the advantages and disadvantages associated with each transient system. Chapter 15 by Whitham et al. presents recombinant plant viruses that are capable of carrying genetic payloads of whole genes or gene fragments that provide convenient platforms as vectors for transient gene expression and silencing in soybean. These authors focus on seven viral vector systems that have been used in this leguminous crop for VOX and/or VIGS applications. They discuss key features of the viral genomes, and future prospects to exploit viral vectors for soybean improvement.

In summary, this volume highlights a wide range of research tools, current methods, and future enabling technologies to improve crop plants to meet the ever increasing global demand for food, feed, and fuel. The editors believe that this book will be an excellent reference source for the scientific community interested in extending model plant systems into valuable applications in crop plants. We sincerely thank all the authors for their hard work and valuable contributions, and colleagues at Springer for the invitation to edit this unique contribution to the literature for the scientific community.

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