Hee-Jong Koh · Suk-Yoon Kwon
Michael Thomson *Editors*

Current Technologies in Plant Molecular Breeding

A Guide Book of Plant Molecular Breeding for Researchers

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Preface

Plant breeding, the science for plant genetic improvement, made great progress in the twentieth century with the rediscovery of Mendelian genetic principles in 1900. Most of the traditional breeding methods were established before the 1960s leading to the development of high-yielding varieties in cereal crops which brought the "Green Revolution" during the 1960s–1980s. Recent progress in biotechnology and genomics has expanded the breeders' horizon providing a molecular platform on the traditional plant breeding, which is now known as "plant molecular breeding." Under a new paradigm of plant breeding in the twenty-first century, breeders try to create new variation through the direct manipulation of target genes instead of phenotype-based trait selection. Genetic resources are extended to the unrelated species because transgenic technologies break through the sexual limit for gene transfer. In addition, selection and genetic fixation in the progeny can be performed by monitoring of genes and genomics information by which breeders can develop new varieties precisely and quickly.

Although diverse technologies for molecular breeding have been developed and applied individually for plant genetic improvement, the common use in routine breeding programs seems to be limited probably due to the complexity and incomplete understanding of the technologies. This book is intended to provide a guide for researchers or graduate students involved in plant molecular breeding by describing principles and application of recently developed technologies with actual case studies for practical use.

This book is organized in nine chapters. In Chap. 1, a brief history and perspectives of plant breeding are presented, including the directions of future development of breeding methods. In Chap. 2, the basics on genetic analysis of agronomic traits are described, including how to construct molecular maps and how to develop DNA markers. In Chap. 3, methods of detecting QTLs are illustrated, while in Chap. 4, the application of molecular markers in actual plant breeding is described in detail with case studies. In Chap. 5, genome sequencing and how to analyze the association between sequencing data and phenotype are introduced, including the epigenome and its possible application to plant breeding. In Chap. 6, genome-wide association studies are explained so that researchers can analyze the data following the manual including the introduction of software for analysis of population structure. In Chap. 7, methods for mutation screening and targeted mutagenesis are described. In Chap. 8, how to isolate the genes of interest and how to analyze the gene function are presented with case studies. In Chap. 9, the basics of gene transfer in major crops and the procedures for commercialization of GM crops are explained.

We attempted to cover most of the molecular tools applicable in plant breeding; however, due to the limitation of the book volume, we had to skip some skills that are still under development. Therefore, in this book, only key technologies which are currently used in plant breeding are mentioned. Since technologies per se are being advanced, we may add newly emerging ones with a chance given later. We hope this book would be a valuable reference for plant molecular breeders and, in addition, will become a cornerstone for the development of new technologies in plant molecular breeding for the future.

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Chapter 1
Brief History and Perspectives on Plant Breeding

Joohyun Lee, Joong Hyoun Chin, Sang Nag Ahn, and Hee-Jong Koh

Abstract Following the rediscovery of Mendel's principles of heredity in 1900, plant breeding has made tremendous progress in developing diverse methodologies to create and select variation by using genetic principles. Since the beginning of the twenty-first century, plant breeding has been systematized with state-of-the-art technologies aided by transgenic and genomics approaches. In the future, breeders will be able to assemble desirable alleles or genes into promising varieties with optimized performance using an approach that integrates scientific fields. Recent concerns about global warming, abnormal weather patterns, and unfavorable environments have pushed breeders to speed up the breeding process. In this chapter, the history of plant breeding, methods for creating variation, selection and generation advance strategies, and challenges and perspectives are briefly reviewed and discussed.

1.1 Brief History of Plant Breeding

Humans began managing wild plants in fields about 12,000 years ago; since then, plants have undergone a series of adaptive changes in production and food-associated traits, called domestication or adaptation syndromes. Early human farmers acted as

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breeders by cultivating and selecting better plants or seeds in anticipation of better performance in the next season (Hancock 2004).

Systematic breeding was not performed until 200 years ago. The existence of sex in plants was first recognized by Rudolf Camerarius in 1694. The first manual crossing was performed in 1717 by Thomas Fairchild, who developed the first artificial hybrid by crossing carnation (*Dianthus caryophyllus*) and sweet William (*Dianthus barbatus*). In the early nineteenth century, Patrick Shirreff developed new varieties of oat and wheat via selection and crossbreeding and are now regarded as the first cereal breeder. Knowledge began to accumulate on plant biology, such as cells, sexual reproduction, and chromosomes. However, because the gene concept was not formulated at that time, plant breeding was performed empirically, without a theoretical background. In 1865, Gregor Mendel published 'Experiments on plant hybridization', his genetic experiment with garden pea that is now the foundation for modern genetics and breeding. However, his hypothesis on plant genetics was not widely accepted scientifically for 40 years (Stoskopf 1993).

After Mendelian genetic law was confirmed in 1900, breeders began to develop new varieties based on these genetic principles. Despite the short history of scientific plant breeding, conventional breeding methods have dramatically improved crop yields in corn, rice, wheat, and other crops. The Food and Agriculture Organization of the United Nations (FAO) reported that in the two decades from 1965 to 1985, crop yield increased 56 % worldwide, whereas from 1985 to 2005, only a 28 % increase was recorded. The rapid yield improvements from 1965 to 1985, called the "Green Revolution", resulted from the introduction of genetically-improved varieties, treatment with fertilizers and pesticides, improved irrigation systems, and mechanization of agriculture. We are now facing new challenges to a stable food supply because of global warming, abnormal weather patterns, water shortages, increased demands on crops for bio-fuel, reduced arable land, and mounting population pressure. The global human population is expected to increase by 1 billion people every 14 years and to reach 10 billion within 25–30 years; stable food supply will require 70–100 % more crop production by then. Moreover, this goal must be achieved under unfavorable environmental conditions (Foley et al. 2011). To overcome these challenges, breeders should use all possible technologies to improve yield. Thus advanced biotechnology which can create new genetic variation, and the molecular technology for selecting superior genotypes will be essential in breeding programs to increase crop yield and provide a stable and sustainable food supply.

Plant breeding comprises two main steps: creating or expanding new variation and selecting and fixing desirable genotypes in the progeny (Fig. 1.1). Variation that meets breeders' goals should primarily exist in the germplasm. In the history of plant breeding, methods for creating useful variation, such as artificial crossing, induced mutation, and polyploidization (chromosome manipulation), were used relatively early. Once tissue-culture techniques were established, cell fusion, tissue culture, and inter-specific hybridization were added to the repertoire of methods. Recently, transgenic technology for introducing foreign genes into crops has

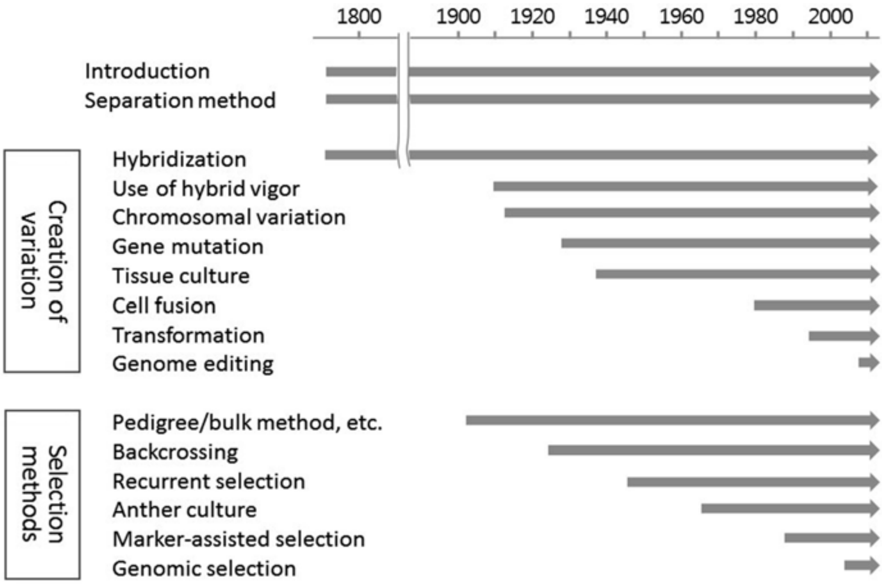

Fig. 1.1 Brief history of plant breeding methods

become available, and targeted mutation and genome editing technologies are under development for crop breeding. Lusser et al. (2011) listed the new plant breeding techniques with a focus on the creation of novel variation.

Even when useful variation exists, without an appropriate method to detect it and to select progeny that contain the target genotypes, breeding goals cannot be accomplished. Thus, the selection method is a critical determinant for successful plant breeding. Pedigree or bulk selection, backcross breeding, recurrent selection, and anther culture are methods for selecting and propagating progeny to fix a desirable trait for the next generation. Several types of molecular marker techniques are available to evaluate selection efficiency and eventually improve breeding efficiency. With accumulated genome sequence information and next-generation sequencing (NGS) techniques for high throughput sequencing, pioneering efforts for sequence-based genomics election have been initiated.

The most important milestone in plant breeding was the Green Revolution, the drastic increase in crop productivity through the development of high-yielding semi-dwarf varieties of wheat and rice. Norman Borlaug, Nobel laureate and father of the Green Revolution indicated that the main reasons for the success of these semi-dwarf varieties were wide adaptation, short plant height, high responsiveness to fertilizer, and disease resistance (1971). The International Rice Research Institute (IRRI) team developed a semi-dwarf rice variety, IR8, in 1962. IR8 had stiff straw to resist lodging and was insensitive to photoperiod, making it widely adaptable. The increase in food production led to more crops being grown per unit of land and with similar effort to that before the Green Revolution. Thus, production costs were reduced, eventually resulting in cheaper food prices at market. Also, this high