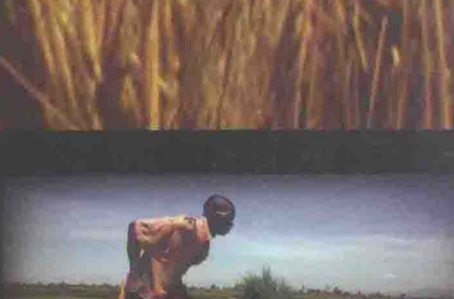
Plant Mutation Breeding and Biotechnology

Edited by Q.Y. Shu, B.P. Forster and H. Nakagawa





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Plant Breeding and Genetics Section
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
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Foreword

Up until the 20th century, spontaneous mutations were the only source of novel genetic diversity that mankind could exploit in selecting plants and animals suitable for domestication and breeding. A leap in plant breeding came when ionizing radiation was discovered to modify the genetic make-up of organisms. The pioneering work of L.J. Stadler in the late 1920s marks the beginning of plant mutation breeding, despite Stadler himself being less than optimistic about its real value. It was not until the establishment in 1964 of the Joint Food and Agricultural Organisation of the United Nations / International Atomic Energy Agency (FAO / IAEA) Division of Nuclear Techniques in Food and Agriculture, with its global coordinating and synergistic roles, that plant mutation breeding became a common tool available to plant breeders worldwide. Since these early days the Joint Division continues to play a considerable role in fostering the use of mutation techniques for crop improvement in FAO and IAEA member states. It does so by coordinating and supporting research, by promoting capacity building and technology transfer, by providing technical services and policy advice, and by collecting, analysing and disseminating information. By the end of 2009 the number of mutant varieties officially released worldwide had reached 3088, up from a mere 77 in 1964.

An early initiative of the Joint Division was the compilation of the *Mutation Breeding Manual*, published by the IAEA in 1975, with a second edition in 1977. The Manual was subsequently translated into several languages and has received wide acclaim as a reference book for plant breeders and as a text book at universities; it has played a pivotal role in educating several generations of plant breeders, including myself. But time does not stand still, and especially the past decade has seen a rapid emergence of new tools of relevance to plant breeders, including bioinformatics, genetic transformation and genomics. With these tools, and with the fast accruing knowledge of plant mutagenesis, the earlier perception of mutation induction as a random, uncontrolled process of empiric nature has also changed, and plant mutagenesis is now fully capitalizing on advances in molecular- and biotechnologies, such as Targetting Induced Local Lesions in Genomes (TILLING), and is an essential tool also in research on gene discovery and gene function. They have surely brought new vigor to plant mutation breeding, and have re-injected this discipline into the mainstream of science-based breeding.

Initially we planned to "merely" update the protocols in the earlier Manual, but quickly realized that this would neither do justice to the vast number of recent scientific and technological developments relevant to plant mutation breeding, nor would it come anywhere close to fulfilling the expectations of modern plant breeders and research scientists. In the book you are now reading we present contemporary knowledge of mutagenesis in plants, state-of-the-art technologies and methodologies and their underlying principles, and provide exemplary case studies on mutation induction, identification and utilization in plant breeding and research. I hope this book will meet your expectations and that you will find it a worthy successor to our earlier *Mutation Breeding Manual*. It is my sincere hope that it will help the global agricultural community to generate more and better crop varieties in its challenging effort to reach global food security and to minimize the currently widening gap between the rich, the poor and the famished.

Qu Liang
Director, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
International Atomic Energy Agency

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Foreword

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Qu Liang
Director, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
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Preface

Evolution and practical breeding both depend on genetic variation. Over the years since Darwin, naturalists and a diversity of scientists have learned how to create, detect and utilize mutations. The development of genomics has more recently increased the power of plant mutagenesis in crop improvement. This unique book elegantly shows how biology, physics and chemistry all interplay to provide the nexus of theory and practice. Clearly written and illustrated, the book provides an up-to-date and comprehensive manual for understanding the application of mutagenesis and its scientific basis. Many inset boxes are included in the text that aid in the explanations of the approach or summarize studies related to the point being addressed. Definitions and glossaries are included and add clarity to the discussion. A sense of history is provided through the listing of milestones in the development of the technology. Useful reference lists and websites are provided in each chapter. Comments also are included at appropriate places relative to safety considerations.

Broad in its scope, many plant species from crops to ornamentals are covered in the book as well as mutant traits such as dwarfing genes, male sterility, disease resistance, chromosome pairing, fatty acid composition and many others. Examples are given that illustrate the need for additional breeding, usually by incorporation of genetic modifier genes, to somewhat change the original mutant phenotype in order for the mutation to become a significant breeding target. The reader is informed early in the book that today's plant mutagenesis not only includes "induced mutagenesis" via the traditional physical or chemical mutagenesis procedures, but also "insertional mutagenesis" and "site-directed mutagenesis". These two latter approaches - which will become even more commonplace in the future - allow greater certainty of obtaining the desired mutant phenotype; these approaches often require considerable molecular genetic information about the trait. Zinc finger nucleases against specific sequences are given as an excellent example of the achievable increased precision. This book explains pertinent molecular genetics aspects from promoters to enhancers, and different types of mutations from insertions/deletions to frameshift mutations. The importance of DNA repair in homologous recombination and mutagenesis also is discussed in detail. Relatively new molecular genetics techniques for detecting genetic variation are changing the precision and frequency of success; TILLING, de-TILLING, and eco-TILLING are discussed as efficient means of finding genetic variation. The next-generation DNA sequencing procedures are providing another leap forward.

Many points learned by experience via plant mutagenesis studies give the reader insights that earlier researchers had to learn the hard way. The outcome of any mutation experiment depends on many factors such as the type of mutagen, dose and dose rate, genotype, growth conditions, etc. Dose and dose responses and how these differ among methods are given in several instances. Success in achieving a high frequency of mutation is a sort of balancing act between maximizing the mutagenic effect and recovering viable/fertile plants; some discussion is allotted as to how to assess plant injury. Another valuable aspect of the book relates to the complications introduced through chimerism. Several mutagenesis methods may lead to a plant where the mutation is not in every cell of the plant. Proper interpretation of the results depends on knowledge of embryo development in that species relative to cell layers and number of primordial cells at the time of treatment.

Mutagenesis techniques - even the newer ones - have resulted in many impressive mutant varieties. The number of such varieties is high across economic plant species, including vegetatively propagated species. The reading of this book provides a lucid review of the basis of mutagenesis, gives many practical tips for the efficient production of mutant types, and portends an important future for such techniques in basic and applied biology. Anyone contemplating the use of mutagenesis as an approach to improving or modifying a trait or achieving basic understanding of a pathway for a trait will find this book an essential reference.

Ronald L. Phillips
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University of Minnesota

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SECTION V – Mutations in Functional Genomics

Section 1

Concepts, Historical Development and Genetic Basis

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Plant Mutagenesis in Crop Improvement: Basic Terms and Applications

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1. Introduction

Before the turn of the 21st century, experiments in plant mutagenesis were driven by the potential use of mutants in plant improvement. During the past ten years, genomics and molecular techniques have become part of plant mutagenesis research and induced mutants have become an established resource in genomics studies. Although plant-induced mutagenesis has been used widely as a tool in basic studies and practical breeding programmes, it is seldom considered to be an independent subject by plant scientists or plant breeders. There are only a very limited number of books or other publications with comprehensive treatments of this subject, particularly its principles and technologies, which sometime leads to ambiguous concepts and misuse of scientific terms. This book describes the underlying principles of plant experimental mutagenesis, its associated enabling technologies and its application to research and plant breeding. Examples and success stories are given to illustrate the practicality of methods. In order to understand these and subsequent subjects the reader must become acquainted with the common terminology of the discipline, which is set out below.

2. Definitions of Basic Terms

2.1. Mutagenesis and Experimental Mutagenesis

Mutagenesis is the process by which the genetic information of an organism is changed in a stable manner. This happens in nature as a result of errors in DNA repair (**see Chapter 5**). Mutagenesis is the process by which mutations are generated. Mutagenesis can be exploited experimentally (experimental mutagenesis) by physical, chemical and biological means (**Box 1.1**).

The following terms are used frequently:

- **Mutation**: This was originally defined in a series of articles by de Vries (1901, 1903 and 1905) as a sudden heritable change in the genetic material not caused by recombination or segregation. De Vries used the word "sudden" to differentiate between subtle changes that could be explained by the normal processes of recombination. "Sudden" changes (mutations) in plant forms (phenotypes) were obvious, apparent and unusual and therefore of interest. Mutation however, especially at the gene sequence (genotype) level can lead to small and subtle changes in phenotype which may not become immediately apparent and these can now be detected using molecular techniques; thus the word "sudden" as applied to phenotype can be deleted from the definition.
- **Mutants**: individuals carrying a mutation that may be revealed using molecular means or identified by phenotyping tools. Different types of mutant can be generated using experimental mutagenesis (**Box 1.1**).

2.2. Mutation Genetics and Breeding

The term mutation breeding ("Mutationszüchtung") was first coined by Freisleben and Lein (1944) to refer to the deliberate induction and development of mutant lines for crop improvement. The term has also been used in a wider sense to include the exploitation of natural as well as spontaneous mutants, and in the development of any variety possessing a known mutation from whatever source. The argument is semantic as all genetic variation is *ipso facto* mutation, in the broad sense mutation breeding can be regarded simply as breeding. However, the term "mutation breeding" has become popular as it draws attention to deliberate efforts of breeders and the specific techniques they have used in creating and harnessing desired variation in developing elite breeding lines and cultivated varieties. Similarly the term mutant variety is simply a variety (var.), but draws attention to the fact that it carries an important trait controlled by a known mutant gene or it has been developed using mutation techniques (**for details see Chapter 24**). In some parts of the world the term cultivar (cv.) is used to describe a cultivated variety, for consistency, this book uses the word "variety". However it should be noted that the word "variety" is also used as a botanical taxonomic descriptor.

Although mutants can be produced using different kinds of experimental mutagenesis, mutation breeding is commonly restricted to the use of physically and chemically induced mutagenesis; other types of experimental mutagenesis are more commonly used in functional genomics studies.

Generation advancement is a key component in both genetic research and in breeding programmes. Here are some generally accepted terms in breeding. P_1, P_2, P_3, P_4,