

Biotechnologies and Genetics in Plant Mutation Breeding

VOLUME 2

Revolutionizing Plant Biology



Tariq Ahmad Bhat
Khalid Rehman Hakeem

EDITORS



CRC Press
Taylor & Francis Group

APPLE ACADEMIC PRESS

BIOTECHNOLOGIES AND GENETICS IN PLANT MUTATION BREEDING

Volume 2

Revolutionizing Plant Biology

Biotechnologies and Genetics in Plant Mutation Breeding, 3-volume set:

ISBN: 978-1-77491-134-1 (hbk)

ISBN: 978-1-77491-135-8 (pbk)

**Biotechnologies and Genetics in Plant Mutation Breeding, Volume 1:
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ISBN: 978-1-77491-136-5 (hbk)

ISBN: 978-1-77491-137-2 (pbk)

ISBN: 978-1-00330-506-4 (ebk)

**Biotechnologies and Genetics in Plant Mutation Breeding, Volume 2:
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ISBN: 978-1-77491-138-9 (hbk)

ISBN: 978-1-77491-139-6 (pbk)

ISBN: 978-1-00330-508-8 (ebk)

**Biotechnologies and Genetics in Plant Mutation Breeding, Volume 3:
Mechanisms for Genetic Manipulation of Plants**

ISBN: 978-1-77491-140-2 (hbk)

ISBN: 978-1-77491-141-9 (pbk)

ISBN: 978-1-00330-510-1 (ebk)

BIOTECHNOLOGIES AND GENETICS IN PLANT MUTATION BREEDING

Volume 2

Revolutionizing Plant Biology

Edited by

Tariq Ahmad Bhat and Khalid Rehman Hakeem



First edition published 2023

Apple Academic Press Inc.

1265 Goldenrod Circle, NE,
Palm Bay, FL 32905 USA

760 Laurentian Drive, Unit 19,
Burlington, ON L7N 0A4, CANADA

CRC Press

6000 Broken Sound Parkway NW,
Suite 300, Boca Raton, FL 33487-2742 USA

4 Park Square, Milton Park,
Abingdon, Oxon, OX14 4RN UK

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Apple Academic Press exclusively co-publishes with CRC Press, an imprint of Taylor & Francis Group, LLC

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Library and Archives Canada Cataloguing in Publication

Title: Biotechnologies and genetics in plant mutation breeding / edited by Tariq Ahmad Bhat and Khalid Rehman Hakeem.

Names: Bhat, Tariq Ahmad, editor. | Hakeem, Khalid Rehman, editor.

Description: First edition. | Includes bibliographical references and indexes. | Contents: Volume II. Revolutionizing plant biology

Identifiers: Canadiana (print) 20220482055 | Canadiana (ebook) 20220482098 | ISBN 9781774911341 (set ; hardcover) | ISBN 9781774911358 (set ; softcover) | ISBN 9781774911389 (v. 2 ; hardcover) | ISBN 9781774911396 (v. 2 ; softcover) | ISBN 9781003305088 (v. 2 ; ebook)

Subjects: LCSH: Plant mutation breeding. | LCSH: Mutagenesis. | LCSH: Plant biotechnology. | LCSH: Plant genetics. | LCSH: Crop improvement.

Classification: LCC SB123 .B56 2023 | DDC 631.5/3—dc23

Library of Congress Cataloging-in-Publication Data

CIP data on file with US Library of Congress

ISBN: 978-1-77491-138-9 (hbk)

ISBN: 978-1-77491-139-6 (pbk)

ISBN: 978-1-00330-508-8 (ebk)

About the Editors



Dr. Tariq Ahmad Bhat, PhD, is serving as lecturer in botany in the Department of Education, Govt. of Jammu and Kashmir, India. He has teaching experience of 16 years. He is engaged in active research of molecular biology, cell biology, mutation breeding, and genetic improvement of legumes and medicinal plants. He has published five international books and over 100 research papers, review articles, and book chapters. Dr. Bhat has participated in 50 conferences and workshops. He is a life member of many scientific bodies across the world. He received

Best Innovative Science Teacher Award 2014 from the Govt. of India to recognize his meritorious and outstanding performance in the field of teaching and research. Dr. Bhat received his MSc and PhD from Aligarh Muslim University, Aligarh, India.



Dr. Khalid Rehman Hakeem, PhD, is Professor at King Abdulaziz University, Jeddah, Saudi Arabia. After completing his doctorate (botany, specialization in plant eco-physiology and molecular biology) from Jamia Hamdard, New Delhi, India, he worked as a lecturer at the University of Kashmir, Srinagar, India, at Universiti Putra Malaysia, Selangor, Malaysia, where he was a Postdoctorate Fellow and Fellow Researcher (Associate Professor) for several years.

Dr. Hakeem has more than 10 years of teaching and research experience in plant eco-physiology, biotechnology and molecular biology, medicinal plant research, plant-microbe-soil interactions, as well as in environmental studies. He is the recipient of several fellowships at both national and international levels. He has served as a visiting scientist at Jinan University, Guangzhou, China. Currently, he is involved with several international research projects with various government organizations. To date, Dr. Hakeem has authored and edited more than 80 books with

international publishers. He also has to his credit more than 170 research publications in peer-reviewed international journals and 65 book chapters in edited volumes. At present, Dr. Hakeem serves as an editorial board member and reviewer for several high-impact international scientific journals. He is included in the advisory board of Cambridge Scholars Publishing, UK. He is also the fellow Royal Society of Biology, London, fellow of the Plantae Group of the American Society of Plant Biologists, member of the World Academy of Sciences, member of the International Society for Development and Sustainability, Japan, and member of the Asian Federation of Biotechnology, Korea.

Dedication

This book is dedicated to



Abu Da'ud Sulayman b. Hassan

Abu Da'ud Sulayman b. Hassan, known as Ibn Juljul, was the most famous Muslim herbalist. He studied grammar and tradition from the age of 10. He began the study of medicine at the age of 15, in which he became very skilful. He was the personal physician to Al-Mu'ayyad Billah Hisham, Caliph from 977 to 1009 CE, during whose reign he wrote most of his medical works.



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Abbreviations

AFLP	amplified fragment length polymorphism
AP	adaptor primer
APX	ascorbate peroxidase
BER	base excision repair
BIR	break-induced replication
BYDV	barley yellow dwarf virus
CAPS	cleaved amplified polymorphic sequence
DGGE	denaturing gradient gel electrophoresis
DDR	DNA damage response
DES	diethyl sulphate
DH	double haploids
DSB	double-stranded break
EI	ethylene imines
EMS	ethyl methane sulphonate
ENU	ethylnitrosourea
FDR	first division restitution
FISH	fluorescence in situ hybridization
GEFs	genetically engineered foods
GISH	genomic in situ hybridization
GM	genetically modified
GMOs	genetically modified organisms
GSP	gene-specific primers
HJs	Holliday junctions
HR	homologous recombination
HRM	high-resolution melting
IAEA	International Atomic Energy Agency
IR	ionizing radiation
ISSR	inter simple sequence repeats
MAS	marker-assisted selection
MMEJ	microhomology-mediated end joining
MMR	mismatch repair
MMS	methyl methanesulfonate
NGS	next-generation sequencing
NHEJ	nonhomologous end joining

NER	nucleotide excision repair
PAC	plant artificial chromosomes
PBGL	plant breeding and genetics laboratory
PCR	polymerase chain reaction
PEG	polyethylene glycol
PFT	pore-forming toxin-like
RAPD	random amplified polymorphic DNA
RFC	replication factor C
RFC	replication fork complex
RFLP	restriction fragment length polymorphism
RNAi	RNA interference
SARP	sequence-related amplified polymorphism
SDR	second division restitutions
SNP	single nucleotide polymorphism
SSBs	single-strand breaks
SSCP	single-strand conformation polymorphism
SSBR	single-strand break repair
SSR	simple sequence repeat
SSII	soluble starch II
STS	sequence tagged site
TCA	tricarboxylic acid
TCR	transcription-coupled repair
UV	ultraviolet

Foreword

Perceived as a fact, the human population along with their demand for food is on the rise. This paradigm, coupled with the depletion of natural resources and the global climate change, present contemporary challenges to the world food security. Over the years, plant breeders have contributed to enhancing food supply using limited traditional tools. With the emergence of biotechnology, breeders are provided with innovative tools to achieve their goals rapidly and efficiently. Recent years have witnessed the development of in vitro methodologies including mutagenesis, molecular techniques, and genetic engineering, which have revolutionized crop genetic improvement qualitatively and quantitatively.

This book, *Biotechnologies and Genetics in Plant Mutation Breeding, Volume 2: Revolutionizing Food Science*, consists of 12 chapters focusing on biotechnological approaches for crop improvement with emphasis on induced mutation technology. The book is an indispensable source for researchers, teachers, and graduate and postgraduate students interested in mutation breeding and genetic engineering. It introduces readers to contemporary knowledge and state-of-the-art technologies in the field of mutation breeding including fundamental mechanisms and applications.

Concepts discussed in this book are mutation induction techniques, applications of mutation breeding, genetic engineering techniques, molecular markers, genetically modified foods, and genotypic applications, haploid mutations, and DNA repair mechanisms.

The editors of this volume, Dr. Tariq Ahmad Bhat, Department of Education, Government of Jammu and Kashmir, India, and Dr. Khalid ur Rehman Hakeem, King Abdul Aziz University, Saudi Arabia, are internationally known book authors and editors. Their research experience in plant biotechnology, molecular biology, and mutagenesis, in particular, attested by their extensive list of publications, makes them an excellent choice to edit the present book. I am honored to be acquainted with the scientific contribution of the editors and for the privilege of collaboration. Their ceaseless efforts in collecting, reviewing, and editing the manuscripts have brought about this valuable reference book, which, I believe, will contribute to breeder's constant striving for further crop improvement to alleviate world food shortage. I anticipate great success for this book and tremendous benefits to the readers.

In nutshell, I extend my cordial thanks and praise to all authors who have contributed their valuable knowledge and expertise in the form of book chapters. It is my pleasure to applaud the editors Dr. Tariq Ahmad Bhat and Prof. Khalid Rehman Hakeem who have put great efforts for compilation and provision of scientific manuscript in the form of book, which will be very productive and useful for the future necessities of the youth and industrialists. It will provide new directions and avenues for enhancement of food security and food quality by using latest techniques of “mutation as breeding approach.”

—**Prof. Jameel M. Al-Khayri**
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Preface

The authors take great pleasure in presenting to readers this enlarged and extensive book on the applications of biotechnologies and genetics in plant mutation breeding. The Volume 2 deals with the use of mutagenesis and biotechnology to explore the variability of mutant genes for crop improvement. This volume contains chapters highlighting the importance of mutagenesis in inducing salt resistance, heat resistance, and draught resistance. The chapters are supported by authentic data of FAO and other international agencies, highlighting the role of mutagenesis in revolutionizing crop science. There is emphasis on improvement of agronomic characters by manipulating the genotype of plant species, resulting in an increase in productivity to combat the hunger in world by induced mutagenesis.

The book is a valuable asset to all the stakeholders, that is, students of biotechnology and genetic engineering. This book is focused on techniques that promise to hunt down hunger and feed humans by combating food scarcity both in quality and quantity in the world. The book has a good market as it is needed to address students and teachers of botany, agriculture, biotechnologies, genetic engineering, and horticulture.

In presenting this book, we wish to express our gratitude to Prof. A. H. Khan and Prof. Samiullah, Department of Botany, Aligarh Muslim University, Aligarh, without whose help and encouragement we would have never become students of cytogenetics, biotechnology, and mutation breeding. We are thankful to the authors who contributed chapters for this book, which is the outcome of their decades of research work. Many of the ideas in the book are the outcomes of teacher training programs, conferences, workshops, and seminars. We wish to express my gratefulness to all teachers and researchers associated with these programs for their suggestions and advice. We are also grateful to our students whom we taught all these years because it is through teaching them in the classroom that we learned much that we know.

We are thankful to the technical editors and board of Apple Academic Press, CRC Press (Taylor and Francis Group) for their wholehearted cooperation and sympathetic assistance whenever it was required.

Finally, we wish to acknowledge a debt to our families whom we left waiting on several evenings, nights, and holidays while we were busy finalizing the manuscript or the illustrations for this book.

—*Editors:*

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CHAPTER 1

Gamma Irradiation

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ABSTRACT

Irradiation technology is in use for developing useful characters or to modify existing characters. Although many irradiations are in use for this purpose but the most used and considered safe is gamma irradiation that is capable of breaking large molecules by hydrolyzing chemical bonds present in the molecule. The morphological, functional, and structural changes in a plant can be caused by using different intensity of irradiation and many countries around the world are using gamma irradiation by developing specific structures like Gamma phytotron and Gamma house. In this chapter, we discussed mechanism and applications of gamma irradiation.

1.1 INTRODUCTION

Ionic irradiation, functional modification agents in agriculture research are continuous to receive attention (Bhat et al., 2005a,c; Gulfishan et al., 2013; Ganai et al., 2005; Piri et al., 2011). These irradiations are considered as one of the natural polysaccharide modification method (Bhat et al., 2005c, 2007a,b,c,d ; Wani et al., 2005; Khan et al., 2005a; Parveen et al., 2006;

Gautam, et al., 2014). The other physical modification methods include UV light (Alves et al., 2011), hydrothermal treatment (Iliev et al., 2010), microwave, and ultra-high hydrostatic pressure (Lee, 2010), but irradiation method is fast, convenient, and has a property to penetrate easily through polysaccharides quicker than other methods (Bhat et al., 2006a,b,c,d, 2007a,b,c; Khan et al., 2005b, 2015; Parveen et al., 2007).

Use of ionic irradiation in agriculture is used to solve many agriculture problems such as control or eradication of insects and pests, reduction of food-born microbes (fungi, bacteria) (Alotaibi and Khan), suppressing postharvest sprouting and contamination (Piri et al., 2011), in developing disease resistant and high yielding crop varieties by mutation using irradiation method (Sharma et al., 2009; Gulfishan et al., 2010, 2011; Bhat et al., 2007a,b,c,d; Raafat, et al., 2012). Mutation (natural or induced) is considered as source of genetic variations in plants and animals (Bhat et al., 2005b, 2007b; Gulfishan et al., 2012, 2015; Springer and Schmitz, 2017). The history of mutation in plants can be traced back in China around 2318 years ago recorded in cereal crops (Razzaq et al., 2019). Gamma irradiation-induced mutation has been effectively used on bud wood, legumes, and citrus plants for the creation of new varieties with improved traits that affect flowering time and fruit ripening, resistant to pathogens, self thinning, fruit color, and self-compatibility (Bhat et al., 2006b, 2017; Bhat and Wani (2017); Wani and Bhat (2017); Barlass and Skene, 2012; Montañola, et al., 2015; Piri et al., 2011).

1.2 SOURCE AND MECHANISM OF GAMMA IRRADIATION

Some of the natural source of gamma rays are ^{40}K (Potassium -40) (El-Batal et al., 2018), ^{60}Co (Cobalt-60) (Obaid et al., 2018), and ^{137}Cs (Cesium-137) (AL-Dhuhaibat, 2015). ^{60}Co and ^{137}Cs are in use for inducing mutation in plants due to their physical parameters like long half-life (Azhar and Ahsanulkhalqin, 2014; Chikelu Mba, 2013). The other physical parameters that are considered are amount required for irradiation as between ^{60}Co and ^{137}Cs , 13 times more ^{137}Cs is needed to produce same activity as ^{60}Co , 0.88 mg of ^{60}Co , and 11.5 both generate 1 curie (Ci) activity (Çelik and Atak, 2017). Similarly, four times more ^{137}Cs is required to produce same dose as produced by ^{60}Co , but Cobalt-60 decay six times faster than Cesium-137 (Tomlekova et al., 2014).

Gamma irradiation facilities around the world are supported by IAEA (International Atomic Energy Agency) (Foulon et al., 2020; Varde et al., 2011), which cooperates support in member states like Asia, Africa, South America, Central America, and Europe (Zaknun et al., 2013). On the basis of mutation induction gamma instruments are classified into two types: acute and chronic irradiation facilities (Foulon et al., 2020). To produce acute irradiation plant parts (propagules) are exposed once only using gamma cells under properly constructed laboratory conditions (Horn and Shimelis, 2013). To produce chronic irradiation plant material are exposed for a prolonged time either by placing a gamma irradiation source in a greenhouse or by placing plant in a chamber or field under controlled conditions (Alvarez and Migliori).

1.2.1 DIFFERENT ADAPTATIONS OF GAMMA SOURCES FOR RADIATION

1.2.1.1 GAMMA PHYTOTRON

A completely closed chamber with controlled environmental conditions for plant growth and chronic irradiation using gamma source is known as Gamma phytotron (Kang et al., 2010; C Mba and Shu, 2012). These chambers use ^{60}Co for ionizing with about 400 Ci radiation activity. Some of the advanced gamma phytotron were built in Japan, Malaysia, Thailand, and Korea (ARTI) (Ibrahim et al., 2018; Joint, 2010a; C Mba and Shu, 2012). These sources are stored 1 m under the ground and controlled using remote to irradiate plant time to time. Phytotrons have housing facility for cultivation of plants from callus to potted plants (Joint, 2010b). The growth conditions like temperature (15–30°C), humidity (50–80%), and light (<30,000 lux) are maintained as per the requirements of target plants (Kumar et al., 2017; Ranga and Kumar, 2020). Different plants including rice, tobacco, and Arabidopsis and many other ornamental plants. The plant callus were exposed to low grad γ irradiation (Downs, 2012; Pugliese et al., 2013; Yudaev et al., 2019).

1.2.1.2 GAMMA HOUSE

A big glass house that use sunlight as source of light. Those γ houses are made of concentric circles covered with translucent polycarbonate roof and an irradiation area of 30 m inside, which have double capsulated with 800 Ci

RSL6050 ^{137}Cs pencil source (C Mba and Shu, 2012; Serizawa et al., 2010; M. Zhang et al., 2010). The source pencil is covered with skyshine hood that protects the source from elements during diffusion of radiation into air. In 30 m irradiation area, security system consists of interlocking system and automatically control exposure of the source and if prerequisite fail then this system automatically return source to safe storage (Diouf et al., 2010; Lanh et al., 2014). Similarly there is an automated system fitted with maze that is fitted with override system, which allow a trapped person inside to break maze and broken system causes source to return to safe storage (Haris et al., 2013; Pestanana et al., 2011).

1.3 USES OF GAMMA IRRADIATION FOR IN VITRO MUTATION INDUCTION AND SELECTION

Mutation can occurs spontaneously or it can be artificially induced (Tanaka et al., 2010). To induce mutation artificially there is need of mutagens like, gamma rays, X-rays, chemical mutagens, and neutrons (Beyaz and Yildiz, 2017; C Mba et al., 2010). The mutagens are broadly classified as physical and chemical mutagens. Physical mutagens include radiations: X-rays, and Gamma rays that are used more often in comparison to chemical mutagens such as ethyl methanesulfonate (Li et al., 2019). Gamma rays as mutagen are more in use in comparison to X-rays as γ -rays are ionizing radiations, which can produce mutation in seed, tissue culture calli, and many other plant parts (Guo et al., 2019; Jain, 2010; Sharma and Singh, 2013) (Fig. 1.1).

Many studies in early 20th century showed that use of radioactive rays increases the frequency of genetic variants in plants and it became a tool for developing new germplasm (Belaj et al., 2018; Henry, 2014). Using radioactive radiation help to develop plant genotype, which is more tolerant to adverse environmental conditions like; high salinity, drought, environmental stress, and high temperature withstanding capacity (Laucou et al., 2011). Plants with both biotic and abiotic tolerance can be obtained with many other desired traits from the media with selected agents (Rai et al., 2011; Sreedharan et al., 2013). In vitro techniques are easy to apply, reliable, and also save time, money, and space in comparison to selection procedures with need greenhouse or fields. *Onobrychis viciifolia* scop. was mutated using irradiation and its *in vitro* selection helped in increasing growth and production of forage legum species widespread in comparison to other species (Beyaz and Yildiz, 2017; Mohajer et al., 2014). The irradiation

was applied to seeds to 0, 400, 500, and 600 Gy using ^{60}Co in SANAEM (Saraykoy Nuclear Research and Training Center), Turkey (Arami et al., 2013; Mehboob et al., 2020). Both treated and nontreated seeds were then grown on MS (Murashige and Skoog) medium with 150 mM NaCl (Beyaz, 2020). The germination was allowed under controlled conditions like temperature (20–21°C), under white fluorescent light, and up to 10 days of

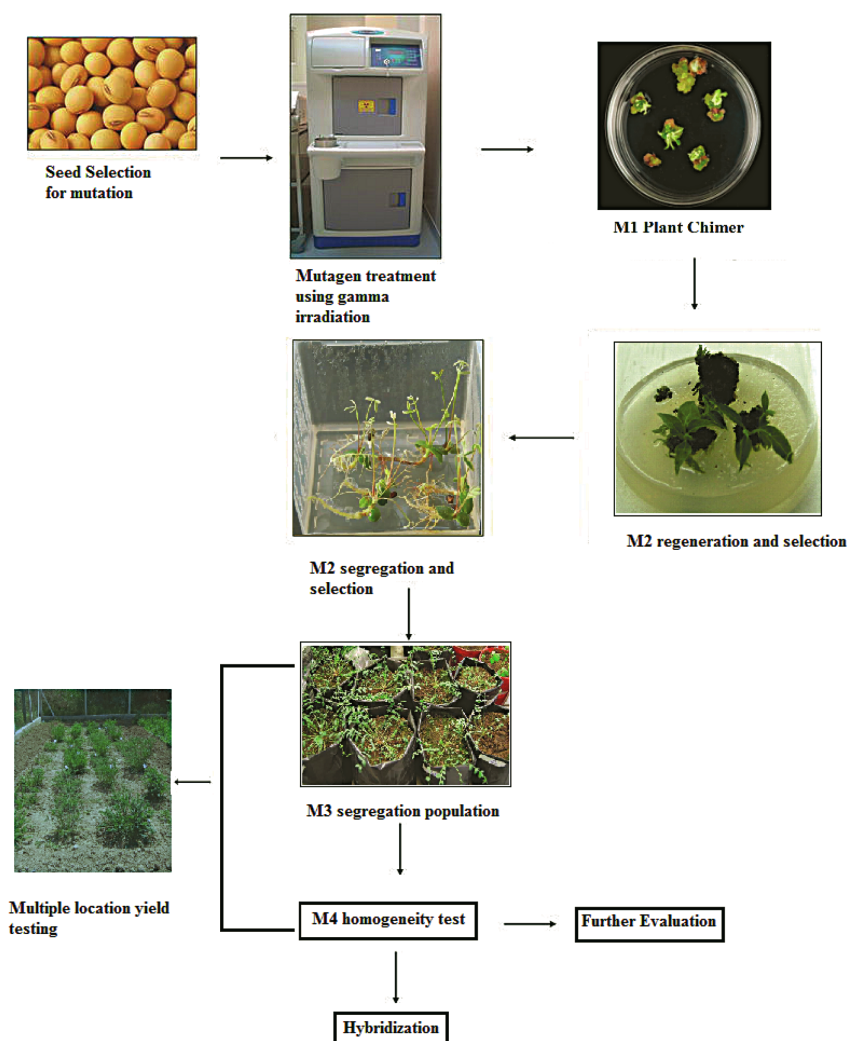


FIGURE 1.1 Method of mutation using gamma irradiation and selection.

illumination period (Arami et al., 2013; Sanam'ian, 2003). Plantlets were then transferred to MS medium without NaCl for more 2 weeks time, then advanced plantlets were transferred to plastic glasses and then to freezer bags, which were then transferred to growth chamber and freezer bags mouth slowly opens in a weeks time (Beyaz, 2020; Beyaz et al., 2016). These plants were watered with 30 mL tap water containing 150 mM NaCl, 1 time in a week for 2 weeks. During this treatment, most of the plantlets dies and the remaining ones were transferred to soil with NaCl. Plantlets that developed salt tolerance are then transferred to growth chambers and then to fields (Sagel, 2017; Yue and Zou, 2012).

TABLE 1.1 Different Mutated Crop Plants with Improved Quality and Nutritional Traits.

Type of crop	Mutant varieties characters	Irradiation	References
Rice	High-resistant starch in rice (RS) preferred by diabetes patients, Giant embryos (containing more plant oils), low amylose content, low protein content (for special dietary needs).	γ -irradiation	(El-Degwy, 2013; Li et al., 2019; Sato et al., 2006)
Barley	Phytate (antinutrient)		(Kurowska et al., 2012)
Soybean	Improved Oil quality, mprovement of protein quality, Oilseed meals low in phytic acid desirable in poultry and swine feed.		(Alikamanoglu et al., 2011; Hanafiah et al., 2010)
Peanut	Oil quality improvement, Dark green obovate leaf pod; increased seed size, higher yield, moderately resistant to diseases, increased oil and protein content		(Benslimani and Khelifi, 2009; Shirasawa et al., 2012; Songsri et al., 2011)
Cassava	High-amylose content preferred by diabetes patients because it lowers the insulin level, which prevents quick spikes in glucose contents		(Ceballos et al., 2008; Kangarasu et al., 2014; Owoseni et al., 2006)

Many researchers studied the application of 20–40 Gy on different species of plants like mandarin and pummelo to develop seedless species (Sutarto et al., 2009). In 1999, Yoshioka et al. reported effect of gamma irradiation in developing black spot disease (caused by *Alternaria alternata*) resistant mutants of Japanese pear, which is one of the most serious disease causing big loss in this plant species (Yoshioka et al., 1999). Khalil et al. successfullly developed sparse seeded species of *Citrus reticulata* Blanco

using gamma irradiation (M. Hussain et al., 2018; Kazmi et al., 2015). To develop mutant species the dormant bud scions of *Citrus reticulata* sp. were treated using 20 Gy gamma irradiation dose, which gave the best result (Hartini, 2016). These irradiated bud scions were then grafted on rough lemon rootstock using side graft method. In another research on seeded *Citrus reticulata* Blanco (“Monreal” or clementine mandarin) by Russo et al., using gamma irradiation developed less seeded fruit species (Del Bosco et al., 2007). During the regeneration process early flowering putative mutant (Joint, 2018; Lagoda, 2009), whose micropopagated vegetative progeny were sent to different countries like South Africa, Malaysia, Honduras, and Australia (Rowe and Rosales, 1993). A dwarf mutant species of papaya was developed by Ram et al., by treating seeds with 15 Kr gamma irradiation (Fermin et al., 2010; Nhat Hang and Chau, 2008; Singh et al., 2008).

1.3.1 GAMMA IRRADIATION FOR FOOD PROTECTION AND STERILITY

A process to ionize food by exposure to ionizing irradiation is known as food irradiation and its use to protect food items from food born pathogens dates back to 20th century in U.S.A., when patents were issued for using ionizing irradiations including gamma irradiation to kill bacteria present in food (Molins, 2001). To facilitate the use of irradiation, U.S.A. did amendments in drug regulations to allow use of irradiation such as X-rays, gamma rays, and electron beam on certain food items for a specific time duration for controlling food-born pathogens (Kume et al., 2009; Thakur and Singh, 1994). With time now 60 countries around the world accepted the use of irradiation of food items to prevent them from damage caused by insects, worms, and microbes including control of sprouting in stored onion and potato (Thayer, 1990; Wilkinson, 1997). As per the joint expert committee of FAO, IAEA, and WHO food irradiation upto 10 kGy is considered safe as this dose do not produce any toxicological, nutritional, and microbial problem in exposed food items (Akram and Kwon, 2010; Organization, 1999). Use of gamma irradiation between 30 and 1000 Gy was found to produce delay in fruit rippling in some fruits and vegetables (Calado, Venâncio, and Abrunhosa, 2014; Piri et al., 2011). The exposure of bread flour to gamma irradiation of 150 Gy can control the growth of mold in bread upto 20 weeks (Enu and Enu, 2014; Piri et al., 2011). Study by Bansa and Appiah reported gamma irradiation of 120 Gy effective in inhibiting sprouting in yams upto 6 months and high doses of 2–4 kGy can reduce rate of infection in beet seeds (Baba, 2015;

Park et al., 2020). Food sterilization at medium (1–10 kGy) and high doses (10–65 kGy) are becoming a choice for immunologically depressed patients as these food items can be easily stored at room temperature and the starch content of this food can be modified with irradiation and physicochemical analysis method (Alexander et al., 2010; Carlson et al., 2011; Mattison et al., 2014; Ugidos-Rodríguez et al., 2018; Vaz Patto et al., 2015).

TABLE 1.2 Improvements in Fruit Crops Using Gamma Irradiation.

Fruit crop	Irradiation type	LD₅₀ dose	Trait improved	References
Apple	Gamma rays	10–20	increase in fruit size, resistant from rust, different fruit color	(Atay et al., 2019; Sasaki et al., 2018; Sharafi and Motallebi-Azar, 2011)
Banana	Gamma rays	30–35	Early fruiting and big bunch size	(Hirimburegama et al., 2012; Pestanana et al., 2011)
Strawberry	Gamma rays	50–100	Fruit size and taste	(Hussain et al., 2012; Murti et al., 2013)
Kiwi	Gamma rays	40–60	Seedless and early production	(Liu et al., 2019; Pathirana et al., 2014)
Plum	Gamma rays	30	Early production	(Aros et al., 2012; Rayan et al.)

1.4 GAMMA IRRADIATION, ROS (REACTIVE OXYGEN SPECIES) AND THEIR DEFENSE SYSTEMS

Gamma irradiation can cause injuries to biological materials on exposure and their first target is water molecules present in an organism. On exposure to gamma irradiation water molecule breaks down to H and OH radicals are produced (Esnault et al., 2010; Maremonti et al., 2020). Radical-like H and OH and nonradical like H₂O₂ and O₂, which are known as ROS (Zhou et al., 2013). ROS control various processes in plants. Plant antioxidant system consists of two machinery, (1) antioxidant enzymes like ascorbate peroxidase (APX) (Beyaz and Yildiz, 2017), SOD (superoxide dismutase) (Bhattacharjee, 2012), GPX (guaiacol peroxidase) (Amist et al., 2019), GR (glutathione reductase) (Tiwari, 2017), MDHAR (monodehydroascorbate reductase), CAT (catalase) (Shin et al., 2013; Y. Zhang et al., 2015), and DHAR (dehydroascorbate reductase) (Habib et al., 2016; Y. Zhang et al., 2015), (2) nonenzymatic antioxidants

like carotenoids (Ahmad et al., 2010), ascorbic acid (Racchi, 2013), flavonoids (Sarker and Oba, 2018), reduced glutathione and osmolyte proline. Many scientific studies investigated the role of ROS in signaling and regulation of growth and development in plants specifically against biotic and abiotic stress (Godic et al., 2014). Esnault et al. hypothesized secondary role of ROS in signaling as observed in plants which become more tolerant to new stress after exposure to first stress due to secondary metabolites signaling (Esnault et al., 2010; Reese et al., 2010). To improve plant varieties against biotic and abiotic stress by creating a permanent gene expression against oxidative stress by starting antioxidative enzyme in first generation plants (Abbott et al., 2017). To investigate the effect of gamma irradiation on permanent production of antioxidant enzyme in M1 plants of *Onobrychis viciifolia* Scop (Williams et al., 2011). Beyaz et al investigated the effect on seeds by exposing them to 0, 400, 500, and 600 Gy from 60 Co at a dose rate of 0.483 kGy h⁻¹ (Beyaz et al., 2016; Beyaz and Yildiz, 2017). Exposed seeds were then transferred to culture plates with MS media for 30 days. The germinated seedlings were again transferred to pots for 30 days. After 60 days, the seedlings were examined for chlorophyll concentration and activities of SOD, CAT, APX, GR, and MDA (Aljoumaa and Khalil, 2018). Result showed an increase in activity of SOD, CAT, and GP after irradiation, whereas APX activity decrease and the concentration of MDA remain unchanged after irradiation using gamma irradiation to 500 Gy but CAT activity reach highest when treated with 600 Gy (Sarropoulou, Dimassi-Theriou, and Therios, 2013; Vandenhove et al., 2010). In case of SOD and GR highest activity was recorded with 400 Gy (Chen et al., 2012; Yang et al., 2014). The studies on effect of gamma irradiation on free radical and ROS found their influence in many biological activities like: aging (Ethemoglu et al., 2017), development and differentiation (Yin et al., 2012), gene regulation (Shi et al., 2011; Yin et al., 2012), degenerative disease, and signal transduction pathway (Alikamanoglu et al., 2011; Gururani et al., 2013). Some researchers hypothesised the role and shifts in antioxidant and ROS equilibrium affects the development and differentiation of various tissues on phylogenetically varied organisms. Similarly over expression of antioxidant enzyme genes are found to rise because of an efficient regulatory mechanism for providing resistance to cell (Zhao et al., 2014). In studies on mutant line of wheat irradiation by 200 Gy, Sen et al. reported effect of gamma irradiation on gene expression of antioxidant enzyme occur permanently on genome of plants (Bidabadi et al., 2020).

1.5 CONCLUSION

Increasing population around the world is increasing at the rate of 34% and will reach 9.1 billion in 2050. Most of the population is concentrated in urban areas and always have a high demand of food products. To fulfill this demand increase in food production is a demand of the century and by creating mutant species with modified and high production rate can be achieved by using gamma irradiation. Gamma irradiation can produce biochemical and physiological change in crop plants such as oxidation of amino acids, development of protein-free radicals, and conformational changes in protein. Gamma irradiation exposure is effective for food protection and sterilization.

KEYWORDS

- **gamma irradiation**
- **antioxidant enzymes**
- **foods sterilized**
- **food sterilization**
- **gamma house**

REFERENCES

- Abbott, H.; Abercrombie, D.; Adams, J.; Clacson, J.; Andrews, R.; Tredennik, H. et al. Le similitudini nell'Inferno dantesco *Conceptual Conflicts in Metaphors and Figurative Language*, Vol. 27; Houghton Mifflin Co: Edinburgh, 2017; pp. xi–xiv.
- Ahmad, P.; Jaleel, C. A.; Salem, M. A.; Nabi, G.; Sharma, S. Roles of Enzymatic And Nonenzymatic Antioxidants in Plants During Abiotic Stress. *Crit. Rev. Biotechnol.* **2010**, 30 (3), 161–175.
- Akram, K.; Kwon, J.-H. Food Irradiation for Mushrooms: A Review. *J. Korean Soc. Appl. Biol. Chem.* **2010**, 53 (3), 257–265.
- AL-Dhuhaibat, M. R. Study of the Shielding Properties for Some Composite Materials Manufactured from Polymer Epoxy Supported by Cement, Aluminum, Iron and Lead Against Gamma Rays of the Cobalt Radioactive Source (Co-60). *Int. J. of Appl. and Eng. Manage.* **2015**, 4 (6).
- Alexander, B.; Warner-Schmidt, J.; Eriksson, T. M.; Tamminga, C.; Arango-Lievano, M.; Ghose, S. et al. Reversal of Depressed Behaviors in Mice by p11 Gene Therapy in the Nucleus Accumbens. *Sci. Transl. Med.* **2010**, 2 (54), 54ra76–54ra76.

- Alikamanoglu, S.; Yalcili, O.; Sen, A. Effect of Gamma Radiation on Growth Factors, Biochemical Parameters, and Accumulation of Trace Elements in Soybean Plants (*Glycine max* L. Merrill). *Biol. Trace Element Res.* **2011**, *141* (1–3), 283–293.
- Aljoumaa, K.; Khalil, A. Evaluations of Red Cabbage (*Brassica oleracea* L. Var. Capitata F. Rubra DC.) Extract Behavior under High Dose Gamma Irradiation. *Int. Res. J. Pure Appl. Chem.* **2018**, 1–9.
- Alotaibi, D. A.; Khan, D. J. Kinetic Study and Basal Media Modification Of Cellulase Enzyme Production By Mutated Strain of *Aspergillus Fumigates* (AF1).
- Alvarez, R. P.; Migliori, A. IAEA Activities in Support of Study and Preservation of Cultural Heritage.
- Alves, P.; Pinto, S.; Kaiser, J.-P.; Bruinink, A.; de Sousa, H. C.; Gil, M. Surface Grafting of a Thermoplastic Polyurethane with Methacrylic Acid by Previous Plasma Surface Activation and by Ultraviolet Irradiation to Reduce Cell Adhesion. *Colloids Surf. B* **2011**, *82* (2), 371–377.
- Amist, N.; Bano, C.; Singh, N. Antioxidative Machinery for Redox Homeostasis During Abiotic Stress. *Mol. Plant Abiotic Stress* **2019**, 65–90.
- Arami, S.; Ahmadi, A.; Haeri, S. A. The Radioprotective Effects of *Origanum Vulgare* Extract Against Genotoxicity Induced by 131I in Human Blood Lymphocyte. *Cancer Biother. Radiopharma.* **2013**, *28* (3), 201–206.
- Aros, D.; Valdés, S.; Olate, E.; Infante, R. Gamma Irradiation on *Alstroemeria aurea* G. In Vitro Rhizomes: An Approach to the Appropriate Dosage for Breeding Purposes. *Revista de la Facultad de Ciencias Agrarias* **2012**, *44* (1), 191–197.
- Atay, N. A.; Atay, E.; Kunter, B.; Kantoglu, Y. K.; Kaplan, N. Determination of Optimal Mutagen Dosage and Its Effects on Morpho-Agronomic Traits in Putative Mutants of ‘Amasya’ Apple. *Genetika* **2019**, *51* (2), 629–639.
- Azhar, M.; Ahsanulkhaliqin, A. W. *Gamma Greenhouse: A Chronic Facility for Crops Improvement and Agrobiotechnology*. Paper presented at the AIP Conference Proceedings, 2014.
- Baba, S. *Effects of Cobalt-60 Gamma Irradiation on Growth and Yield of Pearl Millet (Pennisetum glaucum L.) In The Guinea Savannah Agroecological Zone Of Ghana*, 2015.
- Barlass, M.; Skene, K. Citrus (Citrus species). *Biotechnol. Agric. Forest. Trees I* **2012**, *1*, 207–221.
- Belaj, A.; De La Rosa, R.; Lorite, I. J.; Mariotti, R.; Cultrera, N. G.; Beuzón, C. R. et al. Usefulness of a New Large Set of High Throughput EST-SNP Markers as a Tool for Olive Germplasm Collection Management. *Front. Plant Sci* **2018**, *9*, 1320.
- Benslimani, N.; Khelifi, L. Induction of Dormancy in Spanish Groundnut Seeds (*Arachis hypogaea* L) Using Cobalt-60 Gamma Irradiation. *Induced Plant Mutations in the Genomics Era. FAO, Rome*, 2009; pp 381–384.
- Beyaz, R. Impact of Gamma Irradiation Pretreatment on the Growth of Common Vetch (*Vicia sativa* L.) Seedlings Grown Under Salt and Drought Stress. *Int. J. Radiat. Biol.* **2020**, *96* (2), 257–266.
- Beyaz, R.; Sancak, C.; Yildiz, Ç.; Kuşvuran, Ş.; Yildiz, M. Physiological Responses of the M1 Sainfoin (*Onobrychis viciifolia* Scop) Plants to Gamma Radiation. *Appl. Radiat. Isotopes* **2016**, *118*, 73–79.
- Beyaz, R.; Yildiz, M. The Use of Gamma Irradiation in Plant Mutation Breeding. *Plant Eng.. Intech* **2017**, 33–46.

- Bhat, T. A.; Khan, A. H.; Parveen, S. Comparative Analysis of Meiotic Abnormalities Induced by Gamma Rays, EMS and MMS in *Vicia faba* L. *J. Ind. Bot. Soc.* **2005a**, *84*, 45–48.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Clastogenic Effects of EMS and MMS in *Vicia faba* L. *J. Cyto. Genet.* **2005b**, *6*, 117–122.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by Gamma Rays and EMS in *Vicia faba* L. *Vegetos* **2005c**, *18* (1and2):143–145.
- Bhat, T. A.; Wani, A. A. Mutagenic Effects on Meiosis in Legumes and a Practical Case Study of *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Bhat, T. A.; Gulfishan, M.; Wani, A. A. Cytomixis: Causes and Consequences as a Case Study in *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Effect of Gamma Rays on Certain Cytomorphological Parameters in Two Varieties of *Vicia faba* L. *Adv. Plant Sci.* **2006a**, *19* (1): 227–232.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Wani, N. A. Radiation induced Pod and Seed Mutants and MMS Induced Closed Flower Mutant in Broad Bean (*Vicia faba* L.). *Indian J. Genet.* **2006b**, *66* (3), 249–250.
- Bhat, T. A.; Parveen, S.; Khan, A. H. MMS-induced Cytomixis in Pollen Mother Cells of Broad Bean (*Vicia faba* L.). *Turk. J. Bot.* **2006c**, *30*, 273–279.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Studies on the Effect of EMS on Meiosis and Pollen Fertility in *Vicia faba* L. *Ad. Plant Sci.* **2006d**, *19* (1), 243–247.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by MMS, Gamma Rays and Their Combination in Two Varieties of *Vicia faba* L. *Asian J. Plant Sci.* **2007a**, *6* (3), 558–561.
- Bhat, T. A.; Parveen, S.; Khan, A. H. Meiotic Studies in Two Varieties of *Vicia faba* L. (fabaceae) After MMS Treatment. *Asian J. Plant Sci.* **2007b**, *61* (1):51–55.
- Bhat, T. A.; Sharma, M.; Anis, M. Comparative Analysis of Mitotic Aberrations Induced by Diethyl Sulphide (DES) and Sodium Azide (SA) in *Vicia faba* L. (Fabaceae). *Pak. J. Biol. Sci.* **2007c**, *10* (5): 783–787.
- Bhat, T. A.; Sharma, M.; Anis, M. Comparative Analysis of Meiotic Aberrations Induced by Diethylsulphate and Sodium Azide in Broad Bean (*Vicia faba* L.). *Asian J. Plant Sci.* **2007d**, *6* (7), 1051–1055.
- Bhattacharjee, S. The Language of Reactive Oxygen Species Signaling in Plants. *J. Bot.* **2012**.
- Bidabadi, S. S.; VanderWeide, J.; Sabbatini, P. Exogenous Melatonin Improves Glutathione Content, Redox State and Increases Essential Oil Production in Two Salvia Species Under Drought Stress. *Sci. Rep.* **2020**, *10* (1), 1–12.
- Calado, T.; Venâncio, A.; Abrunhosa, L. Irradiation for Mold and Mycotoxin Control: A Review. *Compreh. Rev. Food Sci. Food Safet.* **2014**, *13* (5), 1049–1061.
- Carlson, G. C.; Talbot, K.; Halene, T. B.; Gandal, M. J.; Kazi, H. A.; Schlosser, L. et al. Dysbindin-1 Mutant Mice Implicate Reduced Fast-Phasic Inhibition as a Final Common Disease Mechanism in Schizophrenia. *Proc. Natl. Acad. Sci.* **2011**, *108* (43), E962–E970.
- Ceballos, H.; Sánchez, T.; Denyer, K.; Tofiño, A. P.; Rosero, E. A.; Dufour, D. et al. Induction and Identification of a Small-Granule, High-Amylose Mutant in Cassava (*Manihot Esculenta* Crantz). *J. Agric. Food Chem.* **2008**, *56* (16), 7215–7222.
- Çelik, Ö.; Atak, Ç. Applications of Ionizing Radiation in Mutation Breeding. *New Insights Gamma Rays*, **2017**, 111–132.

- Chen, Y. J.; Liu, Y. J.; Yang, H. J.; Yuan, Y.; Liu, F. J.; Tian, L. X. et al. Effect of Dietary Oxidized Fish Oil on Growth Performance, Body Composition, Antioxidant Defence Mechanism and Liver Histology of Juvenile Largemouth Bass *Micropterus Salmoides*. *Aquacult. Nutr.* **2012**, *18* (3), 321–331.
- Del Bosco, S. F.; Siragusa, M.; Abbate, L.; Lucretti, S.; Tusa, N. Production and Characterization of New Triploid Seedless Progenies for Mandarin Improvement. *Sci. Hort.* **2007**, *114* (4), 258–262.
- Diouf, M.; Boureima, S.; Tahir, D.; Ilhan Çağırkan, M. Gamma Rays-Induced Mutant Spectrum and Frequency in Sesame. *Turk. J. Field Crops* **2010**, *15* (1), 99–105.
- Downs, R. J. *Environment and the Experimental Control of Plant Growth*, Vol. 6; Elsevier, 2012.
- El-Batal, A. I.; Al-Hazmi, N. E.; Mosallam, F. M.; El-Sayyad, G. S. Biogenic Synthesis of Copper Nanoparticles by Natural Polysaccharides and *Pleurotus Ostreatus* Fermented Fenugreek Using Gamma Rays with Antioxidant and Antimicrobial Potential Towards Some Wound Pathogens. *Microb. Pathogen.* **2018**, *118*, 159–169.
- El-Degwy, I. S. Mutation Induced Genetic Variability in Rice (*Oryza sativa* L.). *Int. J. Agric. Crop Sci.* **2013**, *5* (23), 2789–2794.
- Enu, R.; Enu, P. Sterilization of Grains Using Ionizing Radiation: The Case in Ghana. *Eur. Sci. J.* **2014**, *10* (6).
- Esnault, M.-A.; Legue, F.; Chenal, C. Ionizing Radiation: Advances in Plant Response. *Environ. Exp. Bot.* **2010**, *68* (3), 231–237.
- Ethemoglu, M.; Seker, F.; Akkaya, H.; Kilic, E.; Aslan, I.; Erdogan, C.; Yilmaz, B. Anticonvulsant Activity of Resveratrol-Loaded Liposomes In Vivo. *Neuroscience* **2017**, *357*, 12–19.
- Fermin, G. A.; Castro, L. T.; Tennant, P. F. CP-transgenic and Non-Transgenic Approaches for the Control of Papaya Ringspot: Current Situation and Challenges. *Transgenic Plant J.* **2010**, *4* (S1), 1–15.
- Foulon, F.; Abdelouahed, H. B.; Bogovac, M.; Charisopoulos, S.; Matos, M.; Migliori, A. et al. *IAEA Nuclear Science and Instrumentation Laboratory: Support to IAEA Member States and Recent Developments*. Paper Presented at the EPJ Web of Conferences, 2020.
- Ganai, F. A.; Khan, A. H.; Bhat T. A.; Parveen, S.; Wani, N. A. Cytogenetic Effects of Methyl Methane Sulphonate (MMS) on Varieties of Chickpea (*Cicer arietinum* L.) *J. Cytol. Genet.* **2005**, *6*, 97–102.
- Gautam, R. K.; Mudhoo, A.; Lofrano, G.; Chattopadhyaya, M. C. Biomass-Derived Biosorbents for Metal Ions Sequestration: Adsorbent Modification and Activation Methods and Adsorbent Regeneration. *J. Environ. Chem. Eng.* **2014**, *2* (1), 239–259.
- Godic, A.; Poljšak, B.; Adamic, M.; Dahmane, R. The Role of Antioxidants in Skin Cancer Prevention and Treatment. *Oxidative Med. Cell. Longevity* **2014**, *2014*.
- Gulfishan, M.; Bhat, T. A.; Oves, M. Mutants as a Genetic Resource for Future Crop Improvement. In *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools*, Al-Khayri, J. M. et al., Eds.; © Springer International Publishing: Switzerland, 2015.
- Gulfishan, M.; Jafri, I. F.; Khan, A. H.; Bhat, T. A. Methyl Methane Sulphonate Induced Desynaptic Mutants in *Capsicum annum* L. *Chromosome Bot.* **2013**, *8*, 59–62.
- Gulfishan, M.; Khan A. H.; Gefferi, I. F.; Bhat T. A. Assessment of Mutagenicity Induced by MMS and DES in *Capsicum annum* L. *Saudi J. Biol. Sci.* **2012**, *19*, 251–255.

- Gulfishan, M.; Khan, A. H.; Bhat, T. A. Studies on Cytotoxicity Induced by DES and SA in *Vicia faba* L. var. Major. *Turk. J. Bot.* **2010**, *34*, 31–37.
- Gulfishan, M.; Khan, A. H.; Haneef, I.; Bhat, T.A. Genotoxic Effect of Diethyl Sulphate in Two Varieties of *Capsicum annum* L. *Nucleus* **2011**, *54* (2), 107–111.
- Guo, T.; Yang, G.; Luo, W.; Zhang, J.; Yan, X.; DU, Y. et al. Genome-Wide Comparisons of Mutations Induced by Carbon-Ion Beam and Gamma-Rays Irradiation in Rice via Resequencing Multiple Mutants. *Front. Plant Sci.* **2019**, *10*, 1514.
- Gururani, M. A.; Upadhyaya, C. P.; Baskar, V.; Venkatesh, J.; Nookaraju, A.; Park, S. W. Plant Growth-Promoting Rhizobacteria Enhance Abiotic Stress Tolerance in Solanum Tuberosum Through Inducing Changes in the Expression of ROS-Scavenging Enzymes and Improved Photosynthetic Performance. *J. Plant Growth Regulation* **2013**, *32* (2), 245–258.
- Habib, S. H.; Kausar, H.; Saud, H. M. Plant Growth-Promoting Rhizobacteria Enhance Salinity Stress Tolerance in Okra Through ROS-Scavenging Enzymes. *BioMed. Res. Int.* **2016**, *2016*.
- Hanafiah, D. S.; Trikoesoemoningtyas, T.; Yahya, S.; Wirmas, D. Induced Mutations by Gamma Ray Irradiation to Argomulyo Soybean (Glycine Max) Variety. *Nusantara Biosci.* **2010**, *2* (3).
- Haris, A.; Abdullah, B.; Subaedah, A.; Jusoff, K. Gamma Ray Radiation Mutant Rice on Local Aged Dwarf. *Middle-East J. Sci. Res.* **2013**, *15* (8), 1160–1164.
- Hartini, A. R. *Synthesis of Zinc Oxide Nanostructures and Their Applications as Field Electron Emission Cathodes and Photocatalysts/Hartini Ahmad Rafaie*. University of Malaya, 2016.
- Henry, R. Genomics Strategies for Germplasm Characterization and the Development of Climate Resilient Crops. *Front. Plant Sci.* **2014**, *5*, 68.
- Hirimburegama, K.; Hirimburegama, W.; Dias, W. Banana Improvement Through Gamma Irradiation and Testing for Banana Bract Mosaic Virus in Sri Lanka, 2012.
- Horn, L.; Shimelis, H. Radio-Sensitivity of Selected Cowpea (*Vigna Unguiculata*) Genotypes to Varying Gamma Irradiation Doses. *Sci. Res. Essays* **2013**, *8* (40), 1991–1997.
- Hussain, M.; Raja, N. I.; Rashid, H.; Zia-Ur-Rehman Mashwani, A. M.; Iqbal, M. Establishment of an efficient protocol for plantlets regeneration via direct and indirect organogenesis in Citrus reticulata blanco (kinnow mandarin). *Pak. J. Bot.* **2018**, *50* (3), 1203–1210.
- Hussain, P. R.; Dar, M. A.; Wani, A. M. Effect of Edible Coating and Gamma Irradiation on Inhibition of Mould Growth and Quality Retention of Strawberry During Refrigerated Storage. *Int.J. Food Sci. Technol.* **2012**, *47* (11), 2318–2324.
- Ibrahim, R.; Ahmad, Z.; Salleh, S.; Hassan, A. A.; Ariffin, S. Mutation Breeding in Ornamentals. *Ornamental Crops*; Springer, 2018; pp. 175–211.
- Iliev, V.; Tomova, D.; Rakovsky, S.; Eliyas, A.; Puma, G. L. Enhancement of Photocatalytic Oxidation of Oxalic Acid by Gold Modified WO₃/TiO₂ Photocatalysts Under UV and Visible Light Irradiation. *J. Mol. Catalys. A Chem.* **2010**, *327* (1–2), 51–57.
- Jain, S. M. Mutagenesis in Crop Improvement Under the Climate Change. *Roman. Biotechnol. Lett.* **2010**, *15* (2), 88–106.
- Joint, F. Plant Breeding and Genetics Newsletter, No. 24, January 2010a.
- Joint, F. Plant Breeding and Genetics Newsletter, No. 25, July 2010b.
- Joint, F. FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology. Book of Abstracts: Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, 2018.
- Kang, S.-Y.; Kim, J.-B.; Lee, G.-J.; Kim, D. S. Gamma Phytotron: A New Chronic Gamma Irradiation Facility, 2010.

- Kangarasu, S.; Ganeshram, S.; Joel, A. J. Determination of Lethal Dose for Gamma Rays and Ethyl Methane Sulphonate Induced Mutagenesis in Cassava (*Manihot esculenta* Crantz.). *Int. J. Sci. Res.* **2014**, *3* (1), 3–6.
- Kazmi, S. K.; Khan, S.; Kabir, N.; Mirbahar, A. A.; Raziq, M.; Kauser, N. Embryogenic Callus Induction, Somatic Embryogenesis, Regeneration and Histological Studies of Kinnow Mandarin (*Citrus reticulata* blanco L.) From Nucellar Embryo and Epicotyl Region. *Pak. J. Bot.* **2015**, *47* (1), 305–310.
- Khan, A. A.; Ansari, M. Y. K.; Shahab, D.; Bhat, T. A. Induced Variations for Quantitative Traits in *Vicia faba* L. *Vegetos* **2005a**, *18* (1and2), 83–86.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. DES Induced Variations for Economic Traits in Broad Bean (*Vicia faba* L.). *Biosci. Biotechnol. Res. Asia* **2005b**, *3*(2), 363–366.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. Clastogenic Effects of 8-Hydroxy Quinoline in *Vicia faba* L. *Adv. Plant Sci.* **2007a**, *20* (1), 85–87. ISSN 0970-3586.
- Khan, A. A.; Ansari, M. Y. K.; Shahab, D and Bhat, T. A.; (2007b). Comparative Analysis of Meiotic Abnormalities Induced by Caffeine and DES in *Vicia faba* L. *Adv. Plant Sci.* **2007b**, *20* (1), 73–76.
- Khan, J.; Alotaibi, A.; Dekka, M. Effect of Colchicine Induced Mutation on Cellulose Enzyme Production by *Aspergillus Fumigatus*. *World J. Pharma. Res.* **2015**, *4*, 461–471.
- Kumar, S.; Dhandapani, R.; Kumar, A. Phytotron–Use in Physiological and Climate Change Research. *Manual of ICAR Sponsored Training Programme for Technical Staff of ICAR Institutes on “Physiological Techniques to Analyze the Impact of Climate Change on Crop Plants”*, 2017; p 15.
- Kume, T.; Furuta, M.; Todoriki, S.; Uenoyama, N.; Kobayashi, Y. Status of Food Irradiation in the World. *Radiat. Phys. Chem.* **2009**, *78* (3), 222–226.
- Kurowska, M.; Labocha-Pawłowska, A.; Gnizda, D.; Maluszynski, M.; Szarejko, I. Molecular Analysis of Point Mutations in a Barley Genome Exposed to MNU and Gamma Rays. *Mutat. Res./Fundamental Mol. Mech. Mutagenesis* **2012**, *738*, 52–70.
- Lagoda, P. J.; Maluszynski, M.; Szarejko, I.; Bhatia, C. R.; Nichterlein, K. *Farmer Participation*, 2009; p 159.
- Lanh, D.; Son, P. N.; Son, N. A. In-House Development of an FPGA-based MCA8K for Gamma-Ray Spectrometer. *SpringerPlus* **2014**, *3* (1), 665.
- Laucou, V.; Lacombe, T.; Dechesne, F.; Siret, R.; Bruno, J.-P.; Dessup, M. et al. High Throughput Analysis of Grape Genetic Diversity as a Tool for Germplasm Collection Management. *Theor. Appl. Genet.* **2011**, *122* (6), 1233–1245.
- Lee, K. T. Quality and Safety Aspects of Meat Products as Affected by Various Physical Manipulations of Packaging Materials. *Meat Sci.* **2010**, *86* (1), 138–150.
- Li, F.; Shimizu, A.; Nishio, T.; Tsutsumi, N.; Kato, H. Comparison and Characterization of Mutations Induced by Gamma-Ray and Carbon-Ion Irradiation in Rice (*Oryza sativa* L.) Using Whole-Genome Resequencing. *G3* **2019**, *9* (11), 3743–3751.
- Liu, P.; Yuan, J.; Jiang, Z.; Wang, Y.; Weng, B.; Li, G. A Lower Cadmium Accumulating Strain of *Agaricus Brasiliensis* Produced by ^{60}Co - γ -Irradiation. *LWT* **2019**, *114*, 108370.
- Maremonti, E.; Eide, D. M.; Rossbach, L. M.; Lind, O. C.; Salbu, B.; Brede, D. A. In Vivo Assessment of Reactive Oxygen Species Production and Oxidative Stress Effects Induced by Chronic Exposure to Gamma Radiation in *Caenorhabditis Elegans*. *Free Radical Biol. Med.* **2020**, *152*, 583–596.

- Mattison, C. P.; Desormeaux, W. A.; Wasserman, R. L.; Yoshioka-Tarver, M.; Condon, B.; Grimm, C. C. Decreased Immunoglobulin E (IgE) Binding to Cashew Allergens Following Sodium Sulfite Treatment and Heating. *J. Agric. Food Chem.* **2014**, 62 (28), 6746–6755.
- Mba, C. Induced Mutations Unleash the Potentials of Plant Genetic Resources for Food and Agriculture. *Agronomy* **2013**, 3 (1), 200–231.
- Mba, C.; Afza, R.; Bado, S.; Jain, S. M. Induced Mutagenesis in Plants Using Physical and Chemical Agents. *Plant Cell Culture: Essential Methods* **2010**, 20, 111–130.
- Mba, C.; Shu, Q. Gamma irradiation. *Plant Mutation Breeding and Biotechnology* **2012**, 91–98.
- Mehboob, K.; Alzahrani, Y. A.; Fallatah, O.; Qutub, M.; Younis, H. Radioactivity and Radiation Hazard Indices Assessment for Phosphate Rock Samples from Al-Jalamid, Turaif, Umm Wu'al, and As-Sanam, Saudi Arabia. *Arab. J. Sci. Eng.* **2020**, 1–14.
- Mohajer, S.; Mat Taha, R.; Lay, M. M.; Khorasani Esmaeili, A.; Khalili, M. Stimulatory Effects of Gamma Irradiation on Phytochemical Properties, Mitotic Behaviour, and Nutritional Composition of Sainfoin (*Onobrychis viciifolia* Scop.). *Sci. World J.* **2014**, 2014.
- Molins, R. A. *Food Irradiation: Principles and Applications*; John Wiley and Sons, 2001.
- Montañola, M. J.; Galaz, A.; Gambardella, M.; Mártiz, J. *New Low Seeded Mandarin (Citrus reticulata) and Lemon (C. limon) Selections Obtained by Gamma Irradiation*. Paper Presented at the Proc. XII Intl. Citrus Congress. Acta Horticulturæ, 2015.
- Murti, R. H.; Kim, H. Y.; Yeoung, Y. R. Effectiveness of Gamma Ray Irradiation and Ethyl Methane Sulphonate on In Vitro Mutagenesis of Strawberry. *Afr. J. Biotechnol.* **2013**, 12 (30).
- Nhat Hang, N.; Chau, N. *Radiation Induced Mutation for Improving Papaya Variety in Vietnam*. Paper presented at the II International Symposium on Papaya 851, 2008.
- Obaid, S. S.; Sayyed, M.; Gaikwad, D.; Pawar, P. P. Attenuation Coefficients and Exposure Buildup Factor of Some Rocks for Gamma Ray Shielding Applications. *Radiat. Phys. Chem.* **2018**, 148, 86–94.
- Organization, W. H. *High-Dose Irradiation: Wholesomeness of Food Irradiated with Doses Above 10 kGy*, Vol. 890; World Health Organization, 1999.
- Owoseni, O.; Okwaro, H.; Afza, R.; Bado, S.; Dixon, A.; Mba, C. Radiosensitivity and In Vitro Mutagenesis in African Accessions of Cassava; *Manihot esculenta* Crantz, 2006.
- Park, H.-Y.; Kim, Y.-N.; Lee, Y.-J.; Song, B.-S.; Park, J.-H.; Kim, J.-K.; Son, E.-J. Effects of Gamma Irradiation on the Quality of Manila Clam Porridge and In Vivo Immunomodulatory Activity of Hesperidin as Immunopotentiator for Sterilized Patient Food. *Radiat. Phys. Chem.* **2020**, 109015.
- Parveen, S.; Anis, M.; Bhat, T. A.; and Khan, A. H. Cytological Effects of Physical and Chemical Mutagens in *Trigonella foenum-graecum* L. *Adv. Plant Sci.* **2007**, 20 (11), 633–636.
- Parveen, S.; Anis, M.; Bhat, T. A.; Khan, A. H. Efficiency and Effectiveness of Physical and Chemical Mutagens in M2 Generation of *Trigonella foenum-graecum* L. *J. Cytol. Genet.* **2006**, 7, 1–4.
- Pathirana, R.; Derolles, S.; Hoeata, K.; Montefiori, M.; Tyson, J.; Wang, T. et al. *Fast-Tracking Kiwifruit Breeding Through Mutagenesis*. Paper presented at the XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014), 2015; p 1127.
- Pestana, R. K. N.; Amorim, E. P.; Ferreira, C. F.; de Oliveira Amorim, V. B.; Oliveira, L. S.; da Silva Ledo, C. A.; e Silva, S. d. O. Agronomic and Molecular Characterization

- of Gamma Ray Induced Banana (*Musa* sp.) Mutants Using a Multivariate Statistical Algorithm. *Euphytica* **2011**, 178 (2), 151–158.
- Piri, I.; Babayan, M.; Tavassoli, A.; Javaheri, M. The Use of Gamma Irradiation in Agriculture. *Afr. J. Microbiol. Res.* **2011**, 5 (32), 5806–5811.
- Pugliese, M.; Chitarra, W.; Cogliati, E.; Gullino, M.; Garibaldi, A. Effect of Climatic Factors on Plant Diseases Under Phytotron Conditions. *Protezione delle Colture* **2013**, 2, 31–35.
- Raafat, A. I.; Eid, M.; El-Arnaouty, M. B. Radiation Synthesis of Superabsorbent CMC Based Hydrogels for Agriculture Applications. *Nucl. Instr. Methods Phys. Res. Sect. B Beam Interact. Mater. Atoms* **2012**, 283, 71–76.
- Racchi, M. L. Antioxidant Defenses in Plants with Attention to *Prunus* and *Citrus* spp. *Antioxidants* **2013**, 2 (4), 340–369.
- Rai, M. K.; Kalia, R. K.; Singh, R.; Gangola, M. P.; Dhawan, A. Developing Stress Tolerant Plants Through In Vitro Selection—An Overview of the Recent Progress. *Environ. Exp. Bot.* **2011**, 71 (1), 89–98.
- Ranga, A. D.; Kumar, S. Phytotron: An Unrecongised Masterpiece. *agrinenv. com* **2020**, 81.
- Rayan, A.; Rekab, Z. A. A.; Mohamed, S. In Vitro Studies on Genetic Variation of Some Plum (*Pyrus domestica* L.) Cultivars Using Gamma Irradiation from Cobalt 60.
- Razzaq, A.; Sadia, B.; Raza, A.; Khalid Hameed, M.; Saleem, F. Metabolomics: A Way Forward for Crop Improvement. *Metabolites* **2019**, 9 (12), 303.
- Reese, S.; Vidyasagar, A.; Jacobson, L.; Acun, Z.; Esnault, S.; Hullett, D. et al. The Pin 1 Inhibitor Juglone Attenuates Kidney Fibrogenesis via Pin 1-Independent Mechanisms in the Unilateral Ureteral Occlusion Model. *Fibrogen. Tissue Repair* **2010**, 3 (1), 1–8.
- Rowe, P.; Rosales, F. Breeding Cooking Bananas for Areas with Marginal Growing Conditions by Using Cardaba (ABB) in Cross-Pollinations. *Biotechnol. App. Banana Plantain Improvement* **1993**, 128.
- Sagel, Z. Determination of Effect of Chemical Mutagen EMS on TAEK A-3 and TAEK C-10 Mutant Soybean Varieties in M1 Generation. *Ekin J. Crop Breed. Genet.* **2017**, 3 (1), 19–24.
- Sanam'ian, M. Evaluation of the Effect of Pollen Irradiation on Karyotype Variability in Cotton Plants. *Genetika* **2003**, 39 (7), 947.
- Sarker, U.; Oba, S. Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in *Amaranthus Tricolor*. *Appl. Biochem. Biotechnol.* **2018**, 186(4), 999–1016.
- Sarropoulou, V.; Dimassi-Theriou, K.; Therios, I. Roles of Melatonin in Humans and Plants. *New Dev. Melatonin Res.* **2013**, 1.
- Sasaki, N.; Watanabe, A.; Asakawa, T.; Sasaki, M.; Hoshi, N.; Naito, Z. et al. Biological Effects of Ion Beam Irradiation on Perennial Gentian and Apple. *Plant Biotechnol.* **2018**, 35 (3), 249–257.
- Sato, Y.; Shirasawa, K.; Takahashi, Y.; Nishimura, M.; Nishio, T. Mutant Selection from Progeny of Gamma-Ray-Irradiated Rice by DNA Heteroduplex Cleavage Using Brassica Petiole Extract. *Breed. Sci.* **2006**, 56 (2), 179–183.
- Serizawa, T.; Yamamoto, M.; Sato, Y.; Higuchi, Y.; Nagano, O.; Kawabe, T. et al. Gamma Knife Surgery as Sole Treatment for Multiple Brain Metastases: 2-Center Retrospective Review of 1508 Cases Meeting the Inclusion Criteria of the JLGK0901 Multi-Institutional Prospective Study. *J. Neurosurg.* **2010**, 113 (Special_Supplement), 48–52.
- Sharafi, Y.; Motallebi-Azar, A.-R. Gamma Irradiation Influences on Some Biological Traits in Two Almond (*Prunus amygdalus*, L.) Cultivars. *J. Med. Plants Res.* **2011**, 5 (2), 255–258.

- Sharma, A.; Singh, S. Induced Mutation-a Tool for Creation of Genetic Variability in Rice (*Oryza sativa* L.). *J. Crop Weed* **2013**, *9*, 132–138.
- Sharma, M.; Khan, A. H.; Bhat, T. A. Assessment of Mutagenecity of Individual and Combination Treatment of Gamma Rays and MMS in Broad Bean (*Vicia faba* L.). *Cytologia*. **2009**, *74* (2), 235–241.
- Shi, L.; Xia, Y.; Zhang, M.; Yin, S.-J.; Si, Y.-X.; Qian, G.-Y. et al. Trifluoroethanol-Induced Activity and Structural Changes in Bos Taurus Copper-and Zinc-Containing Superoxide Dismutase. *Protein Peptide Lett.* **2011**, *18* (7), 726–732.
- Shin, S.-Y.; Kim, I.-S.; Kim, Y.-S.; Lee, H.; Yoon, H.-S. Ectopic Expression of Brassica Rapa L. MDHAR Increased Tolerance to Freezing Stress by Enhancing Antioxidant Systems of Host Plants. *SA J. Bot.* **2013**, *88*, 388–400.
- Shirasawa, K.; Hirakawa, H.; Tabata, S.; Hasegawa, M.; Kiyoshima, H.; Suzuki, S. et al. Characterization of Active Miniature Inverted-Repeat Transposable Elements in the Peanut Genome. *Theor. Appl. Genet.* **2012**, *124* (8), 1429–1438.
- Singh, K.; Ram, M.; Kumar, A. *Forty Years of Papaya Research at Pusa, Bihar, India*. Paper presented at the II International Symposium on Papaya 851, 2008.
- Songsri, P.; Suriham, B.; Sanitchon, J.; Srisawangwong, S.; Kesmala, T. Effects of Gamma Radiation on Germination and Growth Characteristics of Physic Nut (*Jatropha curcas* L.). *J. Biol. Sci.* **2011**, *11* (3), 268–274.
- Springer, N. M.; Schmitz, R. J. Exploiting Induced and Natural Epigenetic Variation for Crop Improvement. *Nat. Rev. Genet.* **2017**, *18* (9), 563.
- Sreedharan, S.; Shekhawat, U. K.; Ganapathi, T. R. Transgenic Banana Plants Overexpressing a Native Plasma Membrane Aquaporin M USA PIP 1; 2 Display High Tolerance Levels to Different Abiotic Stresses. *Plant Biotechnol. J.* **2013**, *11* (8), 942–952.
- Sutarto, I.; Agisimanto, D.; Supriyanto, A. Development of Promising Seedless Citrus Mutants Through Gamma Irradiation. *Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations, Rome*, 2009; pp 306–308.
- Tanaka, A.; Shikazono, N.; Hase, Y. Studies on Biological Effects of Ion Beams on Lethality, Molecular Nature of Mutation, Mutation Rate, and Spectrum of Mutation Phenotype for Mutation Breeding in Higher Plants. *J. Radiat. Res.* **2010**, *51* (3), 223–233.
- Thakur, B.; Singh, R. Food Irradiation-Chemistry and Applications. *Food Rev. Int.* **1994**, *10* (4), 437–473.
- Thayer, D. Food Irradiation: Benefits and Concerns. *J. Food Qual* **1990**, *13* (3), 147–169.
- Tiwari, S. Reactive Oxygen Species and Antioxidants: A Continuous Scuffle Within the Cell. *Reactive Oxygen Species in Plants*, 2017; p 1.
- Tomlekova, N.; Panayotov, N.; Kozgar, M.; Wani, M.; Serdaris, P.; Balacheva, E. –Inducing and Exploring New Mutant Donors of Tomato. *Exploring Genetic Diversity of Crops*, 2014; p 283.
- Ugidos-Rodríguez, S.; Matallana-González, M. C.; Sánchez-Mata, M. C. Lactose Malabsorption and Intolerance: A Review. *Food Funct.* **2018**, *9* (8), 4056–4068.
- Vandenhove, H.; Vanhoudt, N.; Cuypers, A.; van Hees, M.; Wannijn, J.; Horemans, N. Life-Cycle Chronic Gamma Exposure of *Arabidopsis thaliana* Induces Growth Effects But No Discernable Effects on Oxidative Stress Pathways. *Plant Physiol. Biochem.* **2010**, *48* (9), 778–786.
- Varde, P.; Joshi, N.; Agarwal, M.; Shrivastava, A.; Bandi, L.; Kohli, A. Development of an Integrated Methodology in Support of Remaining Life Assessment of Co-60 Based Gamma Irradiation Facilities. *Nucl. Eng. Design* **2011**, *241* (1), 328–338.

- Vaz Patto, M. C.; Amarowicz, R.; Aryee, A. N.; Boye, J. I.; Chung, H.-J.; Martín-Cabrejas, M. A.; Domoney, C. Achievements and Challenges in Improving the Nutritional Quality of Food Legumes. *Crit. Rev. Plant Sci.* **2015**, *34* (1–3), 105–143.
- Wani, A. A.; Bhat, T. A. Asynapsis and Desynapsis in Plants: In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Wani, N. A.; Bhat, T. A.; Khan, A. H.; Ansari, M. Y. K. Sodium Azide Induced Variability in Chickpea (*Cicer arietinum* L.). *Vegetos* **2005**, *18* (1and2), 105–107.
- Wilkinson, V. M. *Food Irradiation: A Reference Guide*, Vol. 24; CRC Press, 1997.
- Williams, D.; Norman, G.; Khoury, C.; Metcalfe, N.; Briard, J.; Laporte, A. et al. Evidence for a Second Messenger Function of dUTP During Bax Mediated Apoptosis of Yeast and Mammalian Cells. *Biochim. Biophys. Acta* **2011**, *1813* (2), 315–321.
- Yang, P.-M.; Huang, Q.-C.; Qin, G.-Y.; Zhao, S.-P.; Zhou, J.-G. Different Drought-Stress Responses in Photosynthesis and Reactive Oxygen Metabolism Between Autotetraploid and Diploid Rice. *Photosynthetica* **2014**, *52* (2), 193–202.
- Yin, S.-J.; Lü, Z.-R.; Park, D.; Chung, H. Y.; Yang, J.-M.; Zhou, H.-M. et al. Trifluoroethanol-Induced Changes in Activity and Conformation of Manganese-Containing Superoxide Dismutase. *Appl. Biochem. Biotechnol.* **2012**, *166* (2), 276–288.
- Yoshioka, T.; Masuda, T.; Kotobuki, K.; Sanada, T.; Ito, Y. Gamma-Ray-Induced Mutation Breeding in Fruit Trees: Breeding of Mutant Cultivars Resistant to Black Spot Disease in Japanese Pear. *Japan Agric. Res. Quart.* **1999**, *33* (4), 227–234.
- Yudaev, I.; Stepanchuk, G.; Kaun, O.; Ukraitsev, M.; Ponamareva, N. *Small-Sized Irradiation Structures for Intensive Year-Round Cultivation of Green Vegetable Crops*. Paper Presented at the IOP Conference Series: Earth and Environmental Science, 2019.
- Yue, J.; Zou, J. Study of Radiation Effects on Upland Cotton (*Gossypium hirsutum* L.) Pollen Grain Irradiated by ^{60}Co -[Gamma] Ray. *J. Agric. Sci.* **2012**, *4* (7), 85.
- Zaknun, J. J.; Bodei, L.; Mueller-Brand, J.; Pavel, M.; Baum, R.; Hörsch, D. et al. The Joint IAEA, EANM, and SNMMI Practical Guidance on Peptide Receptor Radionuclide Therapy (PRRT) in Neuroendocrine Tumours. *Eur. J. Nucl. Med. Mol. Imag.* **2013**, *40* (5), 800–816.
- Zhang, M.; Wang, H.; Liao, M.; Chen, X.; Graner, M.; Zhu, X. et al. Diagnosis of Latent Tuberculosis Infection in Bacille Calmette-Guerin Vaccinated Subjects in China by Interferon-Gamma ELISpot Assay. *Int. J. Tuberculosis Lung Dis.* **2010**, *14* (12), 1556–1563.
- Zhang, Y.; Li, Z.; Peng, Y.; Wang, X.; Peng, D.; Li, Y. et al. Clones of FeSOD, MDHAR, DHAR Genes from White Clover and Gene Expression Analysis of ROS-Scavenging Enzymes During Abiotic Stress and Hormone Treatments. *Molecules* **2015**, *20* (11), 20939–20954.
- Zhao, J.; Ma, D.; Luo, M.; Wang, W.; Zhao, C.; Zu, Y. et al. In Vitro Antioxidant Activities and Antioxidant Enzyme Activities in HepG2 Cells and Main Active Compounds of Endophytic Fungus from Pigeon Pea [*Cajanus cajan* (L.) Millsp.]. *Food Res. Int* **2014**, *56*, 243–251.
- Zhou, Y.; Yan, H.; Guo, M.; Zhu, J.; Xiao, Q.; Zhang, L. Reactive Oxygen Species in Vascular Formation and Development. *Oxid. Med. Cell. Longev.* **2013**, 2013.



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CHAPTER 2

Chromosome Manipulations for Crop Improvement

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ABSTRACT

For numerous crops, a series of cytogenetic stocks have been prepared. These stocks were haploids, monosomic, trisomic, autotetraploids, extracted allopolyploids, synthetic allopolyploids, alien chromosome substitution, and alien chromosome addition lines. Before this, the groundwork of cytogenetics was laid, during 1900–1904, on the basis of parallelism recognized between the chromosomal theory of inheritance and Mendel's laws of inheritance. Molecular cytogenetic methods like, genomic in situ hybridization, fluorescence in situ hybridization (FISH), fiber-FISH, chromosome image analyzing system, chromosome banding, chromosome microdissection, chromosomal flow sorting, and plant artificial chromosomes (PACs). Cytogenetic stocks can be characterized cytologically and genetically. These stocks are being used for constructing comparative, physical, genetic, and molecular maps. Numerous breeding lines have been developed by haploid breeding and many varieties have been released. Many varieties have also been prepared using Autotetraploids. A variety of amphiploids and interspecific hybrids have also been produced. Great success has also been achieved in the development of allopolyploids by crossing polyploids or diploids with synthetic allopolyploids and manipulating the alien addition lines and alien substitution lines. With the help of different tools, useful genes have been transferred, for resistance

against many diseases, abiotic stress, insect resistance, and improvement of grain quality, in oats, wheat, rice, cotton, *Brassica*, and many other horticulture plant species. Introgression for disease resistance has also been done.

2.1 INTRODUCTION

For each gene, two or more alleles exist and for only one allele gets transmitted to a gamete. Different alleles do not mix in a zygote and retain their integrity as well as identity in each successive generation. Gregor Mendel's research and theories were ignored from 1865 to 1900 and cytologists continued their discovery in this field. Sutton and Boveri (1903) on the basis of available knowledge proposed the chromosomal theory of hereditary.

The agreement of Mendel's laws of inheritance with the chromosomal behavior during meiosis became the basis of Cytogenesis. Flemming, in 1882, described the process of mitosis and in the same year, Waldeyer introduced the term chromosome. A bulk of studies explaining chromosome manipulation which involves various changes in the structure and number elucidate their usage in breeding research and genetics. Cytogenetic stocks that are produced in large numbers have been characterized. Development of improved crops through; haploid breeding in crops like rice, barley, potato, *Brassica*; tetraploid varieties of ornamentals, rye, forages; triploid sugar beet and seedless watermelon; man-made and extracted allopolyploids, for example, triticale, brassica, and transfer of various useful alien genes in tobacco, oats, wheat, cotton, and other plants. In the past, many years chromosome research has advanced in the areas of chromosome banding, chromosome image analyzing system, microdissection of chromosomes, uptake of isolated chromosomes, flow sorting of chromosomes, computer-aided chromosome analysis, and possibilities of constructing PACs. In the similar way, the advancement in cellular and molecular biology have provided a swift to the chromosome research and development of complete cytological maps, physical and genetic maps, relative genetic maps, genomic differentiation and repatterning of karyotypes, the physical position of centromeres, alien chromosome segment description, and physical location of the gene on the chromosomes with the help of BAC-fluorescence in situ hybridization (FISH) including fiber FISH techniques. With these advancements, new openings have appeared for specific manipulation of chromosomes for genetic modifications of crops have become more available.

2.2 HAPLOID BREEDING

In the development of a homozygous line from a segregating population, haploids are very important. This is faster than any other method for fixing recombinants. They also aid as mapping populations to trace genes that are responsible for economic traits. Below are the regularly used procedures for haploid production:

1. Haploid isolation from a natural population.
2. Anther culture.
3. Interspecific crosses.
4. The approach of Genetic selection.
5. Mutant gene.

In the separation of haploids from the natural population, a big figure of stocks has been known to give high occurrence of haploids. These types of genetic stocks are extensively used for isolation of haploid in corn, flax, *Brassica*, etc.

Anther culture has been used extensively for the production of haploids for various plant species. Many promising cultivars have been produced by from dihaploids derived from anther culture (e.g., brassica, rice, tobacco, and wheat). The application of interspecific crosses has been proven to be an important procedure for the production of haploids in the case of wheat, barley, and potato. A mutant gene “*hap*,” used for the production of haploids in barley, produces progeny with 10–14% haploids. This “*hap*” gene was reported by Hagberg and Hagberg (1980). However, the practical utility of this system is deficient. Alien cytoplasm results into a high frequency of haploid production (Kihara and Tsunewaki, 1962).

2.2.1 INTERPLOID MATING IN POTATO

In cultivated potato crop, haploids offer the benefit of disomic and not tetrasomic inheritance. With this gene transfer from a number of natural and manually cultivated species of tuber, bearing 24 chromosomes has become possible. In potatoes, haploids are obtained through interploidy mating between *Solanum tuberosum* ($2n = 48$) and Phureja ($2n = 24$). The selection of seed parents can be helpful in increasing the frequency of haploids. In the same manner, the selection of *Solanum phureja* and superior pollinators can profoundly affect the haploid frequency. Successful crossings of these haploids have been performed with a number of tuber species with

24-chromosomes. The regular pairing of chromosomes is shown by interhaploids. When there is union of two polar nuclei of *S. tuberosum* with the nucleus of male parent *S. phureja*, they result in the production of dihaploids. The two key features involved are the development of $2n$ gametes and the production of haploids. In such a case of interploid mating in potatoes, both the first division restitution and the second division restitutions are involved. $2x$ males give rise to $2n$ pollens resulting almost exclusively a $4x$ progeny. The abnormal development of the pentaploid endosperm results in the formation of triploid ($3x$) where this pentaploid endosperm is associated with $3x$ embryos. Some of the selected $4x$ clones have been availed as cultivars. The dihaploids of potato which can be crossed with the diploid species are used for the transfer of resistance toward diseases, tuber development under high-temperature environments, and frost resistance.

2.2.2 HAPLOID VIA CHROMOSOME ELIMINATION

A totally different mechanism plays in some interspecific crosses, where subsequent to the fertilization elimination of selected chromosomes of one parent happens. This phenomenon results in to the haploid plant production. Classical examples of this process include; in the crossing of *H. elimination* of *Hordeum bulbosum* and *Hordeum vulgare* in which elimination of bulbosum chromosomes happens, in the cross of wheat and maize, maize chromosomes are eliminated and in the case of crossing between oat and maize, maize chromosomes are eliminated. It is interesting to note that the haploids from these crosses can be produced by embryo rescue after the sexual hybridization. Ho and Kasha (1975) started crossing between the *bulbosum* tetraploids and barley primary trisomes. They successfully located the gene responsible for the chromosome elimination on chromosomes 2 and 3. High-yielding varieties of mingo, rodeo, gwylan have been formed via *bulbosum* technique. No difference was observed in double haploids of barley and homozygous lines of agronomic trait established by pedigree or SSD methods (Park et al., 1976). Therefore, this method shows a very high potential toward barely breeding. Laurie et al. (1990) studied the production of haloids from maize–wheat crosses. Confirmation of hybrid origin of an embryo was done by the observations of zygotes at metaphase. Chromosomes are not connected to spindle and inclined to lag behind the chromosomes, at the anaphase, in wheat. At metaphase, the 2-celled embryos show 4–10 maize chromosomes while there was the lag in the preparation of anaphase and telophase. Further analysis displays that the maize chromosomes were

removed efficiently within three cycles of cellular divisions. It was also evident from wide crosses (maize $\times$ wheat) that the occurrence of fertilization is highly efficient. This may lead to an observation that cell recognition is not important or such signals are conserved between gametes. Cross between *T. durum* and maize can also produce haploids (Almouslem et al., 1998). It was observed that crosses of durum wheat with maize produce haploids of durum wheat Jauhar et al. (2000). The Phi gene is present in plants of durum and their haploids form univalent and possess irregular meiosis. However, some of the haploids possess viable seeds. The formation of seeds having viable embryos might result due to fusion of unreduced female and male gametes. Therefore, it may be said that lack of pairing may be a prerequisite for the process of meiotic restitution and result in a doubling of the chromosomes. Various studies have also reported the substitution of the durum wheat haploid from the cross of maize and wheat (Dogramaci-Altuntepe and Jauhar, 2001). Haploids from the crosses of durum wheat, bread wheat, and triticale with pearl millet have also been developed (Inagaki and Hash, 1998). Alien cytoplasm can also bring out the chromosome elimination. It is reported that 30% of the progenies are haploids in the cross among *Aechmea caudata* and *Triticum aestivum* (strain salmon) (Kihara and Tsunewaki, 1962). From the finding on the haploid production by diverse cross-combinations, it is very clear that the mechanism of elimination of chromosome must be operative in many other crop species as well. The need is to identify such systems for chromosome elimination for the production of haploids and particularly for the crops where anther culture responds poorly or it is restricted to some selected genotypes.

2.3 AUTOPLOID BREEDING

The method of artificial chromosome doubling with treatment of colchicine has been exhaustively employed for a number of crops. As compared to diploids, induced autotetraploids are often genetically enhanced for vegetative features. The reduction in fertility of seeds is common at the initial stages but assortment can progress seed fertility and lessen multivalent development. Many efforts toward increased fertility, autotetraploids have not gained popularity in commercial crops. Few varieties of tetraploid rye have been marketed as Fourex, Tetra Petkux, Steel, and Dubbel Stahl in Germany, Sweden, and the USA. The kernel size of the tetraploid rye is larger in kernel size with a higher ability to grow under antagonistic circumstances and has higher protein content. Many forage crops produce a large number

of autotetraploids. Many of such plant varieties in forage crops, ornamental plants, and fruit trees have been released. Examples of such varieties include red clovers and Alsike, Svalof's Ulva, and Pusa Giant Berseem. Varieties of tetraploids of *Zizania*, *Antirrhinum*, *Chrysanthemum*, spinach, Turnips, radish, snap-dragon, and pierce grapes have been also released. The sterility of seeds in autotetraploids has been used as an benefit in seedless crops. A well-known example of triploid is seedless watermelon. Viable triploid seeds are produced when $4n$ female is crossed with $2n$ male. But only 1% or even less of the all gametes are viable in the case of triploid progenies. Therefore, triploid produce highly sterile seeds and no true seeds are produced. Triploid sugarbeet is one more example of successful triploid breeding. These varieties have larger tubers and more sugar content. The improvement of the allopolyploids has been successful by chromosome doubling.

2.4 SYNTHETIC AMPHIPLOIDS

A number of crops are natural allopolyploids and a number of amphiploids have been formed by the intercross between two or more distantly related taxa which is then followed by chromosome doubling for restoring the chromosomal balance. Since the discovery of colchicine, a number of amphiploids have been produced from hybrids that involve wild or cultivated relatives. In many cases, these amphiploids have different levels of fertility. These kinds of amphiploids do not have genomic harmony and have meiotic instability and many other undesired characteristics which may come due to nuclear–cytoplasmic interactions. Although, a number amphiploids have been produced yet successful instances of artificial amphiploid are very less. It thus combines better agronomic traits of *Secale cereale* which are not existing in *T. aestivum* and permits its farming in areas where wheat is not grown traditionally. Hundred or more varieties of triticale have been released but these are not popular because of poor grain characteristics and infertility despite their perfect chromosome balance. These kinds of man-made amphiploids have been used successfully for crossing with the naturally occurring diploids/polyploids to get desired allopolyploids.

2.5 EXTRACTED ALLOPLOIDS

Amalgamation of a whole alien genome may bring in several objectionable features. So, alternatively, stable allopolyploids can be extracted from the crosses of natural allopolyploids or wild-type varieties with synthetic amphiploids.

Triticale is the best example for the demonstration of the utility of extracted allopolyploids. In the terms of seed yield and meiotic stability, hexaploidy triticales have been proven better. From the progenies of (1) hexaploid triticales $\times$ octaploid triticales and (2) hexaploid triticales $\times$ hexaploid wheat, extracted triticales are much better. They have superiority over the primary triticales in the respect of meiotic stability with yield and quality of grain. This superiority may be attributed to the genetic recombination of genes in the A and B genome of wheat where some of the rye chromosomes are substituted with the D genome of bread wheat. Among 41 varieties of triticales, 29 have RID chromosome substitution. Armadillo strains that carry 2R-2D chromosome substitution are the most popular hexaploidy triticales. Crosses of Armadillo with 8x triticales produce Maya2Armadillo, out of which Mapache was selected and was marketed in Mexico with the name Cananea-79. The extracted allopolyploids are a popular choice for introgression of genes from manmade allopolyploids with natural allopolyploids. *Brassica* is another best example of improved lines where these improved lines are obtained by crossing the natural allopolyploids with the man-made allopolyploid. Cotton in the same way is a good example of fiber strength taken from *Gossypium thurberi* combined with *Gossypium hirsutum* through synthetic allotetraploid *arboretum-thurberi*. In order to develop disease and insect resistance, tolerance toward abiotic stresses and other improved characteristics, a number of genes have been introgressed in to variety of crops through crosses with their wild-type relatives. The F_1 steriles are backcrossed to their recurrent parent so that the necessary alien gene can be put and the stable lines can be developed. A number of examples of introgression of alien genes can be seen in wheat, rice, cotton, barley, tomato, sunflower, etc.

2.6 ANEUPLOID BREEDING

A huge number of aneuploids have been produced. Examples of aneuploids include monosomics, primary trisomies, secondary trisomies, tertiary trisomies, nullisomics, and compensating trisomies. After characterization, these stocks have been employed in genetic analysis and then the mapping of major genes. Such genes are involved in governing quantitatively inherited characters. The modifications in the genetic ratios which involve crosses of aneuploids with normal stocks consisting of consistent chromosome groups are the basis of gene mapping by the use of aneuploid stocks.

2.6.1 MONOSOMICS ($2N-1$)

In many polyploid plant species, for example, wheat, cotton, *Nicotiana*, oats, etc., monosomics have been studied and exhaustively studied in wheat. Sears was the first scientist to find that seven homologous groups of three distinct genomes are constituted from 21 different chromosomes. The chief foundation for this was that every tetrasome has the ability of compensating for the nullisome of the other two chromosomes in the identical group. The deleterious effects of the nullisomes are compensated by the tetrasomes (Sears et al., 1996). Chromosome substitution lines of wheat have been developed by mapping numerous genes with the use of monosomics.

2.6.2 PRIMARY TRISOMIES ($2N+1$)

In addition to the normal diploid set of chromosomes, primary trisomes contain one extra chromosome. Main trisomies are most comprehensively discovered aneuploids for assigning the genes to specific chromosomes in diploid species. Species like tomato, maize, barley, rice, *Datura*, etc., are examples of these trisomies. They have also found applications in the molecular map construction in rice.

2.6.3 SECONDARY TRISOMIES

When the additional chromosome is isochromosome in respect of one of the chromosome arms in the counterpart, these form secondary trisomies. Secondary trisomies are reported in rice, tomato, maize, and barley. These trisomies have found their use in the mapping of some genes on specific arms of tomato, rice, and barley chromosomes (Singh et al., 1996b).

2.6.4 TELO-TRISOMIES

The first telotrisomic was informed in *Zea mays* by Rhoades (1936). After that telotrisomics have been developed for many species including rice, *Datura*, *Triticum*, *Lycopersicon*, *Nicotiana*, and *Secaie*. Secondary and telotrisomics have also been employed in the mapping the centromeres on the rice genetic map (Singh et al., 1996a). A complete set of telotrisomies in rice have been reported to cover all the 24 chromosome arms (Cheng et al., 2001). For the confirmation of telocentric characteristics of additional

chromosome in telotrisomic stocks of rice centromere BAC cloning has been performed.

2.6.5 TERTIARY TRISOMIES

When extra chromosomes consist of two arms of two different (nonhomologous) chromosomes, they form tertiary trisomies. After the assignment of a gene to a particular chromosome using primary trisomies, it becomes likely to bound it to its particular chromosome arm by analyzing the separation in the progeny of primary trisomies. Few genes have been mapped, on the chromosome arms of tomato and barley, with the help of tertiary trisomies.

2.6.6 BALANCED TERTIARY TRISOMIES

Balanced tertiary trisomies were produced by Ramage (1965) for the F_1 hybrid barley production. On the normal chromosome male sterility genes is present in recessive form while an extra chromosome carries a dominant allele associated with the translocation point. Hembar, a barley variety, released was produced with the use of trisomies which were derived from X-ray-induced segmental interchange T2-7d in bonus barley. The system now does not feel the need for the genes responsible to restore fertility in the pollen of the wheat. The balanced tertiary trisomies do not produce sufficient pollens for pollination of male-sterile diploids for an increase in the seed of sterile male parent. Therefore, this system is not practically suitable for commercial-scale breeding of barley.

2.6.7 COMPENSATING TRISOMIES

These are the trisomies having one chromosome missing compensated by the other two modified chromosomes. On the basis of their exclusive segregation patterns, they can have more advantages over other trisomies while indexing gene on arm of specific chromosome. Several genes in tomatoes have been located using these trisomies (Khush and Rick, 1967).

2.7 ALIEN CHROMOSOME ADDITIONAL LINES

There is a lack of recombination between the parental species' alien genes and it plays as a barrier in the introduction of alien genes into the crops. There could also be physiological or genetic imbalance due to the assimilation

of the comprehensive alien genome. Therefore, it is advantageous to add or substitute only singly chromosome and unite small alien chromosome section or gene by means of manipulation of pairing controlling mechanism. A method for single chromosome pair addition from the donor species to the receiver species was described by O'Mara (1940). These alien addition lines have been released in wheat, cotton, maize, oats, potato, rice, sugarbeet, etc.

In 1945, Gerstel produced a mosaic resistant variety of *N. tabacum* and added 11 chromosome pairs from *Nicotiana glutinosa*. Monosomic addition lines of corn-*Tripsacum* were produced from multiple recessive tester stocks of corn. Monosomic Alien Addition Lines (MAALs) in rice have been produced and these lines carry extra chromosome from six species of wild type. Additional lines were used to locate genes of *Neovossia indica* resistance on 6R and 7R of Rye and 1H and 6H of barley (Dhaliwal et al., 1987). In tomato, seven chromosome addition lines, out of 12, were recognized in the background of the potato (Garriga-Caldere et al., 1998). A complete set of MAALs has been produced which represents all the 10 chromosomes in oat when maize and oat were crossed (Kynast et al., 2001). For chromosomes 1–4, 6–7, and 9 of maize, disomic addition lines ($2n = 6x+2 = 44$) have been prepared. For the characterization of MAALs in maize, the use of restriction fragment length polymorphism (RFLP) markers has been done. The oat–maize addition lines have appeared to be a cytogenetic stock of high importance.

Ananeiv et al. (1997) used oat–maize addition lines for producing cosmid library. In order to isolate selective cosmid clones having maize genomic DNA, a highly repetitive maize specific sequence as used as multiprobe. Ananeiv et al. (1998) maize centromere-associated sequence (McS1a) to probe for isolation of cosmid clones out of genomic library of addition line of chromosome 9. A complex organization was revealed by the analysis of six cosmid clones. McS1a constituted from long repeat terminals of repeat elements that are retrotransposon-like. Irradiation method has also been used for the production of radiation hybrid of maize–oat chromosome 9 addition line (Riera-Lizarazu et al., 2000). These derivatives of radiation hybrid are good source of DNA from specific region for the gene cloning. With the help of PCR and oat–maize addition lines, 400 sequences to maize have been mapped (Okagaki et al., 2001). These cytogenetic stocks have an important role, in detecting candidate genes in a number of agronomic traits, when combined with the positional cloning. Although no alien chromosome addition line has become popular in the form of commercial variety as these lines are instable and some undesired features are associated with these alien

chromosomes, they are an important source of genetic variations which carry useful genes.

2.7.1 PROPOSED METHOD OF USING ALIEN ADDITION LINES FOR PRODUCING HYBRID WHEAT

Driscoll (1972) described a novel method for the production of F_1 hybrid seeds of wheat by the exploitation of additional lines. The method includes three lines that are designated as A ($2n = 44$), B ($2n = 43$) and C ($2n = 42$). The recessive allele responsible for the male sterility is not present in wheat chromosomes, Whereas the dominant allele demonstrates its presence on the homologous alien chromosome. The C line is male sterile while A and B lines produce fertile males. The main disadvantage of additional lines is that they as such revert to euploid state and are less stable. In a fertilizing egg cell, the pollen with an additional chromosome is less competitive compared to euploid pollen. There is no practicality in the A, B, and C systems as the scheme is associated with limitations of instability of chromosomes in addition lines and there is inadequate cross-pollination which restricts the seed multiplication.

2.8 ALIEN CHROMOSOME SUBSTITUTION LINES

Many researchers have discussed different methods for the production of substitution lines with the help of monosomic series. In wheat, substitution lines have been studied very exhaustively. In *Nicotiana*, *Triticum*, and *Gossypium* species, alien chromosome substitution lines have been developed. Alien chromosomes from barley, *Agropyron*, *Secale*, and *Aegilops* were used for substitution line of wheat and *Nicotiana glutinosa* and *Nicotiana pumbaginifolia* for the substitution lines of *Nicotiana tabacum*. In the case of tobacco, the production of substitution lines was carried out for the transfer of resistance against tobacco mosaic black-shank disease (Gerstel, 1945). A commercial variety of wheat, known as Weique, containing pair of substituted chromosomes from *Agropyron intermedium* has been developed as a variety resistant against stem rust (Wienhues, 1965). For the production of varieties of oats that are resistant to mildew have been produced with the help of substitution lines. The resistance genes were taken from *Avena barbata* and transferred to *Avena sativa*. In rice cultivars, a succession of overlapping chromosome substitution lines was produced with the molecular markers. Chromosome segments of *Oryza giaberrima* were carried to *Oryza sativa*

(Doi et al., 1997). Despite production of a huge figure of substitution lines of wheat, a few could find their names as commercial cultivars. In many cases, there is no complete compensation for the alien chromosomes for the substituted chromosome and sometimes the resultant may carry undesired genes along with the required genes. Some wheat cultivar varieties produced by Germany and Eastern Europe are the spontaneous substitutions. In such cases, 1R chromosome from rye substitutes the 1B chromosome of wheat (Zeller, 1973). Rajaram et al. (1983) reported several cultivars produced at CIMMYT. In these varieties, 1B chromosome segment was substituted with a segment of 1R from rye (Rajaram et al., 1983). These varieties had a wider adaptability and resistance against *Septoria tritici*. Many of the varieties of wheat cultivars possess wheat-rye (1BL.1RS) translocations.

2.8.1 INTERVARIETAL CHROMOSOME SUBSTITUTION

Sears (1953) developed a series of aneuploids of wheat. These aneuploid stocks have been extensively used for the production of intervarietal, interspecific, and intergeneric chromosome substitution lines. Law (1981) produced chromosome substitution lines of Chinese spring and Cappelle-Desperez; Koga-II and Cappelle-Desperez; and Cappelle-Desperez and Vilmorin-27. Intervarietal chromosome substitutions have found their application in the genetic analysis of agronomic traits. Pairs of home chromosomes have been substituted with the homologous chromosomes in Chinese spring. The outcomes showed that the yield, height of plants, and the chromosome interactions estimations were almost all positive while within the chromosome, interactions were nearly all negative. A lot of information related to the genetics of quantitative features of wheat, cotton, and *Nicotiana* has been available with the help of intervarietal chromosomal substitutions.

2.9 ALIEN GENE TRANSFER AND INDUCED HOMOEOLGOUS PAIRING

Intergenic pairing between chromosomes from A, B, and D genomes was absent in the case of wheat. Okamoto (1957) reported that the 5B chromosome of wheat transports a gene for the suppression of pairing of homoeologous chromosomes. When this gene (Ph, pairing homoeologous) was absent, the homoeologous chromosomes of the genomes (A, B, and D) were found to pair with each other. This was also accurate in the case of pairing among homoeologous genes among related species and genera. Similar to wheat in

hexaploid tall fescue, Jauhar (1975) studied genetic control of diploid-like meiosis. Genetic control was indicated by the observation of multivalents in a monosomic line (90-64-7). Homoeologous pairing can be induced with manipulation *Ph* gene locus. With this, we can obtain recombination of wheat chromosomes with other alien species which otherwise may not be involved in pairing. Other than this, pairing regulators are located in 3A, 3D, and 4D chromosomes in the case of wheat. Wild-type varieties like *Avena longiglumis* and *Aegilops speltoides* have genes for the enhancement of homoeologous pairing in F_1 hybrids. Similar to *Ph* gene B-chromosomes also suppress pairing in some strains (Dover and Riley, 1972). There are three methods for inducing homoeologous and transferring alien genes of high agronomic importance to the commercial varieties of crops.

2.9.1 THE INDUCTION OF HOMOEOLOGOUS PAIRING VIA WILD SPECIES

Homoeologous pairing in wheat and oats is known to be induced by wild species such as *Aegilops speitoides* and *Avena longiglumis* accession Cw57, respectively. For the development of rust-resistant variety of wheat by transferring gene from *Aegilops comosa* to wheat has been done with the application of additional line of *A. comosa* which has an extra chromosome pair (2M) (Riley et al., 1968). This disomic addition line was further crossed with *A. speltoides* having $2n = 14$ chromosomes. Due to the suppression of *Ph* action in this hybrid of 29-chromosomes, the *A. comosa* 2M chromosome gets paired with chromosome-20 of wheat. In this case, the homoeologous pairing takes place as the effect of *Ph* gene is neutralized by the dominant allele of *A. speltoides*. Compare is a selected wheat line that has rust resistance gene transferred from *A. comosa*. With the help of the same technique, gene of brown rust was transferred from *Agropyron elongatum* to wheat (Sears, 1972). *A. longiglumis* Cw57 strain has gene for neutralizing the effect of *A. sativa* gene responsible for suppressing homoeologous pairing. Mildew resistant variety of cultivated oat *A. sativa* has been prepared successfully by transferring genes from *A. barbata* and *Atriplex prostrata* with the help of *A. longiglumis* (Thomas et al., 1980; Griffiths and Thomas, 1983).

2.9.2 USE OF NULLISOMIC 5B SYSTEM

When chromosome 5B is absent, pairing between wheat chromosomes and alien species chromosomes starts taking place. Sears (1973) synthesized

numerous wheat lines carrying leaf rust resistance gene from *A. elongatum* by means of a nulli-5B system. Various wheat–Agropyron transfers were carried out with induced homoeologous pairing (Sears, 1972, 1973).

2.9.3 USE OF INDUCED *Ph* MUTANT

For alien gene transfer, the *Ph* mutant for the induction of homoeologous pairing has not been employed so exhaustively. Wall and group isolate *Ph* mutant after treatment with Ethyl Methane Sulphonate (Wall et al., 1971). However, this mutant has not very high impact on homoeologous pairing as compared to nulli-5B. Sears (1977) isolate another *Ph* mutant by using X-rays on normal pollen. The irradiated pollen was used for pollination of monosomic 5B plants having marker genes (Hairy-Neck). A single mutation with *Ph* deficiency was obtained when 438 M_1 plants were tested for pairing mutations and most of them were crossed with *Trochus kotschyi*. Recombination between 4B chromosome of wheat and streak mosaic virus resistance genes of *A. intermedium* chromosome was induced by using *Ph* mutant (Wang et al., 1977). In order to obtain homoeologous recombinants and transfer genes for high protein from *T. dicoccoides*/*Triticum turgidum* to *T. aestivum*, *Ph1b* and *Ph2* mutants were used (Khushnir and Halloran, 1984). A mutant was isolated in tetraploid wheat variety called Cappelli. This variety has amplified homoeologous pairing toward *S. cereale* and *A. longissimi* (Giorgi and Cuozzo, 1980). Four tetraploid species of *Aegilops* namely *A. kotschyi*, *A. cylindrica*, *A. triuncialis*, and *A. columnaris* were crossed with a *Ph* transform of durum wheat by Giorgi and Barbera (Giorgi and Barbera, 1981). In all these crosses, chromosome pairing was very high compared to those in the control hybrids. *Ph1b* mutants were used for the production of barley–wheat hybrids and recombination was carried out between wheat and barley using isozyme markers (Islam and Shepherd, 1992). A PCR-based, *Ph1b* deletion-specific marker was developed by Gill and Gill (1996). A wheat–barley hybrid with recombination of chromosome 4 and chromosome 7 has also been produced by Sherman et al. (2001). Disomic addition lines of wheat–barley were crossed with the Chinese spring variety of wheat having *Ph1b* mutant for the promotion of homoeologous pairing. For the barley introgression, PCR-based markers were employed. When progenies were tested, they showed that 11 recombinants were found to involve chromosome 7 of barley, whereas 9 recombinants involved chromosome 4. A homoeologous recombinant was induced between wheat and *Thinopyrum*

with the help of *Ph* mutant (Xin et al., 2001). YW642, YW643, and wheat-*Thinopyrum intermedium* translocation lines possess high resistance against barley yellow dwarf virus (BYDV). Genomic in situ hybridization (GISH) revealed that some of the above-mentioned lines are homozygous wheat-*T. intermedium* translocation lines. Dvorak and Dubcovsky (1995) did a genetic analysis of the chromosomes produced as a result of targeted recombination of two pairs of related chromosomes when *Ph1* gene was not present. When the *Ph1* is present, crossing over was a frequent phenomenon in the homologous regions while the frequency of the cross-overs was low in the homoeologous regions. The crossing over frequencies in the case of homoeologous regions was akin to those in the case of crossing over between wheat 1A homologous, when the *Phi* gene was absent. When *Phi* gene was absent, the crossing-over frequencies were reduced at all intervals in case of cross-over between 4B and 4D.

2.10 IRRADIATION OF CYTOGENETIC STOCKS FOR ALIEN GENE INTROGRESSION

For any procedure, like the alien genes introgression, addition or substitution of a single alien chromosome, or incorporation of complete alien genome like in amphiploids, the irradiation of the cytogenetic stocks has not been very successful. In this method, seed or pollen of the MAALs is irradiate and after that, in the successive progenies alien chromosome segment translocations are recovered. The recovery of these alien chromosome segments may be genetical, cytological, or both. This sophisticated method for the transfer of a small chromosomal segment that carries a gene resistant against leaf rust from *A. umbellulata* to chromosome 6B of wheat (Sears, 1956). After this experiment, this method has been used for the transfer of many genes. Using the above method, various genetically enhanced and better strains having useful alien genes for resistance against a numerous disease of wheat have been produced with chromosome 6A of wheat.

The translocation of wheat stem rust resistance genes from *A. elongatum* chromosome to chromosome 6A of wheat has been the most successfully achieved transfer (Knott, 1961). In a variety named as Riley67 produced at Purdue University is soft red winter wheat and has rust resistance against leaf rust taken from *A. umbellulate*. A gene resistant against mildew from *A. barbata*, a tetraploid, into cultivate oat with the help of induced translocation has been successfully produced in 1976 (Aung and Thomas, 1976). A

segment of gene from *A. strigose* was transferred to *A. sativa* with the help of thermal neutrons. This transferred segment carried Pe15 gene and this gene possesses resistance against crown rust. Crasta et al. (2000) irradiated seeds with MAALs and M2 population was then analyzed. These lines carried a gene resistant for BYDV (Crasta et al., 2000). For the identification of wheat-*T. intermedium* translocation lines, RFLP and *Thinopyrum* chromosome-specific repetitive elements were employed. The location of the resistance gene for BYDV was found at the distal end of the introgressed *T. intermedium* chromosome.

2.11 ENHANCEMENT OF CHROMOSOMAL EXCHANGES AND TRANSFER OF GENE AMONG DISTANT GENOMES

There is a strong diversity among genomes, therefore, distant hybrids many of the times lack chromosomal exchange. When there are translocations and breaking of chromosome, changes in karyotype occur in numerous plant species. Among these karyotypic changes, role of translocations is very important for the introgression of beneficial alien genes to the cultivars. The somaclonal variants attained through tissue culture of sterile cross breeds (*H. vulgare* × *Hordeum jubatum*) had increased bivalent formation in comparison with the original asynaptic hybrids (Orton, 1980). In total, 4000 plants of triploid rye grass were produced from plant tissue culturing (Ahloowalia, 1983) in which, many other rearrangements were shown along with chromosomal translocations in the regenerants. In the amphiploids of wheat and rye hybrids regenerated with the help of plant tissue culture, a high degree of structural changes in chromosomes was reported (Lapitan et al., 1984). C-banding of 10 amphiploids was analyzed by karyotype investigation and three wheat × rye, one wheat × wheat, seven deletions, and five intensifications of heterochromatin bands of rye chromosomes were observed (Lapitan et al., 1984). A number of translocation stocks, having gene resistant against BYDV from disomic addition line, were obtained. In similar manner, the gene with resistance against cereal cyst nematode has been introgressed from rye. Enhanced frequency of pairing among regenerates resulted from tissue culture of *H. vulgare* × *Elymus canadensis* was observed (Dahleen, 1999). Intergeneric translocations of chromosomes between *Dasypyrum* and *Triticum* have been reported (Li et al., 2000).

Hybrids of winter wheat and winter barley with the help of tissue culture have been multiplied and after this, it was pollinated with wheat, and back-cross was produced. Meiotic analysis of these hybrids revealed that per

cell association was of 1.59 chromosome arm. But, after in vitro culture in hybrids, the number of chromosomal associations was increased to 4.72 per cell (Molnar-Lang et al., 2000).

For the introgression of alien genes with the use of tissue culture of species, this method can be promising. This method shows a smaller number of homoeologous pairings. The genetic exchange between the cultivated and alien genomes could be enhanced by tissue culture cycles with the use of explants of wide hybrids and somatic hybrids. Tissue culture can also be employed for transferring novel traits through chromosomal segment exchanges. The exchange of genetic material among cultivated and alien species can also be enhanced by the irradiation of fusion products of protoplast. The tissue culture technique has already been used for the production of a number of intergeneric and interspecific somatic hybrids. This technique may be used to bring out desirable translocations between distant genomes.

2.12 STRUCTURAL CHANGES IN CHROMOSOMES

In rice, maize, barley, etc., numerous cytogenetic stocks have been produced which involve chromosomal interchanges, deletions, inversions, and duplications. These stocks have found their role that is the establishment of linkage groups and gene mapping in maize, barley, and other crops.

Chromosomal aberrations have also been reported by different researchers (Bhat et al., 2005a; Gulfishan et al., 2013; Bhat et al., 2005c; Ganai et al., 2005; Bhat et al., 2005c; Wani et al., 2005; Khan et al., 2005a, b) after treatment to plant species by physical and chemical mutagens to induce genetic variability for harvesting the positive variability for improvement of agronomic characters (Bhat et al., 2006a, b, c, d, 2007a, b, c, d; Parveen et al., 2006, 2007; Khan et al., 2005a, b; Sharma et al., 2009; Gulfishan et al., 2010). These chromosomal aberrations include stickiness, laggards, bridges, disturbed polarity, multipolarity, micronuclei, cytomixis, etc. (Bhat et al., 2007; Gulfishan et al., 2011; Bhat et al., 2005b; Gulfishan et al., 2012; Bhat et al., 2007b; Gulfishan et al., 2015; Bhat et al., 2006b; Bhat and Wani, 2017; Wani and Bhat, 2017; Bhat et al., 2017).

2.12.1 TRANSLOCATIONS

In the gene mapping and genetic analysis of traits in barley and maize, all types of structural abnormalities, translocations have been exhaustively

employed (Burnham, 1962; Singh, 1993). Many vegetables and seedless fruits can be produced with multiple chromosome interchange stocks. For the doubling of well-defined segment of chromosomes 6–7 in barley. This seems to increase the yield in the promising manner (Hagberg and Hagberg, 1987). Duplications in the short arm of chromosomes 5 and 6 are also being produced by translocations. The short arm of chromosome 5 has mildew resistance gene while chromosome 6 has alpha-amylase activity. For the permanent heterosis in barley, the doublings of V-v factor can be commercially used.

2.12.2 A-B TRANSLOCATIONS

Among many plant species, commonly known supernumerary chromosome or accessory chromosome, B-chromosomes can be found. In maize and rye, these genes have been studied extensively. These B-chromosomes are highly heterochromatic and contain a minute short arm. The centromere is followed by euchromatin and the long arm is made up of heterochromatic regions. These B-chromosomes have a rare feature of nondisjunction during the second pollen mitosis. The B–A translocation creates a deficiency duplication that can be used for gene mapping.

B chromosomes consist of a heterogeneous cluster of correlated definite sequences. This cluster can be localized to the functional centromere by the derivatives of misdivision. The repeats of (CCCTAAA)*n* motifs are highly specific at the B-specific regions and these centromeric regions are almost characteristics of centromere in many organisms. When an abnormal chromosome 10 is present in maize, a homology is observed to the maize knob and it acts as a kind of facultative centromere. When there are reduced B-specific sequences, it indicates that they are derived from the centromere.

2.13 PRODUCTION OF HOMOZYGOUS LINES VIA SYNTHETIC COMPLEX TRANSLOCATION STOCKS AND SEEDLESSNESS

For the establishment of homozygous lines and gametic selections, numerous interchanges which comprise all the chromosomes of the haploid matches have been produced. Homozygous lines can be produced by using multiple interchanges and this method of gamete selection is called as “*Oenothera*” (Burnham, 1946). In the case of *H. vulgare*, *Triticum monococcum*, *Pennisetum glaucum*, and *Z. mays*, complex interchange stocks have been prepared

by using recurrent irradiation and intercrossing methods. As complex interchange stocks are highly sterile, this method could not be popularized for the development of homozygous lines. This technique has found its application in the commercial production of F_1 seedless watermelons.

2.14 IDENTIFICATION OF ORTHOLOGOUS LOCI AND STRUCTURAL DIFFERENTIATION OF GENOMES: COMPARATIVE MAPS

Employing molecular markers have now made it possible to find the genomic relationships across cross-ability barriers among different species and genera. With the help of conventional cytogenetic methods, such analysis is tough. There is an extensive collinearity among potato and tomato genomes and a karyotypic difference is found to be caused by a few rearrangements in structural chromosomes (Bonierbale et al., 1988). Out of 12 chromosomes, for nine chromosomes the order of loci was the same. But, for three chromosomes, intrachromosomal rearrangements were found. Three were found to be paracentric inversions and had one break point near/at the centromere. In barley, wheat, sugarcane, and foxtail millet, at several regions of genomes, extensive homologues have been found (Ahn et al., 1993). The mapping shows that for all species within the grass family, gene order is highly conserved. However, organization and amount of the repetitive DNA sequences may vary considerably (Devos and Gale, 1997).

Hybrids of *Oryza meyeriana* and *Oryza ridleyi* are highly difficult to be produced due to the strong cross-ability barriers. Therefore, on the basis of meiotic pairing, assigning genomes may not be carried out. Hence, total genomic DNA hybridization-based alternative methods can molecular divergence have been used. With the help of this method, two new genomes allotted to *O. ridleyi* and *O. meyeriana* complexes are GG and HHJJ, respectively (Aggarwal et al., 1997). Comparative RFLP map of *Oryza officinalis* (CC), wild species, and *Oryza sativa* has been constructed (Jena et al., 1994). On different chromosomes of the wild species *O. officinalis*, the linkage order was almost conserved compared to *O. sativa*, nonetheless chromosomal rearrangements were observed. In rice, out of 12 chromosomes, 9 were found to be homosequential with the wild species. Comparative maps of wild rice (*Zizania palustris* $2n = 2x = 30$) and *O. sativa* ($2n = 2x = 24$) were prepared by Kennard et al. (1999). Out of 12 linkage groups, for 11 colinear markers were found in rice, although two species have genomes with different DNA

contents. In order of identification and isolation of orthologous loci from different plant systems, comparative maps provide new opportunities.

2.14.1 CHROMOSOMAL IMAGE ANALYZING SYSTEM

In the area of characterization of plant chromosomes and developing quantitative cytological maps, development of imaging methods has taken place (Fukui and Ijima, 1991). To the existing cytological methods of chromosome analysis, the imaging system has become an important enhancement.

2.15 CHROMOSOME BANDING, CHARACTERIZATION OF KARYOTYPES AND ALIEN INTROGRESSION

For the identification of individual chromosomes, development of chromosome banding methods has become the additional tool. Giesma C-banding has been employed for the identification of chromosomes and in the analysis of evolutionary relationships and for the detection of introgressed sections of alien chromosomes. The banding techniques are useful in identifying the chromosome segments which have AT or GC rich content. The method is also employed for the recognition of constitutive heterochromatins. In barley, identification of all the chromosomes can be done by methods of C- and N-banding, which shows heterochromatin blocks as regions to be dark stained. On the basis of banding in cross between wheat and *A. Intermedium* was identified and alien addition lines were derived by Friebe and group (Friebe et al., 1992). GISH and C-banding procedures were used for the analysis for chromosomal constitution of eight radiation-induced, a rust resistant derivative of wheat-*A. intermedium*. Wheat germplasm having resistance against wheat streak mosaic virus obtained from *A. elongatum* has been analyzed (Jiang et al., 1993). In total, 436 deletions in wheat have also been identified with the use of chromosome banding (Endo and Gill, 1996). Transfer of these deletion chromosomes to the progeny was stable.

2.16 IN SITU HYBRIDIZATION: ALIEN INTROGRESSION AND KARYOTYPIC CHANGES

The development of in situ hybridization procedures has been a key success in the area of chromosome research. Conventional cytogenetic methods

along with the molecular markers and FISH have improved the precision of characterization on changes in karyotypes, determination of differences in the structure of chromosomes, differentiation between genomes from different species, identification of chromosome segments introgressed and alien genes. In situ hybridization analysis, both meiotic and mitotic chromosomes have found their use. The in-situ hybridization analysis involves the hybridization of nucleic acids (RNA or DNA) probes to the cytological preparations and specific nucleotide sequences are directly visualized on the chromosomes. Gall and Perdue developed this method in 1969. Since this method has been developed, in order to map low copy DNA sequences and repetitive DNA sequences, isotopic maps have extensively been used. Nonisotopic procedures for in situ hybridization were tested and developed by Rayburn and Gill (1985). Nowadays, FISH has appeared to be the technique of choice in in situ hybridization analysis. Various other methods like GISH can also be used. A number of studies have been published on the position of the repetitive DNA sequences on the chromosomes of barley, wheat, etc. BAC and YAC libraries have enhanced the potential of physical mapping of small sequences and single copy on chromosomes. In total, 40 kb BAC clones were physically mapped and these clones contained Xa21 on 11th chromosome of rice for the resistance against bacterial blight (Jiang et al., 1995). Now as a main progression, extended DNA fiber-FISH is used for more precise characterization of the genes.

GISH can be effectively used for the detection of introgression of alien chromosomes. Breakpoints in the translocation lines of wheat-rye having resistance against Hessian fly were analyzed using in situ hybridization (Mukai et al., 1993). Introgression lines of tomatoes having chromosome six region from *Lycopersicon peruvianum* having resistance against nematode gene *Mi* were analyzed by Zhong and group (Zhong et al., 1999). The *Mi* on pachytene chromosomes was located with the help of FISH and Mo-BAC clones. The location of *Mi* was found in the short arm (6S) on the border of euchromatin and heterochromatin regions and on the long arm near to euchromatin in Aps-1 of pericentromeric heterochromatin. Introgression lines produced from cross between *T. aestivum* × goat grass were analyzed and introgression alien chromosome sections were found. The recombinants synthesized from barley were analyzed by means of sequential GISH and FISH (Pickering et al., 2000). In barley chromosomes 2H, introgression was located on the long arm with the use of FISH along with a short oligonucleotide sequence as a probe. Intergeneric F_1 hybrids of *Thinopyrum junceiforme* ($J_1J_1J_2J_2$) and durum wheat (*T. turgidum* AABB) were produced

(Jauhar and Peterson, 2000). With the help of FISH, segmental exchange of alien chromosomes was confirmed. To wheat having scab resistance from *Thinopyrum*, some of the derived lines exhibited introgression. GISH was used for the analysis of somatic hybrid (tomato + potato) and MAALs of potato having chromosomes 1 and 8 from tomato (Garriga-Caldere et al., 1999). Lower levels of allosyndetic pairing were observed for monosomic additions; 1.1% for chromosome 1 and 1.3% for chromosome 8.

In a number of species, for example, rice, potato, etc., having somatic chromosomes of small size, there is no possibility of distinguishing between chromosomes on the basis of banding patterns and morphological characteristics. So, the large-insert DNA clones can be used conveniently as DNA markers, where these markers are chromosome-specific. The use of extended DNA fiber has provided a major development in the FISH detection and improved the sensitivity of the technique.

2.16.1 CHARACTERIZATION OF HOMOEOLOGOUS PAIRING THROUGH FISH

It is way harder to differentiate between autosyndetic and allosyndetic pairing among hybrid species using conventional cytogenetic methods. However, FISH comes with an advantage for precise characterization of pairing between the genomes and within the genomes. This technique becomes more useful when the size of the parent chromosomes is similar and there are no cytological markers available for the diagnostics. A comparison of homoeologous pairing, between the wheat and rye derivatives, has been done using GISH and C-banding. The detection of homoeologous binding by GISH was 9.2% while when binding was detected using C-banding it was 2.5%. With this, it was shown that C-banding underestimates the associations between wheat and rye (Begona et al., 1995). In *Oryza*, autosyndetic and allosyndetic pairing was detected by GISH among A and E genomes (Abbasi et al., 1999).

2.17 FLOW SORTING AND CHROMOSOME-MEDIATED GENE TRANSFER

When there is a need for the characterization of materials having different levels of ploidy, flow cytometry has appeared as a very useful tool. The technique has been employed for the characterization of $n + n$, $2n + n$ back-cross progenies that are derivative of crosses among maize and apomictic *Tripsacum*. Flow cytometry has found its application in the sorting of

chromosomes in wheat, *Petunia*, *Haplopappus*, tomato, etc. This practice is competent in the categorization of specific chromosomes and speedy quantifiable karyotyping. In the blend of wheat chromosomes, only 3B chromosome could be identified with help of flow karyotypes. Rest all the chromosomes were observed as three composite peaks and were sorted as groups (Vrana et al., 2000). These sorted chromosomes may be employed for the development of addition lines and also for chromosome-mediated gene transfer methods. These sorted clones can also find applications in the cloning. Chromosomes can be isolated from cultured cells with the use of refined protocols. Szabados and group developed a technique with which protoplasts of other species can uptake the isolated chromosomes. Polyethylene glycol induces the uptake of the isolated chromosomes into the recipient species protoplasts. This method seems promising but it has not found its application for introducing isolated single chromosomes.

2.18 MICRODISSECTION OF CHROMOSOMES

The microdissection method was used for the first time on *Drosophila* polytene chromosomes. After this, the procedure of microdissection of chromosomes have been tried on rye, wheat, barley, oat, etc., in this technique, small copies of specific chromosomes/chromosome regions or arm are isolated with the use of micromanipulator. A very little starting material is required for this method. The isolation of chromosomes could only be done from one single slide made from meristem of a single root tip. Microdissection of chromosomes has also been done in rice (Cheng et al., 2001). By employing microneedle, the additional chromosome arms of $2n + .5S + .5S$ and $2n + .5L$ have successfully been microdissected. The microneedle was controlled by a Leitz micromanipulator. Microsatellites from different chromosome arms and primer pairs from STS were selected. They were amplified with PCR and the PCR products from both control samples and microdissected chromosomes. With the result analysis of this experiment, it was discovered that the amplified DNAs from these microdissected chromosome arms were resulted from 5S and 5L chromosome arms.

2.19 PLANT ARTIFICIAL CHROMOSOMES (PAC)

Major progress has been made for the construction of BAC and YAC. In the physical mapping of plant genomes, both YAC and BAC have been exhaustively used. In the present times, attempts for the construction of the artificial

plant and animal chromosomes are being made. Artificial human chromosomes were constructed by Rosenfeld (1997) and Harrington (1997). In order to create non-natural chromosomes in Human HT1080 cells, elongated synthetic arrays of α -satellite DNA were combined to genomic and telomeric DNA. The resulted linear microchromosomes contain exogenous α -satellite DNA. These are stable at mitotically and cytologically. The construction of PACs has taken a lead in research and three basic prerequisites mandatory for this are (1) origin of replication for duplication of chromosomes, (2) a centromere to permit the division and movement along the microtubules of replicated chromosomes into daughter cells, and (3) telomere at the terminals to confer stability. PACs are useful for the understanding of regulation gene expression which includes probable governing elements and for the conservation of information derived from genes.

KEYWORDS

- **FISH**
- **fiber-FISH**
- **PAC**
- **cytogenetic stock**
- **plant breeding**

REFERENCES

- Abbasi, F. M.; Brar, D. S.; Carpena, A. L.; Fukui, K.; Khush, G. S. Detection of Autosyndetic and Allosyndetic Pairing Among A and E Genomes of *Oryza* Through Genomic In Situ Hybridization. *Rice Genet. Newsl.* **1999**, *16*, 24–25.
- Aggarwal, R. K.; Brar, D. S.; Khush, G. S. Two New Genomes in the *Oryza* Complex Identified on the Basis of Molecular Divergence Analysis Using Total Genomic DNA Hybridization. *Mol. Gen. Genet.* **1997**, *254*, 1–12.
- Aghaee-Sarbarzeh, M.; Singh, H.; Dhaliwal, H. S. *PhI* Gene Derived from *Aegilops speltoides* Induces Homologous Chromosome Pairing in Wide Crosses of *Triticum aestivum*. *J. Hered.* **2000**, 536–5163.
- Ahloowalia, B. S. Spectrum of Variation in Somaciones of Triploid Rye Grass. *Crop Sci.* **1983**, *23*, 1141–1147.
- Ahn, S.; Anderson, A.; Sorrells, M. E.; Tanksley, S. D. Homoeologous Relationship of Rice, Wheat and Maize Chromosomes. *Mol. Gen. Genet.* **1993**, *241*, 483–490.

- Almouslem, A. B.; Jauhar, P. P.; Peterson, T. S.; Bommineni, V. R.; Rao, M. B. Haploid Durum Wheat Production via Hybridization with Maize. *Crop Sci.* **1998**, *38*, 1080–1087.
- Ananiev, E. V.; Lizarazu, O. R.; Rines, H. W.; Phillips, R. L. Oat-Maize Chromosome Addition Lines: A New System for Mapping the Maize Genome. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3524–3529.
- Ananiev, E. V.; Phillips, R. L.; Rines, W. H. Chromosome-Specific Molecular Organization of Maize (*Zea mays*, L.). Centromeric Regions. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 13073–13078.
- Aung, T.; Thomas, H. Transfer of Mildew Resistance from the Wild Oat, *Avena Barbara* Into Cultivated Oat. *Nature* **1976**, *260*, 603–604.
- Banks, P. M.; Larkin P.J.; Bariana, H. S.; Lagudah, E. S.; Appels, R.; Waterhouse, P. M.; Brettell, R. I. S.; Chen, X.; Xu, H. J.; Xin, Z. Y.; Qian, Y. T.; Zhou, X. M.; Cheng, Z. M.; Zhou, G. H. The Use of Cell Culture for Subchromosomal Introgressions of Barley Yellow Dwarf Virus Resistance from *Thinopyrum intermedium* to Wheat. *Genome* **1995**, *38*, 395–405.
- Begona EC.; Benavente, E.; Orellana, J. Meiotic Pairing in Wheat-Rye Derivatives Detected by Genomic In Situ Hybridization and C-Banding: A Comparative Analysis. *Chromosoma* **1995**, *103*, 554–558.
- Bhat, T. A.; Gulfishan, M.; Wani, A. A. Cytomixis: Causes and Consequences as a Case Study in *Vicia faba*, L. In *Chromosome Structure and Aberrations*; Bhat, T. A.; Wani, A. A., Eds.; Springer: Germany, 2017.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Comparative Analysis of Meiotic Abnormalities Induced by Gamma Rays, EMS and MMS in *Vicia faba*, L. *J. Ind. Bot. Soc.* 2005a, *84*, 45–48.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Clastogenic Effects of EMS and MMS in *Vicia faba*, L. *J. Cyto. Genet.* 2005b, *6*, 117–122.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by Gamma Rays and EMS in *Vicia faba*, L. *Vegetos* 2005c, *18* (1&2), 143–145.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Effect of Gamma Rays on Certain Cytomorphological Parameters in Two Varieties of *Vicia faba*, L. *Adv. Plant Sci.* **2006a**, *19* (1), 227–232.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Wani, N. A. Radiation Induced Pod and Seed Mutants and MMS Induced Closed Flower Mutant in Broad Bean (*Vicia faba* L.). *Indian J. Genet.* 2006b, *66* (3), 249–250.
- Bhat, T. A.; Parveen, S.; Khan, A. H. MMS-Induced Cytomixis in Pollen Mother Cells of Broad Bean (*Vicia faba* L.). *Turk. J. Bot.* **2006c**, *30*, 273–279.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Studies on the Effect of EMS on Meiosis and Pollen Fertility in *Vicia faba*, L. *Adv. Plant Sci.* **2006d**, *19* (1), 243–247.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by MMS, Gamma Rays and Their Combination in Two Varieties of *Vicia faba*, L. *Asian, J. Plant Sci.* 2007a, *6* (3), 558–561.
- Bhat, T. A.; Parveen, S.; Khan, A. H. Meiotic Studies in Two Varieties of *Vicia faba*, L. (Fabaceae) After MMS Treatment. *Asian J. Plant Sci.* 2007b, *61* (1), 51–55.
- Bhat, T. A.; Sharma, M.; Anis, M. Comparative Analysis of Mitotic Aberrations Induced by Diethyl Sulphide (DES) and Sodium Azide (SA) in *Vicia faba*, L. (Fabaceae). *Pak. J. Biol. Sci.* 2007c, *10* (5), 783–787.
- Bhat, T. A.; Sharma, M.; Anis, M. Comparative Analysis of Meiotic Aberrations Induced by Diethylsulphate and Sodium Azide in Broad Bean (*Vicia faba*, L.). *Asian J. Plant Sci.* 2007d, *6* (7), 1051–1055.

- Bhat, T. A.; Wani, A. A. Mutagenic Effects on Meiosis in Legumes and a Practical Case Study of *Vicia faba*, L. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Bonierbale, M. W.; Plaisted, R. L.; Tanksley, S. D. RFLP Maps Based on a Common Set of clones reveal. Modes of Chromosomal Evolution in Potato and Tomato. *Genetics* **1988**, *120*, 1095–1103.
- Boveri, Th. Uber mehrpolige Mitosen als Mittel Zur Analyse der Zellkerns. *Verh. Phys.-Med. Ges.* **1902**, *35*, 67–90.
- Brar, D. S.; Khush, G.S. Wide Hybridization and Chromosome Manipulation in Cereals. In *Handbook of Plant Cell Culture*, Vol. 4. Techniques and Applications., 1986; pp 221–263.
- Brar, D. S.; Dalmacio, R.; Elloran, R.; Aggarwal, R. K.; Angeles, R.; Khush, G. S. Gene Transfer and Molecular Characterization of Introgression from Wild *Oryza* Species into Rice. In *Rice Genetics III*; International Rice Research Institute: Manila, Philippines, 1996; pp 477–486.
- Brar, D. S.; Minocha, J. L.; Gill, B. S. Translocations in *Pennisetum typhoides* Involving All the Chromosomes in One Complex. *Curro Sci.* **1973**, *42*, 653–654.
- Burnham, C. R. An “*Oenothera*” or Multiple Translocation Method of Establishing Homozygous Lines. *J. Amer. Soc. Agron.* **1946**, *38*, 702–707.
- Burnham, C. R. *Discussions in Cytogenetics*; Burgess Publishing Company, 1962; p 375.
- Cai, X.; Jones, S. S.; Murray, T. D. Molecular Cytogenetic Characterization of *Thinopyrum* Genomes Conferring Perennial Growth Habit in *Wheat-Thinopyrum* Amphiploids. *Plant Breed.* **2001**, *120*, 21–26.
- Cheng, Z. K.; Yan, H. H.; Yu, H. X.; Tang, S.; Jiang, J.; Gu, M.; Zhu, L. H. Development and Applications of a Complete Set of Rice Telotrisomics. *Genetics* **2001**, *157*, 361–368.
- Crasta, O. R.; Francki, M. G.; Bucholtz, D. B.; Sharma, H. C.; Zhang, J.; Wang, R. C.; Ohm, H. W.; Anderson, J. M. Identification and Characterization of Whet-Wheatgrass Translocation Lines and Localization of Barley Yellow Dwarf Virus Resistance. *Genome* **2000**, *43*, 698–706.
- Brar, D. S.; Khush, G. S. Alien Introgression in Rice. *Plant Mol. Biol.* **1997**, *35*, 35–47.
- Dahleen, L. S. Tissue Culture Increases Meiotic Pairing of Regenerants from Barley x Canada Wild Rye Hybrids. *J. Heredity* **1999**, *90*, 265–269.
- Desel, C.; Lung, C.; Cai, D.; Kleine, M.; Schmidt, T. High-Resolution Mapping of YACs and the Single-Copy Gene *HsI*, *Pro-Ion Beta vulgaris* Chromosomes by Multi-Color Fluorescence In Situ Hybridization. *Plant Mol. Bioi.* **2001**, *45*, 113–122.
- Devos, K. M.; Gale, M. D. Comparative Genetics in the Grasses. *Plant Mol. Biol.* **1997**, *35*, 3–15.
- Dhaliwal, H. S.; Friebe, B.; Gill, K. S.; Gill, B. S. Cytogenetic Identification of *Aegilops squarrosa* Chromosome Additions in Durum Wheat. *Theor. Appl. Genet.* **1990**, *79*, 769–774.
- Dhaliwal, H. S.; Gill, B. S.; Waines IG. Analysis of Induced Homoeologous Pairing in a *Ph* Mutant Wheat x Rye Hybrid. *J. Hered.* **1977**, *68*, 207–209.
- Dhaliwal, H. S.; Multani, D. S.; Singh, B. B. Identification of Rye and Barley Chromosomes Carrying Resistance to Kamal Bunt of Wheat (*Neovossia indica*). *Cereal Res. Comm.* **1987**, *15*, 191–194.
- Dhaliwal, H. S.; Singh, H.; William, M. Transfer of Rust Resistance from *Aegilops ovuta* Into Bread Wheat (*Triticum aestivum*, L.) and Molecular Characterization of Resistant Derivatives. *Euphytica* **2002**, *126*, 153–159.

- Dogramaci-Altuntepe, M.; Jauhar, P. P. Production of Durum Wheat Substitution Haploids from Durum x Maize Crosses and Their Cytological Characterization. *Genome* **2001**, *44*, 137–142.
- Doi, K.; Iwata, N.; Nishimura, Y. The Construction of Chromosome Substitution Lines of African Rice (*Oryza glaberrima* Steud.) in the Background of *offaponica* rice (*O. sativa*, L.). *Rice Genet. Newsl.* **1997**, *14*, 39–40.
- Dong, F.; Song, J.; Naess, S. K.; Helgeson, J. P.; Gabhardt, C.; Jiang, J. Development and Applications of a Set of Chromosome-Specific Cytogenetic DNA Markers in Potato. *Theor. Appl. Genet.* **2000**, *101*, 1001–1007.
- Dover, G. A.; Riley, R. The Prevention of Pairing of Homoeologous Meiotic Chromosomes of Wheat by a Genetic Activity of Supernumerary Chromosomes of *Aegilops*. *Nature* **1972**, *240*, 159–161.
- Driscoll, C. J. X, Y, Z System of Producing Hybrid Wheat. *Crop Sci.* **1972**, *12*, 516–517.
- Dvorak, J.; and Dubcovsky, J. Recombination Between Homoeologous Chromosomes in Wheat in the Absence of the Ph 1 Locus. In *Classical and Molecular Cytogenetic Analysis*; Raupp, W. J., Gill, B. S., Eds.; Kansas State University, Manhattan, 1995; pp 64–75.
- Endo, T. R.; Gill, B. S. The Deletion Stocks of Common Wheat. *J. Hered.* **1996**, *87*, 295–307.
- Fisher, J. A. and Martin, R. Kite, Condor and Egret—Three New Wheats. *Agric. Gaz. NSW* **1974**, *85*, 10–13.
- Flemming, W. Introduction Cited in *Cytology and Cytogenetics* by, C. P. Swanson (1957). Prentice Hall Inc.; New Jersey, 1982; pp. 1–13.
- Friebe, B.; Hatchett, J. H.; Gill, B. S.; Mukai, Y.; Sebesta, E. E. Transfer of Hessian Fly Resistance from Rye to Wheat via Radiation-Induced Terminal and Intercalary Chromosomal Translocations. *Theor. Appl. Genet.* **1991**, *83*, 33–40.
- Friebe, B.; Jiang, L.; Gill, B. S.; Dyck, P. L. Radiation-Induced Nonhomoeologous *Wheat-Agropyron Intermedium* Chromosomal Translocations Conferring Resistance to Leaf Rust. *Theor. Appl. Genet.* **1993**, *86*, 141–149.
- Friebe, B.; Jiang, J.; Raupp, W. J.; McIntosh, R. A.; Gill, B. S. Characterization of Wheat-Alien Translocations Conferring Resistance to Diseases and Pests: Current Status. *Euphytica* **1996**, *91*, 59–87.
- Friebe, B.; Kynast, R. G.; Hatchett, I. H.; Sears, R. G.; Wilson, D. L.; Gill, B. S. Transfer of Rye Derived Hessian Fly Resistance Genes *H21* and *H25* from Bread Wheat Into Durum Wheat. *Crop Sci.* **1999**, *39*, 1692–1696.
- Friebe, B.; Mukai, Y.; Gill, B. S.; Cauderon, Y. C-Banding and In Situ Hybridization Analyses of *Agropyron intermedium*, a Partial Wheat x *Ag. Intermedium* Amphiploid, and Six Derived Chromosome Addition Lines. *Theor. Appl. Genet.* **1992**, *84*, 899–905.
- Fukui, K.; Ijima, K. Somatic Chromosome Map of Rice by Imaging Methods. *Theor. Appl. Genet.* **1991**, *81*, 589–596.
- Galinat, W. C. The Origin of Corn. In *Corn and Corn Improvement*; Sprague, G. F., 1977.
- Gall, J. G.; Pardue, M. L. Formation and Detection of RNA-DNA Hybrid Molecules in Cytological Preparation. *Proc. Natl. Acad. Sci. USA* **1969**, *63*, 378–383.
- Ganai, F. A.; Khan, A. H.; Bhat, T. A.; Parveen, S.; Wani, N. A. Cytogenetic Effects of Methyl Methane Sulphonate (MMS) on Varieties of Chickpea (*Cicer arietinum*, L.). *J. Cytol. Genet.* **2005**, *6*, 97–102.
- Gao, D.; Guo, D.; Jung, C. Monosomic Addition Lines of *Beta corolliflora* Zoss in Sugarbeet: Cytological and Molecular Marker Analysis. *Theor. Appl. Genet.* **2001**, *103*, 240–247.

- Garriga-Calderé, F. et al. Transmission of Alien Tomato Chromosomes from BC1 to BC2 Progenies Derived from Backcrossing Potato (+) Tomato Fusion Hybrids to Potato: The Selection of Single Additions for Seven Different Tomato Chromosomes. *Theor. Appl. Genet.* **1998**, 96, 155–163.
- Garriga-Calderé, F.; Huigen, D. J.; Jacobsen, E.; Ramanna, M. S. Prospects for Introgressing Tomato Chromosomes Into the Potato Genome: An Assessment Through GISH Analysis. *Genome* **1999**, 42, 282–288.
- Gavrilenko, T.; Thieme, R.; Rokka, V. M. Cytogenetic Analysis of *Lycopersicon esculentum* (+) *Solanum tuberosum* Somatic Hybrids and Their Androgenetic Regenerants. *Theor. Appl. Genet.* **2001**, 103, 231–239.
- Gerstel, D. U. Inheritance in *Nicotiana tabacum* II. The Addition of *Nicotiana glutinosa* Chromosomes to Tobacco. *J. Hered.* **1945**, 36, 197–206.
- Gill, B. S.; Kimber, G. Giemsa C-Banding Karyotype of Rice. *Proc. Natl. Acad. Sci. USA* **1974**, 71, 1247–1249.
- Gill, K. S.; Gill, B. S. A DNA Fragment Mapped Within the Submicroscopic Deletion of *Ph1*, a Chromosome Pairing Regulator Gene in Polyploid Wheat. *Genetics* **1991**, 129, 257–259.
- Giorgi, B.; Barbera, F. Use of Mutants That Affect Homoeologous Pairing for Introducing Alien Variation in Both Durum and Common Wheat. In *Induced Mutations—A Tool in Plant Research*; IAEA: Vienna, 1981; pp. 37–47.
- Giorgi, B.; Cuozzo, L. Homoeologous Pairing in a *ph* Mutant of Tetraploid Wheat Crossed with Rye. *Cereal Res. Commun.* **1980**, 8, 485–490.
- Griffiths, N.; Thomas, H. Gene Transfer. In *Annual Report for 1982 Welsh Plant Breeding Station*, Aberystwyth, 1983; pp 145–146.
- Gulfishan, M.; Bhat, T. A.; Oves, M. Mutants as a Genetic Resource for Future Crop Improvement. In *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools*; Al-Khayri, J. M. et al., Eds.; Springer International Publishing Switzerland, 2015.
- Gulfishan, M.; Jafri, I. F.; Khan, A.H.; Bhat, T. A. Methyl Methane Sulphonate Induced Desynaptic Mutants in *Capsicum annum*, L. *Chromosome Bot.* **2013**, 8, 59–62.
- Gulfishan, M.; Khan, A. H.; Gefferi, I. F.; Bhat, T. A. Assessment of Mutagenicity Induced by MMS and DES in *Capsicum annum*, L. *Saudi J. Biol. Sci.* **2012**, 19, 251–255.
- Gulfishan, M.; Khan, A. H.; Bhat, T. A. Studies on Cytotoxicity Induced by DES and SA in *Vicia faba*, L. var. Major. *Turk. J. Bot.* **2010**, 34:31–37.
- Gulfishan, M.; Khan, A. H.; Haneef, I.; Bhat, T. A. Genotoxic Effect of Diethyl Sulphate in Two Varieties of *Capsicum annum*, L. *Nucleus* **2011**, 54(2), 107–111.
- Gupta, P. K. Role of Loss of the Rye Heterochromatin in Improvement of Hexaploid Triticale. *Nucleus* **1984**, 27, 100–107.
- Gupta, P. K. *Cytogenetics*; Rastogi Publications: Meerut, India, 1999; p 418.
- Hagberg, A.; Hagberg, G. High Frequency of Spontaneous Haploids in the Progeny of an Induced Mutation in Barley. *Hereditas* **1980**, 93, 341–343.
- Hagberg, A.; Hagberg, G. Some Vigorous and Productive Duplications in Barley. In *Barley Genetics*; Yasuda, V. S., Konishi, T., Eds.; Okayama: Japan, 1987; pp 423–426.
- Haider Ali, S. N.; Ramanna, M. S.; Jacobson, E.; Visser, R. G. F. Establishment of a Complete Series of a Monosomic Tomato Chromosome Addition Lines in the Cultivated Potato Using RFLP and GISH Analyses. *Theor. Appl. Genet.* **2001**, 103, 687–695.
- Harrington, J. J. Formation of *de novo* Centromeres and Construction of First-Generation Human Artificial Chromosomes. *Nat. Genet.* **1997**, 15, 345–355.

- Ho, K. M.; Kasha, K. J. Genetic Control of Chromosome Eliminating During Haploid Formation in Barley. *Genetics* **1975**, *81*, 263–275.
- Inagaki, M. N.; Hash, C. T. Production of Haploids in Bread Wheat, Durum Wheat and Hexaploid Triticale Crossed with Pearl Millet. *Plant Breed.* **1998**, *117*, 485–487.
- Islam, A. K. M. R.; Shepherd, K. W. Production of Wheat-Barley Recombinant Chromosomes Through Induced Homologous Pairing. Isolation of Recombinants Involving Barley Arms 3HL and 6HL. *Theor. Appl. Genet.* **1992**, *83*, 489–494.
- Jauhar, P. P. Genetic Control of Diploid Like Meiosis in Hexaploid Tall Fescue. *Nature* **1975**, *254*, 595–597.
- Jauhar, P. P.; Peterson, T. S. Hybrids Between Durum Wheat and *Thinopyrum junceiforme*: Prospects for Breeding for Scab Resistance. *Euphytica* **2000**, *00*, 1–10.
- Jauhar, P. P.; Dogramaci-Altuntepe, M.; Peterson, T. S.; Almouslem, A. B. Seedset on Synthetic Haploids of Durum Wheat: Cytological and Molecular Investigations. *Crop Sci.* **2000**, *40*, 1742–1749.
- Jena, K. K.; Khush, G. S.; Kochert, G. Comparative RFLP Mapping of a Wild Rice, *Oryza Officinalis*, and Cultivated Rice, *O. sativa*. *Genome* **1994**, *37*, 382–389.
- Jiang, J.; Friebe, B.; Gill, B. S. Recent Advances in Alien Gene Transfer in Wheat. *Euphytica* **1994**, *73*, 199–212.
- Jiang, J.; Friebe, B.; Dhaliwal, H. S.; Martin, T. J.; Gill, B. S. Molecular Cytogenetic Analysis of *Agropyron elongatum* Chromatin in Wheat Germplasm Specifying Resistance to Wheat Streak Mosaic Virus. *Theor. Appl. Genet.* **1993**, *86*, 41–48.
- Jiang, J.; Gill, B. S.; Wang, G. L.; Ronald, P. C.; Ward, D. C. Metaphase and Interphase Fluorescence In Situ Hybridization Mapping of the Rice Genome with Bacterial Artificial Chromosomes. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 4487–4491.
- Jiang, J.; Morris, K. L. D.; Gill, B. S. Introgression of *Elymus trachycaulus* Chromatin Into Common Wheat. *Chromosome Res.* **1994**, *2*, 3–13.
- Jiang, J.; Nasuda, S.; Dong, F.; Scherrer, C. W.; Woo, S. S.; Wing, R. A.; Gill, B. S.; Ward, D. C. A Conserved Repetitive DNA Element Located in the Centromeres of Cereal Chromosomes. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 14210–14213.
- Kawahara, T.; Taketa, S. Fixation of Translocation 24.4B Infers the Monophyletic Origin of Ethiopian Tetraploid Wheat. *Theor. Appl. Genet.* **2001**, *101*, 705–710.
- Kennard, W.; Phillips, R.; Porter, R.; Grambacher, A. A Comparative Map of Wild Rice (*Zizania palustris* 2n = 2x = 30). *Theor. Appl. Genet.* **1999**, *99*, 793–799.
- Khan, A. A.; Ansari, M. Y. K.; Shahab, D.; Bhat, T. A. Induced Variations for Quantitative Traits in *Vicia faba*, L. *Vegetos* **2005a**, *18* (1&2), 83–86.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. DES Induced Variations for Economic Traits in Broad Bean (*Vicia faba*, L.). *Biosci. Biotechnol. Res. Asia* **2005b**, *3* (2), 363–366.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. Clastogenic Effects of 8-Hydroxy Quinoline in *Vicia faba*, L. *Adv. Plant Sci.* **2007a**, *20* (1), 85–87. ISSN 0970–3586.
- Khan, A. A.; Ansari, M. Y. K.; Shahab, D.; Bhat, T. A. Comparative Analysis of Meiotic Abnormalities Induced by Caffeine and DES in *Vicia faba*, L. *Adv. Plant Sci.* **2007b**, *20* (1), 73–76.
- Khush, G. S. *Cytogenetics of Aneuploids*; Academic Press, New York, 1973; p 301.
- Khush, G. S.; Brar, D. S. Wide Hybridization in Plant Breeding. In *Plant Breeding and Genetic Engineering*; Zakri, A. H., Ed.; Sabrao: Malaysia, 1989; pp 141–188.
- Khush, G. S.; Rick, C. M. Studies on the Linkage Map of Chromosome 4 of the Tomato and on Transmission of Induced Deficiencies. *Genetica* **1967**, *38*, 74–94.

- Khushnir, W.; Halloran, G. M. Transfer of High Kernel Weight and High Protein Content from Wild Tetraploid Wheat to Bread Wheat Using Homologous and Homoeologous Recombination. *Euphytica* **1984**, *33*, 249–256.
- Kihara, H.; Tsunewaki, K. Use of Alien Cytoplasm as a New Method of Producing Haploids. *J. Genet.* **1962**, *37*, 310–313.
- Kjima, K.; Ohmido, T.; Hirose, T.; Mitsui, T.; Fukui, K. Visualization of the DNA Stretches in a Rice Genotype by Extended DNA Fiber-FISH. In *Plant and Animal Genome VII*; San Diego: USA, 1999; p 83.
- Knott, D. R. The Inheritance of Rust Resistance. VI. The Transfer of Stem Rust Resistance from *Agropyron elongatum* to Common Wheat. *Can. J. Plant Sci.* **1961**, *41*, 109–123.
- Kynast, R. G.; Riera-Lizarazu, O.; Vales, M. L.; Okagaki, R. I.; Maquieira, S. B.; Chen, G.; Ananiev, E. V.; Odland, W. E.; Russell, C. D.; Stec, A. O.; Livingston, S. M.; Zaia, H. A.; Rines, H. W.; Phillips, R. L. A Complete Set of Maize Individual Chromosome Additions to the Oat Genome. *Plant Physiol.* **2001**, *125*, 1216–1227.
- Lapitan, N. L. V.; Sears, R. G.; Gill, B. S. Translocations and Other Karyotypic Structural Changes in Wheat x Rye Hybrids Regenerated from Tissue Culture. *Theor. Appl. Genet.* **1984**, *68*, 547–554.
- Larkin, P. I.; Banks, P. M.; Bhati, R.; Brettell, R. I. S.; Davies, P. A.; Ryan, S. A.; Scowcroft, W. R.; Spindler, L. H.; Tanner, G. I. From Somatic Variation to Variant Plants: Mechanisms and Applications. *Genome* **1989**, *31*, 705–711.
- Laurie, D. A.; O'Donoghue, L. S.; Bennett, M. D. Wheat x Maize and Other Wide Sexual Hybrids: Their Potential for Genetic Manipulation and Crop Improvement. In *Gene Manipulation in Plant Improvement II*; Gustafson, I. P., Ed.; 19th Stadler Genetics Symposium; Plenum Press: New York, 1990; pp 95–126.
- Law, C. N.; Snape, J. W.; Worland, A. I. Intraspecific Chromosome Manipulation. *Phil. Trans. R. Soc. Lond.* **1981**, *292*, 509–518.
- Li, H. I.; Guo, B. H.; Li, Y. W.; Du, L. Q.; Jia, X.; Chu, C. C. Molecular Cytogenetic Analysis of Intergeneric Chromosomal Translocations Between Wheat (*Triticum aestivum*, L.) and *Dasyphyrum villosum* Arising from Tissue Culture. *Genome* **2000**, *43*, 756–762.
- McClintock, B. The Origin and Behaviour of Mutable Loci in Maize. *Proc. Natl. Acad. Sci. USA* **1950**, *36*, 344–355.
- McCouch, S. R.; Kochert, G.; Yu, Z. H.; Wang, Z. Y.; Khush, G. S.; Coffman, W. R.; Tanksley, S. D. Molecular Mapping of Rice Chromosomes. *Theor. Appl. Genet.* **1988**, *76*, 815–829.
- Mendel, G. *Versuche uber Pflanzen-Hybriden*. Verh. Naturf Orshung Ver. Briim Verh. **1865**, *4*, 3–47.
- Molnar-Lang, M.; Line, G.; Logojan, A.; Sutka, I. Production and Meiotic Pairing Behaviour of New Hybrids of Winter Wheat (*Triticum aestivum*) x Winter Barley (*Hordeum vulgare*). *Genome* **2000**, *43*, 1045–1054.
- Morgan, W. G.; King, T. P.; Koch, S.; Harper LA.; Thomas, H. Introgression of Chromosomes of *Festuca arundinacea* var. *glaucescens* into *Lotium multiflorum* Revealed by Genomic In Situ Hybridization. *Theor. Appl. Genet.* **2001**, *103*, 696–701.
- Mujeeb-Kazi, A.; Jewell, D. C. CIMMYT's Wide Cross Program for Wheat and Maize Improvement. In *Biotechnology in International Agricultural Research*; International Rice Research Institute: Manila, Philippines, 1985; pp 219–226.
- Mukai, Y.; Fribe, B.; Hatchett, M.; Yamamoto, M.; Gill, B. S. Molecular Cytogenetic Analysis of Radiation-Induced Wheat-Rye Terminal and Intercalary Chromosomal Translocations

- and the Detection of Rye Chromatin Specifying Resistance to Hessian Fly. *Chromosoma* **1993**, 102, 88–95.
- Okagaki, R. J.; Kynast, R. G.; Livingston, S. M.; Russell, C. D.; Rines, H. W.; Phillips, R. L. Mapping Maize Sequences to Chromosomes Using Oat-Maize Chromosome Addition Materials. *Plant Physiol.* **2001**, 125, 1228–1235.
- Okamoto, M. *Asynaptic Effect of Chromosome, V*; Wheat Information Service 5:6, 1957.
- O'mara, J. G. Cytogenetic Studies on Triticinae. I. A method for Determining the Effect of Individual *Secale* Chromosomes on *Triticum*. *Genetics* **1940**, 25, 401–408.
- Orton, T. J. Haploid Barley Regenerated from Callus Cultures of *Hordeum vulgare* x, *H. jubatum*. *J. Hered.* **1980**, 71, 780–782.
- Park, S. J.; Walsh, E. J.; Reinbergs, E.; Song, L. S. P.; Kasha, K. J. Field Performance of Doubled Haploid Barley Lines in Comparison with Lines, 1976.
- Parveen, S.; Anis, M.; Bhat, T. A.; Khan, A. H. Cytological Effects of Physical and Chemical Mutagens in *Trigonella foenum-graecum*, L. *Adv. Plant Sci.* **2007**, 20 (11), 633–636.
- Parveen, S.; Anis, M.; Bhat, T. A.; Khan, A. H. Efficiency and Effectiveness of Physical and Chemical Mutagens in M2 Generation of *Trigonella foenum-graceum*, L. *J. Cytol. Genet.* **2006**, 7, 1–4.
- Peloquin, S.; Hougas, R. W.; Gabert, A. C. Haploidy as a New Approach to the Cytogenetics and Breeding of *Solanum tuberosum*. In *Chromosome Manipulations and Plant Genetics*; Riley, R., Lewis, K. R., Eds.; Oliver and Boyd Ltd.: London, 1964; pp 21–28.
- Pickering, R. A.; Malyshev, S.; Kunzel, G.; Johnston, P. A.; Korzun, V.; Menke, M.; Schubert, I. Locating Introgressions of *Hordeum bulbosum* Chromatin Within the, *H. vulgare* Genome. *Theor. Appl. Genet* **2000**, 100, 27–31.
- Rajaram, S.; Maan, E. E.; Ortiz-Ferrara, G.; Mujeeb-Kazi, A. Adaptation, Stability and High Yield Potential of Certain IB/IR CIMMYT Wheats. In *Sixth Intern. Wheat Genet. Symp.*; Kyoto, Japan, 1983; pp 613–621.
- Ramage, R. T. Balanced Tertiary Trisomies for Use in Hybrid Seed Production. *Crop Sci.* **1965**, 5, 177–178.
- Rayburn, A. L.; Gill, B. S. Use of Biotin Labeled Probes to Map Specific DNA Sequences on Wheat Chromosomes. *J. Hered.* **1985**, 76, 78–81.
- Rhoades, M. M. A Cytogenetic Study of a Chromosome Fragment in Maize. *Genetics* **1936**, 21, 491–502.
- Riera-Lizarazu O.; Rines, H. W.; Phillips, R. L. Cytological and Molecular Characterization of Oat x Maize Partial Hybrids. *Theor. Appl. Genet* **1996**, 93, 123–125.
- Riera-Lizarazu O.; Vales, M. I.; Ananiev, E. V.; Rines, H. W.; Phillips, R. L. Production and Characterization of Maize Chromosome 9 Radiation Hybrids Derived from an Oat-Maize Addition Line. *Genetics* **2000**, 156, 327–339.
- Riley, R.; Chapman, V.; Johnson, R. Introduction of Yellow Rust Resistance of *Aegilops comosa* Into Wheat by Genetically Induced Homoeologous Recombination. *Nature* **1968**, 217, 383–384.
- Roman, H.; Ullstrup, A. J. The Use of A-B Translocations to Locate Genes in Maize. *Agron. Jour.* **1952**, 43, 450–454.
- Rosenfeld, M. Human Artificial Chromosomes Get Real. *Nat. Genet.* **1997**, 15, 333–335.
- Sachan, J. K. S.; Tanaka, R. Variation and Pattern of C-Banding in *Zea* Chromosomes. *Nucleus* **1997**, 20, 61–64.
- Sakaguchi, S.; Nishimura, Y. Breeding Seedless Watermelon by Using Induced Chromosome Translocations. *Jarg.* **1969**, 3, 18–22.

- Scalenghe, F.; Tureo, E.; Edstrom, J. E.; Pirotta, V. Microdissection and Cloning of DNA from a Specific Region of *Drosophila melanogaster* Polytene Chromosomes. *Chromosoma* **1981**, *82*, 205–216.
- Schwarzacher, T.; Anamthawat-Jonsson, K.; Harrison, G. E.; Islam, A. K. M. R.; Jia, J. Z.; King, I. P.; Leitch, A. R.; Miller, T. E.; Reader, S. M.; Rogers, W. J.; Shi, M.; Heslop-Harrison, J. S. Genomic In Situ Hybridization to Identify Alien Chromosomes and Chromosome Segments in Wheat. *Theor. Appl. Genet.* **1992**, *84*, 778–786.
- Sears, E. R. Nullisomic Analysis in Common Wheat. *Amer. Nat.* **1953**, *87*, 245–252.
- Sears, E. R. The Aneuploids of Common Wheat. *Res. Bull.; Mo. Agr. Exp. Stn.* **1954**, *572*, 59pp.
- Sears, E. R. The Transfer of Leaf Rust Resistance from *Aegilops umbellulata* to Wheat. Brookhaven. *Symp. Biol.* **1956**, *9*, 1–22.
- Sears, E. R. Nullisomic-Tetrasomic Combinations in Hexaploid Wheat. In *Chromosome Manipulations and Plant Genetics*; Riley, R., Lewis, K. W.; Oliver and Boyd Ltd.: London, 1966; pp 29–45.
- Sears, E. R. Chromosome Engineering in Wheat. *Stadler Genet. Symp.* **1972**, *4*, 23–38.
- Sears, E. R. *Agropyron-Wheat* Transfers Induced by Homoeologous Pairing. In *4th Intern. Wheat Genet. Symp.*, Columbia, 1973; pp 191–199.
- Sears, E. R. An Induced Mutant with Homoeologous Pairing in Common Wheat. *Can. J. Genet. Cytol.* **1977**, *19*, 585–593.
- Sharma, M.; Khan, A. H.; Bhat, T. A. Assessment of Mutagenecity of Individual and Combination Treatment of Gamma Rays and MMS in Broad Bean (*Vicia faba* L.). *Cytologia* **2009**, *74* (2), 235–241.
- Sherman, J. D.; Smith, L. Y.; Blake, T. K.; Talbert, L. E. Identification of Barley Genome Segments Introgressed Into Wheat Using PCR Markers. *Genome* **2001**, *44*, 38–44.
- Singh, K.; Ishii, T.; Parco, A.; Huang, N.; Brar, D. S.; Khush, G. S. Centromere Mapping and Orientation of the Molecular Linkage Map of Rice (*Oryza sativa*, L.). *Proc. Natl. Acad. Sci.* **1996a**, *93*, 6163–6168.
- Singh, K.; Multani, D. S.; Khush, G. S. Origin, Characterization, and Use in Determining the Orientation of Chromosome Map. *Genetics* **1996b**, *143*, 517–529.
- Singh, R. J. *Plant Cytogenetics*; CRC Press: Boca Raton, 1993; p 391.
- Snowdon, R. J.; Friedrich, T.; Friedt, W.; Kohler, W. Identifying the Chromosomes of the A-C-Genome Diploid *Brassica* Species. *B. rapa* (Syn. *campestris*) and, *B. oleracea* in Their Amphidiploid, *B. napus*. *Theor. Appl. Genet.* **2002**, *104*, 533–538.
- Sutton, W. S. The Chromosomes in Heredity. *Biol. Bull.* **1903**, *4*, 231–251.
- Sutton, W. S.; Boveri, T. *An Appraisal. Cited in Cytogenetics* by, Swanson, C. P., Merz, T., Yong, W. J. (1967); Prentice Hall Inc.: New Jersey, 1903; pp 1–7.
- Swaminathan, M. S. Notes on Induced Polyploids in the Tuber-Bearing *Solanum* Species and Their Crossability with, *S. tuberosum*. *Amer. Potato Jour.* **1951**, *28*, 472–489.
- Szabados, L.; Hadlaczky, G.; Dudits, D. Uptake of Isolated Plant Chromosomes by Plant Protoplasts. *Planta* **1981**, *151*, 141–145.
- Thomas, H.; Leggett, M.; Jones, I. T. The Addition of a Pair of Chromosomes of the Wild Oat, *Avena barabta* (2n = 28) to the Cultivated Oat, *A. sativa*, L. (2n = 42). *Euphytica* **1975**, *24*, 717–724.
- Thomas, H.; Powell, W.; Aung, T. Interfering with Regular Meiotic Behaviour in *Avena sativa* as a Method of Incorporating the Gene for Mildew Resistance from *A. barbata*. *Euphytica* **1980**, *29*, 635–640.

- Unrau, J.; Person, C.; Kuspira, J. Chromosome Substitution in Hexaploid Wheat. *Can. J. Bot.* **1956**, *34*, 629–640.
- Vrana, J.; Kubalakova, M.; Simkova, H.; Chihalikova, J.; Lysak, M. A.; Dolezel, J. Flow sorting of Mitotic Chromosomes in Common Wheat (*Triticum aestivum*, L.). *Genetics* **2000**, *156*, 2033–2041.
- Wall, A. M.; Riley, R.; Chapman, V. Wheat Mutants Permitting Homoeologous Meiotic Chromosome Pairing. *Genet. Res.* **1971**, *18*, 311–328.
- Wang, R. C.; Liang, G. H.; Heyne, E. G.; 1977. Effectiveness of *ph* Gene in Inducing Homoeologous Pairing in *Agrotrichum*. *Theor. Appl. Genet.* **1977**, *51*, 139–141.
- Wang, W. N.; Hang, A.; Hansen, J.; Burton, C.; Mallory-Smith, C. A.; Zemetra, R. S. Visualization of A- and B-Genome Chromosomes in Wheat (*Triticum aestivum*, L.) x Jointed Goat Grass (*Aegilops cylindrica* Host) Backcross Progenies. *Genome* **2000**, *43*, 1038–1044.
- Wani, N. A.; Bhat, T. A.; Khan, A.H.; Ansari, M. Y. K. Sodium Azide Induced Variability in Chickpea (*Cicer arietinum* L). *Vegetos* **2005**, *18* (1&2), 105–107.
- Wani, A. A.; Bhat, T. A. Asynapsis and Desynapsis in Plants: In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Watanabe, K. N.; Orrillo, M.; Vega, S.; Golmirzaie, A. M.; Perez, S.; Crusado, J.; Watanabe, J. A. Generation of Pest Resistant, Diploid Potato Germplasm with Short-Day Adaptation from Diverse Genetic Stocks. *Breed. Sci.* **1996**, *46*, 329–336.
- Wienhues, A. Cytogenetische Untersuchungen tiber die chromosomale Grundlageder Rostresistenz der Weizensortie WeiQue. *Zuchter* **1965**, *35*, 352–354.
- Xin, Z. Y.; Zhang, Z. Y.; Chen, X.; Lin, Z. L.; Ma, Y. Z.; Xu, H. J.; Banks, P. M.; Larkin, P. J. Development and Characterization of Common *Wheat-Thinopyrum Intermedium* Translocation Lines with Resistance to Barley Yellow Dwarf Virus. *Euphytica* **2001**, *119*, 161–165.
- Zeller, F. J. IB/IR Wheat-Rye Chromosome Substitutions and Translocations. In *5th Intern. Wheat Genet. Symp.*, Columbia, Missouri, 1973; pp 209–222.
- Zhong, X. B.; Bodeau, J.; Fransz, P. F.; Williamson, V. M.; Van Kammen, A.; De Jong, J. H.; Zabel, P. FISH to Meiotic Pachytene Chromosomes of Tomato Locates the Root-Know Nematode Resistance Gene *Mi-I* and the Acid Phosphatase Gene *Aps-I* Near the Junction of Euchromatin and Pericentromeric Heterochromatin of Chromosome Arms 6S and 6L, Respectively. *Theor. Appl. Genet.* **1999**, *98*, 365–370.
- Zhong, X. B.; Fransz, P. F.; Van-Wennekes, E.1.; Zabel, P.; Van Kammen, A.; De Jong, J. H. High Resolution Mapping by Fluorescence In Situ Hybridization to Pachytene Chromosomes and Extended DNA Fibres. *Plant Mol. Bioi. Rep.* **1996**, *14*, 232–242.
- Ziolkowski, P. A.; Sadowski, J. FISH-Mapping of rDNAs and *Arabidopsis* BAC on Pachytene Complements of Selected *Brassicac*s. *Genome* **2002**, *45*, 18.



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CHAPTER 3

Molecular Genetics of Symbiotic Plant–Microbe Interactions in a Model Legume, *Lotus japonicus*

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ABSTRACT

The symbiotic association between Rhizobia and *Lotus japonicas* is used as a model to study the molecular genetics of plant–microb association from nodule formation to nitrogen fixation. *Lotus japonicus* is considered the best module for these studies due to many characteristics like plant size, large and profuse flowering, easy manual pollination, high production rate, shorter regeneration time, responsive to mutations, and transformation in tissue culture. Due to more developed genetic tools and techniques, the study of molecular genetics become easy, and to study mutation lines, genetic mapping, and whole-genome sequence study. In this chapter, we will study the symbiotic association in plants and microbes on the basis of the molecular genetics of *L. japonicus*.

3.1 INTRODUCTION

The root nodule formation in legumes is a symbiotic association between the roots and rhizobial bacteria that starts with the exchange of signals between

host and microsymbiont (Djordjevic et al., 2015; Dupont et al., 2012). After the identification of compatible bacteria, the host roots start cell division in cortical tissues for supporting root nodule formation (Charon et al., 1997; Suzaki et al., 2012). The bacteria in the nodule are bounded by plant born membrane, making an organelle-like structure called the symbiosome (Coba de la Peña et al., 2018; Emerich and Krishnan, 2014). The nodules of legumes are broadly classified into two types: (1) indeterminate type, and (2) determinate type (Kutschera and Niklas, 2009).

- a) **Indeterminate type:** These nodules result from cell division of the inner cortex and take persistent apical meristem (Erasmus et al., 2000; Magne et al., 2018). These nodules are cylindrical with the developmental gradient from apex to base of the nodules that can further be divided into different zones, for example, nodules in pea, Medicago, and clovers (Alexander, 2008; Alexander et al., 2012).
- b) **Determinate type:** These nodules originate from cell division of the middle or outer cortex in root, no persistent meristem is formed and they are spherical in shape. The cell division in determinate nodules stops during the early development stage and a mature nodule is formed from the enlargement of cells (Li et al., 2011; Wiener et al., 2015).

Both these nodules are found to undergo genome endoreduplication which is important for cell enlargement and polyploidization (McIver et al., 2014; J. H. Yoon et al., 2010). With nodule development, differentiation of nitrogen-fixing bacteria goes parallel and host environment determines the rate of differentiation and its reversal (Park et al., 2011; K. N. Patel et al., 2018). The process of terminal differentiation is affected by cell elongation, membrane permeability, genome endoreduplication and loss of reproducing capacity, and size of cell and DNA content controls reversible differentiation (Brauner et al., 2015; K. N. Patel et al., 2018). In comparison to the genome of free-living bacteria, nitrogen-fixing bacteria change the transcriptome (Brauner et al., 2015; Moran et al., 2003), cell surface, and metabolic activities, to adopt the environment around and better nitrogen fixation (Burke and Moran, 2011; Moran et al., 2003). Studies show phylogenetic diversity of both leguminous plants and rhizobial bacteria. Early stage of collaboration between bacteria and nodule occurs in such a way that the bacteria cannot infect any other plant nodules (Lardi and Pessi, 2018; Peng et al., 2014).

Inconsistency is also found to occur at the later stage of nodule growth in such a way that nitrogen-fixing effectiveness varies considerably between various plant and bacteria arrangements (Maróti & Kondorosi, 2014; Schlüter et al., 2010). Symbiotic association occurs due to changing signals between

host and bacteria. For recognition, various mechanisms have been developed for better coadaptation (Alloisio et al., 2010; Glick, 1995). Understanding the pathways of the genetic and molecular mechanism of symbiotic specificity, it is important to develop tools for studying genetic recombination and manipulation in host and bacteria to improve nitrogen fixation productivity (Larimer et al., 2004; S. H. Yoon et al., 2011).

3.2 NODULE FORMATION AND SYMBIOTIC MUTATIONS IN *Lotus japonicus*

The process of nodule formation starts with the colonization of Rhizobium bacteria around the root hairs of legume plants, followed by entry of rhizobia into the epidermis of root then to cortical cells by infectious threads of rhizobia (Horváth et al., 2015). With the initiation of infection, cell division in the root cortex starts to take the shape of a nodule primordium, which later develops into a well-developed symbiotic organ as root nodule (Becker et al., 2004; Lurthy et al., 2018).

3.2.1 TYPES OF MUTANTS

Rhizobium bacteria live inside cortical cells surrounded by peribacteroid membrane (PBM) (symbiosis-specific membrane) (Brear et al., 2013; Jones et al., 2007), after differentiation to a bacteriod process of nitrogen fixation starts (Brewin, 2004; Frayse et al., 2003; Santos et al., 2000). Symbiotic mutants in *Lotus japonicas* were mostly produced by ethyl methane sulfonate by T-DNA (Oldroyd et al., 2011) and somatic mutation by regeneration of plant Calli by differentiation (Pumplin and Harrison, 2009). Many researchers provided a differentiation list of symbiotic mutations with their gene map positions (Haney and Long, 2010). Sandal et al. categorized mutation in *L. japonicas* into three types: (1) Nod⁻ (non-nodulating) (Riely et al., 2004), (2) Hist⁻ (defective in cooperative histogenesis) (Gobbato et al., 2012), and (3) Fix⁻ (formation of ineffective nodules) (Høgslund et al., 2009; Sandal et al., 2006).

- a) Nod⁻ type mutations are recognized to develop due to faults in the early stage of symbiotic connections, due to which no bacterial infection or nodule primordium formation cannot occurs (Hungria & Phillips, 1993; Lee et al., 2005; Whitewoods, 2020). Till now, four different genes are identified using allelism test in Nod⁻ mutant lines (Hungria and Phillips, 1993). These genes are Ljsym70 (Senoo et al., 2000), Ljsym71-1 (Kawaguchi et al., 2002), Ljsym71-2 (Pajuelo,

2005), and Ljsym72 (Lee et al., 2006). Some researchers noted that nodule bumps are small in roots with Ljsym70 (Alexander, 2008; Márquez & japonicus Handbook, 2003; Pajuelo, 2005) even after long incubation with M.loti TONO strains (Márquez & japonicus Handbook, 2003), whereas in the case of roots with Ljsym73 gene, the size of nodule is large at low frequency (Kawaguchi et al., 2002; Senoo et al., 2000). For Nod⁻ mutation, no specific strain was found and the possible reason is either mutation in Nod⁻ factor signaling pathway (Geurts et al., 2005; Oldroyd and Long, 2003; Pingret et al., 1998) or lack of nod-gene inducers which is produced from roots (Márquez and Japonicus Handbook, 2003; Oldroyd & Long, 2003).

- b) Hist⁻ type mutation is described as faults in infection threads that are formed during growth, result in imperfect nodule organogenesis (Kawaguchi et al., 2002; Oldroyd & Downie, 2008; van der Hist et al., 1991).
- c) Fix⁻ type mutation causes the formation of normal nodules that contains endosymbiotic bacteria (Spaink, 2000), but these nodules are either not able to do nitrogen fixation partially or completely (Charlesworth, 2020; Rallapalli et al., 2013) (Fig 3.1).

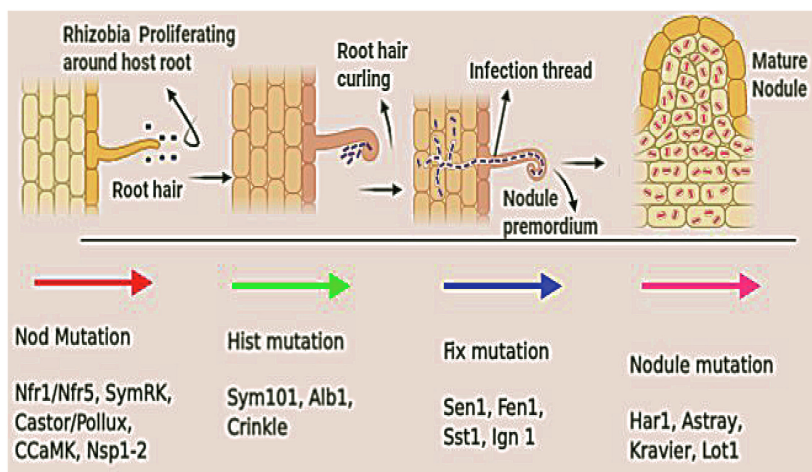


FIGURE 3.1 Different stages of nodule formation and mutations.

Other than above mentioned three mutations, the fourth known category causes a defect in different parameters like the number of nodules and defect in autoregulation (Blackwell & Zhao, 2003; Byers & Waller, 1999; Miura

et al., 2007). This category is known as Nod ++ as it contains hypernodulation mutants (Hojsgaard & Hörandl, 2015; Sobel & Streisfeld, 2013; Voigt, 2014). Nod-mutants are recognized by the defects in the early process of symbiotic associations (Mitra et al., 2004), as Nod factor sensitivity or by instant downstream signal transduction pathways (Amor et al., 2003; Wais et al., 2000), which leads to infection followed by nodulation processes (Ramu et al., 2002; Yan et al., 1997). The nod-mutants known till now half of them are not working in process of symbiosis with arbuscular mycorrhiza (AM) and Myc⁻ (Ramu et al., 2002). The symbiotic association with AM fungi is present in more than 80% of legume plants, which shows legume–Rhizobium association is an evolutionary proceeding of pre-existing mycorrhizal symbiotic mechanism (Markmann et al., 2008; Werner et al., 2014). On the basis of short-term analysis, it was found that no indication of depolarization or any alkalinization was observed by using Nod factors, and no expression in relation to downstream process of genes (Nin, Enod2, LjCBP1) in the signaling pathway was observed (Frederickson, 2013; Osborne, 2007). Nfr1 and 5 are two encode receptor kinase found to be involved in identification of *L. japonicus* Nod factor (Lagunas et al., 2015; Santi et al., 2013).

3.2.2 *Ca*²⁺ SPIKING

*Ca*²⁺ spiking is found to play an important role in symbiotic signaling and function like an oscillator for calcium ion concentration in root hair in reaction to the Nod factor (K. T. Jones, 2005; Santi et al., 2013). Common sym pathway or CSP genes are of two types based on their places around calcium spiking. The mutant that lacks CASTOR and POLLUX (Geurts & Bisseling, 2002; Smit et al., 2005) is putative *Ca*²⁺ gated channel proteins (Durell & Guy, 2001; Wang et al., 2005) and is required for inducing calcium spiking (Chabaud et al., 2011; Wakui et al., 1990). Some studies show CCaMK, a calmoduline-dependent protein kinase as a decoder of calcium spiking (Singh & Parniske, 2012) that was identified as a gene found in symbiotic mutants Ljsym72 (Miller et al., 2013; S. Singh & Parniske, 2012). CCaMK shows kinase activity independently to calcium spiking by substituting single amino acid at the site of autophosphorylation in the kinase domain (Shimoda et al., 2012; Shimoda et al., 2019; Takeda et al., 2012). These studies show CCaMK as a key regulator for activation of downstream signaling cascade, which results in nodule organogenesis. CSP, a novel component of CYCLOPS (Bagchi, 2013; Held et al.; Kang et al.), interacts with CCaMK (Ikeda et al., 2011; Ma et al., 2012).

Further study of nodule formation pathway in relation with genetic approaches of double and triple mutant found CCaMKT265D (Hayashi et al., 2010) to induce spontaneous nodule formation and its transformation in other Nod-mutants can help in investigating inter-relationship between specific symbiotic genes (Lambert, 2017). The obligatory symbiosis between legums and AM fungi make it complicated to study the mycorrhization process (Hohnjec et al., 2005; J. Sun et al., 2015). The only method to overcome these difficulties is by identification of Myc factors (signal molecules) generated by AM fungi followed by isolation and identification of Nod+ Myc- mutants (Corrêa et al., 2015; Duc et al., 1989).

3.3 PROCESS OF INFECTION AND NODULE FORMATION IN *L. JAPONICUS*

The symbiotic association between *L. japonica* and rhizobium bacteria is a nonpathogenic infection found in multicellular organisms (Frank, 2019; Jiménez-Cortés et al., 2018). The nodule formation is under the control of the genetic makeup of host and infection and nodule formation runs parallel to each other. Nodule primordial develops from root cortical cells and reinitiate cell decision (Brewin, 1991; Ding & Oldroyd, 2009), simultaneously bacterial infection process starts in developing primordial (Khan; Khan et al., 2015). The genetic condition required for infection thread development is not clearly found but Nap1 and Pir1 genes (Breakspear et al., 2014; Miyahara et al., 2010; Yokota et al., 2009) are responsible for encoding SCAR and WAVE protein complex (Breakspear et al., 2014; Downie, 2014; Murray, 2011) which causes activation of actin in root hair and Cerberus responsible E3 ubiquitin ligase (Held et al., 2010) required for normal infection thread progression (Breakspear et al., 2014).

3.3.1 MUTANTS AND THEIR EFFECTS

Hist⁻ Mutants

Hist⁻ mutants are considered important genetic loci for infection progression and growth of thread and partial nodule organogenesis (Lawlor et al., 2005; E. P. Price et al., 2013). Alb1 and crinkle are both well-known examples of Hist⁻ mutants found in *L. japonica* (Dalmaso et al., 2008), where Alb1 mutants are responsible for the growth of thick infection thread in root hair cells (Mummidi et al., 1998; Sakthianandeswaren et al., 2009), followed by advance nodulation process but the nodules result from Alb1 mutants are irregular in shape

with immature vascular bundles (Jeffery & Bangham, 2000; Sanglard et al., 1997). Crinkle mutant supported infection thread get blocked at the epidermal layer due to which nodule formation is blocked results in a small bump (AbuOun et al., 2005; Babiker et al., 2001). In comparison to Alb1 size of nodules formed by Ljsym101 (Hist⁻ mutants) are small in size and many in the number of pink color (Chapman & Hill, 2012; K. V. Singh et al., 2007).

The image analysis of these nodules shows areas filled with 77% of infected cells, 21% of uninfected cells, and 2% of intercellular space (Chaube, 2018). In the electron microscope image of nodules, the ultrastructure shows intracellular infection thread with a tubular structure with multilayered fibrillar wall of 139 nm thickness (B. B. Patel and Diao, 2017; F. Sun et al., 2011). The infection thread in *L. japonicas* is wide and can accommodate many vegetative bacteria in it. Each bacterial cell can migrate from infection thread to droplet and from there to cytosol of host cell (Danino & Talmon, 2006; Döring et al., 2013). These bacteria are found to be enclosed in PBM and undergo division by binary fusion forming ring-like structures (Belin et al., 2018; Bolaños et al., 2004) (Fig. 3.2).

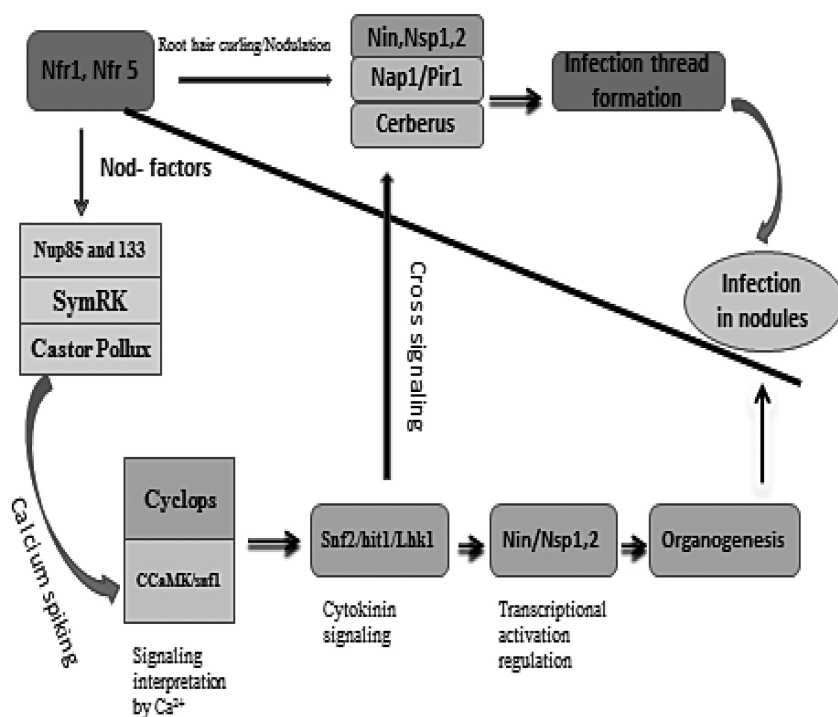


FIGURE 3.2 Different signaling mechanism in nodule formation.

Fix⁻ mutants: These mutants attribute to the final stage of nodule formation, which involves differentiation of rhizobia as bacterioids and induce nitrogenesis (Oldroyd et al., 2011). Nitrogenesis is required for metabolic activity to provide nitrogen for host cells (Masson-Boivin et al., 2009; P. A. Price et al., 2015). The nodules formed by Fix⁻ mutants are mostly found to be normal in physical appearance containing endosymbiotic bacteria with the disadvantage of low rate of nitrogen fixation. Many researchers found genetical interaction in late stages of symbiosis mediated by Fix⁻ mutant (Janczarek et al., 2015). Ljsyms 75 and 81 are the most studied mutants of Fix⁻ category (Kondorosi et al., 2013). The nodules formed from Ljsym75 are morphologically similar to wild plants, but lack nitrogen-fixing ability. These nodules do not have a complete bacterioid due to which they are not able to do nitrogen fixation. In comparison to Ljsym75 nodules (Kondorosi et al., 2013), Ljsym85 nodules carry differentiated bacterioid that is similar to wild type with low nitrogen fixation (Middleton et al., 2007; Provorov, 2013). Ljsym85 or SST1 are recognized as transporters for nodule-specific sulfate (Damiani et al., 2012; Giraud et al., 2007; Pan, 2019). Their protein was found to be a constituent of PBM and helps SO_4^{2-} transport from cystol to bacterioid. For growth and nitrogen fixation supply of SO_4^{2-} ion is important (Franche et al., 2009), and also for electron transport proteins (Pislariu et al., 2012; H. J. Yoon et al., 2014).

3.4 CONCLUSION

Symbiotic association between legume and *L. japonicas* is used as a model to study nodule formation and mutation. Using *L. japonicas* as a model for study helps to identify the gene responsible for mutation and their function, which can help to understand syntony between legume and rhizobium. This can help to clone gene and develop more economically important crop plants. This relationship also helps to understand crop evolution. The similarity between mutant and legume phenotype helps to identify the signal cascade of micro- and macro-symbionts and their gene structure can trace the genetic evolution of legume–rhizobium association.

KEYWORDS

- **symbiosis**
- **symbiotic mutants**
- **legumes**
- **Hist- mutants**
- **Fix- mutants**

REFERENCES

- AbuOun, M.; Manning, G.; Cawthraw, S. A.; Ridley, A.; Ahmed, I. H.; Wassenaar, T. M.; Newell, D. G. Cytolethal Distending Toxin (CDT)-Negative *Campylobacter* Jejuni Strains and Anti-CDT Neutralizing Antibodies Are Induced During Human Infection But Not During Colonization in Chickens. *Infect. Immunity* **2005**, *73* (5), 3053–3062.
- Alexander, E. K. Approach to the Patient with a Cytologically Indeterminate Thyroid Nodule. *J. Clin. Endocrinol. Metabol.* **2008**, *93* (11), 4175–4182.
- Alexander, E. K.; Kennedy, G. C.; Baloch, Z. W.; Cibas, E. S.; Chudova, D.; Diggans, J. et al. Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology. *New England J. Med.* **2012**, *367* (8), 705–715.
- Alloisio, N.; Queiroux, C.; Fournier, P.; Pujic, P.; Normand, P.; Vallenet, D. et al. The Frankia Alni Symbiotic Transcriptome. *Mol. Plant-Microbe Interact.* **2010**, *23* (5), 593–607.
- Amor, B. B.; Shaw, S. L.; Oldroyd, G. E.; Maillet, F.; Penmetsa, R. V.; Cook, D. et al. The NFP Locus of *Medicago truncatula* Controls an Early Step of Nod Factor Signal Transduction Upstream of a Rapid Calcium Flux and Root Hair Deformation. *Plant J.* **2003**, *34* (4), 495–506.
- Babiker, H. A.; Pringle, S.; Abdel-Muhsin, A.; Mackinnon, M.; Hunt, P.; Walliker, D. High-Level Chloroquine Resistance in Sudanese Isolates of *Plasmodium falciparum* Is Associated with Mutations in the Chloroquine Resistance Transporter Gene PFCRT and the Multidrug Resistance Gene PFMDR1. *J. Infect. Dis.* **2001**, *183* (10), 1535–1538.
- Bagchi, R. *Functional Characterization of MtNIP/LATD's Biochemical and Biological Function*. University of North Texas, 2013.
- Becker, A.; Bergès, H.; Krol, E.; Bruand, C.; Rüberg, S.; Capela, D. et al. Global Changes in Gene Expression in *Sinorhizobium meliloti* 1021 Under Microoxic and Symbiotic Conditions. *Mol. Plant-Microbe Interact.* **2004**, *17* (3), 292–303.
- Belin, B. J.; Busset, N.; Giraud, E.; Molinaro, A.; Silipo, A.; Newman, D. K. Hopanoid Lipids: From Membranes to Plant–Bacteria Interactions. *Nat. Rev. Microbiol.* **2018**, *16* (5), 304.
- Blackwell, H. E.; Zhao, Y. Chemical Genetic Approaches to Plant Biology. *Plant Physiol.* **2003**, *133* (2), 448–455.
- Bolaños, L.; Redondo-Nieto, M.; Rivilla, R.; Brewin, N. J.; Bonilla, I. Cell Surface Interactions of *Rhizobium* Bacteroids and Other Bacterial Strains with Symbiosomal and Peribacteroid

- Membrane Components from Pea Nodules. *Mol. Plant-Microbe Interact.* **2004**, *17* (2), 216–223.
- Brauner, E.; Holmes, B. J.; Krane, J. F.; Nishino, M.; Zurakowski, D.; Hennessey, J. V. et al. Performance of the Afirm Gene Expression Classifier in Hürthle Cell Thyroid Nodules Differs from Other Indeterminate Thyroid Nodules. *Thyroid* **2015**, *25* (7), 789–796.
- Breakspear, A.; Liu, C.; Roy, S.; Stacey, N.; Rogers, C.; Trick, M. et al. The Root Hair “Infectome” of Medicago Truncatula Uncovers Changes in Cell Cycle Genes and Reveals a Requirement for Auxin Signaling in Rhizobial Infection. *Plant Cell* **2014**, *26* (12), 4680–4701.
- Brear, E. M.; Day, D. A.; Smith, P. M. C. Iron: An Essential Micronutrient for the Legume-Rhizobium Symbiosis. *Front. Plant Sci.* **2013**, *4*, 359.
- Brewin, N. J. Development of the Legume Root Nodule. *Annu. Rev. Cell Biol.* **1991**, *7* (1), 191–226.
- Brewin, N. J. Plant Cell Wall Remodelling in the Rhizobium–Legume Symbiosis. *Crit. Rev. Plant Sci.* **2004**, *23* (4), 293–316.
- Burke, G. R.; Moran, N. A. Massive Genomic Decay in Serratia Symbiotica, a Recently Evolved Symbiont of Aphids. *Genome Biol. Evol.* **2011**, *3*, 195–208.
- Byers, D.; Waller, D. Do Plant Populations Purge Their Genetic Load? Effects of Population Size and Mating History on Inbreeding Depression. *Annu. Rev. Ecol. System.* **1999**, *30* (1), 479–513.
- Chabaud, M.; Genre, A.; Sieberer, B. J.; Faccio, A.; Fournier, J.; Novero, M. et al. Arbuscular Mycorrhizal Hyphopodia and Germinated Spore Exudates Trigger Ca²⁺ Spiking in the Legume and Nonlegume Root Epidermis. *New Phytol.* **2011**, *189* (1), 347–355.
- Chapman, S. J.; Hill, A. V. Human Genetic Susceptibility to Infectious Disease. *Nat. Rev. Genet.* **2012**, *13* (3), 175–188.
- Charlesworth, B. How Long Does It Take to Fix a Favorable Mutation, and Why Should We Care? *Am. Nat.* **2020**, *195* (5), 753–771.
- Charon, C.; Johansson, C.; Kondorosi, E.; Kondorosi, A.; Crespi, M. enod40 Induces Dedifferentiation and Division of Root Cortical Cells in Legumes. *Proc. Natl. Acad. Sci.* **1997**, *94* (16), 8901–8906.
- Chaube, H. *Plant Disease Management: Principles and Practices*; CRC Press, 2018.
- Coba de la Peña, T.; Fedorova, E.; Pueyo, J. J.; Lucas, M. M. The Symbiosome: Legume and Rhizobia Co-Evolution Toward a Nitrogen-Fixing Organelle? *Front. Plant Sci.* **2018**, *8*, 2229.
- Corrêa, A.; Cruz, C.; Ferrol, N. Nitrogen and Carbon/Nitrogen Dynamics in Arbuscular Mycorrhiza: The Great Unknown. *Mycorrhiza* **2015**, *25* (7), 499–515.
- Dalmasso, C.; Carpentier, W.; Meyer, L.; Rouzioux, C.; Goujard, C.; Chaix, M.-L.; de Senneville, L. D. Distinct Genetic Loci Control Plasma HIV-RNA and Cellular HIV-DNA Levels in HIV-1 Infection: The ANRS Genome Wide Association 01 Study. *PLoS One* **2008**, *3* (12), e3907.
- Damiani, I.; Baldacci-Cresp, F.; Hopkins, J.; Andrio, E.; Balzergue, S.; Lecomte, P.; Hérouart, D. Plant Genes Involved in Harboring Symbiotic Rhizobia or Pathogenic Nematodes. *New Phytol.* **2012**, *194* (2), 511–522.
- Danino, D.; Talmon, Y. Direct-Imaging and Freeze-Fracture Cryo-Transmission Electron Microscopy of Molecular Gels. *Molecular Gels*; Springer, 2006; pp 253–274.
- Ding, Y.; Oldroyd, G. E. Positioning the Nodule, the Hormone Dictum. *Plant Signal. Behav.* **2009**, *4* (2), 89–93.

- Djordjevic, M. A.; Mohd-Radzman, N. A.; Imin, N. Small-Peptide Signals That Control Root Nodule Number, Development, and Symbiosis. *J. Exp. Bot.* **2015**, *66* (17), 5171–5181.
- Döring, A.; Birnbaum, W.; Kuckling, D. Responsive Hydrogels—Structurally and Dimensionally Optimized Smart Frameworks for Applications in Catalysis, Micro-System Technology and Material Science. *Chem. Soc. Rev.* **2013**, *42* (17), 7391–7420.
- Downie, J. A. Legume Nodulation. *Curr. Biol.* **2014**, *24* (5), R184–R190.
- Duc, G.; Trouvelot, A.; Gianinazzi-Pearson, V.; Gianinazzi, S. First Report of Non-Mycorrhizal Plant Mutants (Myc[−]) Obtained in Pea (*Pisum sativum* L.) and Fababean (*Vicia faba* L.). *Plant Sci.* **1989**, *60* (2), 215–222.
- Dupont, L.; Hérouart, D.; Alloing, G.; Hopkins, J.; Pierre, O.; Frendo, P.; El Msehli, S. *The Legume Root Nodule: From Symbiotic Nitrogen Fixation to Senescence*; Intech Open Access Publisher: London, 2012.
- Durell, S. R.; Guy, H. R. A Putative Prokaryote Voltage-Gated Ca²⁺ Channel with Only One 6TM Motif Per Subunit. *Biochem. Biophys. Res. Commun.* **2001**, *281* (3), 741–746.
- Emerich, D. W.; Krishnan, H. B. Symbiosomes: Temporary Moonlighting Organelles. *Biochem. J.* **2014**, *460* (1), 1–11.
- Erasmus, J. J.; McAdams, H. P.; Connolly, J. E. Solitary Pulmonary Nodules: Part II. Evaluation of the Indeterminate Nodule. *Radiographics* **2000**, *20* (1), 59–66.
- Franche, C.; Lindström, K.; Elmerich, C. Nitrogen-Fixing Bacteria Associated with Leguminous and Non-Leguminous Plants. *Plant Soil* **2009**, *321* (1–2), 35–59.
- Frank, A. C. Molecular Host Mimicry and Manipulation in Bacterial Symbionts. *FEMS Microbiol. Lett.* **2019**, *366* (4), fnz038.
- Frayse, N.; Couderc, F.; Poinsot, V. Surface Polysaccharide Involvement in Establishing the Rhizobium–Legume Symbiosis. *Eur. J. Biochem.* **2003**, *270* (7), 1365–1380.
- Frederickson, M. E. Rethinking Mutualism Stability: Cheaters and the Evolution of Sanctions. *Quarterly Rev. Biol.* **2013**, *88* (4), 269–295.
- Geurts, R.; Bisseling, T. Rhizobium Nod Factor Perception and Signalling. *Plant Cell* **2002**, *14* (suppl 1), S239–S249.
- Geurts, R.; Fedorova, E.; Bisseling, T. Nod Factor Signaling Genes and Their Function in the Early Stages of Rhizobium Infection. *Curr. Opin. Plant Biol.* **2005**, *8* (4), 346–352.
- Giraud, E.; Moulin, L.; Vallenet, D.; Barbe, V.; Cytryn, E.; Avarre, J.-C. et al. Legumes Symbioses: Absence of Nod Genes in Photosynthetic Bradyrhizobia. *Science* **2007**, *316* (5829), 1307–1312.
- Glick, B. R. The Enhancement of Plant Growth by Free-Living Bacteria. *Can. J. Microbiol.* **1995**, *41* (2), 109–117.
- Gobbato, E.; Marsh, J. F.; Vernié, T.; Wang, E.; Maillet, F.; Kim, J. et al. A GRAS-Type Transcription Factor with a Specific Function in Mycorrhizal Signaling. *Curr. Biol.* **2012**, *22* (23), 2236–2241.
- Haney, C. H.; Long, S. R. Plant Flotillins Are Required for Infection by Nitrogen-Fixing Bacteria. *Proc. Natl. Acad. Sci.* **2010**, *107* (1), 478–483.
- Hayashi, T.; Banba, M.; Shimoda, Y.; Kouchi, H.; Hayashi, M.; Imaizumi-Anraku, H. A Dominant Function of CCaMK in Intracellular Accommodation of Bacterial and Fungal Endosymbionts. *Plant J.* **2010**, *63* (1), 141–154.
- Held, M.; Hossain, M. S.; Yokota, K.; Bonfante, P.; Stougaard, J.; Szczyglowski, K. Common and Not So Common Symbiotic Entry. *Trends Plant Sci.* **2010**, *15* (10), 540–545.
- Held, M.; Hou, H.; Miri, M.; Huynh, C.; Ross, L.; Hossain, M. S. et al. *Lotus Japonicus* Cytokinin Receptors Work Partially Redundantly to Mediate Nodule Formation
- W OPEN.

- Høgslund, N.; Radutoiu, S.; Krusell, L.; Voroshilova, V.; Hannah, M. A.; Goffard, N. et al. Dissection of Symbiosis and Organ Development by Integrated Transcriptome Analysis of *Lotus Japonicus* Mutant and Wild-Type Plants. *PLoS One* **2009**, *4* (8), e6556.
- Hohnjec, N.; Vieweg, M. F.; Pühler, A.; Becker, A.; Küster, H. Overlaps in the Transcriptional Profiles of *Medicago Truncatula* Roots Inoculated with Two Different Glomus Fungi Provide Insights Into the Genetic Program Activated During Arbuscular Mycorrhiza. *Plant Physiol.* **2005**, *137* (4), 1283–1301.
- Hojsgaard, D.; Hörandl, E. A Little Bit of Sex Matters for Genome Evolution in Asexual Plants. *Front. Plant Sci.* **2015**, *6*, 82.
- Horváth, B.; Domonkos, Á.; Kereszt, A.; Szűcs, A.; Ábrahám, E.; Ayaydin, F. et al. Loss of the Nodule-Specific Cysteine Rich Peptide, NCR169, Abolishes Symbiotic Nitrogen Fixation in the *Medicago Truncatula* dnf7 Mutant. *Proc. Natl. Acad. Sci.* **2015**, *112* (49), 15232–15237.
- Hungria, M.; Phillips, D. Effects of a Seed Color Mutation on Rhizobial Nod-Gene-Inducing Flavonoids and Nodulation in Common Bean. *Mol. Plant-Microbe Interact.*, *6*.
- Ikeda, S.; Okubo, T.; Takeda, N.; Banba, M.; Sasaki, K.; Imaizumi-Anraku, H. et al. The Genotype of the Calcium/Calmodulin-Dependent Protein Kinase Gene (CCaMK) Determines Bacterial Community Diversity in Rice Roots Under Paddy and Upland Field Conditions. *Appl. Environ. Microbiol.* **2011**, *77* (13), 4399–4405.
- Janczarek, M.; Rachwał, K.; Marzec, A.; Grządziel, J.; Palusińska-Szys, M. Signal Molecules and Cell-Surface Components Involved in Early Stages of the Legume–Rhizobium Interactions. *Appl. Soil Ecol.* **2015**, *85*, 94–113.
- Jeffery, K. J.; Bangham, C. R. Do Infectious Diseases Drive MHC Diversity? *Microbes Infect.* **2015**, *2* (11), 1335–1341.
- Jiménez-Cortés, J. G.; García-Contreras, R.; Bucio-Torres, M. I.; Cabrera-Bravo, M.; Córdoba-Aguilar, A.; Benelli, G.; Salazar-Schettino, P. M. Bacterial Symbionts in Human Blood-Feeding Arthropods: Patterns, General Mechanisms and Effects of Global Ecological Changes. *Acta Tropica* **2018**, *186*, 69–101.
- Jones, K. M.; Kobayashi, H.; Davies, B. W.; Taga, M. E.; Walker, G. C. How Rhizobial Symbionts Invade Plants: The Sinorhizobium–Medicago Model. *Nat. Rev. Microbiol.* **2007**, *5* (8), 619–633.
- Jones, K. T. Mammalian Egg Activation: From Ca²⁺ Spiking to Cell Cycle Progression. *Reproduction* **2005**, *130* (6), 813–823.
- Kang, H.; Zhu, H.; Chu, X.; Yang, Z.; Yuan, S.; Yu, D. et al. A Novel Interaction Between CCaMK and a Protein Containing the Scythe_N Ubiquitin-Like Domain in *Lotus japonicus*.
- Kawaguchi, M.; Imaizumi-Anraku, H.; Koiwa, H.; Niwa, S.; Ikuta, A.; Syono, K.; Akao, S. Root, Root Hair, and Symbiotic Mutants of the Model Legume *Lotus japonicus*. *Mol. Plant-Microbe Interact.* **2002**, *15* (1), 17–26.
- Khan, J. Studies on Screening and Strain Improvement of Local Cellulolytic Fungi and Kinetic Studies of Cellulase for Process Standardization.
- Khan, J.; Alotaibi, A.; Deka, M. Effect of Colchicine Induced Mutation on Cellulose Enzyme Production by *Aspergillus Fumigatus*. *World J. Pharma. Res.* **2015**, *4*, 461–471.
- Kondorosi, E.; Mergaert, P.; Kereszt, A. A Paradigm for Endosymbiotic Life: Cell Differentiation of Rhizobium Bacteria Provoked by Host Plant Factors. *Annu. Rev. Microbiol.* **2013**, *67*, 611–628.
- Kutschera, U.; Niklas, K. J. Evolutionary Plant Physiology: Charles Darwin's Forgotten Synthesis. *Naturwissenschaften* **2009**, *96* (11), 1339.

- Lagunas, B.; Schäfer, P.; Gifford, M. Housing Helpful Invaders: The Evolutionary and Molecular Architecture Underlying Plant Root-Mutualist Microbe Interactions. *J. Exp. Bot.* **2015**, *66* (8), 2177–2186.
- Lambert, J. *Nodule Inception NIN Plays a Dual Role in the Establishment of Root Nodule Symbiosis in Lotus japonicus*. Imu, 2017.
- Lardi, M.; Pessi, G. Functional Genomics Approaches to Studying Symbioses Between Legumes and Nitrogen-Fixing Rhizobia. *High-Throughput* **2018**, *7* (2), 15.
- Larimer, F. W.; Chain, P.; Hauser, L.; Lamerdin, J.; Malfatti, S.; Do, L. et al. Complete Genome Sequence of the Metabolically Versatile Photosynthetic Bacterium *Rhodospseudomonas palustris*. *Nat. Biotechnol.* **2004**, *22* (1), 55–61.
- Lawlor, M. S.; Hsu, J.; Rick, P. D.; Miller, V. L. Identification of *Klebsiella pneumoniae* Virulence Determinants Using an Intranasal Infection Model. *Mol. Microbiol.* **2005**, *58* (4), 1054–1073.
- Lee, C.-H.; Chen, Y.-G.; Chen, J.; Reifsnnyder, P. C.; Serreze, D. V.; Clare-Salzler, M. et al. Novel Leptin Receptor Mutation in NOD/LtJ Mice Suppresses Type 1 Diabetes Progression: II. Immunologic Analysis. *Diabetes* **2006**, *55* (1), 171–178.
- Lee, C.-H.; Reifsnnyder, P. C.; Naggert, J. K.; Wasserfall, C.; Atkinson, M. A.; Chen, J.; Leiter, E. H. Novel Leptin Receptor Mutation in NOD/LtJ Mice Suppresses Type 1 Diabetes Progression: I. Pathophysiological Analysis. *Diabetes* **2005**, *54* (9), 2525–2532.
- Li, H.; Robinson, K. A.; Anton, B.; Saldanha, I. J.; Ladenson, P. W. Cost-Effectiveness of a Novel Molecular Test for Cytologically Indeterminate Thyroid Nodules. *J. Clin. Endocrinol. Metabol.* **2011**, *96* (11), E1719–E1726.
- Lurthy, T.; Alloisio, N.; Fournier, P.; Anchisi, S.; Ponsero, A.; Normand, P. et al. Molecular Response to Nitrogen Starvation by *Frankia alni* ACN14a Revealed by Transcriptomics and Functional Analysis with a Fosmid Library in *Escherichia coli*. *Res. Microbiol.* **2018**, *169* (2), 90–100.
- Ma, F.; Lu, R.; Liu, H.; Shi, B.; Zhang, J.; Tan, M. et al. Nitric Oxide-Activated Calcium/Calmodulin-Dependent Protein Kinase Regulates the Abscissic Acid-Induced Antioxidant Defence in Maize. *J. Exp. Bot.* **2012**, *63* (13), 4835–4847.
- Magne, K.; Couzigou, J.-M.; Schiessl, K.; Liu, S.; George, J.; Zhukov, V. et al. MtNODULE ROOT1 and MtNODULE ROOT2 Are Essential for Indeterminate Nodule Identity. *Plant Physiol.* **2018**, *178* (1), 295–316.
- Markmann, K.; Giczey, G.; Parniske, M. Functional Adaptation of a Plant Receptor-Kinase Paved the Way for the Evolution of Intracellular Root Symbioses with Bacteria. *PLoS Biol.* **2008**, *6* (3), e68.
- Maróti, G.; Kondorosi, É. Nitrogen-Fixing Rhizobium-Legume Symbiosis: Are Polyploidy and Host Peptide-Governed Symbiont Differentiation General Principles of Endosymbiosis? *Front. Microbiol.* **2014**, *5*, 326.
- Márquez, A.; Japonicus Handbook, L. Symbiotic Mutants. *Lotus Japonicus Handbook* **2003**, 425, 187.
- Masson-Boivin, C.; Giraud, E.; Perret, X.; Batut, J. Establishing Nitrogen-Fixing Symbiosis with Legumes: How Many Rhizobium Recipes? *Trends Microbiol.* **2009**, *17* (10), 458–466.
- McIver, B.; Castro, M. R.; Morris, J. C.; Bernet, V.; Smallridge, R.; Henry, M. et al. An Independent Study of a Gene Expression Classifier (Afirma) in the Evaluation of Cytologically Indeterminate Thyroid Nodules. *J. Clin. Endocrinol. Metabol.* **2014**, *99* (11), 4069–4077.

- Middleton, P. H.; Jakab, J.; Penmetsa, R. V.; Starker, C. G.; Doll, J.; Kaló, P. et al. An ERF Transcription Factor in *Medicago truncatula* That Is Essential for Nod Factor Signal Transduction. *Plant Cell* **2007**, *19* (4), 1221–1234.
- Miller, J. B.; Pratap, A.; Miyahara, A.; Zhou, L.; Bornemann, S.; Morris, R. J.; Oldroyd, G. E. Calcium/Calmodulin-Dependent Protein Kinase Is Negatively and Positively Regulated by Calcium, Providing a Mechanism for Decoding Calcium Responses During Symbiosis Signaling. *Plant Cell* **2013**, *25* (12), 5053–5066.
- Mitra, R. M.; Shaw, S. L.; Long, S. R. Six Nonnodulating Plant Mutants Defective for Nod Factor-Induced Transcriptional Changes Associated with the Legume-Rhizobia Symbiosis. *Proc. Natl. Acad. Sci.* **2004**, *101* (27), 10217–10222.
- Miura, K.; Jin, J. B.; Hasegawa, P. M. Sumoylation, a Post-Translational Regulatory Process in Plants. *Curr. Opin. Plant Biol.* **2007**, *10* (5), 495–502.
- Miyahara, A.; Richens, J.; Starker, C.; Morieri, G.; Smith, L.; Long, S. et al. Conservation in Function of a SCAR/WAVE Component During Infection Thread and Root Hair Growth in *Medicago truncatula*. *Mol. Plant-Microbe Interact.* **2010**, *23* (12), 1553–1562.
- Moran, N. A.; Plague, G. R.; Sandström, J. P.; Wilcox, J. L. A Genomic Perspective on Nutrient Provisioning by Bacterial Symbionts of Insects. *Proc. Natl. Acad. Sci.* **2003**, *100* (suppl 2), 14543–14548.
- Mummidi, S.; Ahuja, S. S.; Gonzalez, E.; Anderson, S. A.; Santiago, E. N.; Stephan, K. T. et al. Genealogy of the CCR5 Locus and Chemokine System Gene Variants Associated with Altered Rates of HIV-1 Disease Progression. *Nat. Med.* **1998**, *4* (7), 786–793.
- Murray, J. D. Invasion by Invitation: Rhizobial Infection in Legumes. *Mol. Plant-Microbe Interact.* **2011**, *24* (6), 631–639.
- Oldroyd, G. E.; Downie, J. A. Coordinating Nodule Morphogenesis with Rhizobial Infection in Legumes. *Annu. Rev. Plant Biol.* **2008**, *59*, 519–546.
- Oldroyd, G. E.; Long, S. R. Identification and Characterization Of Nodulation-Signaling Pathway 2, a Gene of *Medicago truncatula* Involved in Nod Factor Signaling. *Plant Physiol.* **2003**, *131* (3), 1027–1032.
- Oldroyd, G. E.; Murray, J. D.; Poole, P. S.; Downie, J. A. The Rules of Engagement in the Legume-Rhizobial Symbiosis. *Annu. Rev. Genet.* **2011**, *45*, 119–144.
- Osborne, B. Holy Alliances? *New Phytol.* **2007**, *175* (4), 602–605.
- Pajuelo, E. *A Mutant Catalogue of Lotus Japonicus Lotus japonicus Handbook*; Springer; pp 187–195.
- Pan, H. More Than Antimicrobial: Nodule Cysteine-Rich Peptides Maintain a Working Balance Between Legume Plant Hosts and Rhizobia Bacteria During Nitrogen-Fixing Symbiosis. *Model Legume Medicago truncatula*, 2019; pp 617–626.
- Park, K. T.; Ahn, S. H.; Mo, J. H.; Park, Y. J.; Park, D. J.; Choi, S. I.; Park, S. Y. Role of Core Needle Biopsy and Ultrasonographic Finding in Management of Indeterminate Thyroid Nodules. *Head Neck* **2011**, *33* (2), 160–165.
- Patel, B. B.; Diao, Y. Multiscale Assembly of Solution-Processed Organic Electronics: The Critical Roles of Confinement, Fluid Flow, and Interfaces. *Nanotechnology* **2017**, *29* (4), 044004.
- Patel, K. N.; Angell, T. E.; Babiarz, J.; Barth, N. M.; Blevins, T.; Duh, Q.-Y. et al. Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules. *JAMA Surg.* **2018**, *153* (9), 817–824.

- Peng, J.; Hao, B.; Liu, L.; Wang, S.; Ma, B.; Yang, Y. et al. RNA-Seq and Microarrays Analyses Reveal Global Differential Transcriptomes of Mesorhizobium Huakuii 7653R Between Bacteroids and Free-Living Cells. *PLoS One* **2014**, *9* (4), e93626.
- Pingret, J.-L.; Journet, E.-P.; Barker, D. G. Rhizobium Nod Factor Signaling: Evidence for a G Protein–Mediated Transduction Mechanism. *Plant Cell* **1998**, *10* (5), 659–671.
- Pislariu, C. I.; Murray, J. D.; Wen, J.; Cosson, V.; Muni, R. R. D.; Wang, M. et al. A *Medicago Truncatula* Tobacco Retrotransposon Insertion Mutant Collection with Defects in Nodule Development and Symbiotic Nitrogen Fixation. *Plant Physiol.* **2012**, *159* (4), 1686–1699.
- Price, E. P.; Sarovich, D. S.; Mayo, M.; Tuanyok, A.; Drees, K. P.; Kaestli, M. et al. Within-Host Evolution of Burkholderia Pseudomallei Over a Twelve-Year Chronic Carriage Infection. *MBio* **2013**, *4* (4).
- Price, P. A.; Tanner, H. R.; Dillon, B. A.; Shabab, M.; Walker, G. C.; Griffiths, J. S. Rhizobial Peptidase HrrP Cleaves Host-Encoded Signaling Peptides and Mediates Symbiotic Compatibility. *Proc. Natl. Acad. Sci.* **2015**, *112* (49), 15244–15249.
- Provorov, N. Improvement of Symbiotic Nitrogen Fixation in Plants: Molecular-Genetic Approaches and Evolutionary Models. *Russian J. Plant Physiol.* **2013**, *60* (1), 27–32.
- Pumplin, N.; Harrison, M. J. Live-Cell Imaging Reveals Periarbuscular Membrane Domains and Organelle Location in *Medicago Truncatula* Roots During Arbuscular Mycorrhizal Symbiosis. *Plant Physiol.* **2009**, *151* (2), 809–819.
- Rallapalli, P.; Kemball-Cook, G.; Tuddenham, E.; Gomez, K.; Perkins, S. An Interactive Mutation Database for Human Coagulation Factor IX Provides Novel Insights Into the Phenotypes and Genetics of Hemophilia B. *J. Thromb. Haemostasis* **2013**, *11* (7), 1329–1340.
- Ramu, S. K.; Peng, H.-M.; Cook, D. R. Nod Factor Induction of Reactive Oxygen Species Production Is Correlated with Expression of the Early Nodulin Gene rip1 in *Medicago truncatula*. *Mol. Plant-Microbe Interact.* **2002**, *15* (6), 522–528.
- Riely, B. K.; Ane, J.-M.; Penmetsa, R. V.; Cook, D. R. Genetic and Genomic Analysis in Model Legumes Bring Nod-Factor Signaling to Center Stage. *Curr. Opin. Plant Biol.* **2004**, *7* (4), 408–413.
- Sakthianandeswaren, A.; Foote, S. J.; Handman, E. The Role of Host Genetics in Leishmaniasis. *Trends Parasitol.* **2009**, *25* (8), 383–391.
- Sandal, N.; Petersen, T. R.; Murray, J.; Umehara, Y.; Karas, B.; Yano, K. et al. Genetics of Symbiosis in *Lotus Japonicus*: Recombinant Inbred Lines, Comparative Genetic Maps, and Map Position of 35 Symbiotic Loci. *Mol. Plant-Microbe Interact.* **2006**, *19* (1), 80–91.
- Sanglard, D.; Hube, B.; Monod, M.; Odds, F. C.; Gow, N. A Triple Deletion of the Secreted Aspartyl Proteinase Genes SAP4, SAP5, and SAP6 of Candida Albicans Causes Attenuated Virulence. *Infect. Immunity* **1997**, *65* (9), 3539–3546.
- Santi, C.; Bogusz, D.; Franche, C. Biological Nitrogen Fixation in Non-Legume Plants. *Ann. Bot.* **2013**, *111* (5), 743–767.
- Santos, R.; Hérouart, D.; Puppo, A.; Touati, D. Critical Protective Role of Bacterial Superoxide Dismutase in Rhizobium–Legume Symbiosis. *Mo. Microbiol.* **2000**, *38* (4), 750–759.
- Schlüter, J.-P.; Reinkensmeier, J.; Daschkey, S.; Evguenieva-Hackenberg, E.; Janssen, S.; Jänicke, S. et al. A Genome-Wide Survey of sRNAs in the Symbiotic Nitrogen-Fixing Alpha-Proteobacterium Sinorhizobium Meliloti. *BMC Genom.* **2010**, *11* (1), 245.
- Senoo, K.; Solaiman, M. Z.; Kawaguchi, M.; Imaizumi-Anraku, H.; Akao, S.; Tanaka, A.; Obata, H. Isolation of Two Different Phenotypes of Mycorrhizal Mutants in the Model

- Legume Plant *Lotus japonicus* After EMS-Treatment. *Plant Cell Physiol.* **2000**, 41 (6), 726–732.
- Shimoda, Y.; Han, L.; Yamazaki, T.; Suzuki, R.; Hayashi, M.; Imaizumi-Anraku, H. Rhizobial and Fungal Symbioses Show Different Requirements for Calmodulin Binding to Calcium Calmodulin-Dependent Protein Kinase in *Lotus japonicus*. *Plant Cell* **2012**, 24 (1), 304–321.
- Shimoda, Y.; Imaizumi-Anraku, H.; Hayashi, M. Kinase Activity-Dependent Stability of Calcium/Calmodulin-Dependent Protein Kinase of *Lotus japonicus*. *Planta* **2019**, 250 (5), 1773–1779.
- Singh, K. V.; Nallapareddy, S. R.; Murray, B. E. Importance of the EBP (Endocarditis and Biofilm-Associated Pilus) Locus in the Pathogenesis of *Enterococcus Faecalis* Ascending Urinary Tract Infection. *J. Infect. Dis.* **2007**, 195 (11), 1671–1677.
- Singh, S.; Parniske, M. Activation of Calcium- and Calmodulin-Dependent Protein Kinase (CCaMK), the Central Regulator of Plant Root Endosymbiosis. *Curr. Opin. Plant Biol.* **2012**, 15 (4), 444–453.
- Smit, P.; Raedts, J.; Portyanko, V.; Debellé, F.; Gough, C.; Bisseling, T.; Geurts, R. NSP1 of the GRAS Protein Family Is Essential for Rhizobial Nod Factor-Induced Transcription. *Science* **2005**, 308 (5729), 1789–1791.
- Sobel, J. M.; Streisfeld, M. A. Flower Color as a Model System for Studies of Plant Evo-Devo. *Front. Plant Sci.* **2013**, 4, 321.
- Spaink, H. P. Root Nodulation and Infection Factors Produced by Rhizobial Bacteria. *Annu. Rev. Microbiol.* **2000**, 54 (1), 257–288.
- Sun, F.; Cha, H.-R.; Bae, K.; Hong, S.; Kim, J.-M.; Kim, S. H. et al. Mechanical Properties of Multilayered Chitosan/CNT Nanocomposite Films. *Mater. Sci. Eng. A* **2011**, 528 (21), 6636–6641.
- Sun, J.; Miller, J. B.; Granqvist, E.; Wiley-Kalil, A.; Gobbato, E.; Maillet, F. et al. Activation of Symbiosis Signaling by Arbuscular Mycorrhizal Fungi in Legumes and Rice. *Plant Cell* **2015**, 27 (3), 823–838.
- Suzaki, T.; Yano, K.; Ito, M.; Umehara, Y.; Suganuma, N.; Kawaguchi, M. Positive and Negative Regulation of Cortical Cell Division During Root Nodule Development in *Lotus japonicus* Is Accompanied by Auxin Response. *Development* **2012**, 139 (21), 3997–4006.
- Takeda, N.; Maekawa, T.; Hayashi, M. Nuclear-Localized and Deregulated Calcium- and Calmodulin-Dependent Protein Kinase Activates Rhizobial and Mycorrhizal Responses in *Lotus japonicus*. *Plant Cell* **2012**, 24 (2), 810–822.
- van der Hest, R.; Engdahl, R.; Spakman, W.; Nolet, G. Tomographic Imaging of Subducted Lithosphere Below Northwest Pacific Island Arcs. *Nature* **1991**, 353 (6339), 37–43.
- Voigt, C. A. Callose-Mediated Resistance to Pathogenic Intruders in Plant Defense-Related Papillae. *Front. Plant Sci.* **2014**, 5, 168.
- Wais, R. J.; Galera, C.; Oldroyd, G.; Catoira, R.; Penmetsa, R. V.; Cook, D. et al. Genetic Analysis of Calcium Spiking Responses in Nodulation Mutants of *Medicago truncatula*. *Proc. Natl. Acad. Sci.* **2000**, 97 (24), 13407–13412.
- Wakui, M.; Osipchuk, Y. V.; Petersen, O. H. Receptor-Activated Cytoplasmic Ca²⁺ Spiking Mediated by Inositol Trisphosphate Is Due to Ca²⁺-Induced Ca²⁺ Release. *Cell* **1990**, 63 (5), 1025–1032.
- Wang, Y.-J.; Yu, J.-N.; Chen, T.; Zhang, Z.-G.; Hao, Y.-J.; Zhang, J.-S.; Chen, S.-Y. Functional Analysis of a Putative Ca²⁺ Channel Gene TaTPC1 from Wheat. *J. Exp. Bot.* **2005**, 56 (422), 3051–3060.

- Werner, G. D.; Cornwell, W. K.; Sprent, J. I.; Kattge, J.; Kiers, E. T. A Single Evolutionary Innovation Drives the Deep Evolution of Symbiotic N₂-Fixation in Angiosperms. *Nat. Commun.* **2014**, *5* (1), 1–9.
- Whitewoods, C. A Nod to Their Ancestors: Mutation of MtNOD1 Highlights Conserved Nodule Development. *Plant Cell* **2020**, *32* (6), 1777.
- Wiener, R. S.; Gould, M. K.; Woloshin, S.; Schwartz, L. M.; Clark, J. A. ‘The Thing Is Not Knowing’: Patients’ Perspectives on Surveillance of an Indeterminate Pulmonary Nodule. *Health Expectations* **2015**, *18* (3), 355–365.
- Yan, G.; Fu, Y.; Faustman, D. L. Reduced Expression of Tap1 and Lmp2 Antigen-Processing Genes in the Nonobese Diabetic (NOD) Mouse Due to a Mutation in Their Shared Bidirectional Promoter. *J. Immunol.* **1997**, *159* (6), 3068–3080.
- Yokota, K.; Fukai, E.; Madsen, L. H.; Jurkiewicz, A.; Rueda, P.; Radutoiu, S. et al. Rearrangement of Actin Cytoskeleton Mediates Invasion of *Lotus japonicus* Roots by Mesorhizobium Loti. *Plant Cell* **2009**, *21* (1), 267–284.
- Yoon, H. J.; Hossain, M. S.; Held, M.; Hou, H.; Kehl, M.; Tromas, A. et al. *Lotus japonicus* SUNERGOS 1 Encodes a Predicted Subunit A of a DNA Topoisomerase VI That Is Required for Nodule Differentiation and Accommodation of Rhizobial Infection. *Plant J.* **2014**, *78* (5), 811–821.
- Yoon, J. H.; Kwak, J. Y.; Kim, E.-K.; Moon, H. J.; Kim, M. J.; Kim, J. Y. et al. How to Approach Thyroid Nodules with Indeterminate Cytology. *Ann. Surgical Oncol.* **2010**, *17* (8), 2147–2155.
- Yoon, S. H.; Reiss, D. J.; Bare, J. C.; Tenenbaum, D.; Pan, M.; Slagel, J. et al. Parallel Evolution of Transcriptome Architecture During Genome Reorganization. *Genome Res.* **2011**, *21* (11), 1892–1904.



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CHAPTER 4

Haploid Mutation

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ABSTRACT

Emergence of chromosomal translocation is a multistage process. Chromosomal translocations induced using irradiation, chemical, or physical mutagens are found useful in creating terminal translocations resulting in mutations that help to generate new crop species with new characteristics like disease, drought, and stress resistance species. In this chapter, we focused on type of chromosomal translocations, reasons, and uses as in wheat for the development of leaf rust-resistant species.

4.1 INTRODUCTION

In plants and animals, cells contain genetic information from both parents in the form of chromosome. Some cells exceptionally developed somatic cells with the chromosome number of a gamete are known as haploids. In 1922, Blackslee et al. discovered the presence of haploids in Datura (Datura innoxia) (Furner et al., 1978; Hiraoka & Tabata, 1974; Saxena & King, 1988) that occurred when un-fertilized egg developed to an embryo (parthenogenesis). After this discovery, many researchers worked on the development of haploid plants using hybridization or by exposing pollens to irradiation. Guha and Maheswari

in 1964, produced haploids by culturing anthers of *D. innoxia*, later similar study was done on tobacco plant (*Nicotiana tabacum*) (Gupta & Borthakur, 1987; Thorpe, 1990). After Guha and Maheswari's report, haploids of many crop plants and other economically important plants have been produced like; wheat, maize, rice, and many other fruits and vegetables (Saxena & King, 1988).

Haploids produced from diploid species are known as monoploids (Basu et al., 2011). These monoploids contain half number of diploid donor plant and are sterile due to their inability to pair during meiosis (Georgiev, 2008). These sterile haploids can be converted to fertile plants and can be included in breeding process by inducing chromosome duplication known as double haploids (DH) (Basu et al., 2011; Dunwell, 2010). These DHs are homozygous and can self-pollinate to new plant species. The production of DH helps in saving time used to produce homozygous species and also improves the efficiency of selection (Kelliher et al., 2019; Mishra & Goswami, 2014). When haploid cells are exposed to mutagenic agents like colchicine or irradiation to develop DHs, intermediate predominant recessive mutations can be isolated in the F1 generation, which reduces the time required to develop homozygous mutants (Mishra & Goswami, 2014). The dioecious plant species that are self incompatible, having inbreeding depression for them haploid production is the best way for developing their inbred lines and more adventurous for dioecious plants with regulating gene used for sex determination (Furner et al., 1978; Sopory & Maheshwari, 1976) (Fig. 4.1).

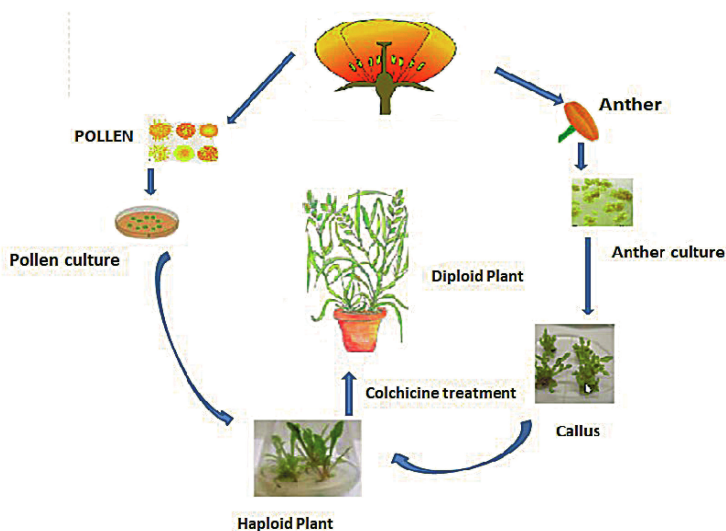


FIGURE 4.1 Process for haploid production using anther culture.

4.2 TECHNIQUES OF HAPLOID AND DOUBLED HAPLOID (DH) PRODUCTION

The haploids have half the number of chromosomes due to which these plants are sterile and to make these plants propagate the procedure applied is by spontaneous or induced mutation the number of chromosome is doubled. These plants are known as double haploids or DH (Chang & Coe, 2009; Ren et al., 2017). The DHs represent a self-pollinating new crop line (El-Hennawy et al., 2011). These DHs are used as a fast method for homozygous production and a selection tool for new and more stable species in different environmental conditions. Many researchers showed several benefits of haploid breeding in their research by using them in genetic and molecular mapping (El-Hennawy et al., 2011; Germana, 2006). DHs are used as a method to develop crop plants with desirable characters in different crops like wheat and rice. Different methods applied for haploid and DHs production are hybridization, anther or microspore culture, ovule culture, haploid inducer genes, and chemical treatments using mutagens (Badu et al., 2017; Bhaskara, 2017).

4.2.1 ANTHR CULTURE

To produce haploid plants anther culture was first used in 1964 by Guha and Maheshwari using *Datura inoxia* plant anthers. After them, many researchers used this technique on different plant species successfully to regenerate plants from calli in wheat, rice, and maize crops. For anther culture in wheat, the spikes of the plant are collected in F1 generation and stored at cold temperature in dark to prevent anthesis (Niroula & Bimb, 2009). In next step, the sterilizations of spike is done using 2% sodium hypochlorite for 20 min with drops of tween 20, followed by rinsing with distilled water (Prem et al., 2009). Anthers are then isolated in aseptic condition and kept in culture tubes with N-6 media and potato-2 medium or W14 medium having different concentration of BAP, NAA, and 2, 4 -D (Germana, 2011; Hiraoka & Tabata, 1974). These culture tubes are stored in dark room for 15 days at 30°C. 2, 4 -D increases embryo development rate and formation of embryonic callus. For regeneration of plant from embryonic calli, the medium is supplied with 17 different mineral salts; thiamine-HCl, sucrose, NAA, glycine, nicotinic acid, myo-inositol, kinetin, and pyridoxine-HCl. After 3 weeks' time, all callus with green shoots are transferred to another medium for rooting

(Ferrie and Caswell, 2011; Ren et al., 2017). This is a semisolid media with a reduced concentration of kinetin and NAA, stored at 27°C and optimum light intensity between 2500 and 3000 lux upto the time of roots development. At fourth week, the plantlets are transferred to pots containing peat and husk of coconut (Savage et al., 1979). The next step is the treatment of haploid plant roots with solution containing 0.1% colchicine and 2% DMSO (dimethyl sulfoxide) and 0.05% of tween 20 for 5 hours at a temperature of 20°C (Aionesei et al., 2005; Sopory and Maheshwari, 1976). The treated plantlets are kept in controlled environment for next 4 weeks and then transferred to external environment. The plant that develops after treatment is a DH plant with seeds containing diploid number of chromosomes. The success of DH development depends on many factors like growth medium type and composition, condition of donor plant, environmental conditions, regeneration, and genotype of anther used (Badu et al., 2017). The selection of plant donor is the most important criteria as the response of culture depends on health and growth of donor plant. The next important factor is culture conditions like temperature, humidity, light, water, fertilizer, and disease control (Gupta and Borthakur, 1987; K.-A. Kim et al., 2012; Saxena & King, 1988). A comparative study between field grown and greenhouse shows variation in culture product. In culture media the carbohydrates components like maltose, amino acids, and nitrogen are important sources for inducing callus formation and differentiation of roots and shoot system (Tai, 2005). The spontaneous mutation that can occur due to incomplete cytokinesis causes doubling of chromosome number in vegetative and generative nuclei. In wheat and barley, the rate of spontaneous mutations is high, the DHs are selected and haploids are discarded (Zulkarnain, 2014). One of the limitations in anther culture is their response to treatment of mutation induction using colchicine, especially some recalcitrant genotypes (Scheller et al., 2009).

4.2.2 MICROSPORE CULTURE

Microspores are usually found in flower buds, inflorescences at a specific age of their development, these parts of plant are collected and homogenized using blender and microspores are collected by filtration followed by washing and centrifugation (Supena et al., 2006). These microspores are finally suspended in an induction medium and incubated at specific temperature conditions (Ferrie & Caswell, 2011). The plants in this process are developed by embryogenesis and it is the most effective system for DHs development in comparison to anther culture (Sopory & Maheshwari, 1973).

The embryo in microspore culture can be easily germinated to plantlets due to their high regeneration potential. The level of gametoclonal variation is very low in microspore culture in comparison to anther culture. The most known disadvantage of microspore culture-derived DHs is high level of albino plantlet formation (Table 4.1).

4.2.3 DHS PRODUCTION BY CROSSING SYSTEM

With time and developments many modifications in culture media for anther culture that caused random genetic changes due to gametoclonal variation, which results in alteration in genetic variations and finally affects selection (Ulukapi & Nasircilar, 2018). Some researchers studied microscopic culture of wheat in Potato-2 medium for DHs development up to F1 generation. The rate of regeneration of calluses from pollen was registered as 47.2% and total plant regeneration rate was recorded 47.9% with spontaneous double haploids (Nagy, 2017). The rate of regeneration is low in microscopic culture that can be because of embryos from anthers fail to regenerate or due to mutation they develop to albino plants. Some comparative studies between anther culture and wheat and maize hybrid cross, claim the rate of regeneration is higher in anther culture than cross culture method (Cheng et al., 2016; C. Song et al., 2020). The anther culture regeneration studied in wheat by Dogramaci et al., in four different culture media and three varied culture condition resulted in 324 (3.7%) out of 86,400 total cultures. Out of 324, 248 were green and 76 were albino plants. They concluded the low regeneration rate was due to high specificity of genotype and environmental condition in which culture are stored (Akhtar & Javed, 2017; Srivastava & Bains, 2018).

The cross culture or inter specific hybridization used for haploid development between different crops like; wheat and maize, wheat and sorghum, wheat and pearls millet, wheat tripsacum, and wheat and barley (Ishizaka, 2018; Morimoto et al., 2019). The intergeneric crosses were found more effective in production of DHs for wheat and other crop plants. In this method, haploids are formed by abolition of chromosomes from pollen of donor plants. The wheat and maize cross developed by pollinating wheat spike with maize pollens result in cross-pollinated embryo formation. The fertilized spike eggs results in zygote formation with haploid number of chromosome from wheat and maize but differences in karyotype make this zygote unstable (Wang et al., 2017). During chromosomal movement toward the poles, the maize chromosomes fail to attach with spindle fibers during cell division due to which the maize chromosome is lost after some divisions

TABLE 4.1 Different Plants Species and Method for Diploid Haploid Production.

Crop type	Species	Technique used for DH production	References
Vegetables	<i>Beta vulgaris</i>	Unfertilized flower culture	(Abbas et al., 2017)
	<i>Brassica campestris</i>	Microspore, anther culture	(Casaletto et al., 2018; Chen et al., 2018)
	<i>Brassica carinata</i>	Microspore, anther culture	(Gu et al., 2004; Khedikar et al., 2020)
	<i>Brassica juncea</i>	Microspore, anther culture	(Inturrisi et al., 2020; J. Yang et al., 2016)
	<i>Brassica oleracea</i>	Microspore, anther culture	(Cheng et al., 2016; Golicz et al., 2016; Sun et al., 2019)
	<i>Brassica rapa</i>	Microspore, anther culture	(Cai et al., 2017; S. Song et al., 2020)
	<i>Solanum tuberosum</i>	Anther culture	(Lou et al., 2007; Mohapatra & Batra, 2017; Reyniers et al., 2020)
Cereal crops	<i>Secale cereale</i>	Microspore, anther culture	(Auinger et al., 2016; Bauer et al., 2017; Mahmood et al., 2020)
	<i>Triticum aestivum</i>	Wide crosses, Microspore, anther culture	(Gao et al., 2018; Miransari & Smith, 2019)
	<i>Oryza sativa</i>	Microspore, anther culture	(Kumarathilaka et al., 2018; Li et al., 2018; Wairich et al., 2019)
	<i>Zea mays</i>	Wide crosses, Microspore, anther culture	(Chen et al., 2018; Hanway & Ritchie, 2019)
	<i>Triticum durum</i>	Wide crosses, Microspore, anther culture	(Ebel et al., 2018; Kabbaj et al., 2017; Tidiane Sall et al., 2019)
	<i>Hordeum vulgare</i>	Wide crosses, Microspore, anther culture	(Beier et al., 2017; Ferchichi et al., 2018; Tombuloglu et al., 2018)
Trees and grasses	Citrus tree (orange, lemon)	Anther culture	(Pan et al., 2016; Wu et al., 2020; Zortea et al., 2018)
	<i>Malus domestica</i> (apple)	Anther culture	(Jadhav et al., 2018; Pires et al., 2018)
	<i>Populus sp.</i> (poplar)	Anther culture	(Chen et al., 2018; Paniagua et al., 2016; Usoltsev et al., 2018)
	<i>Quercus sp.</i> (oak)	Anther culture	(Cuilty et al., 2018; Ghosh & Jung, 2017)
	<i>Lolium perenne</i>	Anther culture	(Jia et al., 2020)
	<i>Phleum pratense</i>	Anther culture	(Cipriani et al., 2018)
	<i>Lolium multiflorum</i>	Anther culture	(Cammarano et al., 2019)

and only haploid cells with wheat chromosomes are left and no endosperm is made and crossing over results only in white fluid with embryo only (Asakura & Hoshino, 2018; Lattier & Contreras, 2017).

4.3 MUTANT SELECTION

The selection of DH mutants is the most important part of development process and it is different than other conventional methods (Ishizaka, 2018). The general method for selection of mutant developed from seed proliferated crop is in M2 generation. The rate of recessive mutants phenotype is very less in M1 progeny, so during screening of quantitative traits like; high yield, tolerance of abiotic stress, disease resistance, and many other characters due to which selection of mutants are carried out after M3 generation (Bhat et al., 2005a,c; Gulfishan et al., 2013; Ganai et al., 2005; Wani et al., 2005; Khan et al., 2005d). To isolate homozygous line of mutants the selection is done after M3 or M4 generation or little longer as per the breeding system and type of crop (Bhat et al., 2006a, c, 2007a,b,c,d; Parveen et al., 2006, 2007; Khan et al., 2005). The technique of DH can help to reduce steps of mutant selection. In DH cells and tissues, as they are recessive in nature, so the selection for quantitative traits can be started at M1 generation. But to see or change the traits should be carried out after M2 or later. The selection of DH mutants are done on the basis of phenotypic characters and fixed genetically, which need to carried out in late stage of development (Golicz et al., 2016; Louvrier et al., 2020). The advantage of using DH for mutant selection is the ration of genetic segregation and genotype are equivalent in these crop species. These species have more efficiency of mutant selection and simple genetic segregation (Sharma et al., 2009; Gulfishan et al., 2010; Bhat et al., 2005b, 2007c,d; Gulfishan et al., 2011).

4.3.1 *IN VITRO* MUTANT SELECTION BY GYNOGENESIS

Gynogenesis is a method to induce maternal haploids or production of haploid embryo from a female gametophyte. To achieve these, the un-pollinated flowers ovules, placenta, or sometime whole flower buds are used for gynogenetic regeneration. Uses of gynogenetic mutants are preferred over androgenetic once as the former is more stable and the numbers of albino plants are also less. Gynogenesis is found successful in all the crop species that fails to get pollinated using other techniques. Method of gynogenic

induction is very successfully applied in many crop plants like cucumber, sunflower, wheat, onion, sugar beet, etc. In both in vivo and in vitro selection of mutants are done after haploid mutagenesis in later stage of DH production (Alotaibi and Khan, 2016). The haploid plants are produced by direct regeneration from callus on media free from selection and differentiation. Seeds of plants collected on maturity in soil contains high rate of spontaneous mutation are used or DH line screening in different growth conditions like, lab, glass rooms, fields, and greenhouse. The seeds that are found with low spontaneous mutation rate are than exposed to colchicine treatment and seed collection for mutation selection is done after 1 generation. In Brassicas, rice and barley many mutants were selected from microspore generated DHs.

A study was conducted using agronomics and fatty acid configuration on microspore production of haploids and DH in Brassica species in Canada. Around 80,000 DHs were produced in a time period of 2 years (Taylor et al., 2018). The study also included growth of DHs with high level of oleic acid and lowered level of α -linolenic acid and other saturated acids. The result of the study showed 5–5.4% decrease in fatty acid, so the plant species with lower fatty acid more than 7.1% were labeled as low saturate. Another study with *Brassica carinata* sp. was conducted on DHs with altered concentration of erucic acid and glucosinolates (Bennett, 2019a, b). Study on 616 DHs of barley regenerated after treatment with sodium azide on microspores. After treatment, different developmental changes were observed in length of plants as some developed as dwarfs, semidwarfs, type of heading (early or late), chlorophyll in seedlings, male sterility, etc. The frequency of these mutants DHs depends on genotype of the plant, which can range between 3.8 and 15.8% (C.-K. Kim et al., 2018). Szarejko et al. in 1995 produced DH lines in M1 generation, which carry mutation at frequency of 25% in morphological and biochemical traits. These were treated with MNU and sodium azide to produce M1 donor plants (Ahmar et al., 2020). The legume species were also treated by physical and chemical mutagens to induce genetic variability, which is harvested to improve agronomic characters (Gulfishan et al., 2012, 2015; Bhat et al., 2006b, 2007b, 2017; Bhat and Wani (2017); Wani and Bhat (2017)).

4.3.2 TREAT IDENTIFICATION FOR IN VITRO SELECTION

Most studies on combining haploids with mutagens found it useful for in vitro selection of mutants that can help to study large population of plant

species. The traits that are modifiable to similar selection for resistance to disease conditions, abiotic stress, salt tolerance, herbicides, and composition of chemical compounds present in seeds. The toxins from herbicides are used to develop tolerant crop species and toxins produced by different pathogens are used to develop disease resistant in mutant species. During selection procedure the selective agents are added to culture media at LD100 to kill all microbes and develop an embryo or calli, which carry the character of tolerance or resistance. These toxins are added to regeneration medium at LD100 and embryo or calli are kept in these media for a prolonged time of 3 to 4 weeks (Osnato et al., 2020). The plants that survive these treatments are then transferred for germination to soil for chromosomal doubling or DHs production. The grown DHs are then screened for tolerance and resistance and further successful mutants are transferred to fields for selection. Many studies with amino acid modifications in plant species with other tolerance and disease-resistant characters were successfully developed using in vitro method as it allows screening of large population in small laboratory space (Shah and Smith, 2020).

4.3.3 ABIOTIC STRESS TOLERANT

With developing plant tissue culture technology and advance knowledge of biochemical pathways that govern disease controlling and signaling can be explored and altered using mutation and DHs development techniques. High level of jasmonic acid, silicic acid, and proline analogs in growth media helps in production of cold temperature tolerant DH mutant crop lines (Parmar et al., 2017). These chemical compounds are found to work as key defense signaling molecules for oxidative stress induced by cold and osmotic stress. For selection of salt tolerant mutant line development, high amount of NaCl is added to the growth media for rapeseed. The tolerance characters are always expressed in calli stage, but it cannot be confirmed even in whole plant stage until laboratory and field condition testing confirms the presence of the character in plant species. In a study by Rahman et al., developed eight million embryos after ENU treatment but only 10 embryos survived the high salinity in medium. Form 10 embryos only two showed changes in salt tolerance related biomarker changes in them like Na and K ratio or increased proline level. It is considered by many researches that these factors are responsible for the salt tolerance character of DHs (Ghosh & Jung, 2017).

4.4 APPLICATIONS OF DH SYSTEMS IN CONNECTION WITH MUTATION TECHNIQUES

The other applications of DHs are in selection of mutants in shortest breeding program especially in plant species, which shows negative affect of mutation on them. DHs can be used to clear mutants and in selection of recombinants that carry desired characters to produce homozygous plant species. Use of DH helps to increase speed for production of true-type mutants and increase their efficiency in anticipated genetic background. DHs are used for production of pure line using F1 offspring's of hybridization between mutant and other varieties to get better breeding lines as produced using conventional breeding techniques but here in less time and with less steps (Sharma et al., 2019).

The DH species retain desired characters for many generations without any change in expression and can be evaluated after many generations (Bauer et al., 2017). DH line of mutants does not follow segregation in progeny and change in phenotypic characters occurs due to environmental factors. Mutation gene mapping using DHs is a recent development in recombination and mutant technology. In this DH mapping population developed by crossing between polymorphic varieties is used to generate molecular marker maps in barley, rice, wheat, and many other species (Martin & Courtier-Orgozozo, 2017). A cross between DH mutant line and other varieties as parents of mapping population, than it can be used to map gene that underline the phenotype of that species (Huu et al., 2020). Similar methods enable chromosomal location for a desired gene for its location on DNA to be used further for genetic reference mapping. To study or analyze in this type of crossing always F2 generation is preferred especially for quantative trait analysis (Sun et al., 2019; Thambugala et al., 2020). Use of microscopic mutagenesis offers an opportunity to generate a huge population of DH with mutation in homozygous phase. The DNA of these varieties are isolated and saved in gene bank (Huang et al., 2020; Peace et al., 2019; S. Yang et al., 2018). These mutants further used as TILLING for mutation identification of mutation in desired gene. Using DHs saves one generation in TILLING and allow direct recovery of pure line in place of looking for homozygous mutant of M3 progeny (Jost et al., 2019; Mohapatra & Batra, 2017).

4.5 CONCLUSION

Haploid and double haploid have lots of application in the development and selection of mutants, germplasm development, and developing species with desired traits in crop plants. In this chapter, we tried to include all different

techniques used for haploid and double haploid production in different crop plants like wheat, maize, rice, etc.

KEYWORDS

- **translocation lines**
- **GISH**
- **chromosome C-banding**
- **chromosome breakages**
- **leaf rust**

REFERENCES

- Abbas, A.; Iqbal, Z.; Abbas, R. Z.; Khan, M. K.; Khan, J. A.; Mahmood, M. S.; Saleemi, M. K. In Vivo Anticoccidial Effects of Beta Vulgaris (Sugar Beet) in Broiler Chickens. *Microb. Pathogen.* **2017**, *111*, 139–144.
- Ahmar, S.; Gill, R. A.; Jung, K.-H.; Faheem, A.; Qasim, M. U.; Mubeen, M.; Zhou, W. Conventional and Molecular Techniques from Simple Breeding to Speed Breeding in Crop Plants: Recent Advances and Future Outlook. *Int. J. Mol. Sci.* **2020**, *21* (7), 2590.
- Aionesei, T.; Touraev, A.; Heberle-Bors, E. Pathways to Microspore Embryogenesis. In *Haploids in Crop Improvement II*; Springer, 2005; pp 11–34.
- Akhtar, S.; Javed, K. Steps and Factors in Double Haploid Wheat Plant Production Through Wheat×Maize Cross-A Review. *J. Agric. Res.* **2017**, *55* (2), 267–278.
- Alotaibi, D. A.; Khan, D. J. Kinetic Study and Basal Media Modification of Cellulase Enzyme Production by Mutated Strain of *Aspergillus fumigatus* (AF1). *Int. J. Pharm. Bio. Sci.* **2016**, *7* (1), 193–200.
- Asakura, I.; Hoshino, Y. Interspecific Hybridization Using Miyama Matatabi (*Actinidia kolomikta*), a Japanese Indigenous Wild Kiwifruit Relative. *Hort. J.* **2018**, *87* (4), 481–489.
- Auinger, H.-J.; Schönleben, M.; Lehermeier, C.; Schmidt, M.; Korzun, V.; Geiger, H. H.; Piepho, H.-P.; Gordillo, A.; Wilde, P.; Bauer, E. Model Training Across Multiple Breeding Cycles Significantly Improves Genomic Prediction Accuracy in Rye (*Secale cereale* L.). *Theor. Appl. Genet.* **2016**, *129* (11), 2043–2053.
- Badu, M.; Tripathy, B.; Sahu, G. S.; Jena, A. K. Role of Doubled Haploids in Vegetable Crop Improvement. *J. Pharmacog. Phytochem.* **2017**, *6* (6), 384–389.
- Basu, S. K.; Datta, M.; Sharma, M.; Kumar, A. Haploid Production Technology in Wheat and Some Selected Higher Plants. *Austr. J. Crop Sci.* **2011**, *5* (9), 1087.
- Bauer, E.; Schmutzer, T.; Barilar, I.; Mascher, M.; Gundlach, H.; Martis, M. M.; Twardziok, S. O.; Hackauf, B.; Gordillo, A.; Wilde, P. Towards a Whole-Genome Sequence for Rye (*Secale cereale* L.). *Plant J.* **2017**, *89* (5), 853–869.

- Beier, S.; Himmelbach, A.; Colmsee, C.; Zhang, X.-Q.; Barrero, R. A.; Zhang, Q.; Li, L.; Bayer, M.; Bolser, D.; Taudien, S. Construction of a map-based reference genome sequence for barley, *Hordeum vulgare* L. *Scientific Data* **2017**, *4* (1), 1–24.
- Bennett, R. *Plants and Seeds of Brassica Carinata Variety dh-040.008*. Google Patents, 2019a.
- Bennett, R. *Plants and Seeds of Brassica Carinata Variety dh-056.149*. Google Patents, 2019b.
- Bhaskara, G. B. Basic Principles and Recent Advances in Anther/Pollen Culture for Crop Improvement. In *Plant Biotechnology*, Vol. 1; Apple Academic Press, 2017; pp 87–123.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Comparative Analysis of Meiotic Abnormalities Induced by Gamma Rays, EMS and MMS in *Vicia faba* L. *J. Ind. Bot. Soc.* **2005a**, *84*, 45–48.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Clastogenic Effects of EMS and MMS in *Vicia faba* L. *J. Cyto. Genet.* **2005b**, *6*, 117–122.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by Gamma Rays and EMS in *Vicia faba* L. *Vegetos* **2005c**, *18* (1&2), 143–145.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Effect of Gamma Rays on Certain Cytomorphological Parameters in Two Varieties of *Vicia faba* L. *Adv. Plant Sci.* **2006a**, *19* (1), 227–232.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Wani, N. A. Radiation Induced Pod and Seed Mutants and MMS Induced Closed Flower Mutant in Broad Bean (*Vicia faba* L.). *Indian J. Genet.* **2006b**, *66* (3), 249–250.
- Bhat, T. A.; Parveen, S.; Khan, A.H MMS-Induced Cytomixis in Pollen Mother Cells of Broad Bean (*Vicia faba* L.). *Turk. J. Bot.* **2006c**, *30*, 273–279.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Studies on the Effect of EMS on Meiosis and Pollen Fertility in *Vicia faba* L. *Adv. Plant Sci.* **2006d**, *19* (1), 243–247.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by MMS, Gamma Rays and Their Combination in Two Varieties of *Vicia faba* L. *Asian J. Plant Sci.* **2007a**, *6* (3), 558–561.
- Bhat, T. A.; Parveen, S.; Khan, A. H. (Meiotic Studies in Two Varieties of *Vicia faba* L. (Fabaceae) After MMS Treatment. *Asian J. Plant Sci.* **2007b**, *61* (1), 51–55.
- Bhat, T. A.; Sharma, M.; Anis, M. Comparative Analysis of Meiotic Aberrations Induced by Diethylsulphate and Sodium Azide in Broad Bean (*Vicia faba* L.). *Asian J. Plant Sci.* **2007c**, *6* (7), 1051–1055.
- Bhat, T. A.; Sharma, M.; Anis, M Comparative Analysis of Mitotic Aberrations Induced by Diethyl Sulphide (DES) and Sodium Azide (SA) in *Vicia faba* L. (Fabaceae). *Pak. J. Biol. Sci.* **2007d**, *10* (5), 783–787.
- Bhat, T. A.; Wani, A. A. Mutagenic Effects on Meiosis in Legumes and a Practical Case Study of *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Bhat, T. A.; Gulfishan, M.; Wani, A. A. Cytomixis: Causes and Consequences as a Case Study in *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Cai, C.; Wang, X.; Liu, B.; Wu, J.; Liang, J.; Cui, Y.; Cheng, F.; Wang, X. Brassica Rapa Genome 2.0: A Reference Upgrade Through Sequence Re-Assembly and Gene Re-Annotation. *Mol. Plant* **2017**, *10* (4), 649–651.
- Cammarano, D.; Hawes, C.; Squire, G.; Holland, J.; Rivington, M.; Murgia, T.; Roggero, P. P.; Fontana, F.; Casa, R.; Ronga, D. Rainfall and Temperature Impacts on Barley (*Hordeum vulgare* L.) Yield and Malting Quality in Scotland. *Field Crops Res.* **2019**, *241*, 107559.

- Casaletto, M. P.; Figà, V.; Privitera, A.; Bruno, M.; Napolitano, A.; Piacente, S. Inhibition of Cor-Ten Steel Corrosion by “Green” Extracts of *Brassica campestris*. *Corros. Sci.* **2018**, *136*, 91–105.
- Chang, M.-T.; Coe, E. H. Doubled Haploids. In *Molecular Genetic Approaches to Maize Improvement*; Springer, 2009; pp 127–142.
- Chen, G.; Ye, X.; Zhang, S.; Zhu, S.; Yuan, L.; Hou, J.; Wang, C. Comparative Transcriptome Analysis Between Fertile and CMS Flower Buds in Wucai (*Brassica campestris* L.). *BMC Genom.* **2018**, *19* (1), 908.
- Cheng, F.; Sun, R.; Hou, X.; Zheng, H.; Zhang, F.; Zhang, Y.; Liu, B.; Liang, J.; Zhuang, M.; Liu, Y. Subgenome Parallel Selection Is Associated with Morphotype Diversification and Convergent Crop Domestication in *Brassica rapa* and *Brassica oleracea*. *Nat. Genet.* **2016**, *48* (10), 1218–1224.
- Cipriani, F.; Mastroiilli, C.; Tripodi, S.; Ricci, G.; Perna, S.; Panetta, V.; Asero, R.; Dondi, A.; Bianchi, A.; Maiello, N. Diagnostic Relevance of IgE Sensitization Profiles to Eight Recombinant Phleum Pratense Molecules. *Allergy* **2018**, *73* (3), 673–682.
- Cuilty, K. R.; Ballinas-Casarrubias, L.; de San Miguel, E. R.; de Gyves, J.; Robles-Venzor, J. C.; González-Sánchez, G. Cellulose Recovery from *Quercus* sp. Sawdust Using Ethanol Pretreatment. *Biomass Bioenergy* **2018**, *111*, 114–124.
- Dunwell, J. M. Haploids in Flowering Plants: Origins and Exploitation. *Plant Biotechnol. J.* **2010**, *8* (4), 377–424.
- Ebel, C.; BenFeki, A.; Hanin, M.; Solano, R.; Chini, A. Characterization of Wheat (*Triticum aestivum*) TIFY Family and Role of Triticum Durum Td TIFY11a in Salt Stress Tolerance. *PLoS One* **2018**, *13* (7), e0200566.
- El-Hennawy, M. A.; Abdalla, A. F.; Shafey, S. A.; Al-Ashkar, I. M. Production of Doubled Haploid Wheat Lines (*Triticum aestivum* L.) Using Anther Culture Technique. *Ann. Agric. Sci.* **2011**, *56* (2), 63–72.
- Ferchichi, S.; Hessini, K.; Dell’Aversana, E.; D’Amelia, L.; Woodrow, P.; Ciarmiello, L. F.; Fuggi, A.; Carillo, P. *Hordeum Vulgare* and *Hordeum Maritimum* Respond to Extended Salinity Stress Displaying Different Temporal Accumulation Pattern of Metabolites. *Funct. Plant Biol.* **2018**, *45* (11), 1096–1109.
- Ferrie, A. M. R.; Caswell, K. L. Isolated Microspore Culture Techniques and Recent Progress for Haploid and Doubled Haploid Plant Production. *Plant Cell, Tissue Organ Culture (PCTOC)* **2011**, *104* (3), 301–309.
- Furner, I. J.; King, J.; Gamborg, O. L. Plant Regeneration from Protoplasts Isolated from a Predominantly Haploid Suspension Culture of *Datura innoxia* (Mill.). *Plant Sci. Lett.* **1978**, *11* (2), 169–176.
- Ganai, F. A.; Khan, A. H.; Bhat, T. A.; Parveen, S.; Wani, N. A. (Cytogenetic Effects of Methyl Methane Sulphonate (MMS) on Varieties of Chickpea (*Cicer arietinum* L.)) *J. Cytol. Genet.* **2005**, *6*, 97–102.
- Gao, X.; Avellan, A.; Laughton, S.; Vaidya, R.; Rodrigues, S. M.; Casman, E. A.; Lowry, G. V. CuO Nanoparticle Dissolution and Toxicity to Wheat (*Triticum aestivum*) in Rhizosphere Soil. *Environ. Sci. Technol.* **2018**, *52* (5), 2888–2897.
- Georgiev, S. Haploids in Genetic and Cytogenetical Research. *Biotechnol. Biotechnol. Equip.* **2008**, *22* (2), 644–651.
- Germana, M. A. Doubled Haploid Production in Fruit Crops. *Plant Cell, Tissue Organ Culture* **2006**, *86* (2), 131.

- Germana, M. A. Anther Culture for Haploid and Doubled Haploid Production. *Plant Cell, Tissue Organ Culture (PCTOC)* **2011**, 104 (3), 283–300.
- Ghosh, S.; Jung, C. Nutritional Value of Bee-Collected Pollens of Hardy Kiwi, *Actinidia arguta* (Actinidiaceae) and Oak, *Quercus* sp. (Fagaceae). *J. Asia-Pacific Entomol.* **2017**, 20 (1), 245–251.
- Golicz, A. A.; Bayer, P. E.; Barker, G. C.; Edger, P. P.; Kim, H.; Martinez, P. A.; Chan, C. K. K.; Severn-Ellis, A.; McCombie, W. R.; Parkin, I. A. The Pangenome of an Agronomically Important Crop Plant *Brassica oleracea*. *Nat. Commun.* **2016**, 7 (1), 1–8.
- Gu, H. H.; Hagberg, P.; Zhou, W. J. Cold Pretreatment Enhances Microspore Embryogenesis in Oilseed Rape (*Brassica napus* L.). *Plant Growth Regulation* **2004**, 42 (2), 137–143.
- Gulfishan, M.; Bhat, T. A.; Oves, M. Mutants as a Genetic Resource for Future Crop Improvement; In *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools*; Al-Khayri, J. M. et al., Eds.; Springer International Publishing Switzerland, 2015.
- Gulfishan, M.; Jafri, I. F.; Khan, A. H.; Bhat, T. A. Methyl Methane Sulphonate Induced Desynaptic Mutants in *Capsicum annum* L. *Chromosome Bot.* **2013**, 8, 59–62.
- Gulfishan, M.; Khan, A. H.; Bhat, T. A. Studies on Cytotoxicity Induced by DES and SA in *Vicia faba* L. var. Major. *Turk. J. Bot.* **2010**, 34, 31–37.
- Gulfishan, M.; Khan, A. H.; Gefferi, I. F.; Bhat, T. A. Assessment of Mutagenicity Induced by MMS and DES in *Capsicum annum* L. *Saudi J. Biol. Sci.* **2012**, 19, 251–255.
- Gulfishan, M.; Khan, A. H.; Haneef, I.; Bhat, T. A. Genotoxic Effect of Diethyl Sulphate in Two Varieties of *Capsicum annum* L. *Nucleus* **2011**, 54 (2), 107–111.
- Gupta, H. S.; Borthakur, D. N. Improved Rate of Callus Induction from Rice Anther Culture Following Microscopic Staging of Microspores in Iron Alum-Haematoxylin. *Theor. Appl. Genet.* **1987**, 74 (1), 95–99.
- Hanway, J. J.; Ritchie, S. W. Zea Mays. In *Handbook of Flowering*; CRC Press, 2019; pp 525–541.
- Hiraoka, N.; Tabata, M. Alkaloid Production by Plants Regenerated from Cultured Cells of *Datura Innoxia*. *Phytochemistry* **1974**, 13 (9), 1671–1675.
- Huang, S.; Hou, L.; Fu, W.; Liu, Z.; Li, C.; Li, X.; Feng, H. An Insertion Mutation in Bra032169 Encoding a Histone Methyltransferase Is Responsible for Early Bolting in Chinese Cabbage (*Brassica rapa* L. ssp. *Pekinensis*). *Front. Plant Sci.* **2020**, 11, 547.
- Huu, C. N.; Keller, B.; Conti, E.; Kappel, C.; Lenhard, M. Supergene Evolution via Stepwise Duplications and Neofunctionalization of a Floral-Organ Identity Gene. *Proc. Natl. Acad. Sci.* **2020**, 117 (37), 23148–23157.
- Inturrisi, F.; Bayer, P. E.; Yang, H.; Tirnaz, S.; Edwards, D.; Batley, J. Genome-Wide Identification and Comparative Analysis of Resistance Genes in *Brassica juncea*. *Mol. Breed.* **2020**, 40 (8), 1–14.
- Ishizaka, H. Breeding of Fragrant Cyclamen by Interspecific Hybridization and Ion-Beam Irradiation. *Breed. Sci.* **2018**, 68 (1), 25–34.
- Jadhav, M. S.; Kulkarni, S.; Raikar, P.; Barretto, D. A.; Vootla, S. K.; Raikar, U. S. Green Biosynthesis of CuO & Ag–CuO Nanoparticles from *Malus Domestica* Leaf Extract and Evaluation of Antibacterial, Antioxidant and DNA Cleavage Activities. *New J. Chem.* **2018**, 42 (1), 204–213.
- Jia, H.; Hou, D.; O'Connor, D.; Pan, S.; Zhu, J.; Bolan, N. S.; Mulder, J. Exogenous Phosphorus Treatment Facilitates Chelation-Mediated Cadmium Detoxification in Perennial Ryegrass (*Lolium perenne* L.). *J. Hazard. Mater.* **2020**, 389, 121849.

- Jost, M.; Szurman-Zubrzycka, M.; Gajek, K.; Szarejko, I.; Stein, N. Tilling in Barley. In *Barley*; Springer, 2019; pp 73–94.
- Kabbaj, H.; Sall, A. T.; Al-Abdallat, A.; Geleta, M.; Amri, A.; Filali-Maltouf, A.; Belkadi, B.; Ortiz, R.; Bassi, F. M. Genetic Diversity Within a Global Panel of Durum Wheat (*Triticum durum*) Landraces and Modern Germplasm Reveals the History of Alleles Exchange. *Front. Plant Sci.* **2017**, *8*, 1277.
- Kelliher, T.; Starr, D.; Su, X.; Tang, G.; Chen, Z.; Carter, J.; Wittich, P. E.; Dong, S.; Green, J.; Burch, E. One-Step Genome Editing of Elite Crop Germplasm During Haploid Induction. *Nat. Biotechnol.* **2019**, *37* (3), 287–292.
- Khan, A. A.; Ansari, M. Y. K.; Shahab, D.; Bhat, T. A. Induced Variations for Quantitative Traits in *Vicia faba* L. *Vegetos* **2005a**, *18* (1&2), 83–86.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. DES Induced Variations for Economic Traits in Broad Bean (*Vicia faba* L.). *Biosci. Biotechnol. Res. Asia* **2005b**, *3* (2), 363–366.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. Clastogenic Effects of 8-Hydroxy Quinoline in *Vicia faba* L. *Adv. Plant Sci.* **2007a**, *20* (1), 85–87. ISSN 0970–3586.
- Khan, A. A.; Ansari, M. Y. K.; Shahab, D.; Bhat, T. A. Comparative Analysis of Meiotic Abnormalities Induced by Caffeine and DES in *Vicia faba* L. *Adv. Plant Sci.* **2007b**, *20* (1), 73–76.
- Khedikar, Y.; Clarke, W. E.; Chen, L.; Higgins, E. E.; Kagale, S.; Koh, C. S.; Bennett, R.; Parkin, I. A. Narrow Genetic Base Shapes Population Structure and Linkage Disequilibrium in an Industrial Oilseed Crop, Brassica carinata A. Braun. *Sci. Rep.* **2020**, *10* (1), 1–11.
- Kim, C.-K.; Seol, Y.-J.; Perumal, S.; Lee, J.; Waminal, N. E.; Jayakodi, M.; Lee, S.-C.; Jin, S.; Choi, B.-S.; Yu, Y. Re-exploration of U's Triangle Brassica Species Based on Chloroplast Genomes and 45S nrDNA Sequences. *Sci. Rep.* **2018**, *8* (1), 1–11.
- Kim, K.-A.; Gu, W.; Lee, I.-A.; Joh, E.-H.; Kim, D.-H. High Fat Diet-Induced Gut Microbiota Exacerbates Inflammation and Obesity in Mice via the TLR4 Signaling Pathway. *PloS One* **2012**, *7* (10), e47713.
- Kumarathilaka, P.; Seneweera, S.; Meharg, A.; Bundschuh, J. Arsenic Accumulation in Rice (*Oryza sativa* L.) Is Influenced by Environment and Genetic Factors. *Sci. Total Environ.* **2018**, *642*, 485–496.
- Lattier, J. D.; Contreras, R. N. Intraspecific, Interspecific, and Interseries Cross-Compatibility in Lilac. *J. Am. Soc. Hort. Sci.* **2017**, *142* (4), 279–288.
- Li, L.; Hou, M.; Cao, L.; Xia, Y.; Shen, Z.; Hu, Z. Glutathione S-Transferases Modulate Cu Tolerance in *Oryza sativa*. *Environ. Exp. Bot.* **2018**, *155*, 313–320.
- Lou, P.; Kang, J.; Zhang, G.; Bonnema, G.; Fang, Z.; Wang, X. Transcript Profiling of a Dominant Male Sterile Mutant (Ms-cd1) in Cabbage During Flower Bud Development. *Plant Sci.* **2007**, *172* (1), 111–119.
- Louvrier, C.; Assrawi, E.; El Khouri, E.; Melki, I.; Copin, B.; Bourrat, E.; Lachaume, N.; Cador-Rousseau, B.; Duquesnoy, P.; Piterboth, W. NLRP3-Associated Autoinflammatory Diseases: Phenotypic and Molecular Characteristics of Germline Versus Somatic Mutations. *J. Allergy Clin. Immunol.* **2020**, *145* (4), 1254–1261.
- Mahmood, K.; Orabi, J.; Kristensen, P. S.; Sarup, P.; Jørgensen, L. N.; Jahoor, A. De novo Transcriptome Assembly, Functional Annotation, and Expression Profiling of Rye (*Secale cereale* L.) hybrids inoculated with ergot (*Claviceps purpurea*). *Scientific Reports* **2020**, *10* (1), 1–16.

- Martin, A.; Courtier-Orgogozo, V. Morphological Evolution Repeatedly Caused by Mutations in Signaling Ligand Genes. In *Diversity and Evolution of Butterfly Wing Patterns*; Springer: Singapore, 2017; pp 59–87.
- Miransari, M.; Smith, D. Sustainable Wheat (*Triticum aestivum* L.) Production in Saline Fields: A Review. *Crit. Rev. Biotechnol.* **2019**, *39* (8), 999–1014.
- Mishra, V. K.; Goswami, R. Haploid Production in Higher Plant. *Int. J. Chem. Biol. Sci.* **2014**, *1*, 26–45.
- Mohapatra, P. P.; Batra, V. K. Tissue Culture of Potato (*Solanum tuberosum* L.), A Review. *Int. J. Curr. Microbiol. Appl. Sci.* **2017**, *6* (4), 489–495.
- Morimoto, T.; Kitamura, Y.; Numaguchi, K.; Akagi, T.; Tao, R. Characterization of Post-Mating Interspecific Cross-Compatibility in *Prunus* (Rosaceae). *Sci. Hort.* **2019**, *246*, 693–699.
- Nagy, M. A. A.-E. A. *Genetical Studies on Bread Wheat (Triticum aestivum L.) Gametoclones* [PhD Thesis]. Minia University, 2017.
- Niroula, R. K.; Bimb, H. P. Overview of Wheat-Maize System of Crosses for Dihaploid Induction in Wheat. *World Appl. Sci. J.* **2009**, *7* (8), 1037–1045.
- Osnato, M.; Cereijo, U.; Sala, J.; Matías-Hernández, L.; Aguilar-Jaramillo, A. E.; Rodríguez-Goberna, M. R.; Riechmann, J. L.; Rodríguez-Concepción, M.; Pelaz, S. The Floral Repressors TEMPRANILLO1 and 2 Modulate Salt Tolerance by Regulating Hormonal Components and Photo-Protection in Arabidopsis. *Plant J.* **2020**.
- Pan, Z.; Lie, D.; Qiang, L.; Shaolan, H.; Shilai, Y.; Yande, L.; Yongxu, Y.; Haiyang, P. Effects of Citrus Tree-Shape and Spraying Height of Small Unmanned Aerial Vehicle on Droplet Distribution. *Int. J. Agric. Biol. Eng.* **2016**, *9* (4), 45–52.
- Paniagua, S.; Escudero, L.; Escapa, C.; Coimbra, R. N.; Otero, M.; Calvo, L. F. Effect of Waste Organic Amendments on *Populus* sp Biomass Production and Thermal Characteristics. *Renew. Energy* **2016**, *94*, 166–174.
- Parmar, N.; Singh, K. H.; Sharma, D.; Singh, L.; Kumar, P.; Nanjundan, J.; Khan, Y. J.; Chauhan, D. K.; Thakur, A. K. Genetic Engineering Strategies for Biotic and Abiotic Stress Tolerance and Quality Enhancement in Horticultural Crops: A Comprehensive Review. *3 Biotech* **2017**, *7* (4), 239.
- Parveen, S.; Anis, M.; Bhat, T. A.; Khan, A. H. (Efficiency and Effectiveness of Physical and Chemical Mutagens in M2 Generation of *Trigonella foenum-graceum* L. *J. Cytol. Genet.* **2006**, *7*, 1–4.
- Parveen, S.; Anis, M.; Bhat, T. A.; Khan, A. H. Cytological Effects of Physical and Chemical Mutagens in *Trigonella foenum-graecum* L. *Adv. Plant Sci.* **2007**, *20* (11), 633–636.
- Peace, C. P.; Bianco, L.; Troggio, M.; Van de Weg, E.; Howard, N. P.; Cornille, A.; Durel, C.-E.; Myles, S.; Migicovsky, Z.; Schaffer, R. J. Apple Whole Genome Sequences: Recent Advances and New Prospects. *Hort. Res.* **2019**, *6* (1), 1–24.
- Pires, T. C.; Dias, M. I.; Barros, L.; Alves, M. J.; Oliveira, M. B. P.; Santos-Buelga, C.; Ferreira, I. C. Antioxidant and Antimicrobial Properties of Dried Portuguese Apple Variety (*Malus Domestica* Borkh. Cv Bravo de Esmolfe). *Food Chem.* **2018**, *240*, 701–706.
- Prem, D.; Gupta, K.; Agnihotri, A. Doubled Haploids in Crop Brassicas: Retrospect and Prospects. *Plant Tissue Culture Mol. Markers* **2009**, 155.
- Ren, J.; Wu, P.; Trampe, B.; Tian, X.; Lübberstedt, T.; Chen, S. Novel Technologies in Doubled Haploid Line Development. *Plant Biotechnol. J.* **2017**, *15* (11), 1361–1370.
- Reynolds, S.; Ooms, N.; Gomand, S. V.; Delcour, J. A. What Makes Starch from Potato (*Solanum tuberosum* L.) Tubers Unique: A Review. *Compreh. Rev. Food Sci. Food Safet.* **2020**, *19* (5), 2588–2612.

- Savage, A. D.; King, J.; Gamborg, O. L. Recovery of a Pantothenate Auxotroph from a Cell Suspension Culture of *Datura Innoxia* Mill. *Plant Sci. Lett.* **1979**, *16* (4), 367–376.
- Saxena, P. K.; King, J. Herbicide Resistance in *Datura Innoxia*: Cross-Resistance of Sulfonylurea-Resistant Cell Lines to Imidazolinones. *Plant Physiol.* **1988**, *86* (3), 863–867.
- Scheller, E. L.; Krebsbach, P. H.; Kohn, D. H. Tissue Engineering: State of the Art in Oral Rehabilitation. *J. Oral Rehab.* **2009**, *36* (5), 368–389.
- Shah, A.; Smith, D. L. Flavonoids in Agriculture: Chemistry and Roles in, Biotic and Abiotic Stress Responses, and Microbial Associations. *Agronomy* **2020**, *10* (8), 1209.
- Sharma, M.; Khan, A. H.; Bhat, T. A. Assessment of Mutagenecity of Individual and Combination Treatment of Gamma Rays and MMS in Broad Bean (*Vicia faba* L). *Cytologia* **2009**, *74* (2), 235–241.
- Sharma, S.; Priya, A. K.; Yadav, M. K. *Development of Drought Tolerant Wheat Through Mutagenic and Plant Tissue Culture Tools*, 2019.
- Song, C.; Zhang, D.; Zheng, L.; Shen, Y.; Zuo, X.; Mao, J.; Meng, Y.; Wu, H.; Zhang, Y.; Liu, X. Genome-Wide Identification and Expression Profiling of the YUCCA Gene Family in *Malus Domestica*. *Sci. Rep.* **2020**, *10* (1), 1–12.
- Song, S.; Arora, S.; Laserna, A. K. C.; Shen, Y.; Thian, B. W.; Cheong, J. C.; Tan, J. K.; Chiam, Z.; Fong, S. L.; Ghosh, S. Biochar for Urban Agriculture: Impacts on Soil Chemical Characteristics and on Brassica Rapa Growth, Nutrient Content and Metabolism Over Multiple Growth Cycles. *Sci. Total Environ.* **2020**, *727*, 138742.
- Sopory, S. K.; Maheshwari, S. C. Similar Effects of Iron-Chelating Agents and Cytokinins on the Production of Haploid Embryos from the Pollen Grains of *Datura Innoxia*. *Zeitschrift Für Pflanzenphysiologie* **1973**, *69* (2), 97–99.
- Sopory, S. K.; Maheshwari, S. C. Development of Pollen Embryoids in Anther Cultures of *Datura innoxia*: II. Effects of Growth Hormones. *J. Exp. Bot.* **1976**, *27* (1), 58–68.
- Sopory, S. K.; Maheshwari, S. C. Morphogenetic Potentialities of Haploid and Diploid Vegetative Parts of *Datura Innoxia*. *Zeitschrift Für Pflanzenphysiologie* **1976**, *77* (3), 274–277.
- Srivastava, P.; Bains, N. S. Accelerated Wheat Breeding: Doubled Haploids and Rapid Generation Advance. In *Biotechnologies of Crop Improvement*, Vol. 1; Springer, 2018; pp 437–461.
- Sun, D.; Wang, C.; Zhang, X.; Zhang, W.; Jiang, H.; Yao, X.; Liu, L.; Wen, Z.; Niu, G.; Shan, X. Draft Genome Sequence of Cauliflower (*Brassica oleracea* L. var. *Botrytis*) Provides New Insights Into the C Genome in Brassica Species. *Hort. Res.* **2019**, *6* (1), 1–11.
- Supena, E. D. J.; Suharsono, S.; Jacobsen, E.; Custers, J. B. M. Successful Development of a Shed-Microspore Culture Protocol for Doubled Haploid Production in Indonesian Hot Pepper (*Capsicum annum* L.). *Plant Cell Rep.* **2006**, *25* (1), 1–10.
- Tai, G. C. Haploids in the Improvement of Solanaceous Species. In *Haploids in Crop Improvement II*; Springer, 2005; pp 173–190.
- Taylor, A.; Rana, K.; Handy, C.; Clarkson, J. P. Resistance to Sclerotinia Sclerotiorum in Wild Brassica Species and the Importance of Sclerotinia Subarctica as a Brassica Pathogen. *Plant Pathol.* **2018**, *67* (2), 433–444.
- Thambugala, D.; Brûlé-Babel, A. L.; Blackwell, B. A.; Fedak, G.; Foster, A. J.; MacEachern, D.; Gilbert, J.; Henriquez, M. A.; Martin, R. A.; McCallum, B. D. Genetic Analyses of Native Fusarium Head Blight Resistance in Two Spring Wheat Populations Identifies QTL Near the B1, Ppd-D1, Rht-1, Vrn-1, Fhb1, Fhb2, and Fhb5 loci. *Theor. Appl. Genet.* **2020**, *133* (10), 2775–2796.

- Thorpe, T. A. The Current Status of Plant Tissue Culture. In *Developments in Crop Science*, Vol. 19; Elsevier, 1990; pp 1–33.
- Tidiane Sall, A.; Chiari, T.; Legesse, W.; Seid-Ahmed, K.; Ortiz, R.; Van Ginkel, M.; Bassi, F. M. Durum Wheat (*Triticum durum* Desf.), Origin, Cultivation and Potential Expansion in Sub-Saharan Africa. *Agronomy* **2019**, *9* (5), 263.
- Tombuloglu, H.; Tombuloglu, G.; Slimani, Y.; Ercan, I.; Sozeri, H.; Baykal, A. Impact of Manganese Ferrite (MnFe₂O₄) Nanoparticles on Growth and Magnetic Character of Barley (*Hordeum vulgare* L.). *Environ. Pollut.* **2018**, *243*, 872–881.
- Ulukapi, K.; Nasircilar, A. G. Induced Mutation: Creating Genetic Diversity in Plants. In *Genetic Diversity in Plant Species-Characterization and Conservation*; IntechOpen, 2018.
- Usoltsev, V. A.; Shobairi, S. O. R.; Chasovskikh, V. P. Climate-Induced Gradients of *Populus* sp. Forest Biomass on the Territory of Eurasia. *J. Ecol. Eng.* **2018**, *19* (1), 206–212.
- Wairich, A.; de Oliveira, B. H. N.; Arend, E. B.; Duarte, G. L.; Ponte, L. R.; Sperotto, R. A.; Ricachenevsky, F. K.; Fett, J. P. The Combined Strategy for Iron Uptake Is Not Exclusive to Domesticated Rice (*Oryza sativa*). *Sci. Rep.* **2019**, *9* (1), 1–17.
- Wang, S.; Ye, X.; Wang, Y.; Chen, Y.; Lin, B.; Yi, Z.; Mao, Z.; Hu, F.; Zhao, R.; Wang, J. A New Type of Homodiploid Fish Derived from the Interspecific Hybridization of Female Common Carp *Cyprinus carpio* L. Male Blunt Snout Bream. *Sci. Rep.* **2017**, *7* (1), 1–12.
- Wani, A. A.; Bhat, T. A. Asynapsis and Desynapsis in Plants: In *Chromosome structure and aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Wani, N. A.; Bhat, T. A.; Khan, A. H.; Ansari, M. Y. K. Sodium Azide Induced Variability in Chickpea (*Cicer arietinum* L.). *Vegetos* **2005**, *18* (1&2), 105–107.
- Wu, Y.; Qu, M.; Pu, X.; Lin, J.; Shu, B. Distinct Microbial Communities Among Different Tissues of Citrus Tree *Citrus Reticulata* cv. Chachiensis. *Sci. Rep.* **2020**, *10* (1), 1–9.
- Yang, J.; Liu, D.; Wang, X.; Ji, C.; Cheng, F.; Liu, B.; Hu, Z.; Chen, S.; Pental, D.; Ju, Y. The Genome Sequence of Allopolyploid Brassica juncea and Analysis of Differential Homoeolog Gene Expression Influencing Selection. *Nat. Genet.* **2016**, *48* (10), 1225–1232.
- Yang, S.; Chen, S.; Zhang, K.; Li, L.; Yin, Y.; Gill, R. A.; Yan, G.; Meng, J.; Cowling, W. A.; Zhou, W. A High-Density Genetic Map of an Allohexaploid Brassica Doubled Haploid Population Reveals Quantitative Trait Loci for Pollen Viability and Fertility. *Front. Plant Sci.* **2018**, *9*, 1161.
- Zortea, M.; Macedo, M. M.; Mattos, A. B.; Ruga, B. C.; Gemignani, B. H. Automatic Citrus Tree Detection from UAV Images Based on Convolutional Neural Networks. *2018 31th SIBGRAPI Conference on Graphics, Patterns and Images (SIBGRAPI)* **2018**, 11.
- Zulkarnain, Z. The In Vitro Response of Soybean Anthers to Plant Growth Regulators. *Jurnal Agroteknologi Tropika* **2014**, *3* (2), 58–67.

CHAPTER 5

Double-Stranded DNA Break, Repair, and Associated Mutations

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ABSTRACT

DNA of living organism face threat from a wide range of factors, which can be natural (UV irradiation, gamma irradiation) or man made (pollution, chemicals from industries), causing DNA damage that remain unrepaired or repaired with error. These errors generate genetic alterations and in severe cases cause death. Double -tranded DNA break (DBS) is one of such severe damages in living cells. In this chapter, we will discuss the type of double-strand break (DSB), repair mechanisms, and mutations associated with DSB.

5.1 INTRODUCTION

Plants are continuous exposure to various factors in environment, which can cause DNA damage. Plants and animals both share the similar basic mechanism of their genome conservation and maintenance; plants developed an advanced mechanism to handle DNA damages and repair. Various factors are responsible for DNA damage, which include endogenous cellular reaction stress, cellular replication stress, and numerous exogenous bases for example irradiation and chemotherapeutics (Balestrazzi et al., 2016). DNA damage occurs in different forms such as cross-linkage between intrastrand and interstrand, DNA-protein crosslinks, base lesions as well as single-strand break (SSBs) and double-strand break (DSBs) (Lindahl, 1993; Dextraze et al., 2010; Cadet et al., 2012). Oxidative damage occurs in DNA bases and are repaired frequently as 10^5 lesions per cell each day. Many numerous factors that include exogenous agents like some particular chemicals and radiation, and endogenous processes are involved in DSBs (Whitaker, 1992; Barker et al., 2005; Cadet and Davies, 2017). However, Meiosis I induce deliberate DSBs that initiates homologous recombination (HR), which helps to confirm normal chromosome isolation (Murakami and Keeney, 2008; Sasaki et al., 2010). In addition to this, the DSBs in programmed formation arises during mating-type switching in yeast, in protozoans it results in developing of somatic nuclei (Ohta et al., 1999), receptor development in T-lymphocytes (Modesti and Kanaar, 2001), and switching of immunoglobulin class in B-lymphocytes (Cannan and Pederson, 2016).

5.1.1 CAUSES OF DSBs IN DNA

Various factors are responsible for causing DNA damage such as endogenous metabolic reactions and replication stress as well as some exogenous sources, which include radiation and chemotherapeutics (Chatterjee and Walker, 2017; Tubbs and Nussenzweig, 2017). Many lethal processes are responsible for DNA damage, which includes SSBs and DSBs (Noll et al., 2006). Various damages occur and are repaired usually 10^5 lesions per cell each day as in case of oxidative damage to DNA bases. For example, various *in vitro* studies demonstrated that base excision repair (BER) enzymes act on various sites on nucleosomes to repair oxidative lesions without changing the host nucleosomes (Hegde et al., 2008; Nemec et al., 2010) (Fig. 5.1). The complementary DNA strands possessing phosphate backbones undergo some breakage, which is considered most cytotoxic

forms of lesion (Tiwari and Wilson III 2019). Exogenous agents who damage DNA (clastogens) constitute anticancer chemotherapeutic drugs and ionizing radiation (IR) (Mavragani et al., 2017; Nickoloff, 2017). Drugs contain DNA-alkylating agents are grouped in chemotherapeutic drugs for example methyl methanosulfonate and temozolomide (Trivedi et al., 2005; Fu et al., 2012). In addition, there are topoisomerase inhibitors like camptothecin and etoposide, which are involved in forming of SSBs and DSBs, respectively. The mode of action of these inhibitors is to trap covalently linked topoisomerase-DNA cleavage complexes (Agnihotri et al., 2012, Xu and Her, 2015). Various drugs responsible for DNA polymerase inhibition are Hydroxyurea and aphidicolin and act in replication impairment process (Clynes et al., 2014; Vesela et al., 2017). IR results into base damage in an extensive way (Adams et al., 2006). Moreover, it leads to DNA SSBs *via* generation of radiolysis radicals, which then targets sugar-phosphate backbone. DSBs result from nicks of two complementary strands within one helical turn due to high irradiation dose (Reisz et al., 2014; Maynard et al., 2009). IR creates 10 SSBs for every DSB. IR results in the generation of “dirty ends,” which consists of phosphoglycolates and terminal nucleotides that cannot be ligated to “clean” ends which constitute 5'-phosphate and 3'-OH group, such as those created by endonucleases (Iyer et al., 2000; Pérez-Gracia et al., 2008).

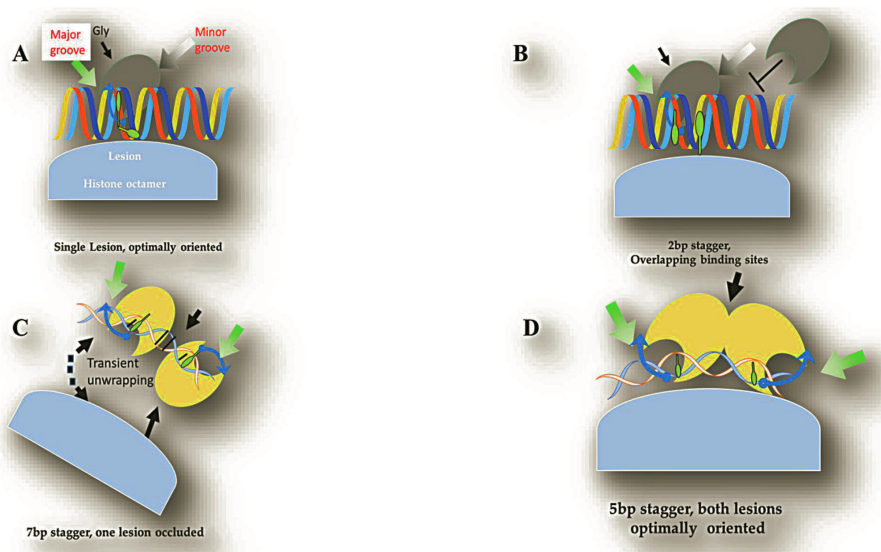


FIGURE 5.1 Nucleosomes suppress BER-mediated double-strand break formation.

DNA is vulnerable for damage during replication, even without exogenous stress-inducing factors (Achanta et al., 2005; Zeman and Cimprich, 2014). Various natural obstacles that cause pause or blockage of replication fork, for instance, unusual DNA and chromatin structures or collisions with transcription machinery or DNA-binding proteins (Rothstein et al., 2000; Mirkin and Mirkin, 2007). One of the ways for estimation of spontaneous DSBs is counting them in cells where DSB prevention has occurred (Leman and Noguchi, 2013; García-Muse and Aguilera, 2016). For example, in budding yeast to analyze the fate of single G_1 cell in which there is absent *RAD52* gene, which is implicated in DSB repair *via* HR (Pâques and Haber, 1999; Cortez, 2015). It implies that there is one spontaneous DSB per 10^8 bp, the genome size being $\sim 1.2 \times 10^7$ bp (Gómez-González and Aguilera, 2019). One of the study reports that $\sim 1\%$ of single-strand lesions in normal human cells are converted to ~ 50 DSBs per cell cycle, which implies that one DSB per 10^8 bp (Hyrien, 2000; Mehta and Haber, 2014). There is another way to calculate DSBs in a cell by monitoring the generation of impairment of foci by using various techniques like indirect staining of immunofluorescent or to perform *via* fluorescent proteins that are involved in insult DNA sites to act as DNA damage (McManus and Hendzel, 2005; Mehta and Haber, 2014).

The indicator of DSBs in vertebrate cells is considered to be γ -H2AX, which is variant of phosphorylation of minor histone H2A variant (Song et al., 2007; Firsanov et al., 2011). It is now established that γ -H2AX is related with other types of DNA damage (Novotna et al.; Bilsland and Downs, 2005; Mehta and Haber, 2014). Various DNA repair proteins such as 53BP1, replication protein A, and Rad51 foci also help to monitor DSBs. Similarly, in budding yeast, DSB damage was mostly live trapped through the involvement of Rad52-YFP into damage-induced foci (Friesner et al.; Lukaszewicz et al., 2010). It has been demonstrated that many DSBs occur in a Rad52 single focus has been revealed as evidence that aggregation of DSBs occurs into a repair center (Ishibashi et al., 2010; Noon and Goodarzi, 2011). Studies have shown that numerous DSBs present in relation with DNA replication can demonstrate that deoxyribonucleotide might be nicked before moving to replication fork, thus it creates a damaged chromatid (Alberts et al., 2002). Moreover, other replication fork arrives in opposite direction, an intact sister chromatid. Furthermore, these breaks might also occur *via* processing of stalled replication forks (Calzada et al., 2005; Atkinson and McGlynn, 2009). Studies have been carried out on stalled forks by proving hydroxyurea or introduction of sequences blocking replication fork, which impair replication (Mirkin and Mirkin, 2007; Scully et al., 2019). Stalled forks could

regress newly synthesized strands through unwinding and annealing process results “chicken foot” structure, commonly known as a Holliday junction (HJ) (Bugreev et al., 2011). Nucleases act on structure of selective or HJ resolvases like Mus81-Mms4 or Yen1, are involved in cleavage of these HJs (Machwe et al., 2007; Wyatt and West, 2014). The damaged end is processed then by 5′ to 3′ resection (Fig. 5.2). Moreover, after it binds with Rad51, involve in break-induced replication (BIR) (Machwe et al., 2006; Machwe et al., 2007).

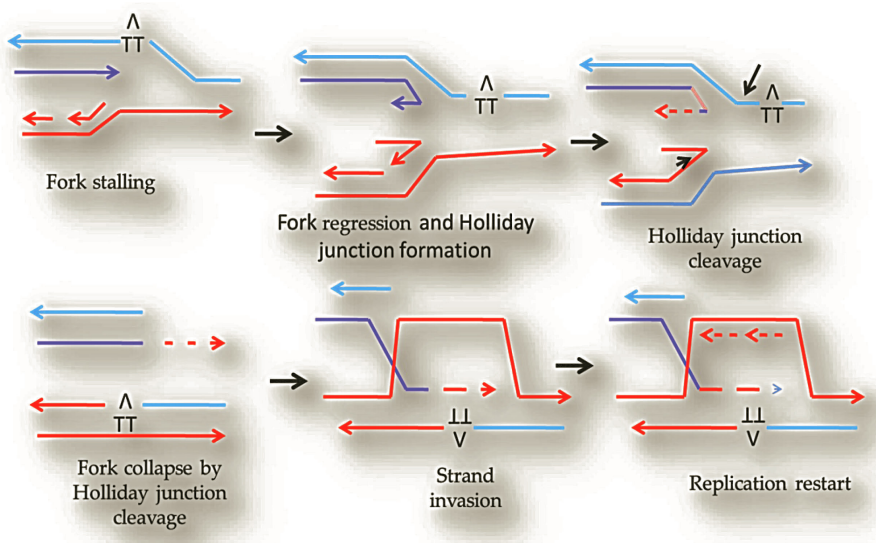


FIGURE 5.2 Replication fork stalling and restart.

5.2 DSBS REPAIR AND ASSOCIATED MUTATIONS

Nonhomologous end joining (NHEJ) and HR are the two mechanistic, well-defined pathways that have advanced to repair DSBs (Helleday et al., 2007; Chiruvella et al., 2013). The process of NHEJ requires ligation and modification of the fragmented DNA ends with almost no or a little homology, thereby creating small insertions or deletions (Pardo et al., 2009; Ceccaldi et al., 2016). However, the process of NHEJ may take place during cell cycle but it is critical in stage of G1 phase, where the important initiating HR process, resection of 5′ to 3′ DSB comes to an end, and is inhibited (Aylon et al., 2004; Wang et al., 2013). For example in budding yeast or a fission process and in particular case of haploid cells G1 cells does not

contain homologous chromosome, thereby making it to use only NHEJ till the replication of chromosome forms a sister chromatid, which could further be used act as template for repairing a DSB (Esashi et al., 2005; Huertas et al., 2008). It can also be seen that NHEJ consists of several related but different process system, which possess numerous genetic requirement and stretch of microhomology at the intersection (Yu et al., 2019). Accurate end joining of almost 30 overhanging ends (e.g., consigning the ends of the restriction endonuclease cleavage) needs Ku70 and Ku80 proteins and also DNA Ligase IV (Fig. 5.3). While on the contrary, many deletions with more long microhomologies at the intersection confirm to be dependent of Ku. It is independent in of mammals DNA Ligase IV in mammals. This alternative NHEJ or microhomology-mediated end joining (MMEJ), course track is apparent present in wild-type cells (Huefner 2012; Held et al., 2016).

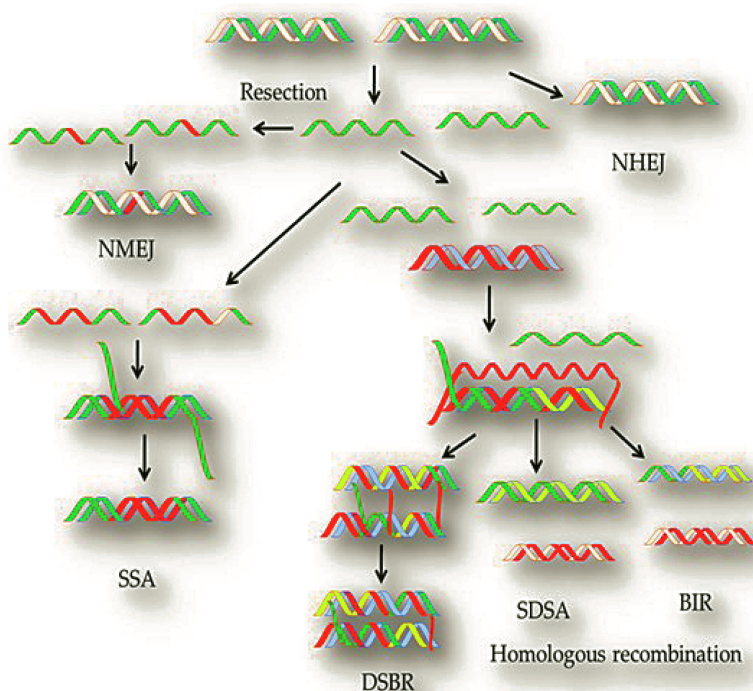


FIGURE 5.3 DNA double-strand breaks (DSBs) repair pathway.

Different mechanism execute to repair DSBs. Rejoined of two ends with little dispensation as NHEJ or restored through homologs sequences (DNA

with red color: HR) after degradation of 5'–3' take place (resection). After DNA strand invasion, a homologous region that can work as a template for prime DNA synthesis later resection and exposed of 3'-OH group. The 5' end of resected DNA can be ligated with the newly synthesized DNA, establish a double HJ or double-strand break repair, or might be moved and reconnected as synthesis-dependent strand annealing or it can continue up to the end of chromosome as BIR. DSB flanked by the two homologous regions, which can anneal by SSA (single-strand annealing) after being exposed by DNA resection, which results in deletion of intervening region. A supplementary mechanism that shares mechanisms with SSA and NHEJ, which use short homology stretches that are usually 2-3 bp long flanking the DSB can also be used as MMEJ.

5.2.1 HR MECHANISM

There are several different HR pathways by which DNA DSBs can be repaired (Pardo et al., 2009; Chapman et al., 2012). The common mechanism is SSA, which permits the deletion among the homologous sequence and hence flanking the DSB (Rothkamm et al., 2003). The other processes of HR depend on identification followed by pairing of homologous sequences of a broken DNA, which are found on the sister chromatid, and allelic locus or an ectopic location in the genome (Jackson 2002; Brandsma and van Gent, 2012). In addition to HR mechanism, the gene targeting could be responsible for the incorporation of foreign DNA in the locus of homologous chromosome. It can further be classified into two distinct pathways of GC, pairing of HJs that leads to crossover results in the repair, whereas in the other case the crossover is rare (Brandsma and van Gent, 2012). This mechanism of DNA repair need special proteins associated to RAD52 conserved epistasis group. It should be noted that RAD52 protein being the crucial recombination gene in fission of yeast, budding yeasts and reported to have shown insignificant role of HR in metazoans (McDonald et al., 1997; Symington 2002). Although it is lacking in flies and worms, however, mammals contain it where it plays an insignificant role in DNA repair as well as strand annealing (Milne and Weaver, 1993; Plate et al., 2008).

The ortholog present in mammals (BRCA2) protein plays major function of RAD52 (Wang et al., 2018; Jalan et al., 2019). It can be seen that the budding or fission yeasts could have displaced their BRCA2 ortholog due to presence of *Ustilago maydis* confined in it and depends on it for HR (Orth

and Sisler, 1990; Wedlich-Soldner et al., 2002). RAD51 recombinase that is DNA-dependent ATPase, is homolog of bacterial RecA is required for GC as well as BIR. It makes a thread-like structure on ssDNA, and facilitates the incursion of single strand of a double-stranded homologous (Hartmann et al., 1999). The major crucial steps in which repair mechanisms act on processing of 5'–3' broken DNA ends, on the contrary NHEJ and MMEJ are implicated in ligation of DNA ends, which is an essential requirement for HR broken ends, cut out to create ssDNA tail with 3' end bounded using RAD51 recombinase develop functionally active agents for searching a homologous templates sequence for healing the DSBs (Muris et al., 1994).

5.2.1.1 HR MECHANISM IN HIGHER PLANTS

In plants, DSB of a chromosome rarely finds its homolog to trigger repair synthesis but Arabidopsis possesses 5 Rad51 paralog genes as found in mammals (Iida and Terada, 2005). Rad51 play important role in gathering and stabilization of Rad51 nucleoprotein filament in place of Rad52 (Abe et al., 2005; Sugiyama et al., 2005; Singh et al., 2010). AtSPO11-1, AtSPO11-2 and AtSPO11-3 are the three SPO11 like genes found in rice and arabidopsis, out of these AtSPO11-1, and AtSPO11-2 advocate a plant-specific metabolism for DSB initiation in meiosis (Terada et al., 2007). Many recent studies suggest no link between HR repair mechanisms responsible for programmed cell cycle of shoot apical meristem of higher plants (Lutz et al., 2007; Cardi and Stewart, 2016). Further studies suggest that to maintain cell cycle control during S and G2, HR repair is important in plant with mutation deficiency of NHEJ (Bennetzen et al., 2005; Sinagawa-García et al., 2009).

5.2.2 SSA REPAIR

SSA repair DNA breaks, which are flanked by the direct repeats of about 30 nt (Sugawara et al., 2000). Homologous sequences of complementary strands exposes by resection, leads to deletion of single copy of repeated sequence of recombine strand (Ivanov and Haber, 1995). Therefore, SSA is known to be a highly lethal mutagenic (Pâques and Haber, 1999; Haber, 2000). The endonuclease Rad (1-10) in connotation with Msh (2-3), the mismatch proteins repair and “scaffold” proteins removed the non-homologous single-stranded tails (Toh et al., 2010; Eichmiller et al., 2018). DNA synthesis must be filled in by the remaining gaps followed by ligation after tail clipping. Rad52

contains strand-annealing activity that is required for SSA and is provided by the Rad52 homolog Rad59 (Eichmiller, 2015; Mehta et al., 2017). However, SSA lacks strand invasion of DNA and thereby self-governing of the Rad51 recombinase (Seol et al., 2018).

5.2.3 GC

The reciprocal genetic information transmission in meiosis from one chromosome to another homolog chromosome was primarily defined for GC; however, the sense has been widened including the events associated with DSB repair processes, a mechanism where homologous template acts for the synthesis of new DNA. Approximately 10–20% of the GCs of interchromosomal alleles in budding yeast are correlated with crossing over (Ira et al., 2004). The major constituents of DNA replication machinery does not need for GC, which include DNA Pol α -primase or a complex made up of proteins like Sld5, Psf (1-3), and Cdc45-GINS helicase complex (Nedelcheva et al., 2005) (Wang et al., 2018). The mutations that has impact on impairment of HR repair mechanism of BIR does not significantly reduce the efficiency of GC and can include the production of hundreds kbs of, the 5'–3' helicase pif1D se pif1D (Schulz and Zakian, 1995; Wilson et al., 2013), and PCNA (Maga and Hübscher, 2003) mutation pol30-FF248, (Kurki et al., 1986) 249AA. The fact that there is no need of major components of DNA replication processivity factors may reflect its sensitivity to mutagenesis. The indication of template switching having in most mutations during repairing of DNA synthesis (Hicks et al., 2010). To explain the GCs and different outcomes, two different mechanisms is supported by the substantial experimental data.

5.3 THE DOUBLE HJ MECHANISM

Resnick (1976), described the dHJ model as DSB repair model (Hollingsworth and Brill, 2004). In intact homologous template, resection followed by invasion, displacement, or D loop structure, which consists of heterologous duplex type DNA together with emigrant ssDNA, is formed via base pairing with the Rad51-coated nucleoprotein filament (Dupaigne et al., 2008). The new DNA synthesis initiates at 3' end of the overrunning strand or the act of helicases can be extended by the D-loop so, DSB possessing ssDNA of opposite side can anneal result in formation of dHJ intermediate

(Iyama and Wilson III, 2013). On the other hand, from both ends of DSB result two independent strand invasions, which is then monitored using DNA synthesis with annealing, that could result in a dHJ transitional model. However, there is lack of experimental data that have proven dependence of recombination on one end or both strands go through invasion ends. Out of number of HJ resolvases cleaved these HJs, and it depends on which pair of strand cleaved, can produce NCO (noncrossover) or CO product. Therefore, as a supernumerary cleavage, dHJs can be “dissolved” to generate exclusive NCO products (Wu and Hickson, 2003). In budding yeast meiosis, dHJs formation has been extensively studied, but also in mitotic cells, they have been envisioned after initiation of DSB using expression of a site-specific (I-SceI) endonuclease enzyme (Bzymek et al., 2010). From ectopic recombination, experiments provide proofs for the occurrence of dHJ intermediates found in yeast cells with budding as the constituents of the dHJ “dissolvase” are deleted. The GC measures carried by a CO as a reverse translocation that increased from 4–12%, (Mundia, 2016) when other factors or Sgs1 are absent. When Sgs1 and related another 3′–5′ helicase, and Mph1 are removed, it results even more increase in proportion of Cos. For example, it looks as deleting MPH1 moves most of the repair procedures into dHJ pathway (Fotheringham and Holloman, 1989; Wedlich-Soldner et al., 2002). Mitchel et al. (2013) studied the position of heterozygous DNA duplex relative with initiating gap found in a gap-based repair system based on plasmid to approximation the fraction of dHJs managed by STR complex. Studies suggested that dHJs fractions managed by “resolution” are approximately equal to that managed by “dissolution” (Šebesta, 2013).

In various evolutionary processes such as ploidy, changing of mating systems, y chromosomes, and elimination of species the process of genomic mutation plays a crucial role (Johnson and Lachance, 2012). They may also cause diseases and this can be broadly being studied for scientific interests to understand the basis that impact the rate of mutation. At this immediate moment, it still remains difficult to track down. Detection of the connection among the number of germ cell divisions (DNA Replication) and the rate of mutation occurring in human and the other organisms suggest that most of the germline mutations are the errors in replication (Castillo and Moyle, 2012).

Precisely, human nucleotide substitution rate of especially the neutral DNA (equalling the mutation rate) and/or the statistics of human epidemiology suggest that larger rate of mutations take place in male germ line than in a female germ line within taxonomy of animals (mice, humans, chicken,

and sheep) and in species of older hierarchy than younger males (humans), patterns that go with hypothesis of cell division. In other words, additionally, DNA replication in males especially in older male hierarchy (Wolf et al., 2014).

Other statistics show that the mutation bias outlined relatively to a gender and age of male do not correlate with the number of germ cell division and the other component can interpret these trends, such as, patterns in methylation, metabolic rate. Transmission of superior mutations to offspring from adults, differential repair (Arnheim and Calabrese, 2009). In point of fact, most of the information present regarding the element that forms the fundamental basis of spontaneous mutation rate is been bounded to the detection of the existence or absence of correlations among the number of the germ cell division and their mutation rate. Therefore, it is obvious that, additionally, empirical data is needed, about the independent replication factors such as age of the cell, the rate of mutation and environmental parameters. The insufficiency and mutations occurring in a repair pathway in the DNA are linked with the process of ageing and various human diseases. The DSBs can be damaging as well as helpful to an organism. It should be noted that molecular features which connect DSB repair to different cellular processes are still evolving. The concept of the role of chromatin structure in HR is yet to adequately inspect. (Tiwari and Wilson III, 2019). Most of our comprehension of HR derives from work in yeast, bacteria, and other system of models. Any insufficiency in HR aims the cell across further to pathways that are prone to error such as NHEJ. It has also been shown in recent studies that HR is itself error prone and can also be a cause of mutagenesis (Rodgers and McVey, 2016). Therefore, the HR is considered to be a double-edged sword whose precise directive is essential. HR elements are quarry to become known to therapies especially in treating cancers. Therefore, further elucidation of mechanism of HR repair, chiefly in mammals is vital (Kelley et al., 2014).

5.4 CONCLUSION

DSB repair mechanisms have many similarities and dissimilarities between higher plants and vertebrates. Arabidopsis has a repaired deficient variable DSB mutant. The process of selection for cell death or repair is different in plants than vertebrates. Some of these mechanisms like HR are discussed in this chapter.

KEYWORDS

- **DSB**
- **NHEJ pathway**
- **double Holliday junction**
- **break-induced replication**

REFERENCES

- Abe, K.; Osakabe, K.; Nakayama, S.; Endo, M.; Tagiri, A.; Todoriki, S.; Ichikawa, H.; Toki, S. Arabidopsis RAD51C Gene Is Important for Homologous Recombination in Meiosis and Mitosis. *Plant Physiol.* **2005**, *139* (2), 896–908.
- Achanta, G.; Sasaki, R.; Feng, L.; Carew, S. J.; Lu, W.; Pelicano, H.; Keating, J. M.; Huang, P. Novel Role of p53 in Maintaining Mitochondrial Genetic Stability Through Interaction with DNA Pol γ . *EMBO J.* **2005**, *24* (19), 3482–3492.
- Adams, K.; Medhurst, A.; Dart, A. D.; Lakin, N. Recruitment of ATR to Sites of Ionising Radiation-Induced DNA Damage Requires ATM and Components of the MRN Protein Complex. *Oncogene* **2006**, *25* (28), 3894–3904.
- Agnihotri, S.; Gajadhar, S. A.; Ternamian, C.; Gorlia, T.; Diefes, L. K.; Mischel, S. P.; Kelly, J.; McGown, G.; Thorncroft, N.; Carlson, L. B. Alkylpurine–DNA–N-Glycosylase Confers Resistance to Temozolomide in Xenograft Models of Glioblastoma Multiforme and Is Associated with Poor Survival in Patients. *J. Clin. Invest.* **2012**, *122* (1), 253–266.
- Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. Integrins. *Molecular Biology of the Cell.*; 4th ed.; Garland Science, 2002.
- Arnheim, N.; Calabrese, P. Understanding What Determines the Frequency and Pattern of Human Germline Mutations. *Nat. Rev. Genet.* **2009**, *10* (7), 478–488.
- Atkinson, J.; P. McGlynn Replication Fork Reversal and the Maintenance of Genome Stability. *Nucl. Acids Res.* **2009**, *37* (11), 3475–3492.
- Aylon, Y.; Liefshitz, B.; Kupiec, M. The CDK Regulates Repair of Double-Strand Breaks by Homologous Recombination During the Cell Cycle. *EMBO J.* **2004**, *23* (24), 4868–4875.
- Balestrazzi, A.; Achary, V.; Macovei, A.; Yoshiyama, O. K.; Sakamoto, N. A. Maintenance of Genome Integrity: DNA Damage Sensing, Signaling, Repair, and Replication in Plants. *Front. Plant Sci.* **2016**, *7*, 64.
- Barker, S.; Weinfeld, M.; Murray, D. DNA–Protein Crosslinks: Their Induction, Repair, and Biological Consequences. *Mutation Res./Rev. Mutat. Res.* **2005**, *589* (2), 111–135.
- Bennetzen, J. L.; Ma, J.; Devos, M. K. Mechanisms of Recent Genome Size Variation in Flowering Plants. *Ann. Bot.* **2005**, *95* (1), 127–132.
- Bilsland, E.; Downs, A. J. Tails of Histones in DNA Double-Strand Break Repair. *Mutagenesis* **2005**, *20* (3), 153–163.
- Brandsma, I.; van, C. D. Gent Pathway Choice in DNA Double Strand Break Repair: Observations of a Balancing Act. *Genome Integrity* **2012**, *3* (1), 9.

- Bugreev, D. V.; Rossi, J. M.; Mazin, V. A. Cooperation of RAD51 and RAD54 in Regression of a Model Replication Fork. *Nucl. Acids Res.* **2011**, *39* (6), 2153–2164.
- Cadet, J.; Davies, J. K. Oxidative DNA Damage & Repair: An Introduction. *Free Radical Biol. Med.* **2017**, *107*, 2–12.
- Cadet, J.; Ravanat, J.-L.; TavernaPorro, M.; Menoni, H.; Angelov, D. Oxidatively Generated Complex DNA Damage: Tandem and Clustered Lesions. *Cancer Lett.* **2012**, *327* (1–2), 5–15.
- Calzada, A.; Hodgson, B.; Kanemaki, M.; Bueno, A.; Labib, K. Molecular Anatomy and Regulation of a Stable Replisome at a Paused Eukaryotic DNA Replication Fork. *Genes Dev.* **2005**, *19* (16), 1905–1919.
- Cannan, and, J. W. Pederson, S. D. Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin. *J. Cell. Physiol.* **2016**, *231* (1), 3–14.
- Cardi, T.; Stewart, N. C. Progress of Targeted Genome Modification Approaches in Higher Plants. *Plant Cell Rep.* **2016**, *35* (7), 1401–1416.
- Castillo, and, M. D. Moyle, C. L. Evolutionary Implications of Mechanistic Models of TE-Mediated Hybrid Incompatibility. *Int. J. Evol. Biol.* **2012**.
- Ceccaldi, R.; Rondinelli, B.; Andrea, D. A. Repair Pathway Choices and Consequences at the Double-Strand Break. *Trends Cell Biol.* **2016**, *26* (1), 52–64.
- Chapman, J. R.; Taylor, R. M.; Boulton, J. S. Playing the End Game: DNA Double-Strand Break Repair Pathway Choice. *Mol. Cell* **2012**, *47* (4), 497–510.
- Chatterjee, N.; Walker, C. G. Mechanisms of DNA damage, repair, and mutagenesis. *Environ. Mol. Mutagen.* **2017**, *58* (5), 235–263.
- Chiruvella, K. K.; Liang, Z.; Wilson, E. T. Repair of Double-Strand Breaks by End Joining. *Cold Spring Harbor Perspectives Biol.* **2013**, *5* (5), a012757.
- Clynes, D.; Jelinska, C.; Xella, B.; Ayyub, H.; Taylor, S.; Mitson, M.; Bachrati, Z. C.; Higgs, R. D.; Gibbons, J. R. ATRX Dysfunction Induces Replication Defects in Primary Mouse Cells. *PLoS One* **2014**, *9* (3), e92915.
- Cortez, D. Preventing Replication Fork Collapse to Maintain Genome Integrity. *DNA Repair* **2015**, *32*, 149–157.
- Dextraze, M.-E.; Gantchev, T.; Girouard, S.; Hunting, D. DNA Interstrand Cross-Links Induced by Ionizing Radiation: An Unsung Lesion. *Mutat. Res./Rev. Mutat. Res.* **2010**, *704* (1–3), 101–107.
- Dupaigne, P.; Le Breton, C.; Fabre, F.; Gangloff, S.; Le Cam, E.; Veaute, X. The Srs2 Helicase Activity Is Stimulated by Rad51 Filaments on dsDNA: Implications for Crossover Incidence During Mitotic Recombination. *Mol. Cell* **2008**, *29* (2), 243–254.
- Eichmiller, R. *Coordination of Rad1-Rad10 with Msh2-Msh3 and Saw1 in the Initiation of 3'non-Homologous Tail Removal*, State University of New York at Buffalo, 2015.
- Eichmiller, R.; Medina-Rivera, M.; DeSanto, R.; Minca, E.; Kim, C.; Holland, C.; Seol, J.-H.; Schmit, M.; Oramus, D.; Smith, J. Coordination of Rad1–Rad10 Interactions with Msh2–Msh3, Saw1 and RPA Is Essential for Functional 3' Non-Homologous Tail Removal. *Nucl. Acids Res.* **2018**, *46* (10), 5075–5096.
- Esashi, F.; Christ, N.; Gannon, J.; Liu, Y.; Hunt, T.; Jasin, M.; West, C. S. CDK-Dependent Phosphorylation of BRCA2 as a Regulatory Mechanism for Recombinational Repair. *Nature* **2005**, *434* (7033), 598–604.
- Firsanov, D. V.; Solovjeva, V. L.; Svetlova, P. M. H2AX Phosphorylation at the Sites of DNA Double-Strand Breaks in Cultivated Mammalian Cells and Tissues. *Clin. Epigenet.* **2011**, *2* (2), 283–297.

- Fotheringham, S.; Holloman, K. W. Cloning and Disruption of *Ustilago Maydis* Genes. *Mol. Cell. Biol.* **1989**, *9* (9), 4052–4055.
- Friesner, J. D.; Liu, B.; Culligan, K.; Britt, B. A. Ionizing Radiation-Dependent γ -H2AX Focus Formation Requires ATM and ATR.
- Fu, D.; Calvo, A. J.; Samson, D. L. Balancing Repair and Tolerance of DNA Damage Caused by Alkylating Agents. *Nat. Rev. Cancer* **2012**, *12* (2), 104–120.
- García-Muse, T.; Aguilera, A. Transcription–Replication Conflicts: How They Occur and How They Are Resolved. *Nat. Rev. Mol. Cell Biol.* **2016**, *17* (9), 553.
- Gómez-González, B.; Aguilera, A. Transcription-Mediated Replication Hindrance: A Major Driver of Genome Instability. *Genes Dev.* **2019**, *33* (15–16), 1008–1026.
- Haber, J. E. Partners and Pathways: Repairing a Double-Strand Break. *Trends Genet.* **2000**, *16* (6), 259–264.
- Hartmann, H. A.; Krüger, J.; Lottspeich, F.; Kahmann, R. Environmental Signals Controlling Sexual Development of the Corn Smut Fungus *Ustilago Maydis* Through The Transcriptional Regulator Prf1. *Plant Cell* **1999**, *11* (7), 1293–1305.
- Hegde, M. L.; Hazra, K. T.; Mitra, S. Early Steps in the DNA Base Excision/Single-Strand Interruption Repair Pathway in Mammalian Cells. *Cell Res.* **2008**, *18* (1), 27–47.
- Held, K. D.; Kawamura, H.; Kaminuma, T.; Paz, S. E. A.; Yoshida, Y.; Liu, Q.; Willers, H.; Takahashi, A. Effects of Charged Particles on Human Tumor Cells. *Front. Oncol.* **2016**, *6*: 23.
- Helleday, T.; Lo van, J.; Gent, C. D.; Engelward, P. B. DNA Double-Strand Break Repair: From Mechanistic Understanding to Cancer Treatment. *DNA Repair* **2007**, *6* (7), 923–935.
- Hicks, W. M.; Kim, M.; Haber, E. J. Increased Mutagenesis and Unique Mutation Signature Associated with Mitotic Gene Conversion. *Science* **2010**, *329* (5987), 82–85.
- Hollingsworth, and, M. N. Brill, J. S. The Mus81 Solution to Resolution: Generating Meiotic Crossovers Without Holliday Junctions. *Genes Dev.* **2004**, *18* (2), 117–125.
- Huefner, N. D. *Recognition and Repair of DNA Double-Strand Breaks in the Model Plant Arabidopsis thaliana*; University of California, Davis, 2012.
- Huertas, P.; Cortés-Ledesma, F.; Sartori, A. A.; Aguilera, A.; Jackson, P. S. CDK Targets Sae2 to Control DNA-End Resection and Homologous Recombination. *Nature* **2008**, *455* (7213), 689–692.
- Hyrien, O. Mechanisms and Consequences of Replication Fork Arrest. *Biochimie* **2000**, *82* (1), 5–17.
- Iida, S.; Terada, R. Modification of Endogenous Natural Genes by Gene Targeting in Rice and Other Higher Plants. *Plant Mol. Biol.* **2005**, *59* (1), 205–219.
- Ira, G.; Pelliccioli, A.; Balijja, A.; Wang, X.; Fiorani, S.; Carotenuto, W.; Liberi, G.; Bressan, D.; Wan, L.; Hollingsworth, M. N. DNA End Resection, Homologous Recombination and DNA Damage Checkpoint Activation Require CDK1. *Nature* **2004**, *431* (7011), 1011–1017.
- Ishibashi, T.; Li, A.; Ausió, J. Histone Variants: Signaling or Structural Modules? *Handbook of Cell Signaling*; Elsevier, 2010; pp 2409–2425.
- Ivanov, L. E.; Haber, E. J. RAD1 and RAD10, But Not Other Excision Repair Genes, Are Required for Double-Strand Break-Induced Recombination in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **1995**, *15* (4), 2245–2251.
- Iyama, T.; Wilson, M. D. III DNA Repair Mechanisms in Dividing and Non-Dividing Cells. *DNA Repair* **2013**, *12* (8), 620–636.
- Iyer, R. R.; Pluciennik, A.; Rosche, A. W.; Sinden, R. R.; Wells, D. R. DNA Polymerase III Proofreading Mutants Enhance the Expansion and Deletion of Triplet Repeat Sequences in *Escherichia coli*. *J. Biol. Chem.* **2000**, *275* (3), 2174–2184.

- Jackson, S. P. Sensing and Repairing DNA Double-Strand Breaks. *Carcinogenesis* **2002**, *23* (5), 687–696.
- Jalan, M.; Olsen, S. K.; Powell, N. S. Emerging Roles of RAD52 in Genome Maintenance. *Cancers* **2019**, *11* (7), 1038.
- Johnson, A. N.; Lachance, J. The Genetics of Sex Chromosomes: Evolution and Implications for Hybrid Incompatibility. *Ann. NY Acad. Sci.* **2012**, *1256*, E1.
- Kelley, M. R.; Logsdon, D.; Fishel, L. M. Targeting DNA Repair Pathways for Cancer Treatment: What's New? *Future Oncol.* **2014**, *10* (7), 1215–1237.
- Kurki, P.; Vanderlaan, M.; Dolbeare, F.; Gray, J.; Tan, E. Expression of Proliferating Cell Nuclear Antigen (PCNA)/Cyclin During the Cell Cycle. *Exp. Cell Res.* **1986**, *166* (1), 209–219.
- Leman, R. A.; Noguchi, E. The Replication Fork: Understanding the Eukaryotic Replication Machinery and the Challenges to Genome Duplication. *Genes* **2013**, *4* (1), 1–32.
- Lindahl, T. Instability and Decay of the Primary Structure of DNA. *Nature* **1993**, *362* (6422), 709–715.
- Lukasiewicz, A.; Howard-Till, A. R.; Novatchkova, M.; Mochizuki, K.; Loidl, J. MRE11 and COM1/SAE2 are Required for Double-Strand Break Repair and Efficient Chromosome Pairing During Meiosis of the Protist Tetrahymena. *Chromosoma* **2010**, *119* (5), 505–518.
- Lutz, K. A.; Azhagiri, K. A.; Tungschat-Huang, T.; Maliga, P. A Guide to Choosing Vectors for Transformation of the Plastid Genome of Higher Plants. *Plant Physiol.* **2007**, *145* (4), 1201–1210.
- Machwe, A.; Xiao, L.; Groden, J.; Orren, K. D. The Werner and Bloom Syndrome Proteins Catalyze Regression of a Model Replication Fork. *Biochemistry* **2006**, *45* (47), 13939–13946.
- Machwe, A.; Xiao, L.; Lloyd, G. R.; Bolt, E.; Orren, K. D. Replication Fork Regression In Vitro by the Werner Syndrome Protein (WRN), Holliday Junction Formation, the Effect of Leading Arm Structure and a Potential Role for WRN Exonuclease Activity. *Nucleic Acids Res.* **2007**, *35* (17), 5729–5747.
- Maga, G.; U. Hübscher Proliferating Cell Nuclear Antigen (PCNA), a Dancer with Many Partners. *J. Cell Sci.* **2003**, *116* (15), 3051–3060.
- Mavragani, I. V.; Nikitaki, Z.; Souli, P. M.; Aziz, Nowsheen, S.; Aziz, K.; Rogakou, E.; Georgakilas, G. A. Complex DNA Damage: A Route to Radiation-Induced Genomic Instability and Carcinogenesis. *Cancers* **2017**, *9* (7), 91.
- Maynard, S.; Schurman, H. S.; Harboe, C.; de Souza-Pinto, C. N.; Bohr, A. V. Base Excision Repair of Oxidative DNA Damage and Association with Cancer and Aging. *Carcinogenesis* **2009**, *30* (1), 2–10.
- McDonald, J. P.; Levine, S. A.; Woodgate, R. The *Saccharomyces Cerevisiae* RAD30 Gene, a Homologue of *Escherichia coli* dinB and umuC, Is DNA Damage Inducible and Functions in a Novel Error-Free Postreplication Repair Mechanism. *Genetics* **1997**, *147* (4), 1557–1568.
- McManus, and, J. K. Hendzel, J. M. ATM-Dependent DNA Damage-Independent Mitotic Phosphorylation of H2AX in Normally Growing Mammalian Cells. *Mol. Biol. Cell* **2005**, *16* (10), 5013–5025.
- Mehta, A.; Beach, A.; Haber, E. J. Homology Requirements and Competition Between Gene Conversion and Break-Induced Replication During Double-Strand Break Repair. *Mol. Cell* **2017**, *65* (3), 515–526. e513.
- Mehta, A.; Haber, E. J. Sources of DNA Double-Strand Breaks and Models of Recombinational DNA Repair. *Cold Spring Harbor Perspect. Biol.* **2014**, *6* (9), a016428.

- Milne, and, T. G. Weaver, T. D. Dominant Negative Alleles of RAD52 Reveal a DNA Repair/ Recombination Complex Including Rad51 and Rad52. *Genes Dev.* **1993**, 7 (9), 1755–1765.
- Mirkin, and, V. E. Mirkin, M. S. Replication Fork Stalling at Natural Impediments. *Microbiol. Mol. Biol. Rev.* **2007**, 71 (1), 13–35.
- Modesti, M.; Kanaar, R. DNA Repair: Spot (Light) s on Chromatin. *Curr. Biol.* **2001**, 11 (6), R229-R232.
- Mundia, M. *Analysis of the Genetic Requirements for Rad51-Mediated 3'Polymerization During DNA Repair by Homologous Recombination*, 2016.
- Murakami, H.; Keeney, S. Regulating the Formation of DNA Double-Strand Breaks in Meiosis. *Genes Dev.* **2008**, 22 (3), 286–292.
- Muris, D. F.; Bezzubova, O.; Buerstedde, J.-M.; Vreeken, K.; Balajee, A.; Osgood, J. C.; Troelstra, C.; Hoeijmakers, H. J.; Ostermann, K.; Schmidt, H. Cloning of Human and Mouse Genes Homologous to RAD52, a Yeast Gene Involved in DNA Repair and Recombination. *Mutat. Res./DNA Repair* **1994**, 315 (3), 295–305.
- Nedelcheva, M. N.; Roguev, A.; Dolapchiev, B. L.; Shevchenko, A.; Taskov, B. H.; Shevchenko, A.; Stewart, F. A.; Stoyanov, S. S. Uncoupling of Unwinding from DNA Synthesis Implies Regulation of MCM Helicase by Tof1/Mrc1/Csm3 Checkpoint Complex. *J. Mol. Biol.* **2005**, 347 (3), 509–521.
- Nemec, A. A.; Wallace, S. S.; Sweasy, B. J. *Variant Base Excision Repair Proteins: Contributors to Genomic Instability*; Seminars in Cancer Biology, Elsevier, 2010.
- Nickoloff, J. A. Paths from DNA Damage and Signaling to Genome Rearrangements via Homologous Recombination. *Mutat. Res./Fundamental Mol. Mech. Mutagen.* **2017**, 806, 64–74.
- Noll, D. M.; Mason, M. T.; Miller, S. P. Formation and Repair of Interstrand Cross-Links in DNA. *Chem. Rev.* **2006**, 106 (2), 277–301.
- Noon, T. A.; Goodarzi, A. A. 53BP1-Mediated DNA Double Strand Break Repair: Insert Bad Pun Here. *DNA Repair* **2011**, 10 (10), 1071–1076.
- Novotna, E.; Tichy, A.; Foltanova, K.; Vavrova, J. Role of γ -H2AX in DNA-Damage Response and Its Possible Clinical Applications.
- Ohta, K.; Wu, T.-C.; Lichten, M.; Shibata, T. Competitive Inactivation of a Double-Strand DNA Break Site Involves Parallel Suppression of Meiosis-Induced Changes in Chromatin Configuration. *Nucl. Acids Res.* **1999**, 27 (10), 2175–2180.
- Orth, B. A.; Sisler, D. H. Mode of Action of Terbinafine in *Ustilago Maydis* and Characterization of Resistant Mutants. *Pesticide Biochem. Physiol.* **1990**, 37 (1), 53–63.
- Pâques, F.; Haber, E. J. Multiple Pathways of Recombination Induced by Double-Strand Breaks in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **1999**, 63 (2), 349–404.
- Pardo, B.; Gómez-González, G.; Aguilera, A. DNA Repair in Mammalian Cells. *Cell. Mol. Life Sci.* **2009**, 66 (6), 1039–1056.
- Pérez-Gracia, V.; García, G. F.; Abad, R. I. GPR Evaluation of the Damage Found in the Reinforced Concrete Base of a Block of Flats: A Case Study. *NDT e Int.* **2008**, 41 (5), 341–353.
- Plate, I.; Hallwyl, C. S.; Shi, I.; Krejci, L.; Müller, C.; Albertsen, L.; Sung, P.; Mortensen, H. U. Interaction with RPA Is Necessary for Rad52 Repair Center Formation and for Its Mediator Activity. *J. Biol. Chem.* **2008**, 283 (43), 29077–29085.
- Reisz, J. A.; Bansal, N.; Qian, J.; Zhao, W.; Furdai, M. C. Effects of Ionizing Radiation on Biological Molecules—Mechanisms of Damage and Emerging Methods of Detection. *Antioxidants Redox Signal.* **2014**, 21 (2), 260–292.

- Rodgers, K.; McVey, M. Error-Prone Repair of DNA Double-Strand Breaks. *J. Cell. Physiol.* **2016**, *231* (1), 15–24.
- Rothkamm, K.; Krüger, I.; Thompson, H. L.; Löbrich, M. Pathways of DNA Double-Strand Break Repair During the Mammalian Cell Cycle. *Mol. Cell. Biol.* **2003**, *23* (16), 5706–5715.
- Rothstein, R.; Michel, B.; Gangloff, S. Replication Fork Pausing and Recombination or “Gimme a Break”. *Genes Dev.* **2000**, *14* (1), 1–10.
- Sasaki, M.; Lange, J.; Keeney, S. Genome Destabilization by Homologous Recombination in the Germ Line. *Nat. Rev. Mol. Cell Biol.* **2010**, *11* (3), 182–195.
- Schulz, P. V.; Zakian, A. V. Modulation of PIF-1-Type Helicases, Google Patents, 1995.
- Scully, R.; Panday, A.; Elango, R.; Willis, A. N. DNA Double-Strand Break Repair-Pathway Choice in Somatic Mammalian Cells. *Nat. Rev. Mol. Cell Biol.* **2019**, *20* (11), 698–714.
- Šebesta, M. Reconstitution of Recombination-Associated DNA Synthesis and Its Regulation, 2013.
- Seol, J.-H.; Holland, C.; Li, X.; Kim, C.; Li, F.; Medina-Rivera, M.; Eichmiller, R.; Gallardo, F. I.; Finkelstein, J. I.; Hasty, P. Distinct Roles of XPF-ERCC1 and Rad1-Rad10-Saw1 in Replication-Coupled and Uncoupled Inter-Strand Crosslink Repair. *Nat. Commun.* **2018**, *9* (1), 1–13.
- Sinagawa-García, S. R.; Tungsuchat-Huang, T.; Paredes-López, O.; Maliga, P. Next Generation Synthetic Vectors for Transformation of the Plastid Genome of Higher Plants. *Plant Mol. Biol.* **2009**, *70* (5), 487–498.
- Singh, S. K.; Roy, S.; Choudhury, R. S.; Sengupta, N. D. DNA Repair and Recombination in Higher Plants: Insights from Comparative Genomics of Arabidopsis and Rice. *BMC Genom.* **2010**, *11* (1), 443.
- Song, X.; Gjonas, E.; Ren, Q.; Taverna, D. S.; Allis, D. C.; Gorovsky, A. M. Phosphorylation of the SQ H2A. X Motif Is Required for Proper Meiosis and Mitosis in Tetrahymena Thermophila. *Mol. Cell. Biol.* **2007**, *27* (7), 2648–2660.
- Sugawara, N.; Ira, G.; Haber, E. J. DNA Length Dependence of the Single-Strand Annealing Pathway and the Role of *Saccharomyces cerevisiae* RAD59 in Double-Strand Break Repair. *Mol. Cell. Biol.* **2000**, *20* (14), 5300–5309.
- Sugiyama, Y.; Watase, Y.; Nagase, M.; Makita, N.; Yagura, S.; Hirai, A.; Sugiura, M. The Complete Nucleotide Sequence and Multipartite Organization of the Tobacco Mitochondrial Genome: Comparative Analysis of Mitochondrial Genomes in Higher Plants. *Mol. Genet. Genom.* **2005**, *272* (6), 603–615.
- Symington, L. S. Role of RAD52 Epistasis Group Genes in Homologous Recombination and Double-Strand Break Repair. *Microbiol. Mol. Biol. Rev.* **2002**, *66* (4), 630–670.
- Terada, R.; Johzuka-Hisatomi, Y.; Saitoh, M.; Asao, H.; Iida, S. Gene Targeting by Homologous Recombination as a Biotechnological Tool for Rice Functional Genomics. *Plant Physiol.* **2007**, *144* (2), 846–856.
- Tiwari, V.; Wilson, M. D. III DNA Damage and Associated DNA Repair Defects in Disease and Premature Aging. *Am. J. Human Genet.* **2019**, *105* (2), 237–257.
- Toh, G. W.-L.; Sugawara, N.; Dong, J.; Toth, R.; Lee, E. S.; Haber, E. J.; Rouse, J. Mec1/Tel1-Dependent Phosphorylation of Slx4 Stimulates Rad1–Rad10-Dependent Cleavage of Non-Homologous DNA Tails. *DNA Repair* **2010**, *9* (6), 718–726.
- Trivedi, R. N.; Almeida, H. K.; Fornsglio, L. J.; Schamus, S.; Sobol, W. R. The Role of Base Excision Repair in the Sensitivity and Resistance to Temozolomide-Mediated Cell Death. *Cancer Res.* **2005**, *65* (14), 6394–6400.

- Tubbs, A.; Nussenzweig, A. Endogenous DNA Damage as a Source of Genomic Instability in Cancer. *Cell* **2017**, *168* (4), 644–656.
- Vesela, E.; Chroma, K.; Turi, Z.; Mistrik, M. Common Chemical Inductors of Replication Stress: Focus on Cell-Based Studies. *Biomolecules* **2017**, *7* (1), 19.
- Wang, H.; Li, S.; Oaks J.; Ren J.; Li, L.; Wu, X. The Concerted Roles of FANCM and Rad52 in the Protection of Common Fragile Sites. *Nat. Commun.* **2018**, *9* (1), 1–14.
- Wang, H.; Shi, Z. L.; Wong, C. C.; Han, X.; Hwang, P. Y.-H.; Truong, N. L.; Zhu, Q.; Shao, Z.; Chen, J. D.; Berns, W. M. The Interaction of CtIP and Nbs1 Connects CDK and ATM to Regulate HR-Mediated Double-Strand Break Repair. *PLoS Genet.* **2013**, *9* (2), e1003277.
- Wedlich-Soldner, R.; Schulz, I.; Straube, A.; Steinberg, G.; Dynein Supports Motility of Endoplasmic Reticulum in the Fungus *Ustilago Maydis*. *Mol. Biol. Cell* **2002**, *13* (3), 965–977.
- Whitaker, S. J. DNA Damage by Drugs and Radiation: What Is Important and How Is It Measured? *Eur. J. Cancer* **1992**, *28* (1), 273–276.
- Wilson, M. A.; Kwon, Y.; Xu, Y.; Chung, W.-H.; Chi, P.; Niu, H.; Mayle, R.; Chen, X.; Malkova, A.; Sung, P. Pif1 Helicase and Pol δ Promote Recombination-Coupled DNA Synthesis via Bubble Migration. *Nature* **2013**, *502* (7471), 393–396.
- Wolf, J.; Oakey, R.; Feil, R. Imprinted Gene Expression in Hybrids: Perturbed Mechanisms and Evolutionary Implications. *Heredity* **2014**, *113* (2), 167–175.
- Wyatt, D. H.; West, C. S. Holliday Junction Resolvases. *Cold Spring Harbor Perspect. Biol.* **2014**, *6* (9), a023192.
- Xu, Y.; Her, C. Inhibition of Topoisomerase (DNA) I (TOP1), DNA Damage Repair and Anticancer Therapy. *Biomolecules* **2015**, *5* (3), 1652–1670.
- Yu, T.-Y.; Garcia, E. V.; Symington, S. L. CDK and Mec1/Tel1-Catalyzed Phosphorylation of Sae2 Regulate Different Responses to DNA Damage. *Nucleic Acids Res.* **2019**, *47* (21), 11238–11249.
- Zeman, K. M.; Cimprich, A. K. Causes and Consequences of Replication Stress. *Nat. Cell Biol.* **2014**, *16* (1), 2–9.

CHAPTER 6

DNA Repair Pathways and Genes in Plants

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ABSTRACT

The integrity of cellular DNA of all organism including plants is constantly at risk and may suffers damage endogenously or exogenously by different types of genotoxins such as chemical mutagens, ultraviolet light, and ionizing radiations. The most common and frequent DNA damage is due to the errors in metabolism; breaks of phosphodiester bond, modifications in nucleotide sequence, and intra- and interstrand cross links. The DNA damage, if not repaired, adversely affects plant developmental processes. Generally, plants are susceptible to the DNA damage as they are surrounded by air, soil, and water, which, sometimes, have DNA damaging factors. The process of DNA damage repair mechanism has been fairly elucidated in the mammalian, bacterial, and fungal model system. Though, the study of DNA damage and repair mechanisms in plants is still in its early stages, and *Arabidopsis thaliana* is at the forefront of the effort. The small genome size, rapid life cycle, and different transformation methods make *Arabidopsis* an absolute model plant to study eukaryotic DNA damage and repair mechanism. With the dawn of new molecular biology techniques, Over the last decade, tremendous progress has been made in understanding DNA damage and repair, as well as the genes involved in the process in different plants

such as rice, wheat, barley, cucumber, tomato, etc. DNA repair techniques include photoreactivation, base excision repair, direct DNA repair by alkyl transferases, mismatch repair, nucleotide excision repair, recombinational repair, and nonhomologous end joining. In this chapter, all these mechanisms and the gene involved have been discussed.

6.1 INTRODUCTION

The genome stability of all living organism is vital for the normal growth and development and transmission of characters from generation to generation. Still, the genome integrity or cells perpetually faces the several environmental and subcellular factors, which lead to the genome damage. Plants, the sessile living organisms of the nature, meet with environmental stress such as extreme temperature, flood, drought, salinity, heavy metals, xenobiotic toxicity, and ionizing radiations chemical mutagens, which results in the DNA damage and destabilization of its integrity (Gulfishan et al., 2010, 2011, 2012, 2013; Jafri et al., 2011; Hu et al., 2016; Nisa et al., 2019; Shahab et al., 2018). Errors in DNA replication and recombination, production of reactive oxygen species due to metabolic disorder also results in damage of DNA (Nimeth et al., 2020). The study of frequency of DNA damage is limited in case of plants. The rate of error during the replication falls in the range of 10^{-7} to 10^{-8} and it is because of the very high accuracy of DNA polymerases, the enzymes with proofreading activity, which itself have very low error chance, that is, 10^{-6} to 10^{-8} and, to the removal of 90–99% mismatched base pairs (Kunkel, 2004). Similarly, errors may occur during DNA repair when polymerases execute proofreading activity at the same rate as replication. Sometimes the rate is higher when the alternative polymerases are involved (Kunkel, 2004; Jain et al., 2018). In human cells, the rate or frequency of DNA damage, due to spontaneous hydrolysis or reactive oxygen species, is quite high and lies between few hundreds to $>10^5$ per cell based on the kind of damage (Bray and West, 2005). In root tips of maize, as a result of 20 h of seed imbibition, the numbers of apurinic/apyrimidic sites formed were estimated to 3.75×10^5 per cell and per genome. However, in plant cell, exact frequency or quantification of DNA damage occurring cells is still not confirmed. Under normal circumstances, DNA damage and repair can be considered as a consistent phenomenon and possibly yet more so in response to different stress environments. The projected mutation rate in plant cells is quite low due to the high levels of DNA damage. *Arabidopsis* lines propagated by whole genome sequencing from the progeny of single

seed for 25–30 years, the genome-wide overall rate of mutation per site calculated was 7×10^{-9} per site (Ossowski et al., 2010; Weng et al., 2019). This number refers to less than only one mutation per generation, which is at least 10 times smaller for a single cell cycle than the replication machinery error rate. It offers striking proof of the efficiency of DNA Damage, in the cell, observed, and repaired. The patching of the DNA lesions via different routes that have been tested elsewhere, and here it will not be defined in depth. (Amiard et al., 2013; Manova and Gruszka, 2015; Spampinato, 2017). If the DNA lesion repaired incorrectly, the replication mechanism get obstructed and leads to DNA double-strand break (DSB) that can be repaired via special pathways for DNA repair such as Homologous Recombination (HR) and Nonhomologous End Joining (NHEJ) (Amiard et al., 2013). In such circumstances, DNA damage response (DDR) is triggered; response of cells to genotoxic stress, an erudite signaling process, activates checkpoints of cell cycle, endoreplication in case of plants, and subsequent mechanism of DNA repair (Yoshiyama et al., 2013; Hu et al., 2016). In order to certify the repair of a number of various forms of lesions in DNA, multiple DNA repair pathways are activated, forming the DDR's DNA repair mechanism. If the DNA damage repair is not suitable, the programmed cell death destroys the affected cell and warrants homeostasis (Manova and Gruszka, 2015; Kim et al., 2019). The plants are highly vulnerable to harsh environmental conditions due to their sessile nature. The researcher has also discovered that temperature and light have an impact on DNA repair mechanisms such as HR and photoreactivation. (Li et al., 2002; Boyko et al., 2005).

6.2 GENOTOXIC AGENTS IN PLANT RESEARCH

In plant breeding research frequently used genotoxic agents are physical and chemical mutagens. Physical mutagens include ionizing radiations and ultraviolet (UV) rays and chemical mutagens include alkylating agents such as methyl methane sulphonate, ethyl methane sulphonate, di-ethyl sulfate; azo dyes like sodium azide; base analogs such as 2-aminopurine and 5-bromouracil; and an intercalating agent ethidium bromide. Different genotoxic agent has been shown in Table 6.1. Ionizing radiation such as carbon particles, gamma rays x-rays, and electron beam causes a extensive range of DNA disruption, which includes DSBs and base modifications in plants (Culligan et al., 2004., Garcia et al., 2003., Osakabe et al., 2006., Yuan et al., 2014). Compounds such as bleomycin and zeocin induces DSB in DNA and are regularly used in DDR research programme (Heitzeberg

et al., 2004., Spadafora et al., 2011). Methyl methanesulfonate (MMS) induces DNA methylation (Yuan et al., 2014., Chen et al., 2008), while DNA crosslinking is caused by cisplatin and mitomycin C (Chen et al., 2008., Reidt et al., 2006). In DNA, UV rays form dimers of pyrimidine (Schonn et al., 2010). The DNA replication or repair mechanism is affected by another type of genotoxic chemicals. DNA polymerase is inhibited by Aphidicolin (Schuermann et al., 2005; Cools et al., 2009), ribonucleotide reductase by hydroxyurea (Weimer et al., 2016; Cools et al., 2009) and poly(ADP-ribose) polymerase (PARP) by 3-aminobenzamide (Schreiber et al., 2002; Adams-Phillips et al., 2010). Topoisomerase I or II are inhibited by camptothecin and etoposide, respectively (Schonn et al., 2010; Li et al., 2014).

TABLE 6.1 Different Genotoxic Agents Causing DNA Damage in Plants.

Genotoxic agent	Action mechanism	Primary cellular DDR
Ionizing radiation (γ -ray, X-ray, and etc.)	Modification of base and Induction of strand breaks	NHEJ and HR
Bleomycin and zeocin	Induction of strand breaks	NHEJ and HR
Methyl methanesulfonate	DNA methylation and induction of strand break	NHEJ and HR
Cisplatin	Induction of crosslinks in DNA, that leads to strand breaks during synthesis of DNA	NER, HR, and BER
Mitomycin C	Induction of crosslinks in DNA	NER, HR, and BER
Ultraviolet	Induction of pyrimidine dimers	Photoreactivation and NER
Aphidicolin	Inhibition of DNA polymerase	Cell cycle arrest and HR
Hydroxyurea	Inhibition of ribonucleotide reductase	Cell cycle arrest
3-aminobenzamide	Inhibition of poly (ADP-ribose) polymerase	DNA repair such as BER
Camptothecin	Inhibition of topoisomerase I and induction of strand breaks	Programmed cell death
Etoposide	Inhibition of topoisomerase II and induction of strand breaks.	Programmed cell death

Source: Adapted from Kim et al. (2019).

6.3 PLANT DNA DAMAGE AND THE GENES INVOLVED

Researchers can get entire genomic sequences and structures through the Arabidopsis and monocot rice genome sequencing programmes, and has offered a new step to study the DNA repair in the higher plants. The nucleic

acid sequences of good quality can be used a strong tool for genetic analysis but the nonannotated sequences are the limitations, mean that some genes that are special part of a DNA repair system can not be detected for the moment. The genome sequence produced through the project of Rice Genome and the outcomes of the full-length cDNA Project are publically available, which results in the identification of genes by database searching and convoluted in DNA repair mechanism (Table 6.1). Also, the genes that are involved in *Arabidopsis* DNA damage and repair can be accessed freely in a database known as DNAMETAB (<http://www.ag.arizona.edu/dnametab/>). Plants have homologs of several genes that are involved in the widely preserved pathways of DNA like nucleotide excision repair (NER) and base excision repair (BER) Table 6.2. Though, few genes with proven role in pathways of DNA repair are absent. In Table 6.1, the genes with parenthesis, such as XPA and DNA polymerase, which are involved in NER and BER, respectively, were not found in the database available for rice. It suggested that the pathway for damage of DNA and repair mechanism in plants and animals have some differences. The plant genome possesses multiple copies of the genes involved in the mechanism of DNA repair, for example, three copies of CSB (Cockayne syndrome, type B) play pivotal role in the transcription-coupled repair (TCR) of nucleotide excision repair, two copies of PCNA (proliferating cell nuclear antigen) and FEN-1 (Flap endonuclease-1) and three copies of RPA (replication protein A), which take part in DNA replication and repair. The same way, *Arabidopsis* have two copies of XPB (AtRad25). Plants, generally, do have more copies of the genes than animals and that is why the genes involved in the mechanism of DNA repair showed the same trend. However, the presence of several DNA repair alleles can also increase the UV tolerance of a plant. In addition, without gene multiplication, any functional distinction will leads to the loss of the original function, which may hinder the opportunity of more effective repair systems to develop due to the absence of any allelic modification. Several genes for DNA repair that seem to be unique to plants, or that are restricted among the eukaryotes to the Plantae, in the completed genome sequences, they were recognized. The nature of such genes indicates plant-specific DNA repair routes, which is of matter of pivotal importance (Kimura and Sakaguchi, 2006).

6.4 DNA DAMAGE REPAIR PATHWAYS

The genome damage caused by various factors/processes should necessarily be repaired for the integrity of the genome and subsequent normal growth and development of the plants. Genome damages could be harmless if the activity

of either RNA or DNA polymerases are not affected by them. Though, the activity of RNA and DNA polymerases can be chocked due to UV-induced pyrimidine dimer, which is a noncoding damage product. Since plants are sessile organism and their exposure to different types of stresses is certain especially to UV radiation, therefore, UV light causes DNA damage, which must be repaired. DNA repair is not only a necessary biological function for normal growth and development, but it also ensures that characters are passed down from generation to generation. The pathways for the repair of DNA in eukaryotes are highly preserved. A number of DNA lesions and repair pathway has been summarised in Table 6.2. The *Arabidopsis* and rice genome sequence projects produced many genes/proteins for repair which, to some extent, were comparable to that of human. Kimura and Sakaguchi (2006) provided a list of DNA repair genes, which took part in different type of pathways for DNA repair. Plants, like their counterpart, that is, animals, possesses different DNA damage repair mechanism and few of them are defined below.

TABLE 6.2 DNA Lesions and Their Repair Pathways.

Lesions of DNA	Pathways for repair	Components for recognizing and repairing major lesions
UV-induced base adducts	Photo reactivation	Photolyases: CPD photolyases and 6-4 photolyase
Base damages: deaminated and alkylated bases, interstrand crosslink's	Nucleotide excision repair (NER): Global genome NER (GG-NER) and transcription-coupled NER (TC-NER) subpathways	XPC(RAD4)-HR23B(RAD23)-CENT2 complex, damaged DNA binding protein (DDB), CSA, CSB, XPA binding protein 2(XAB2), TFIIH complex (XPB and other factors) and cyclin-dependent kinase-activating kinase complex, DNA helicase XPD, XPA, RPA endonucleases (XPG and ERCC1/XPF), PCNA, replication factor C (RFC), DNA polymerase δ , ϵ and κ , ligase 1
Base damages: deaminated, oxidized, methylated, and alkylated bases, AP sites	Base excision repair (BER): "Short"-patch and "long"-patch repair sub pathways	DNA glycosylases/AP lyase, AP endonucleases, polynucleotide kinase 30 phosphatase (PNKP), aprataxin, tyrosyl DNA phosphodiesterase (TDP), XRCC1, poly(ADP-ribose) polymerase 1 (PARP1), FEN1, PCNA and DNA polymerase δ and ϵ , DNA ligase 1
Deaminated bases, replication errors, insertion/deletion loops (IDLs)	Mismatch repair (MMR)	MutS protein complexes (MutSa, MutS β , MutS γ), PCNA, MutL heterodimers, PMS1 endonuclease, replication fork complex (RFC), exonuclease 1 (Exo1), PCNA, RPA, DNA polymerase δ , DNA ligase 1

TABLE 6.2 (Continued)

Lesions of DNA	Pathways for repair	Components for recognizing and repairing major lesions
Single-strand break (SSB)	Single-strand break repair (SSBR)	Extensive overlap with BER, NER, and MMR machineries. PARP1, XRCC1, PNKP, RPA, FEN1, DNA polymerase β , δ and ϵ , ligase 1 and 3
Double-strand break (DSB)	Canonical nonhomologous end joining (cNHEJ) Alternative end joining (altEJ) Single-strand annealing (SSA)	Ku70-Ku80, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), Artemis nuclease, XRCC4, XRCC4 like factor (XLF/Cernunnos), PAXX, poly and μ , DNA ligase 4 MRN complex (nuclease), CtIP/COM1, PARP-1, Exo1, BLM/DNA2 helicase/ nuclease, XRCC1, DNA polymerase θ , DNA ligase 3 MRN complex (nuclease), CtIP/COM1, FANCM, RAD52, Exo1, XPF/ERCC1, RPA, DNA polymerase δ , DNA ligase 1

Source: Adapted from Que et al. (2019).

6.4.1 PHOTOREACTIVATION

This mechanism, shown in Figure 6.1, of DNA repair is also known as direct reversal of damage of DNA. . In this mechanism, the enzyme photolyase reverts the damaged DNA to its normal state the damaged. Photolyases are thought to be advanced from substrate specificity point of view. There are two co-factors in all photolyases and one of which, that is, FADH_2 , a two-electron reduced form, is the common photocatalyst and present in all the photolyases. The photolyases enzymes binds to the lesion in the 300–600 nm range. This reduces dimer to the pyrimidine monomer and releases the enzyme. Photoreactivation mechanism of DNA repair is common in many of eukaryotes and prokaryotes including *Escherichia coli*, yeast, some plant species and animals but in humans it is absent (Tuteja, 2009). The photolyases genes have been isolated from higher plants like rice, spinach, Arabidopsis, and cucumber. In Arabidopsis genome, six gene were identified that were related to photolyases. In *Arabidopsis*, photoreactivation studies results in the identification of two photolyases viz., PHR1/UVR2 and UVR3). PHR1/UVR2 is specific for cyclobutane pyrimidine dimers (CPDs) and UVR3 for pyrimidine (6-4) pyrimidine photoproducts (6-4PPs) (Jiang et al., 1997). Norin 1, a rice cultivar, is inefficient in photo repair of CPDs and it might be due to any mutation in CPD photolyases (Hidema et al., 2000).

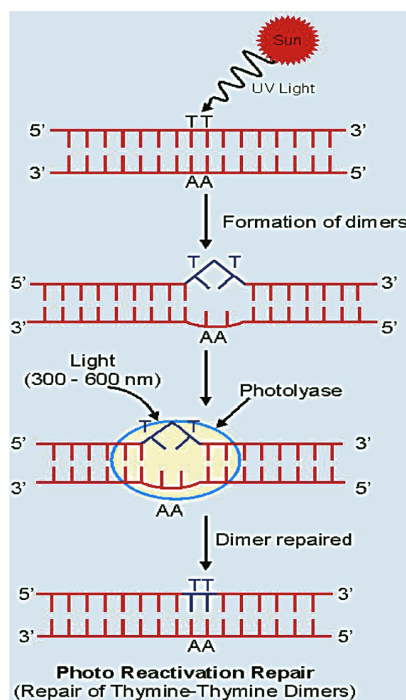


FIGURE 6.1 Photoreactivation is depicted as a diagram.

Source: Adapted from Tuteja et al. (2009).

6.4.2 NUCLEOTIDE EXCISION REPAIR

Nucleotide excision repair (NER) is a general method of DNA repair operated in both eukaryotes and prokaryotes and repair structurally different type of DNA lesions. UV-mediated photoproducts and other massive DNA that create significant structural changes in DNA are the key substrates of NER. The efficacy of NER varies and depends on the form of DNA lesion within the various genomic locations. Global genomic repair (GGR) and transcription-coupled repair are two distinct NER subpathways. Global genomic repair is a pathway for repair of the whole genome affected by the structure of chromatin and the proteins bound to DNA, while TCR precisely increases the removal of transcription-blocking lesions from the highly expressed genes' template DNA strand. The two NER method and involve a common repair proteins, while they are differ in the charter used to feel the damage, while the later phases moves in the same way (Fig. 6.2). The removal of CPD and other types of lesions in DNA from actively transcribed genes by the NER pathway in plants has not been extensively studied, while heterogeneity of

repair has been suggested in maize (Stapleton et al., 1997). Recently, it has been revealed in *Arabidopsis* that the low-rate repair showed, in the dark, at complete genome level, while the house-keeping RPII gene has been effectively reverted within 24 hours of UV irradiation. The key discovery was that better elimination of CPD from the gene's transcribed strand resulted in such repair, thereby providing clear proof of TCR activity in the plant genome (Fidantsef and Britt, 2012). Like these findings, AtCSA-like genes have been involved as part of the CUL4-DDB1^{CSAat1 A and B} complex in *Arabidopsis thaliana* in controlling TCR and UV damage response (Biedermann and Hellmann, 2010). Because AtCSA-1 protein expression was high in all plant tissues, UV-B did not upregulate it. However, the distribution of the AtCSA-1 protein in the nucleus showed patches in areas with significant transcriptional activity, thereby facilitating participation in TCR (Zhang et al., 2010). The selective activity of pathways for excision repair at the level of genes actively transcribed appears to be very important for plants and it would be useful to discover whether gene-specific repair could also make for UV tolerance in crops (Manova1 and Gruszka, 2015).

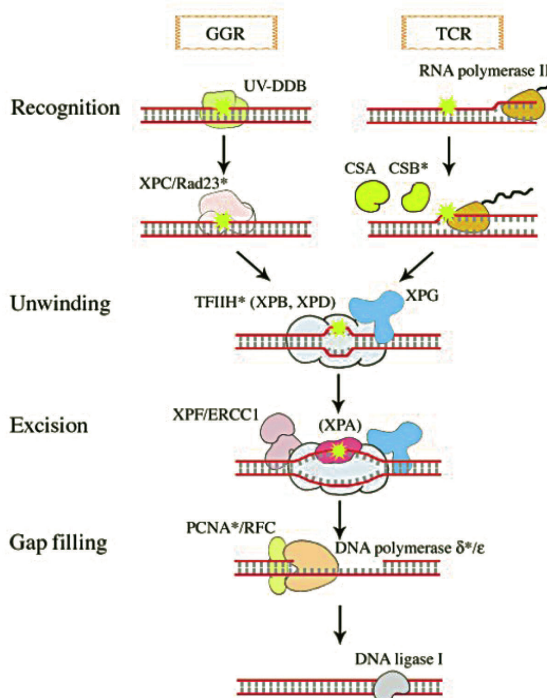


FIGURE 6.2 NER pathway.

Source: Adapted from Kimura and Sagakuchi (2006).

6.4.3 BASE EXCISION REPAIR

Different type of lesions, like injured or altered bases, as well as automatically occurring AP sites has been repaired by excision repair. Glycosylases specific to DNA damage cleave the N-glycosidic bond and eliminate the pretentious base that generates an AP site are initiated. Furthermore, AP site involves AP endonuclease or AP lyase action. Subsequently, based on the enzyme involved and type of lesion the repair response can proceed through short or a long patch mechanism. In plants, so far, a variety of lesion-specific DNA glycosylases has been discovered. The repair gene, for the first time cloned from plant DNA, *Arabidopsis* 3-methyladenine-DNA glycosylase, has been shown to eliminate the lesions in alkylated DNA because of MMS therapy (Santerre and Britt, 1994). The DNA with uracil is repaired in whole-cell extracts extracted from *Arabidopsis* by the BER mechanism using enzymes glycosylase. Particularly, in spite of the absence of pol β and Ligase III homologs in plants, the BER methods can occur after the early slit steps, and the repair reactions are confirmed by the AtLIG1 ligating process (Córdoba-Cañero et al., 2009, 2011). In particular, in vitro observation of the reactions of DNA repair with isolated cell extracts from *Arabidopsis* and other plants has been very helpful in identifying aspects, that is, functional and structural, of BER in plants. Short-patch BER is one of the important pathway for DNA repair in plant mitochondria, at least for uracil removal, where the activity of uracil-DNA glycosylase was found to be primarily correlated with the membrane-bound organs in the plants (Boesch et al., 2009). AtFPG and AtOGG1enzymes in *A. thaliana* undergo glycosylase activities to start the oxidative lesion 8-oxoG and endogenous AP sites's repair and leaves the transitional entity as a substrate of the ZDP DNA3'-phosphatase and ARP endonuclease. Because the AtFPG and AtOGG1 genes were inactive, the number of oxidative DNA damages in both the nuclear and mitochondrial genomes increased, although damage of AtARP and AtZDP functions quicken the aging of the seeds (Córdoba-Cañero et al., 2014). In eukaryotes, the BER is also implicated in epigenetic control because it eliminates the 5-mC, including plants, and its replacement with nonmethylated C. DNA methylation, an epigenetic modification, depends on the state of condensed chromatin that controls the availability of transcription factor or DNA repair protein. Therefore, any complexity in BER pathway may stop demethylation, which subsequently affects regulation of gene and associated activities of other pathways for DNA damage repair. Different types of proteins, such as bifunctional DNA lycosylase/lyaseROS1, which removes

the methylated base while also cutting the strands of DNA, DNA phosphatase ZDP, which is involved in the processing of intermediate product, and AtXRCC1, an Arabidopsis homolog, which starts the cleaning and ligation steps at the 3'-end, have been identified as being required for the demethylation process in plants via BER (Martínez-Macías et al., 2013). A schematic representation of BER has been shown in Figure 6.3.

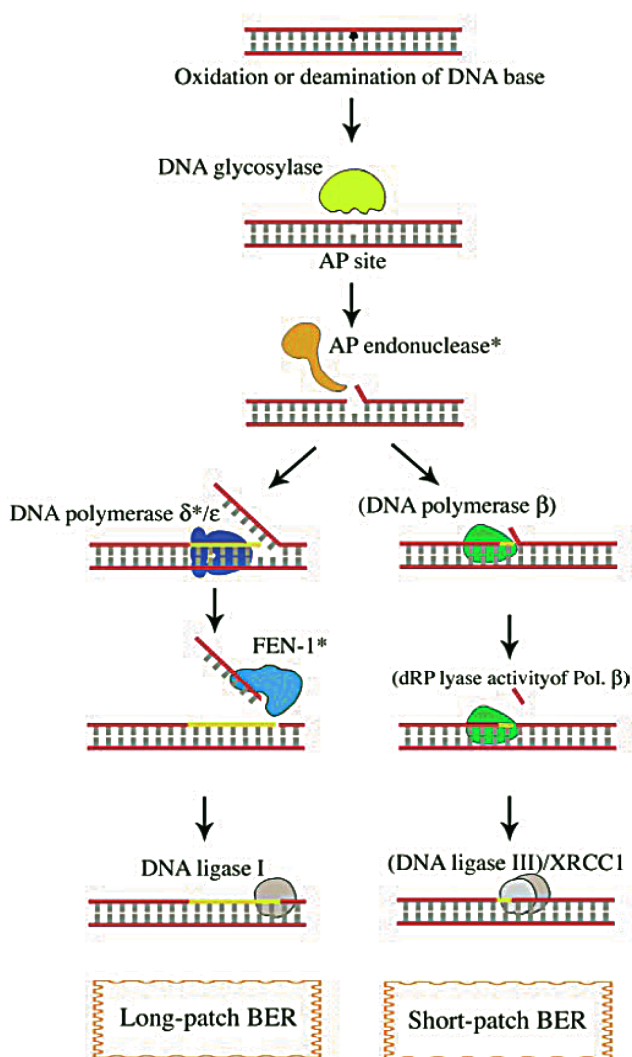


FIGURE 6.3 BER pathway.

Source: Adapted from Kimura and Sagakuchi (2006).

6.4.4 MISMATCH REPAIR (MMR)

The MMR system's main biological function is to precise mistakes like mismatches or mistakenly added or deleted nucleotides through replication. Furthermore, MMR is involved in the rectification of wrongly paired bases and loops in recombination intermediate, excessive heteroduplexes dismissal, elimination of DNA lesions such as crosslinks, psoralen-induced interstrand lesions, oxidative DNA injury, and photoproducts of UV and nucleosome remodeling (Javaid et al., 2009; Honda et al., 2014). Therefore, a successful MMR helps the cell increase DNA replication reliability, decrease mutations rate, regulate the subtleties of short repetitive sequences, preserve integrity of genome, perform homologous high-reliability and prevent homeologous recombination, and perform normal meiosis (Spampinato et al., 2009). The system of MMR is preserved in living organisms as an important protection of genomic stability; however, some differences between the kingdoms were noticed. The MMR gene families MSH and MLH in eukaryotes are homologs of prokaryotic MMR genes MutS and MutL. There are around six MSH genes in eukaryotes, while in plant the seventh gene *MSH7* is conserved (Culligan and Hays, 2000). MSH form different types of heterodimeric complexes like MSH2•MSH3 (MutS β) and MSH2•MSH6 (MutS α) or MSH4•MSH5 are specific for firm DNA structures and their damage. They operate in both either recombination or replicative MMR. Generally, the DNA binding activities and recognition of lesion are accomplished by the MSH heterodimers, while the actual reactions for repair starts with the enlistment of the MLH heterodimer (Culligan et al., 2000). The strand-specific MMR is performed by MutL α (MLH1-PMS2) endonuclease activity. PCNA, RPA, Exo1, RFC, and DNA polymerase δ are other proteins involved in the MMR's downstream stages of (Modrich, 2006). Plants, like other eukaryotes, rely on MMR factors during post-replicative and recombination MMR to maintain genomic stability. MSH2 deficiency in *Arabidopsis* prevents homologous but escalate homeologous recombination (Li et al., 2006) and advances microsatellite uncertainty, chiefly in germline cells (Leonard et al., 2003), and *MSH7* controls meiotic recombination (Lario et al., 2015). Loss in function of *MSH7* weakens meiotic recombination and decreases plant fertility (Lloyd et al., 2007) and the inactivity of AtMLHs disturbed the functions depending on MMR like damage repair, mitotic recombination, and meiosis (Dion et al., 2007). Plant MMR; functional not only in the nucleus but MSH1 protein also found in extranuclear genome of the plants where it affects the different functions of mitochondria and chloroplast (Xu et al., 2012b). There

is a rising need for the expansion of current MMR system awareness and they may directly affect the modern agriculture. Since a long, mutagenesis and hybridization have been used as a powerful method for crop enhancement and these processes have been related to MMR functionality in the plants (Manova and Gruszka, 2015). Attempt has been made to manipulate the MMR system not only in Arabidopsis but crop such as rice, tomato, and tobacco have also been tried (Tam et al., 2011; Xu et al., 2012a; Van Marcke and Angenon, 2013). Different methods like RNAi gene silencing and dominant negative suppression to inactivate the genes for MMR activity has been employed with an aim to ease the barrier between distantly related species and/or to increase variability in mutation (Manova and Gruszka, 2015).

6.4.5 HOMOLOGOUS RECOMBINATION (HR)

HR has been recognized as one of the important mechanism of DNA repair for preserving integrity of the genome and viability in plants where error-free DSB is primarily performed. In HR mechanism, the chromatid is used as template to retrieve the information. In addition, in HR, copying or, in some cases, merging homologous sequences from different places of the genome fills the double-stranded gap created by a ragged DSB. Work on yeast, a reliable model, has contributed to the categorization of HR models of DNA repair into three models; synthesis-dependent strand annealing (SDSA), single-strand annealing (SSA), and DNA double-strand break repair (DSBR) model (Aylon and Kupiec, 2004; Krogh and Symington, 2004). However, in plant somatic cells there are two types of HR-dependent pathways, that is, SSA and SDSA, for DNA repair has been categorized (Puchta, 2005). In short, both SDSA and DSBR start with the 3' splicing of DNA and results in tail of single-stranded DNA, which infest a duplex of homologous and primes synthesis of DNA, while the freshly synthesized DNA in SDSA forges with the other side of DSB, which repairs the break and also stops any joint molecule formation (Bray and West, 2005). SSA mechanism for DNA repair might operate between tandemly repeated sequences and the gaps created during the resection are annealed. The DSBR, SDSA, and SSA HR pathways were found in numerous plant species with a comparable molecular mechanism, and the frequency of recombination was variable depending on the sequences from origin and the donor recipient (Schuermann et al., 2005; Puchta, 2005). The HR-mediated DSB repair rarely found to occur in the somatic cells of plants through allelic sequences (Gisler et al., 2002). The

pathways for SSA and SDSA may also be part of HR and non-HR pathways. Comparatively, The SDSA procedure is around five times inefficient than the SSA procedure. (Manova and Gruszka, 2015). Therefore, the most prominent homology-based pathway for the repair of DSB in higher eukaryotes may be the SSA mechanism.

6.4.6 NONHOMOLOGOUS RECOMBINATION

It is also termed as NHEJ system. This DNA repair process does not require a homologous sequence to repair DNA lesions; instead, it rejoins the two broken ends of the DNA, which, often, comes up with mistakes or deletion and subsequently mutations. While nonhomologous recombination is clearly prone to error and damage or even unsuitable ends can be rejoined, this system of repair seems to be vital in the repair of DSB induced in plants (Friesner and Britt, 2003; Hefner et al., 2003). NHEJ pathway has been categorized into different subpathway like canonical or cNHEJ and the recently aNHEJ subpathway (Mladenov and Iliakis, 2011). In both pro- and eukaryotic species, the cNHEJ system is highly conserved. It is involved in DSBs repair formed by both chemical and physical factors, but also by the recombination of V(D)J in animals and the TDNA incorporation into a genome during transformation (Boboila et al., 2012; Park et al., 2015). In *Arabidopsis*, key players involved in cNHEJ, such as Ku70, Ku80, XRCC4, and ligase IV has identified mutations in the genes Ku70, Ku80, and LigIV that lead to increased sensitivity to factors that cause DSB. A mutation in the genes Ku80, Ku70, and LigIV tends to increased sensitivity to factors that cause DSB (West et al., 2002). Mutants of barley-bearing mutations in the Ku80 gene have been found recently. In these mutants, the DSBs induced by BLM is higher as compared with the parent cultivar. HvKu80 gene plays pivotal role in controlling the telomeres length in barley (Stolarek et al., 2015). In NHEJ, a merely categorized process, the broken ends of DNA are resected. Rejoining of the two single strands contributes to the creation of junctions at locations where there are some complementary nucleotides. Typically, the end pads are clipped, re-joining occurs and at the junction site the micro homologies are revealed. The aNHEJ method normally leads to deletions at the junction site because of resection of DSB (Puchta and Fauser, 2014). Studies suggested that proteins XRCC1 and PARP1 takes part in this process and also conserved in both plant and animals (Jia et al., 2013). The junction site is generally devoid of genetic information, so the process of aNHEJ is known as a highly mutagenic method of restoring DSB (Puchta

and Fauser, 2014). NHEJ has been shown as a significant for DSB repair pathway in somatic tissue. The rejoining of the broken end mediated by NHEJ depends upon the deletions of different sizes and the sequences (filler DNA), though, NHEJ does not require the homology in the sequence to fill the gap (Goettel and Messing, 2009).

6.5 CONCLUSION AND FUTURE PERSPECTIVE

Plant nuclear DNA is an intrinsically unbalanced molecule that can be destroyed by a range of stress causes, including natural, metabolic, and environmental variables. While sunlight is mandatory for plant photosynthesis and survival, it is also one of the key force to the genomic integrity of plants. Such damage and unrecognized and unrepaired DNA accumulation can results in fatal mutations that, in turn, can reduce the growth, productivity, and stability of the plant genome and also threaten the immediate survival of the organism. Various methods are used by plants to tolerate, reverse, or excise the presence of products for DNA damage. Different set of genes and proteins encoded by them which play pivotal roles in DNA repair mechanism has also been discovered. Arabidopsis, one of the major model plant, and other plant genomes have shown many similarities to the set of genes for DNA repair found in other eukaryotes, it will not be difficult to establish the role of proteins encoded by these genes in the plant system. There are also many plant-specific DNA repair genes detected, indicating plant-specific DNA repair pathways that are of great interest. The DNA repair research therefore occupies a central position in modern biology and plays pivotal role in the future development of realistic agricultural applications. An enormous area is currently covered by the field of DNA repair in eukaryotes, and strong connections are being formed between repair mechanism and other areas, including controls of DNA replication, transcription, and cell cycle and its check points. Different models suggested for repairs are essentially conceptual and need more experimental assistance. Cloning all the genes for repair and studying knockouts or over-expression of these genes may be a solution to the long-standing gene targeting problem in plants. In the plant, more research is required to address the many questions that still unanswered about the what are the genes/proteins involved in the mechanisms of DNA repair and their many objectives. Overall, a deeper understanding of the response to DNA damage and the mechanism of repair of DNA in plants can lead to solving environmental and social problems that are impeded.

KEYWORDS

- **DNA damage**
- **DNA repair**
- **genes**
- **mutagens**
- ***Arabidopsis thaliana***
- **NEH**
- **Alkyl transferases**
- **base excision repair**

REFERENCES

- Adams-Phillips, L.; Briggs, A. G.; Bent, A. F. Disruption of poly(ADP-ribosyl)lation Mechanisms Alters Responses of Arabidopsis to Biotic Stress. *Plant Physiol.* **2010**, *152*, 267–280.
- Amiard, S.; Gallego, M. E. E.; White, C. I. I. Signaling of Double Strand Breaks and Deprotected Telomeres in Arabidopsis. *Front. Plant Sci.* **2013**, *4*, 405.
- Aylon, Y.; Kupiec, M. DSB Repair: The Yeast Paradigm. *DNA Repair* **2004**, 797–815,
- Bhat T.A., Gulfishan M., Wani A. A. Cytomixis: Causes and Consequences as a Case Study in *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat T. A., Ed.; Springer, 2017; pp 331–342; doi.org/10.1007/978-81-322-3673-3_15 (Springer)
- Biedermann, S.; Hellmann, H. The DDB1a Interacting Proteins ATCSA-1 and DDB2 Are Critical Factors for UV-B Tolerance and Genomic Integrity in *Arabidopsis thaliana*. *Plant J.* **2010**, *62*, 404–415.
- Boboila, C.; Alt, F. W.; Schwer, B. Chapterone-Classical and Alternative End-Joining Pathways for Repair of Lymphocyte-Specific and General DNA Double-Strand Breaks. In *Advances in Immunology*; W. A. Frederick, Ed.; Academic Press: New York, 2012; pp 1–49.
- Boyko, A.; Filkowski, J.; Kovalchuk, I. Homologous Recombination in plants Is Temperature and Day-Length Dependent. *Mutat. Res.* **2005**, *572*, 1–2.
- Bray, C. M.; West, C. E. DNA Repair Mechanisms in Plants: Crucial Sensors and Effectors for the Maintenance of Genome Integrity. *New Phytol.* **2005**, *168*, 511–528.
- Chen, I. P.; Mannuss, A.; Orel, N.; Heitzeberg, F.; Puchta, H. A Homolog of ScRAD5 Is Involved in DNA Repair and Homologous Recombination in Arabidopsis. *Plant Physiol.* **2008**, *146*, 1786–1796.
- Cools, T.; Veylder, De. L. DNA Stress Checkpoint Control and Plant Development. *Curr. Opin. Plant Biol.* **2009**, *12*, 23–28.
- Córdoba-Cañero, D.; Morales-Ruiz, T.; Roldán-Arjona, T.; Ariza, R. R. Single-nucleotide and long-patch base excision repair of DNA damage in plants. *Plant J.* **2009**, *60*, 716–728.DOI: 10.1111/j.1365-313X.2009.03994.x

- Córdoba-Cañero, D.; Roldán-Arjona, T.; Ariza, R. R. *Arabidopsis* ARP Endonuclease Functions in A Branched Base Excision DNA Repair Pathway Completed by LIG1. *Plant J.* **2011**, *68*, 693–702. DOI: 10.1111/j.1365–313X.2011.04720.x
- Córdoba-Cañero, D.; Roldán-Arjona, T.; Ariza, R. R. *Arabidopsis* ZDPNA3'-Phosphatase and ARP Endonuclease Function in 8 Oxo G Repair Initiated by FPG and OGG1 DNA Glycosylases. *Plant J.* **2014**, *79*, 824–834. DOI: 10.1111/tpj.12588
- Culligan, K. M.; Hays, J. B. *Arabidopsis* MutS Homologs—AtMSH2, AtMSH3, AtMSH6, and a Novel AtMSH7 form Three Distinct Protein Heterodimers with Different Specificities for Mismatched DNA. *Plant Cell.* **2000**, *12*, 991–1002.
- Culligan, K. M.; Meyer-Gauen, G.; Lyons-Weiler, J.; Hays, J. B. Evolutionary Origin, Diversification and Specialization of Eukaryotic MutS Homolog Mismatch Repair Proteins. *Nucl. Acids Res.* **2000**, *28*, 463–471. DOI: 10.1093/nar/28.2.463
- Culligan, K. M.; Robertson, C. E.; Foreman, J.; Doerner, P.; Britt, A. B. ATR and ATM Play Both Distinct and Additive Roles in Response to Ionizing Radiation. *Plant J.* **2006**, *48*, 947–961. DOI: 10.1111/j.1365–313X.2006.02931.x
- Culligan, K.; Tissier, A.; Britt, A. ATR Regulates G2-Phase Cell-Cycle Check Point in *Arabidopsis thaliana*. *Plant Cell.* **2004**, *16*, 1091–1104. DOI: 10.1105/tpc.018903
- Dion, É.; Li, L.; Jean, M.; Belzile, F. An *Arabidopsis* MLH1 Mutant Exhibits Reproductive Defects and Reveals a Dual Role for This Gene in Mitotic Recombination. *Plant J.* **2007**, *51*, 431–440. DOI: 10.1111/j.1365–313X.2007.03145.x
- Fidantsef, A. L.; Britt, A. B. Preferential Repair of the Transcribed DNA Strand in Plants. *Front. Plant Sci.* **2012**, *2*, 105. DOI: 10.3389/fpls.2011.00105
- Friesner, J. D.; Liu, B.; Culligan, K.; Britt, A. B. Ionizing Radiation-Dependent γ -H2AX Focus Formation Requires at Axiatel Angiectasia Mutated and at Axiatel Angiectasia Mutated and Rad3-Related. *Mol. Biol. Cell.* **2005**, *16*, 2566–2576. DOI: 10.1091/mbc.E04–10–0890
- Friesner, J.; Britt, A. B. Ku80- and DNA Ligase IV-Deficient Plants Are Sensitive to Ionizing Radiation and Defective in TDNA Integration. *Plant J.* **2003**, *34*, 427–440.
- Garcia, V.; Bruchet, H.; Camescasse, D.; Granier, F.; Bouchez, D.; Tissier, A. At ATM Is Essential for Meiosis and the Somatic Response to DNA Damage in Plants. *Plant Cell.* **2003**, *15*, 119–132.
- Garcia, V.; Salanoubat, M.; Choisne, N.; Tissier, A. An ATM Homologue from *Arabidopsis thaliana*: Complete Genomic Organisation and Expression Analysis. *Nucl. Acids Res.* **2000**, *28*, 1692–1699. DOI: 10.1093/nar/28.8.1692
- Gisler, B.; Salomon, S.; Puchta, H. The Role of Double-Strand Break-Induced Allelic Homologous Recombination in Somatic Plant Cells. *Plant J.* **2002**, *32*, 277–284.
- Goettel, W.; Messing, J. Change of Gene Structure and Function by Non-Homologous End-Joining, Homologous Recombination, and Transposition of DNA. *PLoS Genet.* **2009**, *5* (6), e1000516. <https://doi.org/10.1371/journal.pgen.1000516>
- Gulfishan M.; Khan, A. H.; Haneef, I.; Bhat, T. A. Genotoxic Effect of Diethyl Sulphate in Two Varieties of *Capsicum annuum* L. The Nucleus. **2011**, *54*, 107–111.
- Gulfishan M.; Khan, A. H.; JAFRI, I. F. Genotoxic Effects Induced by Methyl Methane Sulphonate in 2 Cultivars of *Capsicum annuum* L. *Cytologia* **2012**, *76*, 1–5.
- Gulfishan M.; Khan, A. H.; Bhat, T. A. Studies on Cytotoxicity Induced by DES and SA in *Vicia faba* L. var. *major*. *Turk. J. Bot.* **2010**, *34*, 31–37.
- Gulfishan M.; Jafri, I. F.; Khan, A. H. Methyl Methane Sulphonate Induced Desynaptic Mutants in *Capsicum annuum* L. *Chrom. Bot.* **2013**, *8*, 59–62.

- Hefner, E.; Preuss, S. B.; Britt, A. B. Arabidopsis Mutants Sensitive to Gamma Radiation Include the Homologue of the Human Repair Gene ERCC1. *J. Exp. Bot.* **2003**, *54*, 669–680.
- Heitzberg, F.; Chen, I. P.; Hartung, F.; Orel, N.; Angelis, K. J.; Puchta, H. The Rad17 Homologue of Arabidopsis Is Involved in the Regulation of DNA Damage Repair and Homologous Recombination. *Plant J.* **2004**, *38*, 954–968.
- Hidema, J.; Kumagai, T.; Sutherland, B. M. UV Radiation-Sensitive Norin Rice Contains Defective Cyclobutane Pyrimidine Dimer Photolyases. *Plant Cell.* **2000**, *12*, 1569–1578.
- Hu, Z.; Cools, T.; De Veylder, L. Mechanisms Used by Plants to Cope with DNA Damage. *Annu. Rev. Plant Biol.* **2016**, *67*, 439–462.
- Jafri, I. F.; Khan, A. H.; Gulfishan, M. Genotoxic Effects of 5-Bromouracil on Cytomorphological Characters of *Cichorium intybus* L. *Afr. J. Biotechnol.* **2011**, *10*, 10595–10599.
- Jafri I. F.; Khan, A. H.; Sharma M.; Gulfishan, M. Genomic Damage Induced By Individual and Combination Treatment of Gamma Rays and Ethyl Methane Sulphonate in *Coriandrum sativum* L var. karishma. *Int. J. Bot. Res.* **2013**, *3*, 79–85.
- Jafri I. F.; Khan A. H.; Sharma M.; Gulfishan, M. Comparative Mutagenicity of EMS and HZ in *Hordeum vulgare* L. var. RD2052. *Chrom. Bot.* **2012**, *7*, 79–83.
- Jain, R.; Aggarwal, A. K.; Rechkoblit, O. Eukaryotic DNA Polymerases. *Curr. Opin. Struct. Biol.* **2018**, *53*, 77–87.
- Jia, Q; Dulk-Ras, A; Shen, H; Hooykaas, P. J; dePater, S. Poly(ADP- Ribose) Polymerases Are Involved in Microhomology Mediated Back-Up Non- Homologous End Joining in *Arabidopsis thaliana*. *Plant Mol. Biol.* **2013**, *82*, 339–351.
- Jiang, C. Z.; Yee, J.; Mitchell, D.; Britt, A. Photo Repair Mutants of *Arabidopsis*, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 7441–7445.
- Javaid, S.; Manohar, M.; Punja, N.; Mooney, A.; Ottesen, J. J.; Poirier, M. G. Nucleosome Remodelling by hMSH2-hMSH6. *Mol. Cell.* **2009**, *36*, 1086–1094. DOI: 10.1016/j.molcel.2009.12.010
- Honda, M.; Okuno, Y.; Hengel, S. R.; Martín-López, J. V.; Cook, C. P.; Amunugama, R. Mismatch Repair Protein hMSH2-hMSH6 Recognizes Mismatches and Forms Sliding Clamps Within a D-Loop Recombination Intermediate. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E316–E325. DOI: 10.1073/pnas.1312988111
- Kim, J. H.; Lee, M. H.; Moon, Y. R.; Kim, J. S.; Wi, S. G.; Kim, T. H.; Chung, B. Y. Characterization of Metabolic Disturbances Closely Linked to the Delayed Senescence of *Arabidopsis* Leaves After γ Irradiation. *Environ. Exp. Bot.* **2009**, *67*, 363–371.
- Kim, J. H.; Ryu, T. H.; Lee, S. S.; Lee, S.; Chung, B. Y. Ionizing Radiation Manifesting DNA Damage Response in Plants: An Overview of DNA Damage Signalling and Repair Mechanisms in Plants. *Plant Sci.* **2019**, *278*, 44–53.
- Kim, J. H.; Moon, Y. R.; Lee, M. H.; Kim, J. H.; Wi, S. G.; Park, B. J.; Kim, C. S.; Chung, B. Y. Photosynthetic Capacity of Arabidopsis Plants at the Reproductive Stage Tolerates γ Irradiation. *J. Radiat. Res.* **2011**, *52*, 441–449.
- Kimura S.; Sakaguchi, K. DNA Repair in Plants. *Chem. Rev.* **2006**, *106*, 753–766.
- Krogh, B. O.; Symington, L. S. Recombination Proteins in Yeast. *Annu. Rev. Genet.* **2004**, *38*, 233–271.
- Kunkel, T. A. DNA Replication Fidelity. *J. Biol. Chem.* **2004**, *279*, 16895–16898.
- Lario, L. D.; Botta, P.; Casati, P.; Spampinato, C. P. Role of At MSH7 in UV-B-Induced DNA Damage Recognition and Recombination. *J. Exp. Bot.* **2015**, *66*, 3019–3026. DOI: 10.1093/jxb/eru464

- Leonard, J. M.; Bollmann, S. R.; Hays, J. B. Reduction of Stability of Arabidopsis Genomic and Transgenic DNA-Repeat Sequences (Microsatellites) by Inactivation of AtMSH2 Mismatch-Repair Function. *Plant Physiol.* **2003**, *133*, 328–338. DOI: 10.1104/pp.103.023952
- Li, L.; Jean, M.; Belzile, F. The Impact of Sequence Divergence and DNA Mismatch Repair on Homeologous Recombination in *Arabidopsis*. *Plant J.* **2006**, *45*, 908–916. DOI: 10.1111/j.1365-313X.2006.02657.x
- Li, S.; Paulsson, M.; Bjorn, L. O. Temperature-Dependent Formation and Photorepair of DNA Damage Induced by UV-B Radiation in Suspension Cultured Tobacco Cells. *J. Photochem. Photobiol. B.* **2002**, *66* (1), 67–72.
- Li, Z.; S, B.; Clewell, R. A.; Adeleye, A.; Eerson, M. E.; Zhang, Q. Dose-Response Modeling of Etoposide-Induced DNA Damage Response. *Toxicol. Sci.* **2014**, *137*, 371–384.
- Lloyd, A.; Milligan, A.; Langridge, P.; Able, J. TaMSH7: A Cereal Mismatch Repair Gene That Affects Fertility in Transgenic Barley (*Hordeum vulgare* L.). *BMC Plant Biol.* **2007**, *7*, 67. DOI: 10.1186/1471-2229-7-67
- Manova, V.; Gruszka, D. DNA Damage and Repair in Plants—From Models to Crops. *Front. Plant Sci.* **2015**, *6*:885.
- Martínez-Macias, M. I.; Córdoba-Cañero, D.; Ariza, R. R.; Roldán-Arjona, T. The DNA Repair Protein XRCC1 Functions in the Plant DNA Demethylation Pathway by Stimulating Cytosine Methylation (5-meC) Excision, Gap Tailoring, and DNA Ligation. *J. Biol. Chem.* **2013**, *288*, 5496–5505. DOI: 10.1074/jbc.M112.427617
- Mladenov, E.; Iliakis, G. Induction and Repair of DNA Double Strand Breaks: The Increasing Spectrum of Non-Homologous End Joining Pathways. *Mut. Res.* **2011**, *711*, 61–72.
- Modrich, P. Mechanisms In Eukaryotic Mismatch Repair. *J. Biol. Chem.* **2006**, *281*, 30305–30309. DOI: 10.1074/jbc.R600022200
- Nimeth, B. A.; Riegler, S.; Kalyna, M. Alternative Splicing and DNA Damage Response in Plants. *Front. Plant Sci.* **2020**, *11*, 91.
- Nisa, M. U.; Huang, Y.; Benhamed, M.; Raynaud, C. The Plant DNA Damage Response: Signaling Pathways Leading to Growth Inhibition and Putative Role in Response to Stress Conditions. *Front. Plant Sci.* **2019**, *10*, 653. DOI: 10.3389/fpls.2019.00653
- Osakabe, K.; Abe, K.; Yoshioka, T.; Osakabe, Y.; Todoriki, S.; Ichikawa, H.; Hohn, B.; Toki, S. Isolation and Characterization of the RAD54 Gene from *Arabidopsis thaliana*. *Plant J.* **2006**, *48*, 827–842.
- Ossowski, S.; Schneeberger, K.; Lucas-Lledo, J. I.; Warthmann, N.; Clark, R. M.; Shaw, R. G. The Rate and Molecular Spectrum of Spontaneous Mutations in *Arabidopsis thaliana*. *Science* **2010**, *327*, 92–94.
- Park, S. Y.; Vaghchhipawala, Z.; Vasudevan, B.; Lee, L. Y.; Shen, Y.; Singer, K. Agrobacterium T-DNA Integration Into the Plant Genome Can Occur Without the Activity of Key Non-Homologous End-Joining Proteins. *Plant J.* **2015**, *81*, 934–946.
- Puchta, H. The Repair of Double-Strand Breaks in Plants: Mechanisms and Consequences for Genome Evolution. *J. Exp. Bot.* **2005**, *56*, 1–14.
- Puchta, H.; Fauser, F. Synthetic Nucleases for Genome Engineering in Plants: Prospects for a Bright Future. *Plant J.* **2014**, *78*, 727–741.
- Que, Q.; Chen, Z.; Kelliher, T.; Skibbe, D.; Dong, S.; Mary-Dell Chilton, M. D.; Qi, Y. Plant Genome Editing with CRISPR Systems: Methods and Protocols. In *Methods in Molecular Biology*, Vol. 1917; Springer Science + Business Media, LLC, Part of Springer Nature, 2019. https://doi.org/10.1007/978-1-4939-8991-1_1

- Reidt, W.; Wurz, R.; Wanieck, K.; Chu, H. H.; Puchta, H. A Homologue of the Breast Cancer-Associated Gene BARD1 Is Involved in DNA Repair in Plants. *EMBO J.* **2006**, *25*, 4326–4337.
- Santerre, A.; Britt, A. B. Cloning of a 3-Methyladenine-DNA Glycosylase from *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2240–2244. DOI: 10.1073/pnas.91.6.2240
- Schonn, I.; Hennesen, J.; Dartsch, D. C. Cellular Responses to Etoposide: Cell Death Despite Cell Cycle Arrest and Repair of DNA Damage. *Apoptosis* **2010**, *15*, 162–172.
- Schreiber, V.; Amé, J. C.; Dollé, P.; Schultz, I.; Rinaldi, B.; Fraulob, V.; Murcia, J. M. D.; Murcia, G. D. Polymerase-2 (PARP-2) Is Required for Efficient Base Excision DNA Repair in Association with PARP-1 and XRCC1. *J. Biol. Chem.* **2002**, *277*, 23023–23036.
- Schuermann, D.; Molinier, J.; Fritsch, O.; Hohn, B. The Dual Nature of Homologous Recombination in Plants. *Trends Genet.* **2005**, *21*, 172–181.
- Spadafora, N. D.; Doonan, J. H.; Herbert, R. J.; Bitonti, M. B.; Wallace, E.; Rogers, H. J.; Francis, D. Arabidopsis T-DNA Insertional Lines for CDC25 Are Hyper Sensitive to Hydroxyurea But Not to Zeocin or Salt Stress. *Ann. Bot.* **2011**, *107*, 1183–1192.
- Shahab, D.; Gulfishan, M.; Idrees, M.; Vágvölgyi, C.; Ansari, M. Y. K. Isolation of Mutants From Gamma Ray-Irradiated Population of *Solanum melongena* cultivar. 'Pusa Purple Long'. *Chrom. Bot.* **2018**, *13* (1), 31–36.
- Shahab, D.; Gulfishan, M.; Khan, A. A.; Vágvölgyi, C.; Ansari, M. Y. K. Comparative Mutagenic Effectiveness and Efficiency of Physical and Chemical Mutagens in *Solanum melongena* L. Variety pusa uttam. *Biomed J. Sci. Tech Res.* **2018**, *1-5*. DOI: 10.26717/BJSTR.2018.07.001541
- Spampinato, C. P. Protecting DNA from Errors and Damage: An Over View of DNA Repair Mechanisms in Plants Compared to Mammals. *Cell. Mol. Life Sci.* **2017**, *74*, 1693–1709.
- Spampinato, C. P.; Gomez, R. L.; Galles, C.; Lario, L. D. From Bacteria to Plants: A Compendium of Mismatch Repair Assays. *Mut. Res.* **2009**, *682*, 110–128.
- Stapleton, A. E.; Thorner, C. S.; Walbot, V. UVB Component of Sunlight Causes Measurable Damage in Field-Grown Maize (*Zea mays* L.): Developmental and Cellular Heterogeneity of Damage and Repair. *Plant Cell Environ.* **1997**, *20*, 279–290. DOI: 10.1046/j.1365-3040.1997.d01-81.x
- Stolarek, M.; Gruszka, D.; Braszewska-Zalewska, A.; Maluszynski, M. Functional Analysis of the New Barley Gene HvKu80 Indicates That It Plays a KEY ROLE in Double-Strand DNA Break Repair and Telomere Length Regulation. *Mutagenesis* **2015**, *30*, 785–797.
- Tam, S.; Hays, J.; Chetelat, R. Effects of Suppressing the DNA Mismatch Repair System on Homeologous Recombination in Tomato. *Theor. Appl. Genet.* **2011**, *123*, 1445–1458. DOI: 10.1007/s00122-011-1679-4
- Tuteja N.; Ahmad P.; Panda B. B.; Tuteja R. Genotoxic Stress in Plants: Shedding Light on DNA Damage, Repair and DNA Repair Helicase. *Mut. Res.* **2009**, *681*, 134–149.
- Van Marcke, I.; Angenon, G. Genomic Stability in *Nicotiana* Plants Upon Silencing of the Mismatch Repair Gene MSH2. *Plant Biotechnol. Rep.* **2013**, *7*, 467–480. DOI: 10.1007/s11816-013-0285-0
- Weng, M. L.; Becker, C.; Hildebrandt, J.; Neumann, M.; Rutter, M. T.; Shaw, R. G. Fine-Grained Analysis of Spontaneous Mutation Spectrum and Frequency in *Arabidopsis thaliana*. *Genetics*. **2019**, *211*, 703–714.
- Weimer, A. K.; Biedermann, S.; Harashima, H.; Roodbarkelari, F.; Takahashi, N.; Foreman, J.; Guan, Y.; Pochon, G.; Heese, M.; Van Damme, D.; Sugimoto, K.; Koncz, C.; Doerner, P.; Umeda, M.; Schnittger, A. The plant-specific CDKB1-CYCB1 Complex Mediates Homologous Recombination Repair in Arabidopsis. *EMBO J.* **2016**, *35*, 2068–2086.

- Xu, J.; Li, M.; Chen, L.; Wu, G.; Li, H. Rapid Generation of Rice Mutants via the Dominant Negative Suppression of the Mismatch Repair Protein OsPMS1. *Theor. Appl. Genet.* **2012a**, *125*, 975–986. DOI: 10.1007/s00122–012–1888–5
- Xu, Y.-Z.; Santamaria, R. D. L. R.; Virdi, K. S.; Arrieta-Montiel, M. P.; Razvi, F.; Li, S. The Chloroplast Triggers Developmental Reprogramming When MUTSHOMOLOG1 Is Suppressed in Plants. *Plant Physiol.* **2012b**, *159*, 710–720. DOI: 10.1104/pp.112.196055
- Yao, Y.; Bilichak, A.; Titov, V.; Golubov, A.; Kovalchuk, I. Genome Stability of *Arabidopsis* atm, ku80 and rad51b Mutants: Somatic and Transgenerational Responses to Stress. *Plant Cell Physiol.* **2013**, *56*, 982–989.
- Yoshiyama, K. O.; Sakaguchi, K.; Kimura, S.; Sakagushi, K.; Kimura, S. DNA Damage Response in Plants: Conserved and Variable Response Compared to Animals. *Biology* **2013**, *2*, 1338–1356.
- Yoshiyama, K. O.; Kimura, S.; Maki, H.; Britt, A. B.; Umeda, M. The role of SOG1, a Plant-Specific Transcriptional Regulator, in the DNA Damage Response. *Plant Signal. Behav.* **2014**, *9*, e28889. DOI: 10.4161/psb.28889
- Yuan, D.; Lai, J.; Xu, P.; Zhang, S.; Zhang, J.; Li, C.; Wan, Y.; Du, J.; Liu, Y.; Yang, C. AtMMS21 Regulates DNA Damage Response and Homologous Recombination Repair in *Arabidopsis*. *DNA Repair.* **2014**, *21*, 140–147.
- Zhang, C.; Guo, H.; Zhang, J.; Guo, G.; Schumaker, K. S.; Guo, Y. *Arabidopsis* Cockayne Syndrome Like Proteins 1A and 1B form a Complex with CULLIN4 and Damage DNA Binding Protein 1A and Regulate the Response to UV Irradiation. *Plant Cell.* **2010**, *22*, 2353–2369. DOI: 10.1105/tpc.110.073973



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CHAPTER 7

Herbicide Tolerant Crops Developed from Mutation

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ABSTRACT

Weeds are one of the common problems faced by farmers around the world that hinder good yield. Using genetic engineering techniques different genes are identified to develop herbicide tolerant crops. The herbicide tolerance can be developed due to natural or induced mutations in the genes encoding the herbicide target site or its uptake/metabolism. Cotton, maize, and soybean varieties are developed as transgenic HTCs against bromoxynil and glufosinate. These varieties were reported successful in marked commercially. These commercially available herbicides are mostly incompletely dominant except triazine tolerant mutant species and are a challenge for the researchers to overcome this limitation of HTCs.

7.1 INTRODUCTION

The development of weeds is among the common problems faced in agriculture. They are undesirable plant growth that occurs along with crop plants in the fields, or in garden lawns. The weeds utilize the available nutrients in the soil more readily as compared to food crops and hence grow rapidly. This

competition also occurs for available water and sunlight in the limited space of plant propagation and results in 25–80% loss in yield (Karthik et al., 2020). In ancient times, weeds were largely removed manually from fields. However, this labor-extensive practice was rarely effective. Consequently, the use of oils, mild organic acids, and sea salts as a herbicide was increased in agriculture (Choudhury et al., 2016). This approach too was accompanied by several disadvantages mainly associated with the leaching of soil nutrients. The next generation encouraged the technique of manual or tractor based tillage. The manual tillage process added to the labor extensive inputs of the agricultural processes, whereas the use of tractor-based tillage significantly increased the production of greenhouse gases in the late 1800s (Brookes and Barfoot, 2017). The first chemical herbicide was commercialized in France, in 1896, under the brand name—Sinox containing sodium dinitrocresylate as the main ingredient. This was followed by the development of more than 100 different types of herbicides over the next 20 years due to its extensive demand and magical results (Encyclopaedia Britannica, 2019). Till date, chemical herbicides are the most appropriate choice to actively manage the growth of weeds and increase the crop yield. However, given the herbaceous nature of plants as well as weeds, the selective killing of weeds with absolute protection of desired plants is challenging. For this reason, the development of herbicide tolerance is a desirable trait, especially among industrially important agricultural crops.

Genetic engineering through selective mutation of crops has now made it feasible to develop herbicide tolerant crops (HTCs) that prevent major losses that occur in crop yield. Various genes have been identified in the past few decades that confer herbicide tolerance in agriculturally valuable crops (Zhang et al., 2017). The HTCs can be developed due to mutations in a native gene, or through the insertion of a foreign gene to obtain transgenic plants (Slinkard et al., 2007; Green, 2009). In development of the herbicide tolerance trait, the HTCs modify the target proteins and prevent the binding of herbicides rendering them ineffective. This mechanism of resistance is commonly known as the target site resistance (Delye, 2012). Conversely, the HTCs prevent the uptake and translocation of herbicides or amplify their degradation and detoxification potential. In some cases, these plants secrete molecules that decrease the lethal activity of herbicides or prevent herbicide sequestration. All these alternative mechanisms are characterized as nontarget site resistance (Dill et al., 2008).

The economic advantages of HTCs can be best explained with the help of glyphosate tolerant soybeans, first produced and commercialized in 1996 (Green, 2009). The productivity of these crops was increased by 38%; however, the remaining 62% cost-effectiveness was achieved as a result of the reduction

in the previously required weed management strategies (Barry et al., 1997; Padgett et al., 1995). Based on ISAAA database (2019), 351 HTCs have been approved for cultivation till date. Among these, maize and soybean form a major percentage of commercialized transgenic HTCs (Kumar et al., 2020). In 1995, transgenic HTCs like cotton and canola were introduced that exhibited tolerance to bromoxynil and glufosinate, respectively. They received commercial success and considerable improvement in crop yield was achieved. In the following year, that is, 1996, glyphosate-resistant soybeans were introduced. Together, these crops took over a major share of the North American market and contributed mainly to the success of glyphosate tolerant crops (Duke, 2005).

7.2 GENES AND ENZYMES OF INTEREST FOR HERBICIDE TOLERANT CROP DEVELOPMENT

The herbicides are generally developed by using mechanisms to inhibit key enzymes in the biochemical pathways of plants. Several such enzymes of the amino acid synthesis pathway, photosynthesis, cellular protein synthesis, and growth hormones synthesis pathways have been targeted for the development of HTCs. Among these, the esterases, acetohydroxyacid synthase (AHAS), glutathione S-transferase, UDP-glucosyl transferase, acetyl-CoA carboxylase (ACCase), and D1 protein inhibitor are a few examples of enzymes that develop nontarget site resistance in plants. On the other hand, the glyphosate and glufosinate herbicides confer target site resistance in plants. Details of common herbicides and examples of a few HTCs are represented in Table 7.1.

Different genes and enzymes of interest in the development of HTCs are described in the next page.

7.2.1 5-ENOLPYRUVYL SHIKIMATE3-PHOSPHATE SYNTHASE

Glyphosate is a nontarget site-specific chemical herbicide that inhibits the 5-enolpyruvyl shikimate- 3- phosphate synthase (EPSPS) enzyme of the shikimate (aromatic amino acid) biosynthesis pathway. It was first introduced in 1970 and has been widely used ever since due to its efficient broad-spectrum activity, low toxicity, and cost-effectiveness. The absence of this pathway in members of the animal kingdom renders this herbicide ineffective (harmless) on humans and animals (Schonbrunn et al., 2001). The glyphosate tolerant soybeans express a heterologous and insensitive or mutant EPSPS. Most commonly, these genes are taken from *Agrobacterium tumefaciens* strain CP4. Conversely, they are chemically synthesized by using EPSPS GRG23 gene of *Arthrobacter globiformis* or the mutant EPSPS gene of the maize plant. In a

TABLE 7.1 Commonly Used Herbicides and Their Target Enzymes.

Sr. No.	Brand name	Manufacturer	Active component	Mechanism of action	Example of HTC	Reference
1	Axial	Syngenta	Pinoxaden (phenylpyrazoline)	Inhibition of ACCase enzyme	Wheat, barley	Hofer et al. (2006)
2	Accent Q	Corteva	Nicosulfuron (Sulfonylurea)	Inhibition of ALS enzyme	Sweet corn	Robinson et al. (1994)
3	Acumen	Tenkoz	Pendimethalin (Dinitroaniline)	Inhibition of microtubule assembly	Corn	Pavlista and Cyanamid (1983)
4	Clarity	BASF	Dicamba (Benzoic acid)	It is a synthetic auxin	Maize, soybean, and corn	Nandula (2019)
5	Evik	CIBA-Geigy	Ametryne (Triazine)	Inhibition of Photosystem II	Corn, banana, and sugarcane	Simoneaux and Gould (2008)
6	Prograss	Bayer	Ethofumesate (Benzofuran)	Inhibition of long-chain fatty acids	Corn	Schweizer (1975)
7	TopNotch	Corteva Agriscience	Acetochlor (Chloroacetamide)	Inhibition of long-chain fatty acids	Maize	Jablonkai and Hatzios (1994)
8	Starfighter	United Phosphorus, Inc	Ozadiazon (oxadiazole)	Inhibition of protoporphyrinogen oxidase	Rice	Challam and Thabab (2018)
9	Solicam	Syngenta	Norflurazone (Pyridazinone)	Inhibition of carotenoid biosynthesis at phytoene desaturase	Maize and sorghum	Oorschot (1979)
10	Spartan	FMC	Sulfentrazone (Triazolinone)	Inhibition of protoporphyrinogen oxidase	Corn and soybean	Gehrke et al. (2020)

recent study, tolerance to glyphosate was introduced in japonica rice cultivar known as Zhonghua 11 (ZH11), by transforming it with EPSPS gene of *Pseudomonas fluorescens* G2, to produce two transgenic lines, that is, G2-6 and G2-7. The G2-EPSPS gene conferred tolerance to over 20,000 ppm glyphosate, that is, 20 times higher concentration of the one generally applied in fields (Dong et al., 2017). Previously, the rice cultivars tolerant to 8 ppm and 2.5% herbicide concentrations have been reported (Zhao et al., 2011; Tian et al., 2013; Tian et al., 2015). Even the seedlings tolerated 4 mM glyphosate solution for 24 h before its subsequent transfer to a hydroponic solution and up to five times the concentration applied in fields on maturity (Chandrasekhar et al., 2014; Chhapekar et al., 2015; Khan et al., 2015). Similarly, another agriculturally valuable crop, that is, transgenic glyphosate tolerant Indian cotton cultivar, P8-6, was developed by inserting codon-optimized and modified EPSPS gene with N-terminal chloroplast peptide obtained from *Petunia hybrida*. This plant could tolerate over 4000 ppm glyphosate (Karthik et al., 2020).

7.2.2 GLUTAMINE SYNTHETASE

Another common nontarget site-specific herbicide employed in the development of HTCs, introduced in 1993 in the USA and Canada, is glufosinate. It is a racemic mixture of d, l-phosphinothricin and commonly referred to as glufosinate-ammonium. The l-phosphinothricin is the plant metabolic product of bialaphos (4-[hydroxy (methyl) phosphinoyl] -l-homoalanyl-l-alanyl-l-alanine), which was originally identified as a fermentative product produced by *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*. This chemical compound competitively inhibits glutamine synthetase that results in the accumulation of ammonia in toxic concentrations which, in turn, inhibits photosystems I and II. These compounds cause phytotoxicity by disrupting photorespiration and photosynthesis due to the accumulation of ammonia and reactive oxygen species resulting in lipid peroxidation. The glufosinate tolerant crops are produced by inserting foreign genes, namely, pat and bar obtained from *Streptomyces* sp. (Sauer et al., 1987). It has been reported that the observed resistance to glufosinate, in maize and cotton plants, is directly proportional to the genetic expression of phosphinothricin acetyltransferase (Krenchinski et al., 2018; Carbonari et al., 2016). Also, a recent study confirmed that the amino acid substitution in glutamine synthetase 2 (GS-2) is responsible for glufosinate resistance in Italian ryegrass. They further suggested that the nontarget site resistance mechanisms are active in plant species and do not occur due to an altered target site (Brunharo et al.,

2019). A recent study developed transgenic glufosinate tolerant jute plants capable of growth at 10 ppm glufosinate by transforming the *bar* gene into *Corchorus capsularis* and *Corchorus olitorius* (two species of jute plant) with the help of *Agrobacterium* sp. (Shuvabrata et al., 2018). Although safe and efficient, glufosinate is reported to be inconsistent in its performance in the fields. Moreover, it is highly hydrophilic and hence not easily translocated in plants. They are also degraded rapidly by microorganisms. The major application of glufosinate is for weeds that have developed glyphosate resistance and are majorly utilized in the Midwest and Southern USA and, North and South America (Takano and Dayan, 2020).

7.2.3 D1 PROTEIN

The *psbA* gene encodes the D1 protein which is a vital component of the PS II reaction center of the photosynthetic electron transport chain. The D1 protein facilitates electron transport from plastoquinone A (QA) to plastoquinone B (QB). The inhibition of D1 protein leads to depletion of NADP which is essential for fixation of carbon dioxide. It also forms free radicals, which cause photo-oxidation of essential biomolecules like chlorophylls and unsaturated lipids in the chloroplasts of the cells. Examples of herbicides targeting the D1 protein are triazine, bromoxynil, and phenylurea which bind to the plastoquinone binding site to inactivate the protein (Ryan, 1970). Although a suitable target, it is less commonly used for the development of HTCs. This is because the resistance to D1 protein inhibitors has been observed in several weed species as early as the 1960s in the fields of agriculturally important crops like corn and maize in North America and Europe (LeBaron, 1991). The D1 protein inhibitor-resistant crops are developed through a point mutation of Ser to Gly at amino-acid 264. Other studies have indicated that the point mutations in close proximity at Ser264, Phe265, Phe255, and His215 can also develop resistant varieties in rapeseed (Gronwald, 1994).

7.2.4 ACCASE GENES

ACCase enzyme functions in the catalysis of carboxylation of acetyl-CoA to form malonyl-CoA during the biosynthesis of fatty acids (Rendina and Felts, 1988). Thus, they are a vital enzyme required for growth and cellular integrity. The encoding region of the ACCase gene consists of over 2300 amino acids and occurs as a homomeric or heteromeric enzyme depending on the type of plant. The homomeric ACCase is commonly observed in eukaryotes as a large multidomain protein. The heteromeric enzyme

occurs in prokaryotes and is composed of multiple subunits. Examples of herbicides inhibiting ACCase enzymes are aryloxyphenoxypropionates (haloxyfop), phenylpyrazolines, and cyclohexanedione (cycloxydim and sethoxydim). The dicot species naturally tolerate ACCase inhibiting herbicides in most instances, whereas the grass weeds are susceptible (Powles and Yu, 2010). Although the presence of eukaryotic or altered forms of ACCase enzymes, in few exceptional cases of grass weeds, may result in the development of resistance to these herbicides. Tolerance to the homomeric ACCase inhibitors is reported due to point mutations in the ACCase gene and five such genetic positions, with codon numbers I1781, W2027, I2041, D2078, and G2096 have been identified in *Alopecurus myosuroides*. Among these regions, a point mutation due to amino acid substitution at position I1781 or D2078 confers tolerance to aryloxyphenoxypropionates and cyclohexanediones. However, the mutations in other regions exhibit tolerance to aryloxyphenoxypropionates only. Naturally occurring mutations have been observed to develop tolerance in few cereal crops due to their ability to metabolize the herbicides targeting this enzyme or due to the presence of insensitive form of ACCase (Catanzaro et al., 1993; Stoltenberg et al., 1989). A recent study, from China, reported for the first time that a substitution of isoleucine to threonine at position 2041 confers resistance to ACCase inhibitors in *Alopecurus aequalis* (Guo et al., 2017). The same observation was reported by Ghanizadeh et al. (2020) who suggested that the above target-site mutation in the carboxyltransferase domain of the ACCase enzyme confers resistance to haloxyfop in *Poa annua*, without undergoing any change to the conformational structure.

7.3 ACETOHYDROXY ACID (AHAS) SYNTHASE

AHAS, also known as acetolactate synthase or ALS, is the most common enzyme targeted by herbicides. They catalyze the formation of aceto-hydroxybutyrate and acetolactate and is the first enzyme in the biosynthesis of three branched-chain amino acids, namely, valine, leucine, and isoleucine. Four distinct families of AHAS inhibitors exist and are known as sulfonylurea (fluzasulfuron, imazosulfuron, pyrazosulfuron ethyl, and halosulfuron), imidazolinone (imazethapyr, imazaquin, and imazapyr), triazolopyrimidine sulfonanilide (cloransulam, diclosulam, florasulam, and pyroxsulam), and pyrimidinyl oxybenzoate (bispyribac and pyriminobac) (Barnes and Vans-traelen, 2009). Tolerance to AHAS inhibitor occurs due to substitution mutation of 11 amino acids at Pro-197 which causes a high degree of resistance to sulfonylurea but low or negligible resistance to imidazolinone (Powles and

Yu, 2010). On the contrary, substitution mutation of Trp591 to Leu confers extreme resistance to all AHAS inhibitors. The resistance to imidazolinones also develops on substitution mutation at Ser670 to Asp or Ala122 to Thr while maintaining susceptibility to sulfonylurea and triazolopyrimidines (Bernasconi et al., 1995; Sathasivan et al., 1991). The AHAS enzyme is vital for plant and microbial growth but its activity is absent in animals, making them nontoxic to farm animals as well as humans. Also, the imidazolinone herbicides, in particular, exhibit broad-spectrum activity against the grass as well as broadleaf weeds. Hence, they are a practical choice for developing imidazolinone-resistant crop species. A number of transgenic plants have been produced, including maize, wheat, rice, oilseed, and sunflower, using chemical or physical mutagenesis between 1992 and 2006. All these plants were commercialized under the trademark of CLEARFIELD (Tan et al., 2005; Galon et al., 2014).

7.3.1 PROTOPORPHYRINOGEN IX OXIDASE

Porphyrin is the precursor to the synthesis of tetrapyrrole compounds like chlorophyll. The enzyme protoporphyrinogen oxidase, encoded by PPX2L gene, catalyzes the conversion of protoporphyrinogen to Protoporphyrin IX, and initiates the synthesis of chlorophyll. The two isoforms of protoporphyrinogen oxidase, that is, PPO1 and PPO2 are encoded by PPXI and PPXII, respectively. In instances of inhibition of protoporphyrinogen oxidase, the precursor molecule accumulates in the cytoplasm in large quantities and causes severe photocatalytic damage to the cells and lipid peroxidation. Examples of herbicides that exhibit this mechanism include diphenylethers, thiadiazoles, triazolinones, N-phenyl-phthalimides, and pyrimidinediones and oxidiazoles (Devine and Shukla, 2000). The HTCs with resistance to protoporphyrinogen oxidase inhibitors have been developed commonly in maize (Li and Nicholl, 2005). More than one type of mutation confers resistance to these inhibitors. It has been reported that the deletion mutation at position 210, of glycine residue, and substitution of arginine to glycine/methionine at amino acid position 128 of the PPO2 enzyme results in resistance to fomesafen. Lately, the substitution of glycine to alanine, at position 399, in the catalytic domain of PPXII was identified as a protoporphyrinogen oxidase inhibition mutation in Palmer amaranth species. The demand for protoporphyrinogen oxidase inhibiting herbicides is gradually increasing in recent years. This is because in spite of the widespread use of these herbicides, only six resistant weed species have been reported in six countries.

Four of these six weeds belong to *Amaranthus* species and the other two are grass species (Adhikari et al., 2020).

7.3.2 OTHER GENES OF INTEREST

The comparatively less explored genes for the development of HTC are tubulin and indole acetic acid (IAA) synthesis genes, glutathione S-transferases, and cytochrome P₄₅₀ monooxygenases. The chemical compounds like dinitroaniline inhibit the synthesis of structural proteins like tubulin by binding to the plant tubulin dimers and disrupting microtubule growth. They further cease the cell division due to its irreversible binding that blocks polymerization and formation of spindle fibers. Most commonly, the HTCs for this compound are developed in tobacco by target-site resistance as well as nonspecific mechanisms. In an early study, dinitroaniline resistant transgenic tobacco plant was generated by co-overexpression of a mutant alpha and beta tubulin (Anthony et al., 1999).

Although the mechanism of action and the molecular insights of auxin-type herbicides are not thoroughly explored, they are considered as a potent herbicide that acts by mimicking the endogenous auxin, that is, IAA in the development of HTCs. The observed resistance to these compounds is negligible due to its rare use as herbicide (Webb and Hall, 1995).

The glutathione S-transferases catalyze the conjugative reactions of glutathione with various biochemical substrates and functions in protecting the plant cells from oxidative damage (Powles et al., 1997). The main function, however, is to metabolize and detoxify the herbicides. Studies have suggested that a single amino acid substitution can further improve the efficiency of this enzyme and affect substrate specificity for a number of xenobiotics including herbicides. Most commonly, the triazines are used to control weeds growing in corn fields. However, extensive use of this herbicide has developed resistance among several weed species like *Abutilon theophrasti* (Sinning et al., 1990; Hall et al., 1998).

The cytochrome P₄₅₀ monooxygenases function in the synthesis of sterols, fatty acid derivatives, and hormones. Less commonly, they are also required in the secondary metabolism in plants. Although a suitable target for HTCs, the development of resistance to P450 enzymes in plants and simultaneous increase of these inhibitors on weeds will lead to selection pressure on weeds and resistant varieties will be naturally selected. This is dangerous because it is believed that the P450 enzyme resistance will allow activation of diverse metabolic pathways that will lead to herbicide resistance of various classes,

including the ones that are never used till now (Barr et al., 1992; Christopher et al., 1994).

7.4 DEVELOPMENT OF TOLERANT MUTANT PLANT SPECIES

Spontaneous mutations and variants of the above genes occur in the natural plant population. Hence, few plants develop resistance more easily as compared to others. It has been reported that the frequency of development of resistance to AHAS-inhibiting herbicide in *Arabidopsis thaliana* is 10^{-9} . Such tolerance occurs as a result of selection pressure in the fields that build-up due to continuous but exposure to sublethal concentrations of herbicides in the fields. In order to protect the agriculturally important crops from the lethal effect of herbicides, the development of herbicide-resistant or tolerant varieties is a promising strategy. However, such plants are not widely produced due to regulatory practices, high management, and cost inputs. As described previously in this chapter, the herbicide tolerance can be developed due to natural or induced mutations in the genes encoding the herbicide target site or its uptake/metabolism. The different approaches to induce these mutations are discussed below.

7.4.1 TRANSGENIC APPROACH

Most commonly, the HTC species are developed by transforming the plants with resistant genes. For this purpose, *A. tumefaciens* or *Agrobacterium rhizogenes* are the common vectors that are used as carriers of the foreign gene. These bacteria are common flora of the soil and are responsible for the development of crown galls and hairy roots in plants. They cause infection with the help of plasmid tDNA and introduce the desired genes in plants. However, the greatest drawback of this technique is the species-specificity of the bacteria which limits its application to diverse crops. Although electroporation technique (involving the use of electric field) and protoplast fusion (using poly-ethylene glycol) can largely overcome the limitation of direct gene transfer and development of stable transformations in crops, they are not widely used. This is because the plants produced using transgenic approaches are considered as genetically modified crops (Ramessar et al., 2008). Hence, severe limitations have been posed to the development and commercialization of these varieties since the possibility

of unidentified alteration in protein expression due to genetic modifications cannot be overlooked.

In spite of the limitations posed on transgenic crop production, few crop varieties are produced and reported in the literature. For example, transgenic rice cultivars were developed using genotype-independent gene transfer method. In this method, the transgenic rice plants could be developed using organized tissues from diverse cultivars within 8–10 weeks of gene transfer. Hence, it was a promising approach to produce tolerant as well as high-yielding varieties of rice (Tsaftaris, 1996). Transgenic glufosinate resistant crop varieties of soybean, rice, vineyards, and cotton are the most common HTC in North and South America, Brazil, Canada, and Western USA. During 1993 and 1994, a widespread and continuously increasing resistance to glyphosate herbicides was observed in weeds in the above countries. Hence, the transgenic approach was adapted to replace the use of glyphosate with glufosinate herbicides (since their mechanism of action allows them to be used as alternatives) in fields with glyphosate tolerant crops (Braz et al., 2012; Takano and Dayan, 2020).

7.4.2 MUTAGENESIS

In order to overcome the shortcomings of transgenic approach, the desired trait is more effectively developed in plants with the help of physical (gamma rays) and chemical mutagenesis. Examples of commonly used chemical mutagens include ethyl methane sulfonate (EMS), ethyl nitrosourea, N-nitroso-N methyl urea, and sodium azide. The HTCs generated using these techniques are considered as nongenetically modified crops and hence they are more acceptable in the commercial market. In this approach, the mutagenesis is induced randomly using suitable mutagenic agents in large number of samples to obtain the desired mutations. The mutant varieties are then selected by exposing them to selective agents (herbicides) in different concentrations to examine the level of tolerance exhibited by the mutagens. Most commonly, the resistance to imidazolinones is developed in plants using mutagenesis. The imidazolinone tolerant crops are hence one of the frequently encountered varieties reported in the literature and available commercially. Well known among them are crops produced under the brand name of Clearfield. A few examples of imidazolinone tolerant crops are indicated in Table 7.2.

TABLE 7.2 The Imidazolinone Tolerant Crops Developed Using Mutagenesis.

Sr. No.	Crops and mutant varieties	Part of plant used	Mutagenic technique	Reference
1	Maize (Mutant 1 and 2)	Pollen	Chemical mutagenesis using EMS	Bright et al. (1992)
2	Rice (93AS3510)	Seed	Chemical mutagenesis using EMS	Croughan (2005)
3	Wheat (TealIMI 11A and other mutants)	Seed	Chemical mutagenesis using sodiumazide and EMS	Pozniak et al. (2004)
4	Rapeseed (PM1 and 2)	Microspore	Chemical mutagenesis using Ethyl nitrosourea	Barnes and Vanstraelen (2009)
5	Sunflower (CLHA-PLUS)	Seed	Chemical mutagenesis using EMS	Sala et al. (2008)
6	Soybean (W4-4)	Seed	Chemical mutagenesis using <i>N</i> -nitroso- <i>N</i> -methylurea	Sebastian (1992)
7	Lentil (RH44)	Seed	Chemical mutagenesis using EMS	Slinkard et al. (2007)

In ancient agricultural practices, the plants were selected with desired traits that occurred from spontaneous mutations. These plants were used for selective breeding to increase the population of desirable plants. Several plant species have been developed using these techniques. However, it takes several years in crop plants to decades in higher plants with longer life cycles to produce and select species with such traits. The process of chemical mutagenesis, using seedlings or different parts of plants, mimics the same process that was followed in ancient agriculture, but produces results in a few weeks to months. It also increases the frequency of mutations in plant cultivars and hence significantly increases the chances of selecting species with different desirable mutations at the same time using large number of samples. In addition, the progeny of different plants produced using mutagenesis can be crossed to obtain yet another variety of mutant plant species. This plant may possess superior traits as compared to the parent plants, and hence can be beneficial in modern agricultural practices. The major drawback of this technique, however, is the inability to detect the exact site of mutation using simple techniques. Moreover, whether or not the mutations will produce stable gene products for long-term expression of desired traits is often a question that cannot be answered with absolute surety.

The dose of mutagens and time of exposure for the treatment of plants depend on several factors including the type of mutagen, plant species, and plant organs used for the development of HTC. Similar to chemical mutagens, the use of gamma irradiation is also a popular technique for carrying out mutations and several studies have reported the use of these agents in the development of HTCs. However, till date, there are no reports of commercialized HTCs produced using gamma irradiation. Although it may seem that the chemical mutagens are more effective than physical agents, a number of factors require sincere consideration in this respect. Most importantly, the use of chemical mutagens is less expensive as compared to radiations and much easier to handle. Moreover, the accessibility of radiation sources is limited as compared to chemicals. Hence, these factors may have favored the popularity of chemical agents in practicing mutagenesis.

7.4.3 DIRECT MODIFICATION

The direct modification of the target gene by genetic engineering techniques provides suitable advantages over the transgenic and mutagenic approaches. The molecular breeding techniques use approaches to replace the target gene in wild crop species, with a genetically engineered and homologous gene

sequences with the desired functions. For example, a recently published study used the residue deletion technique to design the AHAS- Δ W548 protein in vitro and engineer the AHAS tolerant cultivars of rice plants. This approach led to the over-expression of the above protein and developed tolerance to several herbicides of the AHAS class in the transgenic lines of rice. Considering the safety and widespread use of AHAS inhibitors, the above study suggested the use of transgenic approach to overcome weed resistance to different herbicides of the same or different classes (Fang et al., 2020). Also, the selectable coediting of acetolactate synthase (Pro-174 codon) and acetyl-coenzyme A carboxylase genes was done by Zhang et al. (2019) to develop transgene-free wheat germplasms. These varieties exhibited tolerance to both AHAS and ACCase inhibitors and thus hold promising potential to improve crop yield in agricultural practices. Hence, the direct modification of wild-type genes not only imparts the desired functions in plants but also confirms its expression by replacing the target gene sequences. Moreover, depending on the extent of modification required, single as well as multiple base-pair substitutions can be engineered at single or multiple sites. However, like transgenic lines, the implementation and commercialization of these cultivars undergo strict laws and regulations of genetically modified foods. These regulations differ considerably from one country to another, as well as from one scholarly group to another. For instance, a single base pair substitution mutation induced using genetic engineering is regarded as nongenetically modified, and similar to chemical or physical mutagenesis, by some scholars (Alotaibi & Khan, 2016). This is based on the reasoning that spontaneous mutations commonly occur in nature. However, other scholars view any human attempts of engineering at the genetic level, as a manipulation of nature and debate the labeling of such products as genetically modified food/crops.

7.5 TOLERANT CROPS DEVELOPED FROM MUTATION

The AHAS, ACCase, and D1 protein of photosynthetic pathway are the most common targets for producing HTCs from mutations, after glyphosate, and glufosinate tolerant varieties. The first HTC was commercialized in 1996 and has since spread in planting area among the biotechnologically produced crops. Currently, over 87.5 million hectares of land are planted with herbicide-tolerant varieties of crops, globally. This contributes to over 45% of the total land area that produces mutant varieties of crops (ISAAA, 2020). Table 7.3 represents examples of a few HTCs developed and commercialized till now.

TABLE 7.3 Herbicide Tolerant Crops Commercialized Globally (ISAAA, 2020; Kumar et al., 2020).

Sr. No.	Crop	Herbicide tolerance	Commercially available brand name	Transgenic	Cultivated in	References
1	Cotton	Bromoxynil	BXN, Calgene	Yes	Argentina, Australia, Brazil, Canada, China, Colombia, Costa Rica, EU, Japan, Malaysia, Mexico, New Zealand, Paraguay, Philippines, Singapore, South Africa, South Korea, Taiwan, USA	Shukla et al. (2009)
		Glyphosate	Roundup Ready, Monsanto	Yes		Shu et al. (2011)
		Isoxaflutole	Liberty Link® GT27™, BASE	Yes		Cheung et al. (2004)
2	Canola	Glyphosate, Glucosinolate	InVigor, Bayer	Yes		Swanson et al. (1989), Shu et al. (2011)
3	Maize	Sulfonylurea, Imidazolinone, Glyphosate	Optimum™ GAT, Corteva Agriscience	Yes		Yi et al. (2016), Chueng et al. (2004)
		Glyphosate, Glucosinolate	Agrisure, Syngenta	Yes		Sundby et al. (1993)
4	Soybeans	ALS inhibitor	Clearfield, BASF	No	Argentina, Australia, Bolivia, Brazil, Canada, Chile, China, Colombia, Costa Rica, EU, India, Indonesia, Iran, Japan, Malaysia, Mexico, New Zealand, Nigeria, Paraguay, Philippines, Russian Federation, Singapore, South Africa, South Korea, Switzerland, Taiwan, Thailand, Turkey, USA, Uruguay, Vietnam	Takano et al. (2020)
		Glyphosate	Roundup Ready, Monsanto	Yes		Zhang et al. (2019)
		Glufosinate	Liberty link, BASF	Yes		Robinson et al. (1994)
		Imidazolinone	Cultivance, BASF	Yes		
4	Wheat	ALS inhibitors	Clearfield, BASF	No	<i>Australia, Colombia, New Zealand, USA</i>	Pozniak et al. (2004)
6	Rice	ALS inhibitors	Clearfield, BASF	No	<i>Australia, Canada, Colombia, Honduras, Mexico, New Zealand, Philippines, Russian Federation, South Africa, USA</i>	Croughan (2005)

TABLE 7.3 (Continued)

Sr. No.	Crop	Herbicide tolerance	Commercially available brand name	Transgenic	Cultivated in	References
7	Sunflower	ALS inhibitors	Clearfield, BASF	No		Sala et al. (2008)
8	Carnation	Sulfonylurea	Moondust, Moonshadow, Moonshade, Moonlite, Moonaqua, Moonvista , FLORIFINE	Yes	<i>Australia, Colombia, EU, Japan, Malaysia</i>	Robinson et al. (1994), Slinkard et al. (2007)
9	Linseed	Sulfonylurea	CDC Triffid Flax, University of Saskatchewan	Yes	<i>Canada, Colombia, USA</i>	Slinkard et al. (2007)
10	Alfalfa	Glyphosate	Roundup Ready, Monsanto	Yes	<i>Argentina, Australia, Canada, Japan, Mexico, New Zealand, Philippines, Singapore, South Korea, USA</i>	Slinkard et al. (2007), Sala et al. (2008)

7.5.1 IMIDAZOLINONE TOLERANT CROPS

The AHAS inhibitor (i.e., imidazolinone) tolerant maize, wheat, soybean, sunflower, lentil, and rice that were developed between 1992 and 2006 have been marketed under the trademark of CLEARFIELD®. These are all nontransgenic crops produced using mutagenesis. For instance, the imidazolinone tolerant maize was produced by chemical mutagenesis as well as cell culture and pollen mutagenesis. In general, the seeds were soaked in suitable chemicals and then the herbicide tolerant varieties were selected. In pollinating crops of maize, the pollens were soaked in chemical mutagens (EMS) and then used for pollinating maize crops. The herbicides were sprayed in the propagation area to kill the undesired plant and select imidazolinone tolerance trait. The XI12 and QJ22 varieties of maize possess an altered form of enzyme AHAS enzyme with an amino acid substitution (from serine to asparagine) at position 653. The imidazolinone tolerant lentil was developed using seed mutagenesis by treating it with EMS and first commercialized as CLEARFIELD® lentil in 2006 (Shu et al., 2011).

The imidazolinone tolerant rice and wheat were also developed using EMS as a chemical mutagen. The commercialized product exhibit a single nucleotide substitution mutation in their AHAS gene at position 654. Exposing rice seeds to gamma rays was also attempted by the CLEARFIELD developers; however, none of the mutant cultivars showed the desired quality. Hence, they were not commercialized. One biotype of imidazolinone tolerant sunflower was produced by CLEARFIELD with the help of seed mutagenesis. The other biotype was identified as naturally tolerant sunflower in a soybean field in Kansas, USA. The tolerant plant was crossed with many other nontolerant sunflower varieties by using traditional breeding techniques to obtain imidazolinone tolerant germplasm lines of sunflower. Few examples of these mutants include IMISUN-1, IMISUN-2, IMISUN-3, and IMISUN-4 (Shu et al., 2011).

The production of imidazolinone tolerant rapeseeds has been particularly of interest to researchers, since a long time. This is because the *Brassica* (rapeseed) species and several weed species are closely related. In addition, the rapeseed plants are the only source of canola and are an agriculturally important crop that suffers huge losses in the crop yield due to attack by several varieties of weed (Swanson et al., 1989). Hence the microspores of *Brassica napus* plant were used to develop two mutants, namely, P1 and P2 that exhibited a high degree of tolerance to commercial AHAS inhibitors (PURSUIT® and ASSERT®). Later, these two varieties were crossed to observe the resulting effect on herbicide tolerance. It was found that the

plant progeny produced by crossing P1 and P2 mutants exhibited higher tolerance to imidazolinone and were commercialized as CLEARFIELD® canola (Chueng et al., 2004).

7.5.2 GLUFOSINATE AND GLYPHOSATE TOLERANT CROPS

In 1995, the first glufosinate tolerant canola was developed. In the following years, glufosinate tolerant varieties of corn (1997), cotton (2004), and soybean (2011) have been commercialized. Apart from these crops, several transgenic lines of rice, sugar beet, and wheat were produced but never marketed. Their use for agricultural purposes has also been discontinued due to GMO regulations. Mutations in the glutamine synthase gene or its overexpression can lead to glufosinate resistance. However, the commercial varieties express high levels of either *pat* or *bar* genes that confer tolerance to glufosinate herbicide (Takano and Dayan, 2020).

The glyphosate tolerant soybeans were first produced and commercialized in 1996 (Green, 2009). It is the most commonly variety of HTC produced and preferred globally. Efforts are still being made to develop and improve new varieties of glyphosate tolerant crops. A recently developed AroAJ variety of rice was reported to tolerate up to 3360 g/ha glyphosate, that is, a fourfold concentration of the dose recommended for agricultural applications (Yi et al., 2016). In another instance, a transgenic rice species was produced that could sprout on a cell culture medium containing 2160 mg/L and transgenic lines another rice variety, in the same study, showed tolerance to glyphosate up to 8400 g/ha (Cui et al., 2016). Many crops exhibiting glyphosate tolerance modulate the enzyme functions of related genes and hence can help in the development of tolerance to other classes of herbicides as well. Considering the widespread interest and potential of glyphosate and ALS inhibiting herbicides, scientists are currently attempting to produce plant varieties tolerant to both these herbicides by combining the molecular mechanisms that metabolically inactivate glyphosate and inhibit ALS genes. Similarly, studies on combining molecular mechanisms of glyphosate resistance with auxin herbicide or ACCase inhibitors in a single crop are also believed to sustain weed management strategies in agriculturally important crops.

7.5.3 OTHER HERBICIDE TOLERANT CROPS

As compared to imidazolinone, glyphosate, and glufosinate tolerant crops, very few mutant species of crops are developed that are tolerant to other

herbicides. The aryloxyphenoxypionate and cyclohexanedione tolerant maize have been produced using callus culture and selection of sethoxydim and haloxyfop tolerant callus lines. In 1990, the potential callus lines were provided to agricultural breeding companies to develop maize hybrids with herbicide tolerant traits. Subsequently, the sethoxydim tolerant and cycloxydim tolerant maize hybrids were marketed in 1996 and are increasingly adopted by farmers. The DNA analysis of these hybrids indicated a single nucleotide substitution mutation at codon 1781 or 1769 in reference to blackgrass or wheat. The resulting alteration in ACCase genes prevents the binding of sethoxydim and cycloxydim herbicides making the crops tolerant toward them (Shu et al., 2011).

Similar to the imidazolinone tolerant sunflower identified as a result of spontaneous mutations, a triazine tolerant rapeseed was discovered in maize fields in Quebec, Canada. The triazine tolerant rapeseed cultivars were produced using the traditional breeding technique by crossing the sensitive crops with resistant species identified above. Several repeated crosses developed the desired trait in rapeseed crop, due to a nucleotide substitution mutation in the chloroplastic *psbA* gene encoding D1 protein from AGT to GGT at codon 264. These crops are marketed since the 1980s till today. However, this desired trait in rapeseed crops is accompanied by lower crop yield and oil content. Hence the requirement of possibly high crop yield over assured moderate yield needs to be weighed stringently before making a suitable choice. The decreased efficiency of the photosynthetic apparatus (due to inhibition of D1 protein) is suggested to be a contributing factor for reduced crop yields in mutant rapeseed crops (Sundby et al., 1993).

A mutation on the 197 codon position of *AHAS1* gene confers resistance to AHAS inhibitors like sulfonylurea. The sulfonylurea tolerant sunflower was also developed by breeding naturally tolerant plant varieties discovered in Kansas, USA, with domestic sunflower lines. Other varieties of sulfonylurea tolerant sunflower have been produced using chemical mutagens like EMS and selecting mutant varieties using thifensulfuron-methyl or rimsulfuron. Similarly, the sulfonylurea tolerant soybeans were produced by soaking the seeds in EMS or N-nitroso-N-methylurea and marketed since 1994 (Kolkman et al., 2004).

7.6 CONCLUSION

A large number of researches on the development of HTC plant have taken place and still continuously to improve production. All herbicide mutants are

developed from a single nucleotide substitution gene responsible for protein encoding. Commercially available herbicides are mostly incompletely dominant except triazine tolerant mutant species. These herbicide tolerant traits should be included in all selected varieties using crossing between trait donor and selected species.

KEYWORDS

- **herbicide tolerant crops**
- **cotton**
- **maize**
- **Zhonghua 11**
- **protoporphyrinogen IX oxidase**

REFERENCES

- Adhikari, P.; Goodrich, E.; Fernandes, S. B.; Lipka, A. E.; Tranel, P.; Brown, P.; Jamann, T. M. Genetic Variation Associated with PPO-Inhibiting Herbicide Tolerance in Sorghum. *Plos One* **2020**, *15* (10), e0233254. <https://doi.org/10.1371/journal.pone.0233254>.
- Alotaibi, A.; Khan, J. Kinetic Study and Basal Media Modification of Cellulase Enzyme Production by Mutated Strain of *Aspergillus Fumigatus* (AF1). *Int. J. Pharma. Bio. Sci.* **2016**, *7* (1), 193–200.
- Anthony, R. G.; Reichelt, S.; Hussey, P. J. Dinitroaniline Herbicide-Resistant Transgenic Tobacco Plants Generated by Co-Overexpression of a Mutant Alpha-Tubulin and a Beta-Tubulin. *Nat. Biotechnol.* **1999**, *17* (7), 712–716. <https://doi.org/10.1038/10931>.
- Barnes, S.; Vanstraelen, S. Assay for Imidazolinone Resistance Mutations in *Brassica* Species. US Patent 7595177, 2009.
- Barr, A. R.; Mansoorji, A. M.; Holtum, J. A. M.; Powles, S. B. The Inheritance of Herbicide Resistance in *Avena sterilis* ssp. *Ludoviciana*, Biotype SAS. *Proc. 1st Int. Weed Control Congress* **1992**, *2*, 70.
- Barry, G. F.; Kishore, G. M.; Padgett, S. R.; Stallings, W. C. (1997) Glyphosate Tolerant 5-Enolpyruvylshikimate-3-Phosphate Synthases. US Patent 5633435, 1997.
- Bernasconi, P.; Woodworth, A. R.; Rosen, B. A.; Subramanian, M. V.; Siehl, D. L. A Naturally Occurring Point Mutation Confers Broad Range Tolerance to Herbicides That Target Acetolactate Synthase. *J. Biol. Chem.* **1995**, *270* (29), 17381–17385. <https://doi.org/10.1074/jbc.270.29.17381>.
- Braz, G. B. P.; Oliveira, R. S.; Jr, Constantin, J.; Raimondi, M. A.; Franchini, L. H. M.; Biffe, D. F.; Arantes, J. G. Z. I.; Takano, H. K. Selectivity of Ammonium-Glufosinate Applied

- Alone or in Mixture with Pyriithiobac Sodium in Transgenic Liberty Link Cotton. *Planta Daninha* **2012**, 30, 853–860.
- Bright, S. W. J.; Chang, M. T.; Evans, I. J. Herbicide Resistant Plants. Patent Application of World Intellectual Property Organization, 1992. WO92/08794.
- Brookes, G.; Barfoot, P. Environmental Impacts of Genetically Modified (GM) Crop Use 1996–2015: Impacts on Pesticide Use and Carbon Emissions. *GM Crops Food* **2017**, 8, 117–147. <https://doi.org/10.1080/21645698.2017.1309490>.
- Brunharo, C. A. C. G.; Takano, H. K.; Mallory-Smith, C. A.; Dayan, F. E.; Hanson, B. D. Role of Glutamine Synthetase Isogenes and Herbicide Metabolism in the Mechanism of Resistance to Glufosinate in *Lolium perenne* L. spp. Multiflorum Biotypes from Oregon. *J. Agric. Food Chem.* **2019**, 67 (31), 8431–8440. <https://doi.org/10.1021/acs.jafc.9b01392>.
- Carbonari, C.A.; Latorre, D.O.; Gomes, G.L.; Velini, E.D.; Owens, D.K.; Pan, Z.; Dayan, F. E. Resistance to Glufosinate Is Proportional to Phosphinothricin Acetyltransferase Expression and Activity in LibertyLink (®) and WideStrike (®) Cotton. *Planta* **2016**, 243 (4), 925–933. <https://doi.org/10.1007/s00425-015-2457-3>.
- Catanzaro, C. J.; Burton, J. D.; Skroch, W. A. Graminicide Resistance of Acetyl-CoA Carboxylase from Ornamental Grasses. *Pesticide Biochem. Physiol* **1993**, 45 (2), 147–153. <https://doi.org/10.1006/pest.1993.1017>.
- Challam, T.; Thabab, S. Studying the Impact of Four Pre-Emergence Herbicides on Paddy-Field Soil. *Int. Res. J. Manage. Sci. Technol.* **2018**, 9 (3), 404–416.
- Chandrasekhar, K.; Reddy, G. M.; Singh, J.; Vani, K.; Vijayalakshmi M.; Kaul, T.; Reddy, M. K. Development of Transgenic Rice Harboursing Mutated Rice 5-Enolpyruvylshikimate 3-Phosphate Synthase (Os-mEPSPS) and *Allium sativum* Leaf Agglutinin (ASAL) Genes Confering Tolerance to Herbicides and Sap-Sucking Insects. *Plant Mol. Biol. Rep.* **2014**, 32 (6), 1146–1157. <https://doi.org/10.1007/s11105-014-0715-3>.
- Cheung, W. Y.; Gagnon, M. J.; Laforest, M.; Landry, B. S. Composition and Methods for Identifying Plants Having Increased Tolerance to Imidazolinone Herbicides, 2004. 20040142353.
- Chhapekar, S.; Raghavendrarao, S.; Pavan, G.; Ramakrishna, C.; Singh, V.K.; Phanindra, M. L.; Dhandapani, G.; Sreevathsa, R.; Kumar, A. Transgenic Rice Expressing a Codon-Modified Synthetic CP4-EPSPS Confers Tolerance to Broad-Spectrum Herbicide, Glyphosate. *Plant Cell Report* **2015**, 34 (5), 721–731. <https://doi.org/10.1007/s00299-014-1732-2>.
- Choudhury, P. P.; Singh, R.; Ghosh, D.; Sharma, A. R. Herbicide Use in Indian Agriculture. Indian Centre of Academic Research—Directorate of Weed Research, Jabalpur, Madhya Pradesh, 2016; 110 p. <https://doi.org/10.13140/RG.2.2.20822.75848>.
- Christopher, J. T.; Preston, C.; Powles, S. B. Malathion Antagonizes Metabolism-Based Chlorsulfuron Resistance in *Lolium rigidum*. *Pesticide Biochem. Physiol* **1994**, 49 (3), 172–182. <https://doi.org/10.1006/pest.1994.1045>.
- Croughan, T. P. Resistance to Acetohydroxyacid Synthase-Inhibiting Herbicides. US Patent 6943280, 2005.
- Cui, Y.; Huang, S.; Liu, Z.; Yi, S.; Zhou, F.; Chen, H.; Lin, Y. Development of Novel Glyphosate-Tolerant Japonica Rice Lines: A Step Toward Commercial Release. *Front. Plant Sci* **2016**, 7, 1218. <https://doi.org/10.3389/fpls.2016.01218>.
- Delye, C. Unravelling the Genetic Bases of Non-Target-Site Based Resistance (NTSR) to Herbicides: A Major Challenge for Weed Science in the Forthcoming Decade. *Pest Manage. Sci.* **2012**, 69 (2), 176–187. <https://doi.org/10.1002/ps.3318>.

- Devine, M. D.; Shukla, A. Altered Target Sites as a Mechanism Of Herbicide Resistance. *Crop Protection* **2000**, *19* (8–10), 881–889. [https://doi.org/10.1016/S0261-2194\(00\)00123-X](https://doi.org/10.1016/S0261-2194(00)00123-X).
- Dill, G. M.; CaJacob, C. A.; Padgett, S. R. Glyphosate-Resistant Crops: Adoption, Use and Future Considerations. *Pest Manage. Sci.*, *64* (4), 326–331. <https://doi.org/10.1002/ps.1501>.
- Dong, Y.; Jin, X.; Tang, Q.; Zhang, X.; Yang, J.; Liu, X.; Cai, J.; Zhang, X.; Wang, X.; Wang, Z. Development and Event-Specific Detection of Transgenic Glyphosate-Resistant Rice Expressing the G2-EPSPS Gene. *Front. Plant Sci* **2017**, *8*, 885. <https://doi.org/10.3389/fpls.2017.00885>.
- Duke, S. O. Taking Stock of Herbicide-Resistant Crops Ten Years After Introduction. *Pest Manage. Sci.* **2005**, *61* (3), 211–218. <https://doi.org/10.1002/ps.1024>.
- Fang, J.; Wan, C.; Wang, W.; Ma, L.; Wang, X.; Cheng, C.; Zhou, J.; Qiao, Y.; Wang, X. Engineering Herbicide-Tolerance Rice Expressing an Acetohydroxyacid Synthase with a Single Amino Acid Deletion. *Int. J. Mol. Sci* **2020**, *21* (4), 1265. <https://doi.org/10.3390/ijms21041265>.
- Galon, L.; Concenco, G.; Pinto, J. J. O.; Marques, R. F.; Andres, A. Imidazolinone-Tolerant Maize as a Tool for Weed Control in Flooded Rice Production Systems. *Maydica* **2014**, *59* (2): 129.
- Gehrke, V. R.; Camargo, E. R.; Avila, L.A. Sulfentrazone: Environmental Dynamics and Selectivity. *Planta Daninha* **2020**, *38*. <http://dx.doi.org/10.1590/s0100-83582020380100032>.
- Ghanizadeh, H.; Mesarich, C. H.; Harrington, K. C. Molecular Characteristics of the First Case of Haloxypop-Resistant Poa Annu. *Sci. Rep.* **2020**, *10* (1), 4231. <https://doi.org/10.1038/s41598-020-61104-0>
- Green, J. Review of Glyphosate and ALS-Inhibiting Herbicide Crop Resistance and Resistant Weed Management. *Weed Technol.* **2007**, *21* (2), 547–558. <https://doi.org/10.1614/WT-06-004.1>.
- Green, J. Evolution of Glyphosate-Resistant Crop Technology. *Weed Sci.* **2009**, *57* (1), 108–117. <https://doi.org/10.1614/WS-08-030.1>.
- Gronwald, J. W. Resistance to Photosystem II Inhibiting Herbicides. In *Herbicide Resistance in Plants: Biology and Biochemistry*; Powles, S. B., Holtum, J. A. M., Eds.; CRC Press: Boca Raton, 1994; pp. 27–60. https://doi.org/10.1007/978-94-011-5538-0_5.
- Guo, W.; Zhang, L.; Wang, H.; Li, Q.; Liu, W.; Wang, J. A Rare Ile-2041-Thr Mutation in the ACCase Gene Confers Resistance to ACCase-Inhibiting Herbicides in Shortawn Foxtail (*Alopecurus aequalis*). *Weed Sci.* **2017**, *65* (2), 239–246. <https://doi.org/10.1017/wsc.2016.32>.
- Hall, L. M.; Stromme, K. M.; Horsman, G. P.; Devine, M. D. Resistance to Acetolactate Synthase Inhibitors and Quinclorac in a Biotype of False Cleavers (*Galium spurium*). *Weed Sci.* **1998**, *46* (4), 390. <https://doi.org/10.1017/S0043174500090780>.
- Hofer, U.; Muehlebach, M.; Hole, S.; Zoschke, A. Pinoxaden—For Broad Spectrum Grass Weed Management in Cereal Crops. *J. Plant Dis. Protection* **2006**, *113* (20).
- ISAAA Database, 2019. GM Approval Database. <https://www.isaaa.org/gmapprovaldatabase/default.asp> (accessed Dec 14, 2020).
- ISAAA database, 2020. GM Approval Database. <https://www.isaaa.org/resources/publications/pocketk/10/default.asp> (accessed Dec 16, 2020).
- Jablonskai, I.; Hatzios, K. K. Microsomal Oxidation of the Herbicides EPTC and Acetochlor and of the Safener MG-191 in Maize. *Pesticide Biochem. Physiol.* **1994**, *48* (2), 98–109. <https://doi.org/10.1006/pest.1994.1011>.

- Jabran K. Weed Flora, Yield Losses and Weed Control in Cotton Crop. *Julius Kühn Arch.* **2016**, 452, 177–182. <https://doi.org/10.5073/JKA.2016.452.023>.
- Karthik, K.; Nandiganti, M.; Thangaraj, A.; Singh, S.; Mishra, P.; Rathinam, M.; Sharma, M.; Singh, N. K.; Dash, P. K.; Sreevathsa, R. Transgenic Cotton (*Gossypium hirsutum* L.) to Combat Weed Vagaries: Utility of an Apical Meristem-Targeted in Planta Transformation Strategy to Introgress a Modified CP4-EPSPS Gene for Glyphosate Tolerance. *Front. Plant Sci.* **2020**, 11, 768. <https://doi.org/10.3389/fpls.2020.00768>.
- Khan, J.; Alotaibi, A. and Deka, M. Effect of Colchicine Induced Mutation on Cellulose Enzyme Production by *Aspergillus fumigatus*. *World J. Pharma. Res.* **2015**, 4, 461–471.
- Kolkman, J. M.; Slabaugh, M. B.; Bruniard, J. M.; Berry, S.; Bushman, B. S.; Olungu, C.; Maes, N.; Abratti, G.; Zambelli, A.; Miller, J. F.; Leon, A.; Knapp, S. J. Acetohydroxyacid Synthase Mutations Conferring Resistance to Imidazolinone or Sulfonylurea Herbicides in Sunflower. *Theor. Appl. Genet.* **2004**, 109 (6), 1147–1159. <https://doi.org/10.1007/s00122-004-1716-7>.
- Krenchinski, F. H.; Carbonari, C. A. S.; Cesco, V. J. P.; Albrecht, A. J.; Campos Arcuri, M. L.; de Godoy Maia, I. and Velini, E. D. Glufosinate Resistance Level Is Proportional to Phosphinothricin Acetyltransferase Gene Expression in Glufosinate-Resistant Maize. *J. Agric. Food Chem.* **2018**, 66 (48), 12641–12650. <https://doi.org/10.1021/acs.jafc.8b04823>.
- Kumar, K.; Gambhir, G.; Dass, A.; Tripathi, A. L.; Singh, A.; Jha, A. K.; Yadava, P.; Choudhary, M.; Planta, S. R. Genetically Modified Crops: Current Status and Future Prospects. *Planta* **2020**, 251, 91. <https://doi.org/10.1007/s00425-020-03372-8>.
- LeBaron, H. M. Distribution and Seriousness of Herbicide-Resistant Weed Infestations Worldwide. In *Herbicide Resistance in Weeds and Crops*; Caseley, J. C.; Cussans, G. W.; Atkin, R. K., Eds.; Butterworth-Heinemann: Oxford, UK, 1991; pp 27–43.
- Li, X.; Nicholl, D. Development of PPO Inhibitor-Resistant Cultures and Crops. *Pest Manage. Sci.* **2005**, 61 (3), 277–285. <https://doi.org/10.1002/ps.1011>.
- Nandula, V. K. Herbicide Resistance Traits in Maize and Soybean: Current Status and Future Outlook. *Plants* **2019**, 8 (9), 337. <https://doi.org/10.3390/plants8090337>.
- Padgett, S. R.; Kolacz, K. H.; Delannay, X.; Re, D. B.; LaVallee, B. J.; Tinius, C. N.; Rhodes W. K.; Otero, Y. I.; Barry, G. F.; Eichholz, D. A.; Peschke, V. M.; Nida, D. L.; Taylor, N. B.; Kishore, G. M. Development, Identification and Characterization of a Glyphosate-Tolerant Soybean Line. *Crop Sci.* **1995**, 35 (5), 1451–1461. <https://doi.org/10.2135/cropsci1995.0011183x003500050032x>.
- Pavlista, A. D.; Cyamid. Tolerance of Corn to Pendimethalin as Affected by Planting Depth, 1983.
- Powles, S. B.; Yu, Q. Evolution in Action: Plants Resistant to Herbicides. *Annu. Rev. Plant Biol.* **2010**, 61, 317–347. <https://doi.org/10.1146/annurev-arplant-042809-112119>.
- Powles, S. B.; Preston, C.; Bryan, I. B.; Jutsum, A. R. Herbicide Resistance: Impact and Management. *Adv. Agron.* **1997**, 58, 57–93. [https://doi.org/10.1016/S0065-2113\(08\)60253-9](https://doi.org/10.1016/S0065-2113(08)60253-9).
- Pozniak, C. J.; Birk, I. T.; O'Donoghue, L. S.; Ménard, C.; Hucl, P. J.; Singh, B. K. Physiological and Molecular Characterization of Mutation-Derived Imidazolinone Resistance in Spring Wheat. *Crop Sci.* **2004**, 44 (4), 1434–1443. <https://doi.org/10.2135/cropsci2004.1434>.
- Ramessar, K.; Capell, T.; Twyman, R. M.; Quemada, H.; Christou, P. Trace and Traceability—A Call for Regulatory Harmony. *Nat. Biotechnol.* **2008**, 26 (9), 975–978. <https://doi.org/10.1038/nbt0908-975>.

- Rendina, A. R.; Felts, J. M. Cyclohexanedione Herbicides Are Selective and Potent Inhibitors of Acetyl-CoA Carboxylase from Grasses. *Plant Physiol.* **1988**, *86* (4), 983–986. <https://doi.org/10.1104/pp.86.4.983>.
- Robinson, D. K.; Monks, D. W.; Schultheis, J. R. Effect of Nicosulfuron Applied Postemergence and Post-Directed on Sweet Corn (*Zea mays*) Tolerance. *Weed Technol.* **1994**, *8* (3), 630–634.
- Ryan, G. F. Resistance of Common Groundsel to Simazine and Atrazine. *Weed Sci.* **1970**, *18* (5), 614–616. <http://www.jstor.org/stable/4041888>.
- Sala, C. A.; Bulos, M.; Echarte, M. Molecular and Biochemical Characterization of an Induced Mutation Conferring Imidazolinone Resistance in Sunflower. *Theor. Appl. Genet.* **2008**, *118*, 105–112.
- Sathasivan, K.; Haughn, G. W.; Murai, N. Molecular Basis of Imidazolinone Herbicide Resistance in *Arabidopsis thaliana* var Columbia. *Plant Physiol.* **1991**, *97* (3), 1044–1050. <https://doi.org/10.1104/pp.97.3.1044>.
- Sauer, H.; Wild, A.; Rühle, W. The Effect of Phosphinothricin (Glufosinate) on Photosynthesis II. The Cause of Inhibition of Photosynthesis. *Zeitschrift für Naturforschung C* **1987**, *42* (3), 270–278. <https://doi.org/10.1515/znc-1987-0317>.
- Schonbrunn, E.; Eschenburg, S.; Shuttleworth, W. A.; Schloss, J. V.; Amrhein, N.; Evans, J. N.; Kabsch, W. Interaction of the Herbicide Glyphosate with Its Target Enzyme 5-Enolpyruvylshikimate 3-Phosphate Synthase in Atomic Detail. *Proc. Natl. Acad. Sci. USA* **2001**, *98* (4), 1376–1380. <https://doi.org/10.1073/pnas.98.4.1376>.
- Schweizer, E. E. Crop Response to Soil Applications of Ethofumesate. *Weed Sci.* **1975**, *23* (5), 409–413. <https://doi.org/10.1017/S0043174500062779>.
- Sebastian, S. A. Soybean Plants with Dominant Selectable Trait for Herbicide Resistance. US Patent 5084082, 1992.
- Shu, Q. Y.; Forster, B. P.; Nakagawa, H., Eds. Plant Mutation Breeding and Biotechnology. In *Plant Breeding and Genetics. Division of Nuclear Techniques in Food and Agriculture International Atomic Energy Agency*, Vienna, Austria, 2011.
- Shukla, V. K.; Doyon, Y.; Miller, J. C.; DeKever, R. C.; Moehle, E. A.; Worden, S. E. et al. Precise Genome Modification in the Crop Species *Zea Mays* Using Zinc-Finger Nucleases. *Nature* **2009**, *459* (7245), 437–441.
- Shuvobrata, M.; Datta, S.; Sarkar, C.; Saha, S. C.; Datta, S. K. The Development of *Macrophomina phaseolina* (Fungus) Resistant and Glufosinate (Herbicide) Tolerant Transgenic Jute. *Front. Plant Sci.* **2018**, *9*, 920. <https://doi.org/10.3389/fpls.2018.00920>.
- Simoneaux, B. J.; Gould, T. J. Plant Uptake and Metabolism of Triazine Herbicides. In *The Triazine Herbicides*, 2008. <https://doi.org/10.1016/B978-044451167-6.50010-6>.
- Sinning, I.; Koepke, J.; Michel, H. Recent Advances in the Structure Analysis of *Rhodospseudomonas viridis* Mutants Resistant to the Herbicide Terbutryn. In *Reaction Centers of Photosynthetic Bacteria*; Michel-Beyerle, M. E., Eds.; Springer-Verlag: Berlin, 1990; p 199.
- Slinkard, A. E.; Vanderberg, A.; Holm, F. Lentil Plants Having Increased Resistance to Imidazolinone Herbicides. US Patent 7232942, 2007.
- Stoltenberg, D. E.; Gronwald, J. W.; Wyse, D. L.; Burton, J. D.; Somers, D. A.; Gengenbach, B. G. Effect of Sethoxydim and Haloxypop on Acetyl-Coenzyme A Carboxylase Activity in Festuca Species. *Weed Sci.* **1989**, *37* (4), 512–516. <https://doi.org/10.1017/S0043174500072325>.

- Sundby, C.; Chow, W. S.; Anderson, J. M. Effects on Photosystem II Function, Photoinhibition, and Plant Performance of the Spontaneous Mutation of Serine-264 in the Photosystem II Reaction Center D1 Protein in Triazine-Resistant *Brassica napus* L. *Plant Physiol.* **1993**, *103* (1), 105–113. <https://doi.org/10.1104/pp.103.1.105>.
- Swanson, E. B.; Herrgesell, M. J.; Arnoldo, M.; Sippell, D. W.; Wong, R. S. C. Microspore Mutagenesis and Selection: Canola Plants with Field Tolerance to the Imidazolinones. *Theor. Appl. Genet.* **1989**, *78*, 525–530. <https://doi.org/10.1007/BF00290837>.
- Takano, H. K.; Dayan, F. E. Glufosinate-Ammonium: A Review of the Current State of Knowledge. *Pest Manage. Sci.* **2020**, *76* (12), 3911–3925. <https://doi.org/10.1002/ps.5965>.
- Tan, S.; Evans, R. R.; Dahmer, M. L.; Singh, B. K.; Shaner, D. L. Imidazolinone-Tolerant Crops: History, Current Status and Future. *Pest Manage. Sci.* **2005**, *61* (3), 246–257. <https://doi.org/10.1002/ps.993>.
- The Editors of Encyclopaedia Britannica. Herbicide, 2019. <https://www.britannica.com/science/herbicide> (accessed Dec 10, 2020).
- Tian, Y. S.; Xu, J.; Peng, R. H.; Xiong, A. S.; Xu, H.; Zhao, W.; Fu, X. Y.; Han, H. J.; Yao, Q. H. Mutation by DNA Shuffling of 5-enolpyruvylshikimate-3-Phosphate Synthase from *Malus domestica* for Improved Glyphosate Resistance. *Plant Biotechnol. J.* **2013**, *11* (7), 829–838. <https://doi.org/10.1111/pbi.12074>.
- Tian, Y.; Xu, J.; Xing, X.; Zhao, W.; Fu, X.; Peng, R.; Yao, Q. H. Improved Glyphosate Resistance of 5-Enolpyruvylshikimate-3-Phosphate Synthase from *Vitis vinifera* in Transgenic Arabidopsis and Rice by DNA Shuffling. *Mol. Breed.* **2015**, *35* (7), 148. <https://doi.org/10.1007/s11032-015-0327-0>.
- Tsaftaris, A. The Development of Herbicide-Tolerant Transgenic Crops. *Field Crops Res.* **1996**, *45* (1–3), 115–123. [https://doi.org/10.1016/0378-4290\(95\)00064-X](https://doi.org/10.1016/0378-4290(95)00064-X).
- Van Oorschot, J. L. P. Types of Selective Action by Herbicides Which Inhibit Photosynthesis. *Z. Naturforsch.* **1979**, *34*, 900–904. <https://doi.org/10.1515/znc-1979-1103>.
- Webb, S. R.; Hall, J. C. Auxinic-Herbicide Resistant and Susceptible Wild Mustard (*Sinapis arvensis* L.) Biotypes: Effect of Auxinic Herbicides on Seedling Growth and Auxin-Binding Activity. *Pesticide Biochem. Physiol.* **1995**, *52* (2), 137–148. <https://doi.org/10.1006/pest.1995.1038>.
- Xu, L.; Liu, H.; Kilian, A.; Bhoite, R.; Liu, G.; Si, P.; Wang, J.; Zhou, W.; Yan, G. QTL Mapping Using a High-Density Genetic Map to Identify Candidate Genes Associated with Metribuzin Tolerance in Hexaploid Wheat (*Triticum aestivum* L.). *Front. Plant Sci.* **2020**, *11*, 573439. <https://doi.org/10.3389/fpls.2020.573439>.
- Yi, S. Y.; Cui, Y.; Zhao, Y.; Liu, Z. D.; Lin, Y. J.; Zhou, F. A Novel Naturally Occurring Class I 5-Enolpyruvylshikimate-3-Phosphate Synthase from *Janibacter* sp. Confers High Glyphosate Tolerance to Rice. *Sci. Rep.* **2016**, *6*, 19104. <https://doi.org/10.1038/srep19104>.
- Zhang, R.; Liu, J.; Chai, Z.; Chen, S.; Bai, Y.; Zong, Y.; Chen, K.; Li, J.; Jiang, L.; Gao, C. Generation of Herbicide Tolerance Traits and a New Selectable Marker in Wheat Using Base Editing. *Nat. Plants* **2019**, *5*, 480–485. <https://doi.org/10.1038/s41477-019-0405-0>.
- Zhang, X. B.; Tang, Q. L.; Wang, X. J.; Wang, Z. X. Development of Glyphosate-Tolerant Transgenic Cotton Plants Harboring the G2-aroA Gene. *J. Integrat. Agric.* **2017**, *16*, 551–558. [https://doi.org/10.1016/s2095-3119\(16\)61458-2](https://doi.org/10.1016/s2095-3119(16)61458-2).
- Zhao, T.; Lin, C.; Shen, Z. Development of Transgenic Glyphosate-Resistant Rice with G6 Gene Encoding 5-Enolpyruvylshikimate-3-Phosphate Synthase. *J. Integrat. Agric.* **2011**, *10*, 1307–1312. [https://doi.org/10.1016/S1671-2927\(11\)60123-5](https://doi.org/10.1016/S1671-2927(11)60123-5).



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CHAPTER 8

Plant Mutagenesis: Terms and Applications

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ABSTRACT

Feeding the exponentially growing populations around the globe under changing climatic conditions is going to be a cumbersome task. The enhancement in food production is seriously and urgently needed to meet this huge demand as the world's population is expected to be 9 billion by 2050. One of the most effective tools to enhance food production by creating useful genetic variations in plants, i.e., induced mutagenesis, has been widely used to generate genetic diversity and identify the essential genetic variants for economically significant features for crop development. Numerous methods, including insertional, chemical, and physical mutagen treatments, can be utilized to cause mutations in crop plants. Induced mutagenesis has come a long way since the epoch-making discoveries by Muller (1928) and Stadler (1928) in *Drosophila* and maize by using X-rays. More than 3000 mutant varieties, with one or more improved characteristics, of different crop plants, such as grain crops, oil crops, horticultural and ornamental crops, have been developed and released in different parts of the world. In the 21st century, induced mutagenesis, coupled with ultramodern biological techniques, plays

a pivotal role in creating useful genetic variation and its subsequent utilization in crop improvement programmes.

8.1 INTRODUCTION

By 2050, human population on earth is expected to increase up to 9 billion. To mitigate the challenge of possible food scarcity due to the expected increase in the human population, there is an urgent need to increase food production. Apart from the population, there are other factors that daunt the agricultural productivity, such as varying climatic conditions, depleted arable lands and water resources, insects and pest attack. Further, the increasing use of agrochemicals costs for environment and health. The only viable option for increased production is the development of smart crops with high yield and resistant to biotic and abiotic stresses. Although many new crop varieties are prone to the same kind of stresses because of genetic similarities amongst them which limits the unmasking of novel alleles of the genes and subsequently the new combination of genes that can break the yield barrier and enhance resistance toward abiotic and biotic factors. Mutation-based breeding mainly focuses on increased yield and improved quality by modifying one or more underlying trait (Bhat et al., 2021; Raina et al., 2021; Goyal et al., 2021; Laskar et al., 2021). Induced mutation played pivotal role in the development of smart crop varieties resilient to changing climatic conditions. Over the years, by using chemical and physical mutagens, more than 3200 mutant varieties of different crop plants have been released worldwide for cultivation. Furthermore, these mutant varieties could be used as a genomics and proteomics resource for future crop improvement programs. The use of induced mutation over the past six decades has played a major role in the development of superior plant varieties all over the world. After the epoch-making discoveries made by Mullar (1927) and Stadler (1928), a large amount of genetic variability has been induced by various mutagens in the most economically important plant species, and a small portion of those induced variations has resulted in the development of more than 3364 mutant varieties worldwide in about 180 plant species during the past 70 years (MVD, 2021). Among the mutant varieties, the majority are of food crops. Induced mutations are considered as an alternative to naturally occurring genetic variation that serves as the source of germplasm for crop improvement program and also an alternative to hybridization and recombination in plant breeding since mutations give rise to nonexisting variations (Brock, 1970). It is an effective tool in hands of plant breeders especially in crop with narrow genetic base. Many mutants have been identified as donors of desirable traits in breeding programs.

8.2 MUTATION, MUTAGENESIS, AND MUTANTS

8.2.1 MUTATION

Mutation, a term coined by De Vries (1901) for the appearance of a new phenotype in *Oenothera lamarckiana*, is the sudden heritable change in the genotype of an organism. Mutation is the ultimate source of all genetic changes which provides the raw material for evolution and is a very valuable approach for the improvement of economic characters in plants. It is a process by which the genetic information is changed in a stable manner either in nature or experimentally by the use of chemicals or radiations. These chemicals and radiations, with the quality of acting upon the genetic material, are called mutagens. Radiations, also called physical mutagens, include ionizing radiations, for example, α -rays, β -rays, fast neutrons, thermal neutrons, X-rays, and γ -rays, and nonionizing radiation, for example, ultraviolet (UV) radiation. Chemical mutagens include alkylating agents such as ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), ethylene imines, diethyl sulfate (DES), etc., azides, for example, sodium azide, acridine dyes, for example, acriflavine, acridine orange, proflavin, etc., base analogues, for example, 5 Bromouracil, 2 Aminopurine, 5 chlorouracil, etc., and other direct-acting chemicals, such as nitrous acid, mustard gas, etc.

Physical mutagens predominantly induce chromosomal aberrations either through ionization of the target itself or indirectly through mutagenic free radicals as a result of ionization of background components. Chemical mutagens have shown evidence of more specific action than the physical mutagens, which act at random. Many cases of mutagenic specificity have been found to be regional (with reference to the chromosome) rather than genic. Though there were several attempts to induce mutations by chemical agents (Westergard, 1957; Gustafsson, 1969), the first definite evidence that chemical agents can induce mutations was given by Auerbach and Robson (1942) in *Drosophila* by using mustard gas and Oehlkers (1943) in *Oenothera* by urethane. It was clear from the pioneering studies in Sweden that alkylating agents are particularly suited for mutagenic studies in plants (Ehrenberg and Gustafsson, 1957; Ehrenberg, 1960). USA, Europe, Japan, and China emerged as great centers for induced mutagenesis work in the 1950s on this planet (Chopra, 2005).

8.2.2 MUTAGENESIS

In the beginning of the 20th century, the researchers indicated that the comparatively slow rate of usual mutation in cereals and *Drosophila* could

be amplified several times with the treatment of X-rays. These experiments of mutation induction came before the evidences proving about the DNA as a heritable material. It also provides the advantage of induced mutagenesis in crop improvement programs; the prior information about any genes or its function is not necessary for the successful creation of plants having improved economic characters and subsequent release of such plants, after multilocal trial, as a new variety. Mutagenesis can be accomplished by the use of chemical and physical mutagens and other biological methods. From the beginning, induced mutagenesis has been achieved by different types of alkylating agents, azo dyes, and radiation especially the gamma radiations. This type of mutation induction is random and the mutants produced by this process are called “induced mutant.” When induction of mutation is achieved by the insertion of T-DNA by genetic transformation or transposon elements activation then it is called insertion mutagenesis and transposon mutagenesis, respectively, and the mutant are known as insertion mutants. When the mutations are created at a specific locus in the DNA by the genetic transformation it is called site-directed mutagenesis. It is now well established that mutagenic agents are effective for inducing genetical changes in the treated population of different plant species including *Triticale* (Viswanathan and Reddy, 1998), *Lens culineris* (Reddy and Annadurai, 1992; Verma et al., 1999), *Vigna radiata* (Khan et al, 1999), *Triticum aestivum* (Kalia et al., 2000), *Vicia faba* (Bhat et al., 2005a, b; 2006a, b, 2007; Khurshed et al., 2015, 2016, 2017, 2018), *Trigonella foenum-graecum* (Jabee et al., 2007), *Helianthus annuus* (Khurshed et al., 2009), *Capsicum annum* (Abdul Salam et al., 2010; Sri Devi et al., 2011; Gulfishan et al., 2010, 2011, 2012, 2013, 2015), Chickpea (Laskar et al., 2015; Raina et al., 2017), Lentil (Laskar et al., 2018), *Hordeum vulgare* (Jafri et al., 2011, 2012, 2013), *Solanum melongena* (Shahab et al., 2018), *Vigna mungo* (Goyal et al., 2019), *Vigna unguiculata* and cowpea (Raina et al., 2018, 2020) and produced mutants with improved characteristics over their wild relatives.

8.2.3 MUTANTS

Individual plants developed through induced mutagenesis, insertion mutagenesis, or site-directed mutagenesis strategies and with improved economic characteristics over their parents. The mutation in such plants can be verified by molecular methods or by phenotyping tools. There are 3364 mutants of different plants that have been released so far and India has contributed with 341 mutant varieties of different plants (MVD, 2021).

In any genetic research program or breeding programs, the generation progression is a crucial factor. P_1, P_2, P_3, P_4 are usually accepted terms that marks the parent 1, 2, 3, and 4, etc. In cross-breeding or hybridization program, the P_1 becomes F_1 , that is, the first filial generation or F_1 generation, and so on. In the same pattern, the first generation of mutated plants is termed as M_1 generation and so on but the untreated seeds are termed as M_0 . The seeds of M_1 generation are used to grow M_2 generation and so on. The embryo which is generated by such cross where the embryo sac or pollens have undergone mutagenic treatment; the subsequent generation is also known as the first generation of mutation and is represented as M_1 . The code M_1V_1, M_2V_2 is used for the first generation of vegetatively propagated plants, though, sometime it may be presented as MV_1, MV_2 , etc. In the same line of notation, SV_1, SV_2 represents the variants obtained through somaclonal mutagenesis. In the case of mutants obtained through transformation, techniques are represented as T_0 in the first generation and later on denoted as T_1, T_2 , etc. The selection of mutants, that is, identification of target mutant phenotype can be achieved by using two methods; mutant screening and mutant confirmation. Mutants screening is the isolation of mutants with the desired character from a large population, for example, M_2 plants mature 5 days before their parent plant and can be regarded as potential early maturing mutants. Since maturity is dependent on genotypic and environmental factors than it may be considered as putative mutants means that they may or not be a true mutants. The same may happen with the disease resistance as the pathogen may be absent in the surrounding environment of the crop plants. A putative mutant undergoes for confirmation by a process called re-evaluation where selected putative M_2 mutant lines are grown and the selection has been made in M_3 lines. Sometimes many mutants such as disease resistance, yield, and quality may be proved false mutants.

8.3 APPLICATION OF MUTATION TECHNIQUES IN CROP BREEDING

8.3.1 ENHANCEMENT OF GENETIC VARIABILITY

The induction of mutation through chemical or physical mutagen occurs randomly without any choice of specific genes or alleles in the whole genome. This is one of the most significant characteristics of mutagenesis as it offers the chance of creating mutations for any desired gene/s as well as allows the multiple mutation development for any specific gene in a prognostic method. These several mutant alleles are the source of the genetic variability for plant breeding and targeted gene analysis. Mutations, the change in genetic material, provided the basic raw material for species evolution

without any impact on its wild form or wild relative. The newly evolved species slowly adjusted to the natural environment and later on can be used by man in the process of agricultural domestication and crop improvement. In nature, the rate of spontaneous mutations with the trait or characters of economic importance is quite low yet the success in the breeding program was accomplished by harnessing these very few beneficial natural mutations in crop plants. One of the great examples of such practice was the development of semidwarf mutants of rice and wheat during the “Green Revolution.” The genes responsible for the dwarfism in rice and wheat were developed spontaneously from Norin-10 and Dee-geo-woo-gen varieties of wheat and rice, respectively. These two mutants cross-breed for short height and stiff stem which produced high grain yield and enhanced mechanical strength when supplied with suitable fertilizer. Exceptional enhancements in yield were recorded which solve the scarcity of food in many parts of the world at that time. India’s status changed from “importer” to “exporter” of wheat after this remarkable discovery of wheat and rice mutants.

Since its discovery, the process of induced mutagenesis has come to a long way and added genetic variability, without affecting its wild gene pool, in many crop plants with narrow genetic base. It also supplements the wild germplasm and improves the existing varieties in specific traits of economic importance. Several researchers used induced mutagenesis in different crop plants, such as *V. faba* L. (Bhat et al., 2005, 2006, 2007, 2015, 2017, 2019; Khursheed et al., 2018, 2019), Cowpea (Raina and Khan, 2020), Lentil (Wani et al., 2021, Laskar, 2018,), *V. mungo* (Goyal et al., 2019, 2020), *Trigonella* (Hasan et al., 2018), *Nigella* (Raina et al., 2019), etc., and increase the genetic variability along with their wild genotypes.

8.3.2 DEVELOPMENT OF MUTANTS IN DIFFERENT CROP PLANTS

Induced mutagenesis involves a three-step process, namely, (1) induction of mutation, (2) screening and identification of possible or putative mutant, and (3) testing of mutants and official release (Fig. 8.1). The testing and official release of mutants is specific for a country and not dependent on the research and development activities to improve efficiencies (Jankowicz-Cieslak et al., 2017). Generally, the choice of mutagen used in mutation breeding programs has been based on the past successful report of the particular mutagen for a particular crop plant species as well as the accessibility of mutagens, expenses, and infrastructure facilities (Bado et al., 2015; Mba, 2013; Gulfishan, 2015; MVD, 2021). Among the chemical and physical mutagens,

the ionizing radiation, that is, gamma rays proved to be one of the powerful mutagen in terms of mutant development in different crop plants (Table 8.1) while among the physical mutagens, EMS proved to be the most efficient mutagen with the highest number of mutants (Table 8.2). (MVD, 2021). In India, 341 mutants of different crop plants have been released. The mutants with improved traits (general and specific) have been presented in Tables 8.3 and 8.4. The data on mutant varieties on MVD are mainly categorized into five different categories: quality and nutrition, agronomic and botanic trait, resistance to biotic stress, tolerance to abiotic stress, and yield and contributors. For 3364, officially registered mutant varieties show improvement in more than one character, for example, a mutant with enhanced yield also showed resistance to biotic stress (Fig. 8.1).

TABLE 8.1 Mutants Developed by Various Physical Mutagens in Crop Plant (MVD, 2021).

S.N.	Name of mutagens	Numbers of mutants
1.	Gamma rays	1703
2.	X-rays	561
3.	Aerospace	46
4.	Laser	26
5.	Neutrons	24
6.	Ion beams	20
7.	Beta rays	16
8.	Electron	10
9.	Ultrasound	01
10.	UV light	01

TABLE 8.2 Mutants Developed by Various Chemical Mutagens in Crop Plant (MVD, 2021).

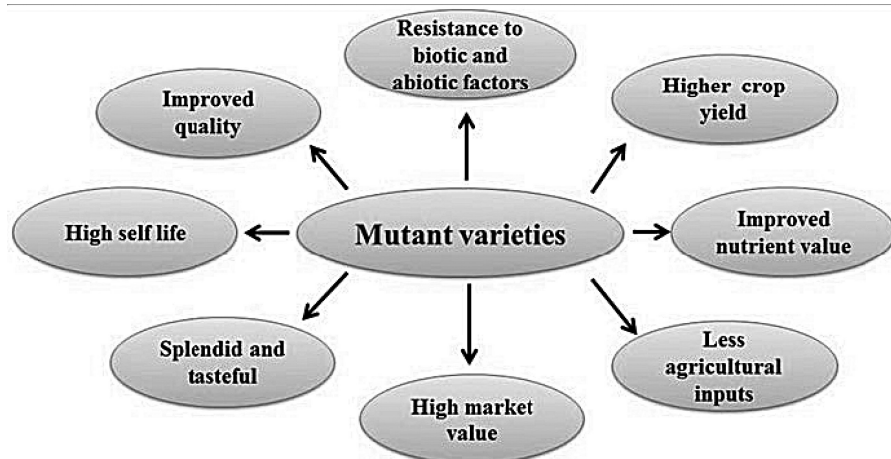
S.N.	Name of mutagens	Numbers of mutants
1.	EMS	108
2.	NEU	57
3.	Calcium	47
4.	E1	36
5.	DMS	19
6.	DES	14
7.	Tissue culture induced	06
8.	NMU+NEU	02
9.	SA	02
10.	ENH	01

TABLE 8.3 Mutants with Improved Character (General) (MVD, 2021).

S.N.	Improved character	No. of mutants
1	Agronomic and botanic traits	2987
2	Quality and nutrition	1174
3	Yield and contributors	1029
4	Resistant to biotic stress	558
5	Tolerance to abiotic stress	248

TABLE 8.4 Mutants with Improved Character (Specific) (MVD, 2021).

S.N.	Improved character	No. of mutants
1	Drought tolerant	103
2	Disease resistance	34
3	Salinity tolerance	32
4	Early maturity	31
5	Fiber quality	02

**FIGURE 8.1** A mutant with more than one improved characters (Gulfishan et al., 2015).

8.4 MUTATION BREEDING MILESTONE

Sufficient genetic variations for the desired character are the prerequisite for any crop breeding program. The required genetic variations, for example, tolerance to abiotic stress, disease resistance, etc., is accessible from the wild gene pool of the crop plants. Sometimes, however, the required wild gene pool is present in distantly related gene pool or wild relative or old

landraces or outdated varieties. Recovering and using these variations to develop a genetically stable new variety is long-drawn-out process with the least interest of the scientist or breeders.

Hugo de Vries in his mutation theory documented the prospective of induced mutation in plant breeding and stated: “We may search for mutable plants in nature, or we may hope for species to become mutable by artificial methods. The first promises to yield results most quickly, but the scope of the second is much greater and it may yield results of far more importance. Indeed, if it once should become possible to bring plants to mutate at our will and perhaps even in arbitrarily chosen directions, there is no limit to the power we may finally hope to gain over nature.” Since its inception, the milestone in the history of mutation breeding has been shown in Table 8.5.

TABLE 8.5 Milestones in Mutation Breeding (Forster and Shu, 2012).

300 BC	“Lulan” an old Chinese book, for the first time, described the selection of mutants in plant breeding
1590	“Lulan” was the first spontaneous mutant of greater celandine
1667	Graft-chimera; Bizarria-orange was the first described mutant in Florence, Italy
1672	In Waare Oefeninge der Planten, an oldest publication, describe the variability in shrubs, trees, and herbaceous plants
1774 onwards	Linnaeus described numbers of mutants in wild and cultivated species
1865	Mendel’s law published
1900	Mendal’s laws are approved by the scientific community
1901–1904	Induction of mutation by radiation was described by de Vries
1907	Examples of spontaneous mutations were published by Cramer
1927	Induction of mutation in <i>Datura</i> by using radium ray was the first proof of induction of mutation
1927	Muller described the induction of mutation in <i>Drosophila</i> by using X-rays
1927	First mass selection method for mutants was given by von Sengbusch
1928	Stadler, for the first time, described induction mutation in crop plants such as wheat, barley, oat, and maize
1930s	USA, Sweden, and Germany setup mutation breeding programs
1936	“Chlorina” was the first mutant variety of tobacco. X-ray was used as mutagen

TABLE 8.5 *(Continued)*

1942	Induction of disease resistance in barley by using X-ray
1944	Freisleben and Lein coined the term mutation breeding (“Mutationszüchtung”)
1944/46	Induction of mutation by chemical (Auerbach and Robson, 1944)
1949	Use of ⁶⁰ Co started for induction of mutation in crop plants
1954	First mutant variety of tulip with improved color of flower and pattern released
1964	To enhance the production of induced mutagenesis, a joint division of FAO/IAEA was build-up to ensure the food security specially for developing countries
1966	“Luther” a mutant variety of barley was produced by chemical mutagenesis
1972	FAO/IAEA started publishing “Mutation Breeding Newsletter”
1993	FAO/IAEA started the register of plant mutant varieties which, in 2008, changed to mutant variety genetic stock database (http://mvgs.iaea.org)
2000 onwards	Site directed mutagenesis, development of TILLING populations, development of modern genome editing tools, high-throughput genotyping, and phenotyping

8.5 CONCLUSION AND FUTURE PROSPECTS

With 3364 mutant varieties developed in different crop plants round the globe, mutation breeding program proved successful and provide food to the world. The unprecedented growth in population which is supposed to touch 9 billion by 2050 certainly might create food shortage and to cope with this challenge induced mutagenesis definitely be one of the forefront strategies. Within the past two Freisleben and Lein decades, the advent of new biological techniques supported the mutation breeding research. The genetic analysis of population or individual leads to the enhancement in experimental mutagenesis and helps in gene function determination. We assumed that induced mutagenesis coupled with ultramodern techniques, such as genotyping, phenotyping, site-directed mutagenesis will play a pivotal role to ensure food security in the different parts of the world. New technologies will also provide rapid and cost-effective approaches for the generation and analysis of new alleles which can be used to create new mutant varieties with improved general or specific characters.

KEYWORDS

- **mutations**
- **mutagenesis**
- **mutant**
- **crop improvement**
- **chemical mutagen**
- **physical mutagen**

REFERENCES

- Aamir, R.; Amin, R. L.; Shahnawaz, K.; Samreen, K.; Kouser, P.; Ruhul, A. Induce Physical and Chemical Mutagenesis for Improvement of Yield Attributing Traits and Their Correlation Analysis in Chickpea. *Int. Lett. Nat. Sci.* **2017**, *61*, 14–22.
- Abdul Salam, C. M.; Thoppil, J. E. Mutagen Induced Cytological Aberrations in *Capsicum annum* L. *Inter. J. Sci. Res.* **2010**, *2*, 52–55.
- Abdul Salam, C. M.; Thoppil, J. E. Isolation of Induced Morphological Mutants in *Capsicum annum* L. *J. Phytol.* **2010**, *2*, 57–63.
- Amin, R.; Wani, M. R.; Raina, A.; Khursheed, S.; Khan, S. Induced Morphological and Chromosomal Diversity in the Mutagenized Population of Black Cumin (*Nigella sativa* L.) Using Single and Combination Treatments of Gamma Rays and Ethyl Methane Sulfonate. *Jordan J. Biol. Sci.* **2019**, *12* (1), 23–33.
- Auerbach, C.; Robson, J. M. Experiments on the Action of Mustard Gas in *Drosophila*. Report to the Ministry of Supply 1942, W.3979 W: 11831.
- Bado, S.; Forster, B. P.; Till, B. J.; Nielen, S.; Ghanim, A. M. A.; Lagoda, P. J. L. Plant Mutation Breeding: Current Progress and Future Assessment. *Plant Breeding Rev.* **2015**, *39*, 23–88.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Clastogenic Effect of EMS and MMS in *Vicia faba* L. *J. Cytol. Genet.* **2005**, 24–33.
- Bhat, T. A.; Gulfishan, M. Mutations Affecting Plant Morphology. In *Mutagenesis and Cytotoxicity: A Review*; Bhat, T. A., Ed.; 2019; pp 64–74.
- Bhat, T. A.; Gulfishan, M.; Wani, A. A. Cytomixis: Causes and Consequences as a Case Study in *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer, 2017; pp 331–342.
- Bhat, T. A.; Gulfishan, M.; Zahid, M. T. Induced Mutagenesis. In *Mutagenesis and Cytotoxicity: A Review*; Bhat, T. A., Ed.; 2019; pp 11–22.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Comparative Analysis of Meiotic Abnormalities Induced by Gamma Rays, EMS and MMS in *Vicia faba* L. *J. Indian Bot. Soc.* **2005a**, *84*, 45–48.
- Bhat, T. A.; Parveen, S.; Khan, A. H. Clastogenic Effect of EMS and MMS in *Vicia faba* L. *J. Cytol. Genet.* **2005b**, *6*, 117–122.

- Bhat, T. A.; Khan, A. H.; Parveen, S. Effect of EMS on Certain Cytomorphological Parameters in Two Varieties in *Vicia faba* L. *Adv. Plant Sci.* **2006a**, *19*, 227–232.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Studies on the Effect of EMS on Meiosis and Pollen Fertility in *Vicia faba* L. *Adv. Plant Sci.* **2006b**, *19*, 243–247.
- Bhat, T. A.; Parveen, S.; Khan, A. H. Meiotic Studies in Two Varieties of *Vicia faba* L. (Fabaceae) After EMS Treatment. *Asian J. Plant Sci.* **2007**, *6*, 51–55.
- Bhat, T. A.; Shakoori, A. R.; Zahid, M. T.; Gulfishan, M.; Wani, M. R.; Qadri, I. H. Induced Cytological Aberrations Through Mutagenesis: A Review. In *Mutagenesis, Cytotoxicity and Crop Improvement: Revolutionizing Food Science*; Bhat, T. A., Ed.; 2021; pp 115–158.
- Bhat, T. A.; Sharma, M.; Anis, M. Comparative Analysis of Mitotic Aberrations Induced by Diethyl Sulphate (DES) and Sodium Azide (SA) in *Vicia faba* L. (Fabaceae). *Pak. J. Biol. Sci.* **2007**, *10*, 783–787.
- Bhat, T. A.; Wani, A. A. Mutagenic Effects on Meiosis in Legumes and a Practical Case Study of *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer, 2017; pp 219–244.
- Bhat, T. A.; Zahid, M. T.; Gulfishan, M.; Wani, M. R. Exploration, Applicability and Conclusion of Induced Mutagenesis on Broad Bean. A Review. In *Mutagenesis, Cytotoxicity and Crop Improvement: Revolutionizing Food Science*; Bhat, T. A., Ed.; 2021; pp 265–285.
- Bhat, T. A.; Shahzad, A.; Wani, M. R. Isolation of Promising Mutants of M3 Generation in Broad Bean and Their Protein Patterns Through SDS-PAGE. In *Mutagenesis, Cytotoxicity and Crop Improvement: Revolutionizing Food Science*; Bhat, T. A., Ed.; 2021; pp 377–412.
- Brook, R. D. Mutations in Quantitatively Inherited Traits Induced by Neutron Radiation. *Rad. Bot.* **1970**, *10*, 209–223.
- Chopra, V. L. Mutagenesis: Investigating the Process and Processing the Outcome for Crop Improvement. *Curr. Sci.* **2005**, *89*, 353–359.
- De Vries, H. *Die Mutations Theorie*; Leipzig. (Steite V). **1901**.
- Ehrenberg, L. Chemical Mutagenesis: Biochemical and Chemical Points of View on Mechanism of Action. *Erwin-Baur-Gedachtnis-Vorlesungen I. Abh. Dt. Akad. Wiss. Berl. (Med.)* **1960**, *1*, 124–136.
- Ehrenberg, L.; Gustafsson, A. On the Mutagenic Action of Ethylene Oxide and Diepoxybutane in Barley. *Hereditas* **1957**, *43*, 595–602.
- Forster, B. P.; Shu, Q. Y. Plant Mutagenesis in Crop Improvement: Basic Terms and Applications. In *Plant Mutation Breeding and Biotechnology*; Shu, Q. Y., Ed.; 2012; pp C01. 09 20.
- Freisleben, R. A.; Lein, A. Über die auf induktion einer mehltau resistenten mutante in einer chrotengebestrahlungseiner anfalligen reinenlinie von sommergerste. *Naturwissenschaften*. **1942**, *30*, 608.
- Gager, C. S.; Blakeslee, A. F. Chromosome and Gene Mutations in *Datura* Following Exposure to Radium Rays. *Proc. Natl. Acad. Sci. USA* **1927**, *13*, 75–59.
- Goyal, S.; Wani, M. R.; Laskar, R. A. Induction of Morphological Mutations and Mutant Phenotyping in Black Gram [*Vigna mungo* (L.) Hepper] Using Gamma Rays and EMS. *Vegetos* **2019**, *32*, 464–472.
- Goyal, S.; Wani, M. R.; Laskar, R. A.; Khan, S.; Bhat, T. A. Individual and Simultaneous Treatments of Gamma Rays and Ethylmethan Sulphate Induced Genetic Variability for Plant Height in Urdbean (*Vigna mungo* (L.) Hepper). In *Mutagenesis, Cytotoxicity and Crop Improvement: Revolutionizing Food Science*; Bhat, T. A., Ed.; 2021; pp 159–170.
- Gulfishan, M.; Khan, A. H.; Jafri, I. F.; Bhat, T. A. Assessment of Mutagenicity Induced by MMS and DES in *Capsicum annum* L. *Saudi J. Biol. Sci.* **2012**, *19*, 251–255.

- Gulfishan, M.; Khan, A. H. Induced Desynapsis in *Capsicum annuum* L. *Trends Biosci.* **2012**, *5*, 14–16.
- Gulfishan, M.; Jafri, I. F.; Khan, A. H. Methyl Methane Sulphonate Induced Desynaptic Mutants in *Capsicum annuum* L. *Chrom. Bot.* **2013**, *8*, 59–62.
- Gulfishan, M.; Bhat, T. A.; Oves, M. Mutants as a Genetic Resource for Future Crop Improvement. In *Advances in Plant Breeding Strategies: Volume 1 Breeding, Biotechnology and Molecular Tools*; Al-Khayri, J. M., et al., Eds.; 2015. DOI: 10.1007/978-3-319-22521-0_4
- Gulfishan, M.; Khan, A. H.; Bhat, T. A. Studies on Cytotoxicity Induced by DES and SA in *Vicia faba* L. var. Major. *Turk. J. Bot.* **2010**, *34*, 31–37.
- Gulfishan, M.; Khan, A. H.; Haneef, I.; Bhat, T. A. Genotoxic Effect of Diethyl Sulphate in Two Varieties of *Capsicum annuum* L. *Nucleus* **2011**, *54*, 107–111.
- Gulfishan, M.; Khan, A. H.; Jafri, I. F. Genotoxic Effects Induced by Methyl Methane Sulphonate in 2 Cultivars of *Capsicum annuum* L. *Cytologia.* **2012**, *76*, 1–5.
- Gustafsson, A. A Study of Induced Mutations in Plants “Induced Mutations in Plants”. *Proc. Ser. IAEA* **1969**; pp 9–31.
- Hasan, N.; Laskar, R. A.; Raina, A.; Khan, S. Maleic Hydrazide Induced Variability in Fenugreek (*Trigonella foenum-graecum* L.) Cultivars CO1 and Rmt-1. *Res Rev: J. Botanical Sci.* **2018**, *7* (1), 19–28.
- Huang, C.; Liang, J. Plant Breeding Achievements in Ancient China. *Agron. History Res.* **1980**, *1*, 1–10 (in Chinese).
- Jabee, F.; Ansari, M. Y. K.; Shahab, D. Maleic Hydrazide (MH) Induced Variations for Economic Traits in *Trigonella foenum-graecum* L. *Biosci. Biotech. Res. Asia.* **2007**, *4*, 141–144.
- Jafri, I. F.; Khan, A. H.; Sharma, M.; Gulfishan, M. Comparative Mutagenicity of EMS and HZ in *Hordeum vulgare* L. var. RD2052. *Chrom. Bot.* **2012**, *7*, 79–83.
- Jafri, I. F.; Khan, A. H.; Gulfishan, M. Genotoxic Effects of 5-Bromouracil on Cytomorphological Characters of *Cichorium intybus* L. *Afr. J. Biotechnol.* **2012**, *10*, 10595–10599.
- Jafri, I. F.; Khan, A. H.; Sharma, M.; Gulfishan, M. Genomic Damage Induced by Individual and Combination Treatment of Gamma Rays and Ethyl Methane Sulphonate in *Coriandrum sativum* L. var. karishma. *Int. J. Bot. Res.* **2013**, *3*, 79–85.
- Jankowicz-Cieslak, J.; Mba, C.; Till, B. J. Mutagenesis for Crop Breeding and Functional Genomics. In *Biotechnologies for Plant Mutation Breeding*; Jankowicz-Cieslak, J. et al., Eds.; 2012; pp 3–18. DOI: 10.1007/978-3-319-45021-6_1
- Kalia, C. S.; Kharkwal, M. C.; Singh, M. P. Recovery of Desirable Mutations in Wheat. *Indian J. Genet. Plant Breed.* **2000**, *60*, 465–470.
- Khan, S.; Siddiqui, B. A.; Rehman, M. U. Mutation Genetic Studies in Mungbean III. Screening of High Yielding Mutants. *J. Cytol. Genet.* **1999**, *34*, 75–78.
- Khursheed, S.; Raina, A.; Parveen, K.; Khan, S. Effect of Gamma Radiation and EMS on Mutation Rate: Their Effectiveness and Efficiency in Faba Bean (*Vicia faba* L.) *Caryologia* **2018**, *71*, 397–404.
- Khursheed, S.; Raina, A.; Parveen, K.; Khan, S. Induced Phenotypic Diversity in the Mutagenized Populations of Faba Bean Using Physical and Chemical Mutagenesis. *J. Saudi Soc. Agric. Sci.* **2017**, 52–60.
- Khursheed, S.; Raina, A.; Khan, S. Improvement of Yield and Mineral Content in Two Cultivars of *Vicia faba* L. Through Physical and Chemical Mutagenesis and their Character Association. *Analys. Arch. Curr. Res. Int.* **2016**, *4*, 1–7.

- Khursheed, S.; Raina, A.; Wani, M. R.; Amin, R.; Khan, S. Comparative Analysis of Cytological Abnormalities Induced in *Vicia faba* L. Genotypes Using Physical and Chemical Mutagenesis. *Chrom. Sci.* **2015**, *18*, 3–7.
- Khursheed, T.; Ansari, M. Y. K.; Shahab, D. Studies on the Effect of Caffeine on Growth and Yield Parameters in *Helianthus annuus* L. *Variety Modern. Biol. Medi.* **2009**, *1*, 56–60.
- Laskar, R. A.; Khan, S.; Khursheed, S.; Raina, A.; Amin, R. Quantitative Analysis of Induced Phenotypic Diversity in Chickpea Using Physical and Chemical Mutagenesis. *J. Agron.* **2015**, *14*, 102.
- Laskar, R. A.; Raina, A.; Khan, S.; Younus, H. Induced Mutation Analysis with Biochemical and Molecular Characterization of High Yielding Lentil Mutant Lines. *Int. J. Biol. Macromol.* **2018**, *109*, 167–179.
- Laskar, R. A.; Wani, M. R.; Khan, S.; Deb, C. R.; Khan, T. U.; Bhat, T. A. Induced Chromosomal in Grain Legumes: *Lens culinaris* Medik. In *Mutagenesis, Cytotoxicity and Crop Improvement: Revolutionizing Food Science*; Bhat, T. A., Ed.; 2021; pp 244–264.
- Mba, C. Induced Mutations Unleash the Potentials of Plant Genetic Resources for Food and Agriculture. *Agronomy* **2013**, *3*, 200–231.
- Muller, H. J. Artificial Transmutation of the Gene. *Science* **1927**, *66*, 84.
- MVD. <https://mvd.iaea.org/>, 2021 (accessed Feb 25, 2021).
- Oehlkers, F. Die Auslösung Von Chromosomen Mutation in der meiosis durch Einwirkung von Chemikalein. *Z. Ind. Abst. U. VerserLehre.* **1943**, *81*, 313.
- Raina, A.; Ansari, S. B.; Khursheed, S.; Wani, M. R.; Khan, S.; Bhat, T. A. Mutagens, Their Types and Mechanism of Action with an Emphasis on Sodium Azide and Gamma Radiations. In *Mutagenesis, Cytotoxicity and Crop Improvement: Revolutionizing Food Science*; Bhat, T. A., Ed.; 2021; pp 159–117.
- Raina, A.; Khan, S. Mutagenic Effectiveness and Efficiency of Gamma Rays and Sodium Azide in M2 Generation of Cowpea [*Vigna unguiculata* (L.) Walp.] *Bio Rxiv* **2020**. <https://doi.org/10.1101/2020.03.09.983486>
- Raina, A.; Khursheed, S.; Khan, S. Optimisation of Mutagen Doses for Gamma Rays and Sodium Azide in Cowpea Genotypes. *Trends Biosci.* **2018**, *11*, 2386–2389.
- Raina, A.; Laskar, R. A.; Tantray, Y. R.; Khursheed, S.; Wani, M. R.; Khan, S. Characterization of Induced High Yielding Cowpea Mutant Lines Using Physiological, Biochemical and Molecular Markers. *Sci. Rep.* **2020**, *10*, 3687.
- Raina, A.; Laskar, R. A.; malik, S.; Wani, M. R.; Bhat, T. A.; Khan, S. Plant Mutagenesis: Principle and Application in Crop Improvement. In *Mutagenesis, Cytotoxicity and Crop Improvement: Revolutionizing Food Science*; Bhat, T. A., Ed.; 2021; pp 38–65.
- Reddy, V. R. K.; Annadurai, M. Cytological Effects of Mutagens in Lentil (*Lens culinaris* Medik). *Cytologia* **1992**, *57*, 213–216.
- Shahab, D.; Gulfishan, M.; Idrees, M.; Vágvölgyi, C.; Ansari, M. Y. K. Isolation of Mutants from Gamma Ray-Irradiated Population of *Solanum melongena* Cultivar. ‘Pusa Purple Long’. *Chrom Bot.* **2018**, *13* (1), 31–36.
- Shahab, D.; Gulfishan, M.; Khan, A. A.; Vágvölgyi, C.; Ansari, M. Y. K. Comparative Mutagenic Effectiveness and Efficiency of Physical and Chemical Mutagens in *Solanum melongena* L. Variety Pusa Uttam. *Biomed. J. Sci. Tech. Res.* **2018**, 1–5. DOI: 10.26717/BJSTR.2018.07.001541
- Sparrow, A. H.; Singleton, W. R. The Use of Radio Cobalt as a Source of Gamma Rays and Some Effects of Chronic Irradiation on Growing Plants. *Am. Naturalist* **1953**, *87*, 29–48.

- Sri Devi, A.; Mullainathan, L. Physical and Chemical Mutagenesis for Improvement of Chilli (*Capsicum annum L.*). *World Appl. Sci. J.* **2011**, *15*, 108–113.
- Stadler, L. J. Mutation in Barley Induced by X-Rays and Radium. *Science* **1929**, *9*, 186–187.
- Van Harten, A. M. *Mutation Breeding. Theory and Practical Applications*; Cambridge University Press: Cambridge, 1998.
- Van Harten, A. M.; Broertjes, C. Induced Mutations in Vegetatively Propagated Crops. *Plant Breeding Rev.* **1989**, *6*, 55–91.
- Verma, R. P.; Srivastava, G. K.; Kumar, G. Comparative Radiocytological Studies in Three Varities on *Lens culinaris*. *J. Cytol. Genet.* **1999**, *34*, 49–56.
- Vishwanathan, P.; Reddy, V. R. K. Induced Desirable Agronomical Mutants in Wheat and Triticale. *J. Indian Bot. Soc.* **1998**, *77*, 67–69.
- Wani, M. R.; Laskar, R. A.; Raina, A.; Khan, S.; Khan, T. Application of Chemical Mutagenesis for Improvement of Productivity Traits in Lentil (*Lens culinaris* Medik). *Ann. Biol.* **2021**, *37* (1), 69–75, 2021.
- Wani, M. R.; Tomlekova, N.; Raina, A.; Laskar, R. A.; Khursheed, S.; Khan, S.; Tak, M. A.; Bhat, T. A. Mutation Breeding Technique for the Improvement of Pulse Crop with Special Reference to Faba Bean (*Vicia faba L.*). In *Mutagenesis, Cytotoxicity and Crop Improvement: Revolutionizing Food Science*; Bhat, T. A., Ed.; 2021; pp 222–243.
- Westergard, M. Chemical Mutagenesis in Relation to the Concept of the Gene. *Experimentia* **1957**, *13*, 224–234.



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CHAPTER 9

Genetically Engineered Foods (GEFs)

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ABSTRACT

Global climate change, urbanization, unwise resource utilization, and ever-exceeding population have taken on threat the global food security. Keeping in view the global 2050 rise in human population, the food security is going to be a serious challenge. Efficient resource utilization, securing the food reserves, and protecting the plants from environmental stressors are key components of sustainable agricultural development. To meet these challenges, genetically modified organisms are being developed for useful traits like herbicide resistant and pest control. The genetically engineered foods (GEFs) are proving as strong candidates in ensuring global food security. Although these organisms are proving fruitful in several instances but at the same time, the ecological integrity and public health are on compromise. In this chapter, the overview of GEFs is being discussed. The pros and cons of these foods are also the part and parcel of the study.

9.1 INTRODUCTION

9.1.1 GEFS ARE THE DEMAND OF CURRENT SCENARIOS TO ENSURE GLOBAL FOOD SECURITY

Food is one of the basic necessities for the existence of life on this planet. The past few years have brought a lot of challenges in the form of population

increase, global climate change, deforestation, and loss of biological integrity of the ecosystem. The environmental stresses in various biotic and abiotic forms have negatively impacted the yield attributes of the cereal and millets. So keeping in view, the current scenarios the genetically engineered foods (GEFs) are the need of the hour (Gurău and Ranchhod, 2016). The food produced by the genetically modified organisms (GMOs) through various biotechnological interventions is termed as GEFs. World Health Organization (WHO) defines GMOs as the organisms which contain genetically altered genome not founded naturally (Hudson, 2017). The first genetically modified (GM) crop was introduced in 1994 and since then over 300 various food crops have been introduced. In 2015, first GM animal has been approved by the Food and Drug Administration (Wong and Chan, 2016). Recombinant DNA technology brings a set of tools to modify the genome. The genome modification results in the production of desirable traits in the organism such as high-yielding varieties and pest resistance strains (Moglia and Portis, 2016). Technically, a GMO may possess the improved qualities related to yield parameters or the product, more pest resistance, and better agronomical traits in terms of herbicide tolerance (Lüthy, 1999). However, there are various pros and cons of the GEFs. In the coming lines, we will be discussing the merits and demerits of the GEFs.

9.2 MERITS OF GEFS

9.2.1 RECOMBINANT DNA PRACTICES HAVE LED TO FORMATION OF HERBICIDE RESISTANT PLANTS

Using the recombinant DNA technology, herbicide-resistant strains are being engineered. The unwanted plants present in the crop surroundings lead to around 20–60% yield losses on average. As weed killers are used, they may negatively affect the crops as well. Using the tools of genetic engineering, the herbicide-tolerant varieties have been prepared leading to increased yields and agronomic vigor in a cost-effective manner (Holst-Jensen, 2009). Insects and pest resistant varieties have transformed the plants increasing their utility in terms of environmental and economic gains. Boulter (1993) stated the qualities of an ideal insecticide gene as follows:

- An ideal insecticide gene does not interfere with the metabolism of the host.
- An ideal insecticide gene is active against the harmful pests but beneficial insects are not harmed.
- It does not affect the animals including human being.

Various food crops like canola, corn, cotton, and potato have been modified by giving them insect-resisting genes (Phipps and Park, 2002).

9.2.2 GENETIC ENGINEERING HAS TRANSFORMED PLANTS INCREASING DISEASE RESISTANCE

The departure of plants from normal homeostatic state leads to incalculable yield losses. The environmental or climatic factors further enhance the issue. Genetic engineering updates have led plant to a new direction making them disease resistant and tolerable to fluctuating weather. Transfer of an antifrost gene from a cold-water fish in certain varieties of potato and tobacco has made them frost resistant (Godfray et al., 2010). The disease preventive measures can be practiced in various ways using the genetic engineering strategies. One of the strategies includes dominant-negative expression. The expression of negative dominant genes leads to synthesis of certain proteins like cell surface receptor against the pathogen blocking the disease progression. Similarly RNA decoys destroy the biosynthetic machinery of a disease-causing entity checking the disease progression. Other ways of disease prevention may involve the transgenic production of antibodies and using RNA interferences (Whitelaw and Sang, 2005).

9.2.3 ENVIRONMENTAL STRESSORS IN THE FORM OF CLIMATIC AGENTS SIGNIFICANTLY REDUCE THE AGRONOMIC VIGOR OF THE FOOD CROPS

Wang et al. (2003) narrated that the high temperature leads to ROS-induced damages to the plants and the heats disrupts the structures of proteins. The plants produce heat shock proteins in response to heat stress. The activity of antioxidant enzymes like superoxide dismutase also regulates the stress mitigation activity of the plants. Wang et al. (2003) reported that the prolonged-expression of plastidic SOD leads to compensate the damages caused by the high light intensity and the cold environment.

9.2.4 GENETIC ENGINEERING STRATEGIES ARE PROVING TO ENABLE THE PLANTS IN MITIGATING WATER AND SALT STRESS

The saline environment and water-scarce conditions are major culprits behind the productivity losses. The soils experiencing saline crust and water shortage may lead to abundant field losses to the farmers however the

genetically programmed varieties of maize, cotton, and other crops make the plants to grow under the hostile environments (Zhu, 2001). Saline conditions may lead to development of rosette condition of a plant and may also induce the oxidative damages (Zhu, 2003). The genetic engineering for the salt tolerating traits is being carried out to minimize the yield losses (Gregorio and Senadhira, 1993).

9.2.5 NUTRIENT ACQUISITION PATTERNS OF THE PRODUCER ARE DIRECTLY LINKED TO NUTRIENT UPTAKE BY THE CONSUMERS

As the research in the field of genetics is expanding the food crops are being engineered to uptake the nutrients efficiently and at the same time, the nutritional quality of the food is also evolving. For instance, the rice is a staple food of millions of people across the globe; however, the paddies do not contain the sufficient nutrients. The genetic engineering of the rice to increase its food value may lead to nutrient deprivation in rice consumers (Whitman, 2000). Several food crops have been engineered to do the job and studies have shown that GM foods are safe to use (Aulrich et al., 2001; Flachowsky et al., 2005).

9.3 WIDELY USED GM PLANTS FOR GEF

9.3.1 POTATOES (SOLANUM TUBEROSUM L.—FAMILY: SOLANACEAE)

Potatoes are the staple foods worldwide due to their high nutritive value in the form of starch. The amylopectin starch is a healthy source of caloric intakes; however, the fraction of amylopectin is not sufficient in natural varieties. Moreover, the natural potato produces a number of secondary metabolites as well such as alkaloids, solanine, and phenols. Using the genetic engineering tactics, the potatoes have been GM to produce less secondary metabolite and increased amylopectin starch contents (Domingo, 2007). Similarly, potatoes face insect attacks and their yield quality is decreased both qualitatively and quantitatively. To make them insect-resistant potatoes have been treated genetically with “delta-endotoxin.” However, the treatment has safety reservations for the consumers (Domingo, 2007).

9.3.2 MAIZE (*ZEAMAYS L.—FAMILY POACEAE*)

Maize is the second most important GM food crop in European Union and used in different forms as source of food and nutrition (Fernandez et al., 2014). The prime objective is to make GM maize varieties that are resistant to broad-spectrum herbicides and insecticides (Novak and Haslberger, 2000). The experiments of Séralini et al. (2007) have depicted that transgenic maize cultivars are potentially harmful and may induce certain maladies in rats, such as hepato-renal toxicity and increase in triglyceride contents. So, the GM maize cultivar (MON863) tested in the experiment raised biosafety issues. The experiments of Domingo (2007), however, produced encouraging results in using maize transgenic variety CBH 351. The rats fed with GM CBH 351 had no significant differences in terms of fatty acid composition and phytate contents as compared to control. Moreover, they reported no incident of any abnormality related to histology of kidney and bone marrow.

9.3.3 RICE (*ORYZA SATIVA L.—FAMILY POACEAE*)

Rice is used as staple food worldwide and it is among those food crops that are most cultivated on a hectare basis (Lu and Snow, 2005). Rice genome was mapped out prior to any other crop species (Singh et al., 2014). Domingo (2007) carried out a study to analyze the food quality obtained from herbicide-tolerant rice plants. No adverse effects were reported. Junhua et al. (2005) carried out a study to understand the nutritional composition of GM rice foods and showed that the nutritive value of the GM rice was equal to parental cultivars (Table 9.1).

TABLE 9.1 Comparison of Nutrients in Parental Rice and GM Rice (g/100 g of Edible Portion).

Nutrient	Parental rice	GM rice
Moisture		
Protein	There was no significant nutritional value difference found between parental (original variety) and GM rice variety in Experiment of Junhua et al. (2005).	
Lipid		
Fiber		
Ash		
Carbohydrate		

Source: Junhua et al. (2005).

9.3.4 SOYBEANS (*GLYCINE MAX*—FAMILY *LEGUMINOSAE*)

Soybean is a pulse plant that produces certain secondary metabolites which become harmful in terms of caloric intake (Novak and Haslberger, 2000). Teshima et al. (2000) carried out a research study to illustrate the comparative nutritional contents of GM soybeans and parental soybeans. The nutritional values along with the impacts of GM soybeans in terms of immune functions were part of their study. They concluded that GM soybeans have no adverse impacts on immune functions. Zhu et al. (2004) performed a similar study on rats and found no toxic inferences.

9.3.5 TOMATOES (*LYCOPERSICUM ESCULENTUM L.*—FAMILY *SOLANACEAE*)

Tomatoes are also among notable GM plants. The “Flavr Savr” tomatoes find their history back in 1992 produced by Calgene and represent the first generation of GM tomatoes. The “Flavr Savr” tomatoes were fed to rats and the rats showed signs of stomach erosion and necrotic lesions (Dona and Arvanitoyannis, 2009). A potential secondary metabolite tomatine is produced in excess and GM tomatoes were raised to control the production of tomatine; however, the genetic modification did not alter the contents of tomatine in tomatoes (Dona and Arvanitoyannis, 2009).

9.4 GM ANIMALS AND GEFS

A GM animal is the animal whose genome has been modified using the techniques of genetic engineering and recombinant DNA for the development of better traits (Seney et al., 2015). WHO along with Organization for Economic Co-operation and Development lays greater emphasis on biosafety issues that arise due to the introduction of a transgenic breed of animals. The safe introduction of a transgenic animal in natural environment requires safety measurement and analysis related to public health (Kleter and Kuiper, 2002). The animals are GM for better traits like increased immunity to different diseases and engineering the animals for enhanced quality and quantity of the products they produce (Pandey et al., 2016). The animals with modified genomes can be used for research related to genetics (Seney et al., 2015). The animal models can be used to access the nature and symptomatology of diseases related to human as well. The

analogous genes can be traced and their function in disease development and control can be analyzed. In Alzheimer's disease, for instance, transgenic mice overexpressing the human Alzheimer precursor protein seven-fold. The animal shows neurotic changes and congophilic plaques and this mouse line can be used to comprehend the role of a particular substrate in disease pathogenesis and to test drugs for inhibiting this process (Rudolph and Möhler, 1999).

9.5 RISK ASSESSMENT FOR THE GEFs

Despite having a lot of utilities, GEFs have raised several biohazardous and biosafety-related crises and they are not accepted with an open heart in the scientific and public health communities worldwide. Several forms of toxicities along with genetic alteration related expression of genes and their behavior in the different hosts is still a point to focus. There are concerns in the GEFs use as some people experience several types of allergies and stomach erosion issues (Zhang et al., 2016a,b).

The unknown effects of the exogenous genes are estimated to raise certain threats of known and unknown nature. The worldwide scientific community considers the GMOs and GEFs a challenge for the already under stress natural environment (Zhang et al., 2012). The unintended impacts of the GEFs are the area of particular concern. The unintended effects of the GEFs are in context with significant change in external appearance of the transgenic organism as compared to the naturally found parental organism. The composition of the GEFs is an area of concern too. The comparative study of GEFs with natural counterparts may evaluate the advantages or demerits of the GEFs. Certain effects related to GM development are of intended nature. The analysis of GEFs whether they have developed characters that were intended or not is a major focus of the research. Certain effects of the GEFs are not properly understood and they are expected to pose unknown threats to human and nature. The risk assessment is the part of biosafety measures to be taken against a particular GM crop or animal as these plants and animals may lead to serious irregularities in the nature and natural ecosystems (Cellini et al., 2004). For instance, a study has shown that pollen grains produced from a *Bt* corn (transgenic variety of corn) are source of mortality in butterfly caterpillars. These caterpillars feed on the milkweed plants. The risk is as those *Bt* corn pollens are blown by wind to the milkweeds the feeding caterpillars will experience high mortality (Whitman, 2000).

The pesticides are the chemicals that are manipulated to kill harmful insects, weeds, and fungal pathogens for sustainable agricultural productivity. The insect-resistant transgenic crops are engineered to overcome yield losses caused by the harmful insects (Qaim, 2009). Although the transgenic varieties of crops engineered for insect resistant are proving fruitful yet some researchers worldwide are of the view that insects may become resistant to *Bt* with the passage of time as mosquitoes developed DDT resistance (Whitman, 2000). The use of pest killers is harmful to ecosystem integrity and as the pesticides become the part of the food chains, these become potentially more harmful (Lusk et al., 2004).

Another area of concern is that crop species that have been engineered genetically for the pesticide and herbicide tolerance may cross-breed with wild weeds and in the process the genes may be transmitted to those wild weeds transforming them to be pesticide tolerant (Whitman, 2000). The insect resistant varieties of the crops when taken as food by the human and other animals are, however, safe as the DNA of these GM crops is degraded in stomach in seconds (Dale et al., 2002). The development of herbicide-resistant weeds will again pose productivity-related issues with the crops. As the NPK fertilizers used to nourish the crops will be used by these weeds raising once again the same problems for which the genetically engineered crops were used (Whitman, 2000).

9.5.1 GENETICALLY ENGINEERED FOOD MAY LEAD TO DEPARTURE FROM NORMAL HOMEOSTATIC STATE IN HUMAN BEINGS

Probably the biggest concern with the human health is that a person may show allergic reactions in response to GEFs. Some people may show severe allergic reaction in response to GEFs (Whitman, 2000). The Codex Committee on Food Labeling has given a list of types of allergies due to the use of GEFs and several children have shown a set of allergic reaction in response to peanuts and other GEFs. The GEFs pass through various sets of testing practices before being approved as standard food. If a GEF has been manufactured from a gene extracted from a well-defined allergenic medium, the food is subjected to testing to understand the type of allergen it may contain and if the food contains any allergen, it is rejected before going through the other testing steps. On the other hand, if a GEF does not contain any allergen or GEF has not been derived from an allergen source then it is subjected to quality assurance criteria. The testing includes the protocols to

comprehend the allergen nature in susceptible persons, the pepsin resistance, and immunogenicity (Taylor, 1997). However, it is a proven fact that a nucleic acid piece or gene derived from a reputable allergen medium will show allergic reactions. For a food to be safe, it must have a gene source from a nonallergen medium (Goodman et al., 2008).

GEFs bring some socioeconomic risk factors with them. Certain developing nations like Argentina, Pakistan, India, South Africa, and Brazil have nominated some of the GEFs as safe to use; however, there are some countries that are against the GEFs. For instance, Latin America people are against the soybeans obtained from the herbicide-tolerant crops (Pandey et al., 2016). Introducing a GEF for the public requires a thought process with a lot of testing and skill. It requires a lot of expenditures and time for a GEF to pass the certification criteria (Akumo et al., 2013). The developing countries including the third-world farmers are striving to get the high yielding variety seeds; however, the farmers have financial crises and debts that is why they are unable to access these better seeds. The far-reach scenarios are creating hostile conditions for the farmers (Whitman, 2000). The economic gains due to the use of high-yielding transgenic varieties have been reported to be 42% (Zhang et al., 2016a, b). Certain high-yielding varieties have been developed across the globe by the intellect of farmers and researchers working in the field. The developments are intellectual properties of the developers and field workers and they got these patents. However, the patent infringement is an alarming problem and misuse of intellectual property rights is posing a lot of tensions. To protect the patent rights, it was suggested to put a suicide gene in the GM crop specie. The plants having suicide genes will not produce the viable seeds in the next growing season but this all will no doubt may protect the patent rights but for a farmer to buy the high yielding variety seeds in every season will be a problem due to financial liabilities (Whitman, 2000). The advantages of GEFs have been reported by some researchers (Gurău and Ranchhod, 2016). By comparing the normal traditional foods and GEFs following advantages can be easily conferred:

1. GEFs are cheaper than traditional food as GM plants have increased productivity.
2. GEFs can be more fruitful in terms of nutritional contents.
3. GEFs can be used to transmit some useful metabolites and medicinal items in contrast to traditional foods.
4. The GM plants can prove to be environment friendly by mitigating the pollutants.

5. Keeping in view the global 2050 food demand for nearly 10 billion people, the GEFs promise for the food security.

9.6 CONCLUSIONS

GEFs have a bright future prospect keeping in view of the hunger and food crises arising due to population explosion, unwise utilization of the resources, urbanization, and global climate change; however, the biological safety issues are arising. The risk of introducing transgenic specie in natural environment is not beyond threat. GEFs can be developed and used but the health of masses an ecological integrity should not be harmed. In the coming decades, we will see an enhanced impact of biotechnology in our lives; however, for sustainable agriculture and eco-friendly developments, the risk assessment and biosafety measures should be on top priority.

KEYWORDS

- **global climate change**
- **genetically engineered foods**
- **public health**
- **efficient resource utilization**
- **herbicide resistant**
- **global food security**

REFERENCES

- Akumo, D. N.; Riedel, H.; Semtanska, I. *Social and Economic Issues—Genetically Modified Food*; Intech Open Access Publisher, 2013.
- Aulrich, K. et al. Genetically Modified Feeds in Animal Nutrition. 1st Communication: *Bacillus thuringiensis* (Bt) Corn in Poultry, Pig and Ruminant Nutrition. *Arch. Tierernahr.* **2001**, *54* (3), 183–195.
- Aumaitre, A. et al. New Feeds from Genetically Modified Plants: Substantial Equivalence, Nutritional Equivalence, Digestibility, and Safety for Animals and the Food Chain. *Livest. Sci.* **2002**, *74* (3), 223–238.
- Bawa, A.; Anilakumar, K. Genetically Modified Foods: Safety, Risks and Public Concerns—A Review. *J. Food Sci. Technol.* **2013**, *50* (6), 1035–1046.

- Benbrook, C. M. Impacts of Genetically Engineered Crops on Pesticide Use in the U.S.—The First Sixteen Years. *Environ. Sci. Eur.* **2012**, *24* (1), 24.
- Betz, F. S.; Hammond, B. G.; Fuchs, R. L. Safety and Advantages of *Bacillus Thuringiensis*-Protected Plants Tocontrol Insect Pests. *Regul. Toxicol. Pharm.* **2000**, *32* (2), 156–173.
- Boulter, D. Insect Pest Control by Copying Nature Using Genetically Engineered Crops. *Phytochemistry* **1993**, *34* (6), 1453–1466.
- Cellini, F. et al. Unintended Effects and Their Detection in Genetically Modified Crops. *Food Chem. Toxicol.* **2004**, *42* (7), 1089–1125.
- Chheda, A.; Vernekar, M. A Natural Preservative ϵ -Poly-L-Lysine: Fermentative Production and Applications in Food Industry. *Int. Food. Res. J.* **2014**, *22*, 23–30.
- Chinnusamy, V.; Zhu, J.-K. *Plant Salt Tolerance*; Springer: Berlin, pp 241–270.
- Conner, A. J.; Glare, T. R.; Nap, J. P. The Release of Genetically Modified Crops Into the Environment. *Plant J.* **2003**, *33* (1), 19–46.
- Dale, P. J.; Clarke, B.; Fontes, E. M. Potential for the Environmental Impact of Transgenic Crops. *Nat. Biotechnol.* **2002**, *20* (6), 567–574.
- Domingo, J. L. Toxicity Studies of Genetically Modified Plants: A Review of the Published Literature. *Crit. Rev. Food Sci. Nutr.* **2007**, *47* (8), 721–733.
- Dona, A.; Arvanitoyannis, I. S. Health Risks of Genetically Modified Foods. *Crit. Rev. Food Sci. Nutr.* **2009**, *49* (2), 164–175.
- Eastham, K.; Sweet, J. *Genetically Modified Organisms (GMOs): The Significance of Gene Flow Through Pollen Transfer*; European Environment Agency: Copenhagen, 2002.
- Engel, K.-H.; Frenzel, T.; Miller, A. Current and Future Benefits from the Use of GM Technology in Food Production. *Toxicol. Lett.* **2002**, *127* (1), 329–336.
- Fernandez, T. J. R. et al. A Survey on Genetically Modified Maize in Foods Commercialized in Portugal. *Food Control* **2014**, *35* (1), 338–344.
- Firbank, L. et al. An Introduction to the Farm-Scale Evaluations of Genetically Modified Herbicide-Tolerant Crops. *J. Appl. Ecol.* **2003**, *40* (1), 2–16.
- Flachowsky, G. et al. Studies on Feeds from Genetically Modified Plants (GMP): Contributions to Nutritional and Safety Assessment. *Anim. Feed Sci. Technol.* **2007**, *133* (1), 2–30.
- Flachowsky, G.; Chesson, A.; Aulrich, K. Animal Nutrition with Feeds from Genetically Modified Plants. *Arch. Anim. Nutr.* **2005**, *59* (1), 1–40.
- Flowers, T. Improving Crop Salt Tolerance. *J. Exp. Bot.* **2004**, *55* (396), 307–319.
- Frewer, L. et al. Societal Aspects of Genetically Modified Foods. *Food Chem. Toxicol.* **2004**, *42* (7), 1181–1193.
- Garcia-Yi, J. et al. What Are the Socio-Economic Impacts of Genetically Modified Crops Worldwide? A Systematic Map Protocol. *Environ. Evid.* **2014**, *3* (1), 24.
- Gewin, V. Genetically Modified Corn—Environmental Benefits and Risks. *Plos Biol.* **2003**, *1* (1), e8.
- Godfray, H. C. J. et al. Food Security: The Challenge of Feeding 9 Billion People. *Science* **2010**, *327* (5967), 812–818.
- Goodman, R. E. et al. Allergenicity Assessment of Genetically Modified Crops—What Makes Sense? *Nat. Biotechnol.* **2008**, *26* (1), 73–81.
- Gregorio, G.; Senadhira, D. Genetic Analysis of Salinity Tolerance in Rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **1993**, *86* (2–3), 333–338.
- Grieve, C. M.; Grattan, S. R.; Maas, E. V. Plant Salt Tolerance. In *Agricultural Salinity Assessment and Management*; Wallender, W. W., Tanji, K. K., Eds. 2nd ed.; ASCE: Reston, VA, 2012; pp 405–459.

- Gurău, C.; Ranchhod, A. The Futures of Genetically-Modified Foods: Global Threat or Panacea? *Futures*, **2016**, 83, 24–36.
- Hodgson, E. Genetically Modified Plants and Human Health Risks: Can Additional Research Reduce Uncertainties and Increase Public Confidence? *Toxicol. Sci.* **2001**, 63 (2), 153–156.
- Holst-Jensen, A. Testing for Genetically Modified Organisms (GMOs): Past, Present, and Future Perspectives. *Biotechnol. Adv.* **2009**, 27 (6), 1071–1082.
- Hudson, J. Genetically Modified Products and GMO Foods: A Game of Chance? In *Developing New Functional Food and Nutraceutical Products*; Nair, S., Bagchi, D., Eds.; Academic Press: San Diego, 2017; pp 481–494.
- Junhua, H. et al. Comparison of Nutrient Composition of Parental Rice and Rice Genetically Modified with Cowpea Trypsin Inhibitor in China. *J. Food Compos. Anal.* **2005**, 18 (4), 297–302.
- Kennedy, G. G. *Integration of Insect-Resistant Genetically Modified Crops Within IPM Programs*; Springer Science + Business Media B.V, 2008.
- Key, S.; Ma, J. K.; Drake, P. M. Genetically Modified Plants and Human Health. *J. R. Soc. Med.* **2008**, 101 (6), 290–298.
- Kleter, G. A.; Kuiper, H. A. Considerations for the Assessment of the Safety of Genetically Modified Animals Used for Human Food or Animal Feed. *Livest. Sci.* **2002**, 74 (3), 275–285.
- Ladics, G. S. et al. Genetic Basis and Detection of Unintended Effects in Genetically Modified Crop Plants. *Transgenic Res.* **2015**, 24 (4), 587–603.
- Lievens, A. et al. Genetically Modified Animals: Options and Issues for Traceability and Enforcement. *Trends Food Sci. Technol.* **2015**, 44 (2), 159–176.
- Lin, C.-H.; Pan, T.-M. Perspectives on Genetically Modified Crops and Food Detection. *J. Food Drug Anal.* **2016**, 24 (1), 1–8.
- Lu, B.-R.; Snow, A. A. Gene flow from Genetically Modified Rice and Its Environmental Consequences. *Bioscience* **2005**, 55 (8), 669–678.
- Lusk, J. L. et al. Effect of Information About Benefits of Biotechnology on Consumer Acceptance of Genetically Modified Food: Evidence from Experimental Auctions in the United States, England, and France. *Eur. Rev. Agric. Econ.* **2004**, 31 (2), 179–204.
- Lüthy, J. Detection Strategies for Food Authenticity and Genetically Modified Foods. *Food Control*, **1999**, 10 (6), 359–361.
- Metcalf, D. D. et al. Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants. *Crit. Rev. Food Sci. Nutr.* **1996**, 36 (S1), 165–186.
- Metcalf, D. D. Introduction: What Are the Issues in Addressing the Allergenic Potential of Genetically Modified Foods? *Environ. Health Prospect.* **2003**, 111 (8), 1110.
- Meyerson, L. A.; Reaser, J. K. Biosecurity: Moving Toward a Comprehensive Approach: A Comprehensive Approach to Biosecurity Is Necessary to Minimize the Risk of Harm Caused by Non-Native Organisms to Agriculture, the Economy, the Environment, and Human Health. *Bioscience* **2002**, 52 (7), 593–600.
- Moglia, A.; Portis, E. *Genetically Modified Foods. Encyclopedia of Food and Health*; Academic Press, 2016; pp 196–202.
- Moura-Melo, S. et al. Targeting Helicase-Dependent Amplification Products with an Electrochemical Genosensor for Reliable and Sensitive Screening of Genetically Modified Organisms. *Anal. Chem.* **2015**, 87 (16), 8547–8554.
- Novak, W. K.; Haslberger, A. G. Substantial Equivalence of Antinutrients and Inherent Plant Toxins in Genetically Modified Novel Foods. *Food Chem. Toxicol.* **2000**, 38 (6), 473–483.

- O'Callaghan, M. et al. Effects of Plants Genetically Modified for Insect Resistance on Nontarget Organisms. *Annu. Rev. Entomol.* **2005**, *50*, 271–292.
- Pandey, A. et al. Genetically Modified Food: Its Uses, Future Prospects, and Safety Assessments. *Biotechnology* **2010**, *9* (4), 444–458.
- Pandey, R. et al. Genetically Modified Food Animals: An Overview. In *Genetically Modified Organisms in Food*; Preedy, V. R., Ronald, W. R., Eds.; Academic Press: San Diego, 2016; pp 19–26.
- Pauwels, K. et al. Next-Generation Sequencing as a Tool for the Molecular Characterization and Risk Assessment of Genetically Modified Plants: Added Value or Not? *Trends Food Sci. Technol.* **2015**, *45* (2), 319–326.
- Phipps, R.; Park, J. Environmental Benefits of Genetically Modified Crops: Global and European Perspectives on Their Ability to Reduce Pesticide Use. *J. Anim. Feed Sci.* **2002**, *11* (1), 1–18.
- Pryme, I. F.; Lembcke, R. In Vivo Studies on Possible Health Consequences of Genetically Modified Food and Feed—with Particular Regard to Ingredients Consisting of Genetically Modified Plant Materials. *Nutr. Health*, **2003**, *17* (1), 1–8.
- Pusztai, A.; Bardocz, S.; Ewen, S. Genetically Modified Foods: Potential Human Health Effects; Food Safety CAB International: Aberdeen, 2003; p 347.
- Qaim, M. The Economics of Genetically Modified Crops. *Ann. Rev. Resour. Econ.* **2009**, *1* (1), 665–694.
- Qaim, M.; Zilberman, D. Yield Effects of Genetically Modified Crops in Developing Countries. *Science* **2003**, *299* (5608), 900–902.
- Ramezani, B. et al. Vector-Based Genetically Modified Vaccines: Exploiting Jenner's Legacy. *Vaccine* **2016**, *34* (50), 6436–6448.
- Rischer, H.; Oksman-Caldentey, K.-M. Unintended Effects in Genetically Modified Crops: Revealed by Metabolomics? *Trends Biotechnol.* **2006**, *24* (3), 102–104.
- Rudolph, U.; Möhler, H. Genetically Modified Animals in Pharmacological Research: Future Trends. *Eur. J. Pharmacol.* **1999**, *375* (1), 327–337.
- Sandmann, G.; Römer, S.; Fraser, P. D. Understanding Carotenoid Metabolism as a Necessity for Genetic Engineering of Crop Plants. *Metab. Eng.* **2006**, *8* (4), 291–302.
- Savulescu, J. Genetically Modified Animals: Should There Be Limits to Engineering the Animal Kingdom. In *The Oxford Handbook of Animal Ethics*; Beauchamp, T. L., Frey, R. G., Eds.; Oxford University Press: Oxford, 2013.
- Seney, M.; Edgar, N.; Sibille, E. Genetically Modified Animals. In *Encyclopedia of Psychopharmacology*; Stolerman, I. P., Price, L. H., Eds.; Springer Verlag GmbH: Heidelberg, 2015; pp 702–709.
- Séralini, G.-E.; Cellier, D.; de Vendomois, J. S. New Analysis of a Rat Feeding Study with a Genetically Modified Maize Reveals Signs of Hepatorenal Toxicity. *Arch. Environ. Contam. Toxicol.* **2007**, *52* (4), 596–602.
- Shannon, M.; Grieve, C. Tolerance of Vegetable Crops to Salinity. *Sci. Hortic.* **1998**, *78* (1), 5–38.
- Shelton, J. A. M.; Kennedy, G. G., Eds. *Progress in Biological Control*; Springer: Netherlands, 2010; pp 1–26.
- Singh, A.; Kumar, V.; Poonam, G. H.; Genetically Modified Food: A Review on Mechanism of Production and Labeling Concern. *Adv. Plants Agric. Res.* **2014**, *1* (4), 00020.
- Slater, A.; Scott, N. W.; Fowler, M. R. *The Genetic Manipulation of Plants*. *Plant Biotechnology*; Oxford University Press: Oxford, 2003.

- Smith, I. F.; Green, K. N.; LaFerla, F. M. Calcium Dysregulation in Alzheimer's Disease: Recent Advances Gained from Genetically Modified Animals. *Cell Calcium* **2005**, *38* (3), 427–437.
- T. H. et al. Insect-Resistant Transgenic Plants. *Trends Biotechnol.* **1998**, *16* (4), 168–175.
- Taylor, S. L. Food from Genetically Modified Organisms and Potential for Food Allergy. *Environ. Toxicol. Pharmacol.* **1997**, *4* (1–2), 121–126.
- Taylor, S. L.; Hefle, S. L. Will Genetically Modified Foods Be Allergenic? *J. Allergy Clin. Immunol.* **2001**, *107* (5), 765–771.
- Teshima, R. et al. Effect of GM and Non-GM Soybeans on the Immune System of BN Rats and B10A Mice. *Shokuhin Eiseigaku Zasshi* **2000**, *41*, 188–193.
- Tufarelli, V. et al. Genetically Modified Feeds in Poultry Diet: Safety, Performance, and Product Quality. *Crit. Rev. Food Sci. Nutr.* **2015**, *55* (4), 562–569.
- Uzogara, S. G. The Impact of Genetic Modification of Human Foods in the 21st Century: A Review. *Biotechnol. Adv.* **2000**, *18* (3), 179–206.
- Wang, W.; Vinocur, B.; Altman, A. Plant Responses to Drought, Salinity, and Extreme Temperatures: Towards Genetic Engineering for Stress Tolerance. *Planta* **2003**, *218* (1), 1–14.
- Watkinson, A. et al. Predictions of Biodiversity Response to Genetically Modified Herbicide-Tolerant Crops. *Science* **2000**, *289* (5484), 1554–1557.
- Whitelaw, C.; Sang, H. Disease-Resistant Genetically Modified Animals. *Rev. Sci. Tech.* **2005**, *24* (1), 275.
- Whitman, D. B. Genetically Modified Foods: Harmful or Helpful? CSA Discovery Guides, 2000; pp 1–13. <https://biomed.brown.edu/arise/resources/docs/GM%20foods%20review.pdf>
- Wong, A. Y.-T.; Chan, A. W.-K. Genetically Modified Foods in China and the United States: A Primer of Regulation and Intellectual Property Protection. *Food Sci. Hum. Well.* **2016**, *5* (3), 124–140.
- Zhang, C.; Wohlhueter, R.; Zhang, H. Genetically Modified Foods: A Critical Review of Their Promise and Problems. *Food Sci. Hum. Well.* **2016a**, *5* (3), 116–123.
- Zhang, C.; Wohlhueter, R.; Zhang, H. Transgenic Food, Benefits, Safety, and More. *Food Sci. Hum. Well.* **2016b**, *5*, 116–123.
- Zhang, H.-C. et al. Unintended Effects and Their Detection in Genetically Modified Crops. *Acta Agriculturae Zhejiangensis* **2012**, *1*, 024.
- Zhu, J.-K. Genetic Analysis of Plant Salt Tolerance Using Arabidopsis. *Plant Physiol.* **2000**, *124* (3), 941–948.
- Zhu, J.-K. Plant Salt Tolerance. *Trends Plant Sci.* **2001**, *6* (2), 66–71.
- Zhu, Y. et al. Nutritional Assessment and Fate of DNA of Soybean Meal from Roundup Ready or Conventional Soybeans Using Rats. *Arch. Anim. Nutr.* **2004**, *58* (4), 295–310.

CHAPTER 10

Uses of TILLING for Crop Improvement

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ABSTRACT

Climate changes cause temperature extremes and uneven rain spells which induce severe abiotic/biotic stresses that collectively hampering the global crop production. These inevitable threats pose serious threats to global food security. Mutation breeding is an excellent strategy that exploited various genome manipulated toolkits for crop improvement. But transgenic crops are less acceptable in many countries due to strict regulatory protocols and public concerns. There is a need to develop cost-effective and risk-free tools to fast-track the breeding process to boost up crop yield. One such nontransgenic tool is targeting Induced local lesions in genomes (TILLING) that can bypass the regulatory sanctions imposed on GMOs. Tilling has emerged as a powerful alternative to develop mutagenesis and works on the principle of reverse genetics. It can integrate with conventional and modern mutagenic

agents to produce point mutations in any desired gene for crop improvement. Also, it offers a robust, economical, and precise technique to detect genome-wide single nucleotide mutations in autopolyploids, allopolyploids, allohexaploid, monocots, dicots, and triploids. It has significant potential to cope with the huge task of associating the sequencing data to gene functions and fine-tune vital traits in different crop species. In this study, we highlight the vast applications of TILLING for the improvement, especially its role in gene functional studies for abiotic/biotic stress resistance and improvement of key agronomic traits.

10.1 INTRODUCTION

The exponentially burgeoning population and unprecedented climate changes are the biggest challenges to guarantee the global food security. The stakeholders and policymakers are striving hard to eradicate the hunger and fulfill the food requirements of people around the globe (Verma et al., 2020). As quoted by Norman Borlaug “Food is the basic requirement for all the people in the world.” It is forecasted that the population is going to exceed 10 billion by the end of 2050, which will further aggravate the scenario of food security. The detrimental impact of climate change has put great pressure on modern agriculture and limiting the crop yield sustainably (Leng and Huang, 2017).

The climate change causes uneven spells of rainfall and continuous increase in global temperature, which enhance the vulnerability of crops against different abiotic and biotic stresses. These environmental constraints hampering the crop growth and crops failed to adapt under harsh climatic conditions that accelerate the food crisis in some countries (Raza et al., 2019). The annual yield of cereals needs to fast-track twofold from the current production to match the annual increase of cereal demands. Therefore, quick action must be needed to lessen the impacts of climate change of agriculture and develop climate-resilient crop cultivars that can withstand the adverse abiotic/biotic stresses and ensure the food needs of people for sustainable agriculture (Kukal and Irmak, 2018).

Conventional breeding has been extensively used to develop improved crop cultivars with the desired traits since thousands of years ago. But the development of these cultivars is time-consuming and carried out by applying various approaches. Intensive selection and screening are the methods that have been used to select the superior traits by crossing the

elite crop cultivars (Brozynska et al., 2016). Hybridization is another method frequently exploited in addition to the selection process. Hybridization also called introgression employs the genetic variation produced during a crossing between two parents for crop improvement. The crossing can be between the different species known as interspecific or between the species called intraspecific (Kashtwari et al., 2019).

However, these intensive breeding platforms have reduce the genetic diversity and eliminate several unique genes that are linked with important agronomic traits and tolerant mechanisms. The crop natural selection and evolution have certain drawback such as the extinction of wild relatives and landraces and less genetic variation in the genomic pool of cultivated crop species. It hinders the generation of new crop species having better quality and resistant against abiotic/biotic stresses (Smýkal et al., 2018). Also, genetic erosion produces obstacles in enhancing the yield and nutritive value to connect the gap between need and production. Therefore, conventional breeding approaches are no longer beneficial to create climate-smart crops with greater adaptability to acclimatize in diverse environments with increased production to overcome the problem of food insecurity (Zhang et al., 2017).

This study describes the current mutation breeding approaches used for crop improvement and their possible drawbacks. We focus on the reserve genetic technology “Targeting Induced Local lesions In Genomes” (TILLING) and comprehensively spotlight its principle. We also highlight the applications of tilling to develop abiotic/biotic stress resistance and improve the agronomic traits in major crop species.

10.2 GENOMIC TOOLS FOR CROP IMPROVEMENT

Modern genomics approaches have widened the exciting roadmap for crop improvement. Advanced sequencing technologies have sequenced the complete genome of major crops and still many of genomes are yet to be completed (Till et al., 2003). However, the utilization of whole-genome sequencing databases is restricted by the complex nature of several agronomic and stress-tolerant traits. The data available for these crops have enlightened the prerequisite to design genomic-based reverse genetic tools for functional investigation of any trait (Parry et al., 2009). Although reverse genetic techniques permit advancement to be attained on the crucial task of associating sequence datasets to the gene functions and deciphering their

linkage to specific traits (Kannan et al., 2020). Generally, these techniques depend on the manipulation of the gene of interest via RNA interference (RNAi), T-DNA tagging, transgenic, transposons, and mutagenesis (chemical and physical mutagens).

The genetic engineering approaches came to rescue and have been widely applied for crop improvement. Although transgenic technologies, they encountered several problems on the drive to get access the farm, which has led to out-of-the-box approaches for crop improvement (Kashtwari et al., 2019). The total area under cultivation of transgenic crops in six big countries including the USA, Mexico, Australia, Canada, Argentina, and China was 1.7 million hectares since the commercialization of the first biotech crop in 1996. The area increased to 191.7 million hectares in 2018 with 70 countries cultivating the biotech crops. In addition, after 23 years of the first plantation, the transgenic crops yield rise 113-fold, covering 2.5 billion hectares around the world (ISAAA, 2020).

However, there are several disadvantages of the transgenic techniques that minimizing its application in many countries, and a complete ban on biotech crops in Europe and some other countries due to its societal and human safety concerns. The major bottleneck is the inefficient transformation techniques both chemical and physical methods for *Agrobacterium* transformation (Waltz, 2016). Tissue culturing protocols for some crops are not present to regenerate the transformed tissues and stable integration of foreign genes is also the big problem associated with transgenic techniques. Furthermore, there is strict regulatory mechanism for the approval of any genetically modified (GM) crop that makes it laborious and time-consuming and costly method for crop improvement (Razzaq et al., 2019b).

To overcome these limitations, clustered regularly interspaced short palindromic repeats (CRISRP), CRISPR-associated protein 9 (Cas9)-based genome editing provide an excellent platform for targeted insertion of deletion of any gene in crop. CRISPR/Cas9 has vast application in crop breeding and is applied to develop the crop varieties having desired traits (Zhu et al., 2020). But still, there are some concerns that divided the world into two halves on GM regulations. USA, Canada, China, Argentina, and Brazil are approving the crops to develop through CRISPR/Cas9 technology without any stringent GM rules and called in non-GM crops. On the other hand, Europe and other countries have raised some serious question on the safe consumption of these crops (Pickar-Oliver and Gersbach, 2019).

10.3 MUTATION BREEDING

Mutation breeding is recognized as one of the earliest strategy for trait improvement without modifying the natural genetic make-up of crops and has a long history in several important crops as many of them have their origin in genetic resources produced by a genetic mutation (Raina et al., 2016). Induce mutagenesis is a vital technique for targeted manipulation of candidate gene for crop improvement. Mutation breeding helps to make a strong connection between the gene product and its function in vivo (Jankowicz-Cieslak et al., 2017).

For many years, mutagenesis has been applied to identify the genes and determine their functions by examining mutants that are nonfunctional for a desire trait of interest through forwarding genetics. The forward genetics tools allow the selection of unique phenotypes that can be employed in traditional breeding programs for trait improvement (Gajewska et al., 2018). Conventional mutagenesis strategies have been extensively applied in forward genetics and have developed about 2000 mutant crop cultivars. With innovation in molecular biology, various methods have been designed to produce or screen mutants in cloned genes in order to elucidate the gene function (Jankowicz-Cieslak et al., 2017). The reverse genetics methods enable the capturing of point mutations in the whole gene pool and can overcome the main drawback of bridging whole genome sequencing data to the specific gene function and also detect unique genetic variations within the crop species (Raina et al., 2017).

Mutagenesis can be targeted or random and induce naturally or artificially by scientists. Induced mutagenesis are of two major types, such as chemical mutagenesis and physical mutagenesis. The later one includes gamma rays, X-rays, and fast neutrons to develop greater number of variations and have crucial role in genetic analysis of crops (Tadele, 2016). On the other hand, chemical mutagens are very significant to produce efficient and precise mutations in diverse crop species. Ethyl methane sulphonate (EMS) is the extensively applied for induced mutagenesis due to its tremendous efficacy at producing point mutations and can offer vast phenotypes. Scientists are keen to detect the polymorphism or mutations within the genome and elucidate the gene function and its applications for crop improvement (Chaudhary et al., 2019).

Although the applications of mutation breeding are vast and different mutagenic agents like chemicals and radiations offer a nontransgenic and a safe route to create mutants lines that assist to fine-tune the certain traits

including increased tolerance to abiotic and biotic stresses, enhanced nutritive value, and higher yield production. But, these methods have several pitfalls which make their uses only limited to some cases. For example, it is tough to detect a single mutant line having unique traits in a huge pool of mutant population and numerous mutants have no evident signs because of genetic redundancy induced by polyploidy or gene duplication (Parry et al., 2009).

10.4 TILLING FOR CROP IMPROVEMENT

The mutation breeding has attained substantial advancement which allowed the development of numerous improved varieties. Recently, great improvement has been achieved in the genomics approaches that led to the introduction of highly sophisticated tools for next-generation sequencing (NGS). It enabled the scientist to sequence numerous crop cultivars and map the genes existing in the genomic reservoir of a crop. Availability of gene sequences make the reverse genetics approaches more appealing and facilitated the development of nontransgenic tools that have been widely used to probing the gene functions and detect the genetic variation between the crop species that can be employed for crop improvement (Sestili et al., 2010; Verma et al., 2020).

TILLING has come out as a promising reverse genetics modern technology that uses a mismatch-specific endonuclease to screen natural or induced polymorphism within any candidate gene (McCallum et al., 2000). It assists to localize induced mutation within the genotypes harboring mutations in the candidate genes. Also, TILLING offers the rapid and cost-effective discovery of a point mutation or single nucleotide polymorphism (SNP) through unmatched cleavage in a group of plant mutants, generated by employing chemical mutagens (Tadele, 2016). TILLING is used to interrupt or silence the individual genes of interest, offering the chance to connect sequence variation data to capture unique allelic variants for determining the gene functions and trait phenotypes (Parry et al., 2009). SNPs are detected in candidate genes via population screening of mutated genotypes applying any technique of mutant screening. The DNA-based mutant identification technique bypasses the troubles of screening unique phenotypic traits in polyploidy crops. The collection of alleles that can be identified through TILLING to produce novel characteristics is incomparable and unlikely to be present anywhere in genomic pool including crop wild relatives and landraces available for plant breeders (Slade et al., 2012).

The first step of TILLING technique consists of conventional mutagenesis application in optimum concentration that enables to produce nonchimeric individuals. In the second step, DNA extracted from all the genomic pool and mutant screen is achieved through various methods. The final step is the assessment and validation of acquired results for the specific phenotype (Parry et al., 2009). In addition, TILLING generates a wide-range of mutations such as splicing, missense, and nonsense mutation that can also be applied to annotate protein domains. TILLING has been efficiently used for numerous organisms including animals, microorganisms, and plants, and has emerged as a commanding approach for screening the beneficial mutant lines and functional studies of candidate genes in major crop species (Tadele, 2016).

TILLING is largely applied to all types of genomes whether large or small, diploid, polyploid, and allohexaploid and offers an excellent platform to deal with the big task of bridging the gap between sequence information and their respective sequence information in order to fine-tune important traits for crop improvement (Jankowicz-Cieslak et al., 2011). Many projects have been carried out for 25 crop species since the initial depiction of TILLING in the 1990s (Jankowicz-Cieslak and Till, 2016; Serrat et al., 2014). For example, a series of economical and simple methods have been developed by Plant Breeding and Genetics Laboratory in Austria for quick and efficient mutant characterization. These approaches do not need sophisticated tools and do not depend on harmful substances. These comprise techniques for DNA extraction, single standard nuclease extraction, and mutant identification using agarose gel-based assays (Manzanares et al., 2016; Jankowicz-Cieslak et al., 2017).

In the TILLING procedure, the selection of chemical mutagens is very crucial which has been exploited for many years in mutation breeding of different crops. The chemical mutagens enable efficient point mutations with higher frequency and series of allelic mismatches (Irshad et al., 2020). There are numerous chemical mutagens that can be applied to produce desired mutations and EMS is recognized as the most important mutagenic agent (Rout, 2020). The EMS an alkylating mutagenic agent is especially efficient because it produces adducts with DNA bases, leading to damage the nucleotide complementation, hence modifying the base pairs. It causes G/C-to-A/T transitions that give a higher rate of random mutations all over the genome (Schreiber et al., 2019). Endonuclease cleave successfully with several missenses in the unknown sequence of DNA heteroduplex and duplex to that of known sequence unveils the polymorphism at different positions.

Hence, both indels mutations and nucleotide modifications can be detected with cost-effective sequencing techniques (Sestili et al., 2019). In addition, other mutagenic agents like MNU (N-methyl-N-nitrosourea) and ethylnitrosourea (ENU) have also been applied in the mutation breeding of many crop species. The best TILLING agent is the one that induces insertions/deletions within less than 30 bases random single-base substitutions can be identified with TILLING (Sestili et al., 2010).

TILLING is a more sophisticated and advanced approach of mutation breeding and has numerous advantages as compared to other reverse genetic approaches including its suitability to any crop regardless of its ploidy level and its capability for high-throughput detection of natural or induced variation in many crops (Raina et al., 2016). TILLING approach is quite useful where the transformation is cumbersome or impossible and the examination of an array of unknown alleles is needed. It offers an excellent platform for mutant screening to elucidate the gene function escaping both laborious tissue culture protocols and confounding gene screening approaches as are required in RNA silencing (RNAi) and antisense RNA (Sestili et al., 2019). TILLING is superior as compared to other reverse genetics approaches that are applied to capture the SNP. For example, the other mutant identification strategies such as single-strand conformation polymorphism and denaturing gradient gel electrophoresis are gel-based assays that unable to detect position and polymorphism type. Different hybridization approaches are only effective in determining of about 50% SNPs in any DNA sample (Raina et al., 2017).

Latest innovations in CRISPR/Cas mediated genome editing revamping the modern breeding but have serious biosafety concerns related to foreign gene introduction (Razzaq et al., 2019b). TILLING is a nontransgenic approach and provides an alternative safer platform to overcome the limitations faced by transgenic and genome editing technologies. Because in TILLING, there is no introduction of any foreign DNA into the host cell which make, it more preferable and address all the regulatory, societal, and consumer concerns for their safe use (Schreiber et al., 2019; Sestili et al., 2019). Also due to is greater effectiveness in mutation screening, TILLING has attained more acceptability in public since, the crops designed by employing this method are released from the strict biosafety regulations enforced by GM regulatory bodies (Sestili et al., 2010).

TILLING approaches have achieved hundreds of thousands of mutation to the plant breeding around the globe. Progress in advance mutation screening approaches assure to enhance more applicability and efficacy of TILLING

in several crops. With the successful reports of TILLING mediated induce mutations in monocots, dicots, triploids, allopolyploids, and autopolyploids, this strategy will assist in producing elite crop cultivars through induced mutation breeding in the coming decades (Kashtwari et al., 2019).

10.5 TILLING MODE OF ACTION

The protocol of TILLING-based induced mutation comprises some major steps including generation of mutant population by using mutagenic agents, followed by disintegration of chimeric cells produced by mutagens, DNA isolation from the mutant population, pooling and preservation of germplasm, followed by mutant identification, screening, and phenotype determination (Till et al., 2004, 2006).

10.5.1 MUTANT DEVELOPMENT

In the initial steps of TILLING technique in plants, a large pool of mutants is developed through induced mutations by treating the multicellular organs like seeds and pollens with mutagenic agents (Triques et al., 2007). When multicellular tissues are targeted, the chimeric plants are produced due to the random mutations in a different part of the plant cells. These chimeric cells must be disintegrate via in vitro approaches or self-fertilization prior to mutant detection. In addition, a diverse spectrum of mutants can be developed applying various kinds of mutagenic agents (Li et al., 2017). Like, rice seed treated with MNU generates 20% A:T to G:C and 67% G:C to A:T indels mutations. The first nonchimeric plants that develop through sexual propagation are used to produce M2 generation which is selected to develop mutant lines. A single seed descent approach is generally applied to develop a mutant line from a single M2 plant developed from M1 parental lines (Till et al., 2007).

10.5.2 DNA EXTRACTION AND POOLING

The M2 progeny is selected to extract DNA from each sample for mutant identification. These M2 generations are used for self-fertilization to develop M3 lines for phenotype determination in the later stage. These DNA libraries are pooled and preserved for future use in microtiter plates. Double haploidy,

tissue culture, and in vitro propagation can be useful for generating mutants that are unresponsive to the technique presented before. Since mutagen agents like EMS create random mutations in the whole genome and each mutant will hold diverse mutations (Till et al., 2003, 2007).

10.5.3 MUTANT SCREENING

Denaturing high-performance liquid chromatography was the first commercial technique applied to discover mismatches through the PCR process. There are some other methods developed such as capillary gel electrophoresis, enzymatic mismatch cleavage with slab and direct sequencing (Colbert et al., 2001; Perry et al., 2003; Wienholds et al., 2003; Suzuki et al., 2008). A most economical and robust amendment of TILLING technique was developed that uses a LI-COR gel analyzer platform combined with mismatch-specific celery nuclease (CEL1) for mutant screening. In this technique, fluorescent-labeled gene-specific primers such as forward primer and reverse primer are employed to amplify the target region of DNA having the size of about 1.5 kb. The fluorescent dye used to label the primers is detected at 700 nm for forward primer and at 800 nm for reverse primer (Till et al., 2006). Heteroduplexed DNA molecules are formed from amplicons by denaturing and annealing the samples during PCR. At the positions of induced mutation mismatches are produced in double-standard DNA that is cleaved during DNA incubation with CEL (Till et al., 2004). These cleaved DNA sequences are size fractionated via gel electrophoresis and fluorescent signals perceived in two isolated chambers through LI-COR system. The molecular size of PCR amplicons equal to whole sliced sequences showed mutation in the sample and location of mutation (Greene et al., 2003). The screened mutants can be subjected to NGS for confirmation of the exact mutation in DNA sequence and then evaluated through functional assays (Till et al., 2003, 2004, 2006).

PCR primers designing is an important decision for TILLING and choice of an appropriate primers gives a greater opportunity to detect variation in the nucleotides with an effect on the function of protein in TILLING-based detection (Uauy et al., 2009). It is beneficial to select a sequence within the coding region and can be chosen using different bioinformatics tools like Codons Optimized to Discover Deleterious Lesions (Stemple, 2004) and Project Aligned Related Sequences and Evaluate SNPs (Taylor and Greene, 2003). In polyploid wheat, coding regions frequently show functional domains with greater resemblance among homologous alleles. High-resolution melting

(HRM) examination of diverse PCR amplicons can also identify mismatch in three genes altogether (Dong et al., 2009). NGS can significantly increase the opportunities for mutant screening at whole-genome due to the low cost, enhanced accuracy, and improved capability in TILLING.

10.6 APPLICATIONS OF TILLING IN CROP IMPROVEMENT

The unprecedented events of climate change hampering crop production and increasing serious fears of food insecurity all over the world. Mutation breeding through the advanced TILLING technique has the excellent potential to mitigate the different abiotic and biotic stresses to develop the climate-resilient crop varieties (Gruner et al., 2020b; Zhong et al., 2020). TILLING also has vast applications in improving the various important agronomic traits related to crop yield and nutritive value to produce superior crop genotypes to deal with the major concerns of climate change and food security (Okabe et al., 2012; Lakhssassi et al., 2020).

10.6.1 TILLING FOR IMPROVING AGRONOMIC TRAITS IN MAJOR CROPS

Improving agronomic traits including yield-related traits, nutrition value, and shelf life is the ultimate goal of plant breeders. TILLING has been broadly applied to improving the several traits in major crop species (Okabe et al., 2012; Reddy et al., 2012; Yano et al., 2019).

10.6.1.1 WHEAT

Wheat is ranked second and is one of the most important staple crop to fulfill the food requirements of people. It is the essential part of human diet to provide the daily calories (Kanwal et al., 2019). Essential nutrients like macro and micro nutrients are crucial to maintain the metabolic balance in humans by up-taking them through food. Wheat contains a large of important protein and carbohydrates, but lack the essential micronutrients, such as provitamin A. Its deficiency results in the prevalence of many severe disease around the globe (Sestili et al., 2019). Hence, biofortification of wheat is vital to deal with nutrient deficiencies and effective way to prevent serious human diseases. TILLING has been executed to target the b-carotene, lycopene epsilon cyclase that regulation of carotenoids pathways in wheat. The knock-out mutants of this enzyme demonstrated down-regulation in *lcyE* gene

expression and biochemical analysis showed 75% increase in the carotenoids production in wheat mutants as compared to normal lines (Sestili et al., 2019).

Durum wheat is important to make the couscous and pasta and cultivated on large scale in different countries. The quality of flour for making pasta is effected by the ration of amylose to amylopectin. High amylose starch consumption is beneficial for human health due to the abundance of resistant starch, such as dietary fibers. The durum wheat flour only contains 25% of amylose starch, but it can be enhanced by mutating the starch branching enzyme IIa (*SBEIIa*) and soluble starch II (*SSII*) genes responsible for amylopectin production. A TILLING protocol was established to knock down the *SBEIIa* and *SSII* genes in durum wheat. The successful knock-outs produce pleiotropic effects on the ratio of amylopectin and amylose. The results indicated a higher accumulation of amylose (up to ~1.95-fold) in the double mutant's grains which increased the nutritive value of durum wheat (Sestili et al., 2015). These similar types of experiments were carried out by Slade and colleagues by using TILLING techniques in bread wheat to disrupt the *SBEIIa* gene. The mutation breeding resulted in the production of enhance amylose concentration 47–55% in wheat as compare to wild-type (Slade et al., 2012). Botticella and coworkers identified *SBEIIa-A* (56), *SBEIIa-B* (14), *SBEIIa-D* (53) that led to modify the structure and functions of proteins. TILLING-based mutant of *SBEIIa* genes showed 21% high amylose production in *Triticum aestivum* (Botticella et al., 2011), and 22% in tetraploid wheat (Bovina et al., 2014) as compared to control wheat lines.

In addition, TILLING is also used to create unique allelic variation within the wheat genome to enhance the genetic variations which is very crucial for wheat improvement programs (Colasuonno et al., 2016). Starch synthase II (*Sgp-I*) and *waxy* genes were mutated via TILLING and results unveiled novel allelic variations that can be applied to fine-tune the agronomic traits in wheat (Sestili et al., 2010). Harrington et al. (2019) exploited the series of unique induced variations in durum wheat via TILLING to detect a dominant chlorosis phenotype Yellow Early Senescence 1 (*YES-1*) located on chromosome 3A. Mutant lines harbor little chlorophyll during their life cycle and presented early flag lead senescence. This showed that mutant population have induced variation that can offer vital dominant variations in crops of large size genome, emphasizing their significance in future genetic breeding programs (Harrington et al., 2019).

In wheat, starch granules have two types, like A- and B-type having bimodal size. TILLING-based mutagenesis was conducted for B-GRANULE

CONTENT 1 (*BGCI*) gene which controls the starch synthesis in polyploid wheat. The mutant lines stop the A-granules in the initial phase of grain development while induce the B-granules in the intermediate phase of grain formation. It showed that *BGCI* gene regulate the starch granules formations and effect granule number and size in wheat (Chia et al., 2020). Another study reported the increased grain size in both bread and durum wheat by mutating the *TaGW2-A1* gene which is negatively associated with grain weight and size. The mutant wheat lines revealed increase grain length (2.1%), width (2.8%), and weight (6.6%) in contrast to wild variety (Simmonds et al., 2016).

TILLING has also applied to identify the novel wheat q' allele associated with *APETALA2* transcription factor which govern the development of square-head and free threshing spikes of modern wheat cultivars. The detection of novel q' allele assists to characterize Q and the mutagenesis resulted in the Ala → Thr substitution *APETALA2* (Madsen and Brinch-Pedersen, 2020). Li and coworkers demonstrated the TILLING-based mutagenesis in wheat to improve the quality by disrupting the *Pinb*, *SSIIa-A* and *Agp2* genes related to kernel hardness and starch biosynthesis (Li et al., 2017).

10.6.1.2 RICE

Rice is the most widely cultivated and consumed crop in the world and has great potential to meet food demands and guarantee global food security. The nutritional value and quality are the major traits that have been exploited by the breeders to develop more nutritious and healthier rice. In 2018, Li and colleagues demonstrated the TILLING mediated induced mutations by using γ radiation to mutate the two genes such as *OsLCT1* and *SPDT* which are directly linked with rice quality and nutritive value. HRM analysis was employed to screen the indel knockouts from the wild type. The mutagenesis led to single base substitution (G→A) and three bases deletion (TTC) was reported in *SPDT* while one base addition (A) was detected in *OsLCT1* gene. These mutant lines showed increased nutrition characteristics and can be used in different breeding programs for rice improvement (Li et al., 2018). TILLING by sequencing was performed to detect the unique mutations to improve the quality of rice by increasing the amylose content in mutant lines. Three genes *SBEI*, *SBEIIa*, and *SBEIIb* that have different amylose contents were targeted to determine the amylose to amylopectin ratio. Physiological analysis showed an increase biosynthesis of amylose to 19% and reduce the

amylopectin concentration from 9.2 to 4.8%. Hence, the detection of unique *SBEI* knockouts will be beneficial in deciphering its function and can be applied to improve the quality related traits in rice (Kim et al., 2018).

10.6.1.3 SOYBEAN

Soybean (*Glycine max* L.) is an essential oilseed plant and has great economical value. Its oil generally consists of α -linolenic acid in which it is oxidized very easily and highly unstable in nature. This feature of soybean oil makes it undesirable for domestic usage and industrial purposes. Genetic improvement of soybean oil having reduced biosynthesis of α -linolenic acid can increase the market value. TILLING approach was applied to mutate the *GmFAD3-1a*, *GmFAD3-2a*, and *GmFAD3-1b* genes that regulate the omega-3-fatty acid desaturase (FAD3) pathway. The mutant lines showed significant decrease in α -linolenic acid level of about 6% in the seed. This can be used to improve the soybean with better quality oil production (Hoshino et al., 2014). Recently, TILLING by sequencing was used to increase the health benefits of soybean oil by increasing stearic acid concentration in oil which is the major objective of soybean industry. On the other hand, polyunsaturated fatty acids like palmitic acid concentration was decreased because it cause many health-related issues and undesirable for nutrition. The mutagenesis created mutant lines for *GmSACPD-A*, *GmSACPD-B*, and *GmSACPD-D* genes that demonstrated unprecedented role in the biosynthesis of unsaturated fatty acids. This novel research will allow the higher production of stearic acid and increase the nutritional value of soybean (Lakhssassi et al., 2020). Moreover, oil consumption of soybean seeds is also used as a part of animal feed. But the soybean seed comprises of some antinutritional compounds like stachyose and raffinose that are indigestible by some animals. TILLING-by-sequencing was executed to create missense mutations in *RAFFINOSE SYNTHASE3* gene which resulted in the elimination of stachyose and raffinose from the seed as carbohydrates and enhance sucrose biosynthesis (Thapa et al., 2019).

10.6.1.4 TOMATO

Parthenocary is a desirable characteristic for fruit set under environmental conditions that are unfavorable for pollination. It enables to produce seedless fruit and increase the commercial value and consumer preference of the specific fruit. TILLING was used to target the *Aux/IAA9* gene which

negatively regulate the auxin role in tomato fruit set. Total three mutations were found in the coding region with one nucleotide deletion and two single nucleotide substitution that led to premature stop codon. This resulted in the reduction of leaf compoundness, changed axillary growth, loss of *IAA9* function, and induced parthenocarpic fruit set. Mutant tomato lines have larger fruit size and high brix value as compared to wild type. These findings will help to develop seedless tomatoes having good quality (Mazzucato et al., 2015). TILLING technology was applied to develop improved quality tomato lines having increased concentration of lycopene that transforms into carotene during fruit ripening by mutating the *Cyc-B* allele. The results unveiled that the mutant lines harbor the increased accumulation of lycopene without any negative impact on other agronomic and yield-related traits. The result highlighted the utility of *Cyc-B* allele to improve the agronomic traits like carotenoid contents in tomato through breeding process and produce elite commercial varieties to get maximum economic gains (Silletti et al., 2013). The shelf life of tomato fruits was increased by disrupting the ETHYLENE RESPONSE1 (*SlETR1*) gene via TILLING technique (Schreiber et al., 2019).

TILLING has also been applied to improve the legumes such as chickpea (Amri-Tiliouine et al., 2018), mungbean (Varadaraju et al., 2020), sorghum (Xin et al., 2008), and barley (Schreiber et al., 2019) (Table 10.1).

10.7 APPLICATION OF TILLING TO DEVELOP ABIOTIC AND BIOTIC STRESS TOLERANCE CROPS

10.7.1 ABIOTIC STRESSES

Climate change is affecting the crop productivity by inducing the extreme abiotic stresses. TILLING has achieved tremendous progress in coping the different stresses and successfully developed stress-resilient crops.

10.7.1.1 SALT TOLERANCE

For example, TILLING technology has been applied to tackle the salinization of soil which hamper the crop productivity and causes huge economic losses every year (Negrão et al., 2017). Recently, a study was conducted to investigate the salinity tolerance by developing the *CAX1a* mutants of *Brassica rapa*. The results unravel higher salt tolerance in *CAX1a* mutant

TABLE 10.1 Applications of TILLING To Improve Agronomic Traits.

Crop species	Trait improvement	Mutagenic agent	Target gene	Year	References
Durum wheat	Provitamin A biofortification	EMS	<i>lcyE</i>	2019	Till et al. (2004)
Durum wheat	Amylose production increase ~1.95 fold	EMS	<i>SBEIIa</i> , <i>SSII</i>	2015	Sestili et al. (2015)
Bread wheat	Amylose production increase 45–55%	EMS	<i>SBEIIa</i>	2012	Slade et al. (2012)
Bread wheat	Amylose production increase 21%	EMS	<i>SBEIIa-A</i> , <i>SBEIIa-B</i> , <i>SBEIIa-D</i>	2011	Botticella et al. (2011)
Durum wheat	Amylose production increase 22%	EMS	<i>SBEIIa-A</i> , <i>SBEIIa-B</i> ,	2014	Bovina et al. (2014)
Wheat cultivar Cadenza	Different agronomic traits	EMS	<i>Sgp-1</i> , <i>waxy</i>	2010	Sestili et al. (2010)
Durum wheat	Dominant chlorosis phenotype	EMS	<i>YES-1</i>	2019	Harrington et al. (2019)
Bread wheat	B-starch granules formation	EMS	<i>BGC1</i>	2020	Chia et al. (2020)
Durum wheat, Bread wheat	Grain size, width, weight	EMS	<i>TaGW2-A1</i>	2016	Simmonds et al. (2016)
Bread wheat	square-head, free threshing spikes	EMS	<i>APETALA2</i>	2020	Madsen and Brinch-Pedersen (2020)
Bread wheat	Improve kernel hardness and starch biosynthesis	EMS	<i>Pinb</i> , <i>SSIIa-A</i> , <i>Agp2</i>	2017	Li et al. (2017)
Rice	Enhance nutrition and health benefits	γ radiation	<i>OsLCT1</i> , <i>SPDT</i>	2018	Li et al. (2018)
Rice	Improve amylose content	EMS	<i>SBEI</i> , <i>SBEIIa</i> , <i>SBEIIb</i>	2018	Kim et al. (2018)
Soybean	Reduce α -linolenic acid level	EMS	<i>GmFAD3-2a</i>	2014	Hoshino et al. (2014)
Soybean	Increased stearic acid level	EMS	<i>GmSACPD-A</i> , <i>GmSACPD-B</i> , <i>GmSACPD-D</i>	2020	Lakhssassi et al. (2020)

TABLE 10.1 (Continued)

Crop species	Trait improvement	Mutagenic agent	Target gene	Year	References
Soybean	Reduce stachyose, raffinose level	EMS	<i>RAFFINOSE SYNTHASE3</i>	2019	Thapa et al. (2019)
Tomato	Seedless fruit	EMS	<i>IAA9</i>	2015	Mazzucato et al. (2015)
Tomato	Improved carotenoid content	EMS	<i>Cyc-B</i>	2013	Silletti et al., (2013)
Tomato	Increased shelf life	EMS	<i>SIETR1</i>	2012	Okabe et al. (2012)
Mungbean	Improved yield	EMS	<i>GI, RMS, TFL1</i>	2020	Varadaraju et al. (2020)
Chickpea	Agronomic traits	γ radiation	Different genes	2018	Amri-Tiliouine et al. (2018)
Sorghum	Altered lignin content	EMS	<i>COMT</i>	2008	Xin et al. (2008)

lines having increased ascorbate redox state, enhanced ROS scavenging, low Na^+/K^+ ratio, higher accumulation of K^+ and Ca^{2+} in shoots, and greater biomass production. In addition, the higher concentration of chloroplastic and cytosolic HSP70 isoforms could assist to provide salinity tolerance to *B. rapa*. Hence, this research screened a potential *B. rapa* line that can be applied to develop salt-tolerant crops (Navarro-León et al., 2020a).

In another experiment, TILLING was performed in *B. rapa* to study the salt tolerance mechanism under high concentrations of Ca^{2+} . The chief goal of this research was to decipher the impact of various mutations and treatment of Ca^{2+} on crop metabolism. The mutated lines gave tolerance against higher Ca^{2+} concentration by hyperaccumulating the abscisic acid, indole-3-acetic acid, malate, and glutamate, and improved tricarboxylic acid cycle and nitrogen metabolism in *B. rapa* (Navarro-León et al., 2019b). A similar study was demonstrated by Navarro-León and colleagues to study the CAX1a mutant lines of *B. rapa* develop through TILLING which could be used as specific traits for biofortification with Fe, Mg, and Ca. The higher level of these element do not affect the crop growth and help to provide higher Ca tolerance (Navarro-León et al., 2018).

The salinity tolerance mechanism was also investigated in rice mutant genotypes developed via TILLING technique. Gamma-ray irradiation-induced mutations were achieved in 2961 M2 rice population and 41 targeted SNPs in candidate genes were detected. The expression level of five coding regions including *OsSOS1*, *OsHAK11*, *OsNSCC2*, *OsHKT6*, and *OsAKT1* was changed that headed to enhance salinity tolerance in rice (Hwang et al., 2016).

10.7.1.2 DROUGHT AND HEAT TOLERANCE

Drought is another major abiotic stress which severely affects the growth and yield of different crops. This leucine zipper transcription factor *Abscisic Acid INSENSITIVE 5* (*ABI5*) regulates the ABA pathway and trigger abiotic tolerance response in barley. TILLING was applied to create mutagenesis in *HvABI5* gene which showed maximum water storage in seed under drought stress as compared to the wild variety. Also, mutant lines displayed rapid stomatal closure, increase biosynthesis of flavonoids, improved membrane protection, and increase drought tolerance in barley (Collin et al., 2020). *Enhanced Response to ABA1* (*ERA1*) gene has been reported to present hypersensitivity against ABA during seed germination. It helps to provide

increase drought tolerance in various crops including soybean and wheat. The regulatory function of ERA1 gene in barley was determined under drought stress conditions. The mutant lines of barley for ERA1 gene were produced through TILLING which exhibited an increase metabolism of galactolipids to make the chloroplast membranes. The mutant lines also showed improved photosynthesis performance and enhance drought tolerance in barley (Marzec et al., 2020).

Sucrose nonfermenting 1-related protein kinases 2 (SnRK2) confer abiotic stress tolerance in various crops. TILLING technology was applied to examine the function of a SnRK2 gene family, *OsSAPK8* in rice under multiple stress conditions including salinity, cold, and drought stress. The results revealed that the *OsSAPK8* gene confer resistance to multiple stresses. Hence, the mutant lines showed susceptibility under to salinity, cold, and drought stresses (Zhong et al., 2020).

The development of heat tolerance cultivars is vital to guarantee global security under climate change conditions. The crop genotypes having varied heat tolerance mechanisms are beneficial for crop improvement. TILLING was employed to develop *Heat Shock Binding Protein 1 (HSBPI)* mutants of tomato. The effects of mutant allele on tomato thermo-tolerance capacity and protoplasts have been examined in contrast to the wild tomato lines. The *HSBPI* mutant seedlings and mature plants demonstrated increase heat tolerance and these finding can be employed to produce heat stress-tolerant tomato genotypes (Marko et al., 2019).

10.7.1.3 METAL STRESS TOLERANCE

Increased level of different toxic metalloids contents in different crop species causes harmful effects to human health. Arsenic stress distress the aerial parts and grain of the crops and considered as a major target to for breeding stress-resilient crops. TILLING approach was applied to target the *arsenic-tolerant type 1 (ATT1)* gene to develop rice mutant through γ radiation. The *ATT1* lines displayed higher shoot and root biomass via enhancing cell size and cell proliferation. Enhanced biomass of root cells and transcriptional richness of arsenic transporters allowed greater arsenic tolerance by reducing the arsenic translocation from root to shoots (Lim et al., 2020).

Cadmium is another toxic metal that poses serious threat to human health and crop productivity. Phytoremediation is crucial to clean up the soil and environment from heavy metals. Scientists are introducing novel

crop species that have the ability for phytoremediation. *CAX1a* gene was targeted to produce the mutant lines of *Brassica rapa* under high cadmium concentration via TILLING technology. The results indicated that the mutant lines showed increased tolerance to cadmium stress and this was due to a high accumulation of Mg and Ca which improved the photosynthesis performance, increased ROS scavenges, lower ethylene, and high malate level (Navarro-León et al., 2020b). The role of *HMA4a* gene was studied by employing TILLING technology which demonstrated the increased tolerance to cadmium stress in *Brassica rapa* mutant lines (Navarro-León et al., 2019a).

10.7.2 BIOTIC STRESS RESISTANCE

Likewise, TILLING has also vast applications to produce the biotic stress-resistant crop varieties (Taheri et al., 2017). For example, powdery mildew is a devastating pathogen to cereals crops especially produces severe disease in wheat cultivars. So the development of resistant wheat is the prime objective of plant breeders to ensure the food security. To achieve *Mlo*-based resistance, TILLING was executed to identify mutant population of durum wheat. The result revealed increase resistance against powdery mildew in durum wheat (Ingvarsdén et al., 2019). In an experiment, *mlo*-based resistance was acquired in *T. aestivum* by using TILLING against powdery mildew. Partial knockout mutants of *TaMlo* were generated and screened 16 missense induce mutations, such as *TaMlo-A1*, *TaMlo-B1*, and *TaMlo-D1* alleles. Double and triple homozygous mutants displayed better resistant to powdery mildew and can be a step forward to produce nontransgenic commercial resistant varieties of wheat (Acevedo-Garcia et al., 2017).

Recently, Gruner and coworker investigated the infection phenotypes of TILLING and TALEN-based *mlo* lines against two hemibiotrophic fungi, including *Magnaporthe oryzae* pv. *Triticum* and *Zymoseptoria tritici* cause wheat blast disease and leaf blotch of wheat, respectively. However, *Tamlo* lines exhibited unchanged results against *Z. tritici* and detected allele-specific increased vulnerability to *M. oryzae* pv. *Triticum* has higher resistance to powdery mildew associated strong attack of *M. oryzae*. Unexpectedly, mutant lines showed no signs of pleiotropic effects like early leaf senescence or callose deposition in leaf mesophyll tissues (Gruner et al., 2020a). TILLING was also used to produce resistance against *Fusarium graminearum* that causes fusarium head blight in barley and wheat. *Fhb1* gene mutation

revealed the *pore-forming toxin-like (PFT)* gene confers greater resistance against fusarium head blight (Rawat et al., 2016).

TILLING can also be used to develop resistant against viral pathogens. A TILLING technique was demonstrated to produce broad-spectrum resistance in tomato cultivars against potyviruses by disrupting the *eIF4E* gene (Gauffier et al., 2016). A nontransgenic herbicide-tolerant wheat was developed by targeting the *EPSPS* gene to produce *T102I* and *P106S* mutations. The results showed glyphosate resistance in mutant lines as compared to wild ones. These findings can be vital to developing the commercial lines of wheat for glyphosate resistance (Moebs et al., 2020) (Table 10.2).

10.8 SUMMARY

Mutation breeding through high throughput reverse genetics approach like TILLING has been playing crucial part in manipulating the genetic make-up of major crop species by introducing the desired traits into the genome. TILLING is a nontransgenic strategy that offered more safe and acceptable road-map to overcome the limitations posed by transgenic and other genome-edited techniques. TILLING is providing more robust and cost-effective technique to develop superior crop cultivars without any regulatory rules that are imposed on transgenic crops due to the public concerns and safety-related issues. Numerous TILLING programs are nowadays used for many major crop species harboring the specific traits and integrated it with breeding platforms for crop improvement. TILLING has got attraction since it can efficiently incorporate mutations or increase genetic variation in crops that is crucial for developing future crops.

There are numerous applications of TILLING technology in agriculture and has been extensively exploited for many decades for crop improvement. In many applications, the development of stress-resilient crops are the major one that assist to develop abiotic and biotic stress resistance in major crop species. This helps to mitigate the harmful effects of climate change on crop productivity and makes crops more resistant to harsh climatic conditions. Also, TILLING is also used to introduce desirable agronomic traits in crop species that provide more nutritive value and increase yield. Hence, TILLING is helping the scientists to cope with the current issue of unpredictable environmental conditions and assisting to develop climate-smart crops having improved yield and better stress tolerance.

TABLE 10.2 Applications of TILLING To Develop Abiotic and Biotic Stress Tolerance.

Crop species	Stress tolerance	Mutagenic agent	Target gene	Year	References
Abiotic stresses					
<i>Brassica rapa</i>	Salinity tolerance	EMS	<i>CAX1a</i>	2020	Navarro-León et al. (2020a)
<i>Brassica rapa</i>	High Ca ²⁺	EMS	<i>CAX1a</i>	2019	Navarro-León et al. (2019b)
<i>Brassica rapa</i>	High Ca ²⁺	EMS	<i>CAX1a</i>	2018	Navarro-León et al., (2018)
<i>Oryza sativa</i>	Salinity tolerance	γ radiation	<i>OsSOS1</i> , <i>OsHAK11</i> , <i>OsNSCC2</i> , <i>OsHKT6</i> , <i>OsAKT1</i>	2016	Hwang et al. (2016)
<i>Hordeum vulgare</i>	Drought tolerance	EMS	<i>ABI5</i>	2020	Collin et al. (2020)
<i>Hordeum vulgare</i>	Drought tolerance	EMS	<i>ERA1</i>	2020	Marzec et al. (2020)
<i>Solanum lycopersicum</i>	Heat tolerance	EMS	<i>HSBP1</i>	2019	Marko et al. (2019)
<i>Oryza sativa</i>	Drought, salinity, cold tolerance	EMS	<i>OsSAPK8</i>	2020	Zhong et al. (2020)
<i>Oryza sativa</i>	Arsenic tolerance	γ radiation	<i>ATT1</i>	2020	Lim et al. (2020)
<i>Brassica rapa</i>	Cadmium tolerance	EMS	<i>CAX1a</i>	2020	Navarro-León et al. (2020b)
<i>Brassica rapa</i>	Cadmium tolerance	EMS	<i>HMA4a</i>	2020	Navarro-León et al. (2019a)
Biotic stresses					
<i>Triticum turgidum</i>	Powdery mildew resistance	EMS	<i>mlo</i>	2019	Ingvarsdén et al. (2019)
<i>Triticum aestivum</i>	Powdery mildew resistance	EMS	<i>TaMlo</i>	2017	Acevedo-Garcia et al. (2017)
<i>Triticum aestivum</i>	Susceptibility to <i>Zymoseptoria tritici</i> and <i>Magnaporthe oryzae</i>	EMS	<i>TaMlo</i>	2020	Gruner et al. (2020a)
<i>Triticum aestivum</i>	Resistance to fusarium head blight	EMS	<i>Fhb1</i>	20016	Rawat et al. (2016)
<i>Solanum lycopersicum</i>	Potyvirus	EMS	<i>eIF4E</i>	2016	Gauffier et al. (2016)
<i>Triticum aestivum</i>	Glyphosate resistance	EMS	<i>EPSPS</i>	2020	Moehs et al. (2020)

The innovations in TILLING have introduced the eco-TILLING and TILLING-by-sequencing technologies which makes this technique more powerful and effective. Some pitfalls in TILLING technology such as the random mutations, undesired knockouts, lower mutation rate, and development of NGS techniques that can rapidly capture the mutation need to be addressed. If these hurdles will overcome, the TILLING technology can revolutionized the crop breeding and will be ensuring the food security. In the future, TILLING can be integrated with other modern omics tools such as metabolomics (Razzaq et al., 2019a) to characterize the climate-ready crops developed through TILLING technology to ensure public safety.

KEYWORDS

- **TILLING**
- **mutant screening**
- **abiotic stress tolerance**
- **biotic stress tolerance**
- **climate change**
- **food security**

REFERENCES

- Acevedo-Garcia, J.; Spencer, D.; Thieron, H.; Reinstädler, A.; Hammond-Kosack, K.; Phillips, A. L.; Panstruga, R. mlo-Based Powdery Mildew Resistance in Hexaploid Bread Wheat Generated by a Non-Transgenic TILLING Approach. *Plant Biotechnol. J.* **2017**, *15* (3), 367–378.
- Amri-Tiliouine, W.; Laouar, M.; Abdelguerfi, A.; Jankowicz-Cieslak, J.; Jankuloski, L.; Till, B. J. Genetic Variability Induced by Gamma Rays and Preliminary Results of Low-Cost TILLING on M2 Generation of Chickpea (*Cicer arietinum* L.). *Front. Plant Sci.* **2018**, *9*, 1568.
- Botticella, E.; Sestili, F.; Hernandez-Lopez, A.; Phillips, A.; Lafiandra, D. High Resolution Melting Analysis for the Detection of EMS Induced Mutations in Wheat Sbella Genes. *BMC Plant Biol.* **2011**, *11*, (1), 156.
- Bovina, R.; Brunazzi, A.; Gasparini, G.; Sestili, F.; Palombieri, S.; Botticella, E.; Lafiandra, D.; Mantovani, P.; Massi, A. Development of a TILLING Resource in Durum Wheat for Reverse-and Forward-Genetic Analyses. *Crop Pasture Sci.* **2014**, *65* (1), 112–124.

- Brozynska, M.; Furtado, A.; Henry, R. J. Genomics of Crop Wild Relatives: Expanding the Gene Pool for Crop Improvement. *Plant Biotechnol. J.* **2016**, *14* (4), 1070–1085.
- Chaudhary, J.; Alisha, A.; Bhatt, V.; Chandanshive, S.; Kumar, N.; Mir, Z.; Kumar, A.; Yadav, S. K.; Shivaraj, S.; Sonah, H. Mutation Breeding in Tomato: Advances, Applicability and Challenges. *Plants* **2019**, *8* (5), 128.
- Chia, T.; Chirico, M.; King, R.; Ramirez-Gonzalez, R.; Saccomanno, B.; Seung, D.; Simmonds, J.; Trick, M.; Uauy, C.; Verhoeven, T. A Carbohydrate-Binding Protein, B-Granule Content 1, Influences Starch Granule Size Distribution in a Dose-Dependent Manner in Polyploid Wheat. *J. Exp. Bot.* **2020**, *71* (1), 105–115.
- Colasuonno, P.; Incerti, O.; Lozito, M. L.; Simeone, R.; Gadaleta, A.; Blanco, A. DHPLC Technology for High-Throughput Detection of Mutations in a Durum Wheat TILLING Population. *BMC Gene.* **2016**, *17* (1), 1–10.
- Colbert, T.; Till, B. J.; Tompa, R.; Reynolds, S.; Steine, M. N.; Yeung, A. T.; McCallum, C. M.; Comai, L.; Henikoff, S. High-Throughput Screening for Induced Point Mutations. *Plant Physiol.* **2001**, *12* (2), 480–484.
- Collin, A.; Daszkowska-Golec, A.; Kurowska, M.; Szarejko, I. Barley *ABI5* (*Abscisic Acid Insensitive 5*) Is Involved in Absciscic Acid-Dependent Drought Response. *Front. Plant Sci.* **2020**, *11*, 1138.
- Dong, C.; Dalton-Morgan, J.; Vincent, K.; Sharp, P., A Modified TILLING Method for Wheat Breeding. *Plant Genome* **2009**, *2*, (1), 39–47.
- Gajewska, P.; Janiak, A.; Kwasniewski, M.; Kędzierski, P.; Szarejko, I. Forward Genetics Approach Reveals a Mutation in *bHLH* Transcription Factor-Encoding Gene as the Best Candidate for the Root Hairless Phenotype in Barley. *Front. Plant Sci.* **2018**, *9*, 1229.
- Gauffier, C.; Lebaron, C.; Moretti, A.; Constant, C.; Moquet, F.; Bonnet, G.; Caranta, C.; Gallois, J. L. A TILLING Approach to Generate Broad-Spectrum Resistance to Potyviruses in Tomato Is Hampered by *eIF4E* Gene Redundancy. *Plant J.* **2016**, *85* (6), 717–729.
- Greene, E. A.; Codomo, C. A.; Taylor, N. E.; Henikoff, J. G.; Till, B. J.; Reynolds, S. H.; Enns, L. C.; Burtner, C.; Johnson, J. E.; Odden, A. R. Spectrum of Chemically Induced Mutations from a Large-Scale Reverse-Genetic Screen in *Arabidopsis*. *Genetics* **2003**, *164* (2), 731–740.
- Gruner, K.; Esser, T.; Acevedo-Garcia, J.; Freh, M.; Habig, M.; Strugala, R.; Stukenbrock, E.; Schaffrath, U.; Panstruga, R. Evidence for Allele-Specific Levels of Enhanced Susceptibility of Wheat *mlo* Mutants to the Hemibiotrophic Fungal Pathogen *Magnaporthe oryzae* pv. *Triticum*. *Genes* **2020a**, *11* (5), 517.
- Gruner, K.; Esser, T.; Acevedo-Garcia, J.; Freh, M.; Habig, M.; Strugala, R.; Stukenbrock, E.; Schaffrath, U.; Panstruga, R. Wheat *mlo* Mutants Show Allele-Specific Levels of Enhanced Susceptibility to the Hemibiotrophic Fungal Pathogen *Magnaporthe oryzae* pv. *Triticum*, **2020b**.
- Harrington, S. A.; Cobo, N.; Karafiátová, M.; Doležel, J.; Borrill, P.; Uauy, C. Identification of a Dominant Chlorosis Phenotype Through a Forward Screen of the *Triticum turgidum* cv. Kronos TILLING Population. *Front. Plant Sci.* **2019**, *10*, 963.
- Hoshino, T.; Watanabe, S.; Takagi, Y.; Anai, T. A novel *GmFAD3–2a* Mutant Allele Developed Through TILLING Reduces α -Linolenic Acid Content in Soybean Seed Oil. *Breed. Sci.* **2014**, *64* (4), 371–377.
- Hwang, J. E.; Jang, D.-S.; Lee, K. J.; Ahn, J.-W.; Kim, S. H.; Kang, S.-Y.; Kim, D. S.; Kim, J.-B., Identification of Gamma Ray Irradiation-Induced Mutations in Membrane Transport Genes in a Rice Population by TILLING. *Genes Genet. Syst.* **2016**, *15*–00052.

- Ingvaridsen, C. R.; Massange-Sánchez, J. A.; Borum, F.; Uauy, C.; Gregersen, P. L. Development of mlo-Based Resistance in Tetraploid Wheat Against Wheat Powdery Mildew. *Theor. Appl. Genet.* **2019**, *132* (11), 3009–3022.
- Irshad, A.; Guo, H.; Zhang, S.; Liu, L. TILLING in Cereal Crops for Allele Expansion and Mutation Detection by Using Modern Sequencing Technologies. *Agronomy* **2020**, *10* (3), 405.
- International Services for the Acquisition of Agri-biotech Applications (ISAAA), 2020. <http://www.isaaa.org/kc/cropbiotechupdate/article/default.asp?ID=17936>
- Jankowicz-Cieslak, J.; Huynh, O. A.; Bado, S.; Matijevic, M.; Till, B. J. Reverse-Genetics by Tilling Expands Through the Plant Kingdom. *Emir. J. Food Agric.* **2011**, 290–300.
- Jankowicz-Cieslak, J.; Mba, C.; Till, B. J. Mutagenesis for Crop Breeding and Functional Genomics. In *Biotechnologies for Plant Mutation Breeding*; Springer: Cham, 2017; pp 3–18.
- Jankowicz-Cieslak, J.; Till, B. J. Chemical Mutagenesis of Seed and Vegetatively Propagated Plants Using EMS. *Curr. Protoc. Plant Biol.* **2016**, *1* (4), 617–635.
- Kannan, M.; Zainal, Z.; Ismail, I.; Baharum, S. N.; Bunawan, H. Application of Reverse Genetics in Functional Genomics of *potyvirus*. *Viruses* **2020**, *12* (8), 803.
- Kanwal, M.; Razaq, A.; Maqbool, A. Characterization of Phytase Transgenic Wheat Under Salt Stress. *Biol. Bull.* **2019**, *46* (4), 371–380.
- Kashtwari, M.; Wani, A. A.; Rather, R. N. TILLING: An Alternative Path for Crop Improvement. *J. Crop Imp.* **2019**, *33* (1), 83–109.
- Kim, H.; Yoon, M.-r.; Chun, A.; Tai, T. H. Identification of Novel Mutations in the Rice *Starch Branching Enzyme I* Gene via TILLING by Sequencing. *Euphytica* **2018**, *214* (6), 94.
- Kukul, M. S.; Irmak, S. Climate-Driven Crop Yield and Yield Variability and Climate Change Impacts on the US Great Plains Agricultural Production. *Sci. Rep.* **2018**, *8* (1), 1–18.
- Lakhssassi, N.; Zhou, Z.; Liu, S.; Piya, S.; Cullen, M. A.; El Baze, A.; Knizia, D.; Patil, G. B.; Badad, O.; Embaby, M. G. Soybean TILLING-by-Sequencing+ Reveals the Role of Novel *GmSACPD* Members in the Unsaturated Fatty Acid Biosynthesis While Maintaining Healthy Nodules. *J. Exp. Bot.* **2020**.
- Leng, G.; Huang, M. Crop Yield Response to Climate Change Varies with Crop Spatial Distribution Pattern. *Sci. Rep.* **2017**, *7* (1), 1–10.
- Li, S.; Liu, S.M.; Fu, Hw.; Huang, J.Z.; Shu, Q.Y. High-Resolution Melting-Based TILLING of γ Ray-Induced Mutations in Rice. *J. Zhejiang Univ. Sci. B.* **2018**, *19* (8), 620–629.
- Li, W.; Guo, H.; Wang, Y.; Xie, Y.; Zhao, L.; Gu, J.; Zhao, S.; Zhao, B.; Wang, G.; Liu, L. Identification of Novel Alleles Induced by EMS-Mutagenesis in Key Genes of Kernel Hardness and Starch Biosynthesis in Wheat by TILLING. *Genes Genom.* **2017**, *39* (4), 387–395.
- Lim, S. D.; Lee, S. C.; Choi, S. Y.; Lee, J.; Hwang, S.-G.; Jang, C. S. A TILLING rice *ATT1* Enhances Arsenic Tolerance by Increasing Vacuolar Sequestration Capacity of Arsenic. *Environ. Exp. Bot.* **2020**, 104057.
- Madsen, C. K.; Brinch-Pedersen, H. A Novel Wheat Q'allele Identified by Forward Genetic in Silico TILLING. *J. Plant Physiol.* **2020**, *251*, 153221.
- Manzanares, C.; Yates, S.; Ruckle, M.; Nay, M.; Studer, B. TILLING in Forage Grasses for Gene Discovery and Breeding Improvement. *New Biotechnol.* **2016**, *33* (5), 594–603.
- Marko, D.; El-Shershaby, A.; Carriero, F.; Summerer, S.; Petrozza, A.; Iannacone, R.; Schleiff, E.; Fragkostefanakis, S. Identification and Characterization of a Thermotolerant TILLING Allele of Heat Shock Binding Protein 1 in Tomato. *Genes* **2019**, *10* (7), 516.

- Marzec, M.; Daszkowska-Golec, A.; Collin, A.; Melzer, M.; Eggert, K.; Szarejko, I. Barley Strigolactone Signalling Mutant *hvd14.d* Reveals the Role of Strigolactones in Absciscic Acid-Dependent Response to Drought. *Plant Cell Environ.* **2020**, *43* (9), 2239–2253.
- Mazzucato, A.; Cellini, F.; Bouzayen, M.; Zouine, M.; Mila, I.; Minoia, S.; Petrosza, A.; Picarella, M. E.; Ruii, F.; Carriero, F. A TILLING Allele of the Tomato *Aux/IAA9* Gene Offers New Insights Into Fruit Set Mechanisms and Perspectives for Breeding Seedless Tomatoes. *Mol. Breed.* **2015**, *35* (1), 22.
- McCallum, C. M.; Comai, L.; Greene, E. A.; Henikoff, S. Targeting Induced Locallesions in Genomes (TILLING) for Plant Functional Genomics. *Plant Physiol.* **2000**, *123* (2), 439–442.
- Moehs, C. P.; Austill, W. J.; Facciotti, D.; Holm, A.; Loeffler, D.; Lu, Z.; Mullenberg, J. C.; Slade, A. J.; Steine, M. N.; van Boxtel, J. Development of Non-Transgenic Glyphosate Tolerant Wheat by TILLING. *bioRxiv* **2020**.
- Navarro-León, E.; López-Moreno, F. J.; de la Torre-González, A.; Ruiz, J. M.; Esposito, S.; Blasco, B. Study of Salt-Stress Tolerance and Defensive Mechanisms in *Brassica rapa* *CAX1a* TILLING Mutants. *Environ. Exp. Bot.* **2020a**, 104061.
- Navarro-León, E.; Oviedo-Silva, J.; Ruiz, J. M.; Blasco, B. Possible Role of *HMA4a* TILLING Mutants of *Brassica rapa* in Cadmium Phytoremediation Programs. *Ecotoxicol. Environ. Saf* **2019a**, *180*, 88–94.
- Navarro-León, E.; Ruiz, J. M.; Albacete, A.; Blasco, B. Effect of *CAX1a* TILLING Mutations and Calcium Concentration on Some Primary Metabolism Processes in *Brassica rapa* Plants. *J. Plant Physiol.* **2019b**, *237*, 51–60.
- Navarro-León, E.; Ruiz, J. M.; Albacete, A.; Blasco, B. Tolerance to Cadmium Toxicity and Phytoremediation Potential of Three *Brassica rapa* *CAX1a* TILLING Mutants. *Ecotoxicol. Environ. Saf* **2020b**, *189*, 109961.
- Navarro-León, E.; Ruiz, J. M.; Graham, N.; Blasco, B. Physiological Profile of *CAX1a* TILLING Mutants of *Brassica rapa* Exposed to Different Calcium Doses. *Plant Sci.* **2018**, *272*, 164–172.
- Negrão, S.; Schmöckel, S.; Tester, M. Evaluating Physiological Responses of Plants to Salinity Stress. *Ann. Bot.* **2017**, *119* (1), 1–11.
- Okabe, Y.; Asamizu, E.; Ariizumi, T.; Shirasawa, K.; Tabata, S.; Ezura, H. Availability of Micro-Tom Mutant Library Combined with TILLING in Molecular Breeding of Tomato Fruit Shelf-Life. *Breed. Sci.* **2012**, *62* (2), 202–208.
- Parry, M. A.; Madgwick, P. J.; Bayon, C.; Tearall, K.; Hernandez-Lopez, A.; Baudo, M.; Rakszegi, M.; Hamada, W.; Al-Yassin, A.; Ouabbou, H. Mutation Discovery for Crop Improvement. *J. Exp. Bot.* **2009**, *60* (10), 2817–2825.
- Perry, J. A.; Wang, T. L.; Welham, T. J.; Gardner, S.; Pike, J. M.; Yoshida, S.; Parniske, M. A TILLING Reverse Genetics Tool and a Web-Accessible Collection of Mutants of the Legume *Lotus japonicus*. *Plant Physiol.* **2003**, *131* (3), 866–871.
- Pickar-Oliver, A.; Gersbach, C. A. The Next Generation of CRISPR–Cas Technologies and Applications. *Nat. Rev. Mol. Cell Biol.* **2019**, *20* (8), 490–507.
- Raina, A.; Laskar, R. A.; Khursheed, S.; Amin, R.; Tantray, Y. R.; Parveen, K.; Khan, S. Role of Mutation Breeding in Crop Improvement-Past, Present and Future. *Asian Res. J. Agric.* **2016**, 1–13.
- Raina, A.; Laskar, R. A.; Khursheed, S.; Khan, S.; Parveen, K.; Amin, R. Induce Physical and Chemical Mutagenesis for Improvement of Yield Attributing Traits and Their Correlation Analysis in Chickpea. *Int. Lett. Nat. Sci.* **2017**, 61.

- Rawat, N.; Pumphrey, M. O.; Liu, S.; Zhang, X.; Tiwari, V. K.; Ando, K.; Trick, H. N.; Bockus, W. W.; Akhunov, E.; Anderson, J. A. Wheat *Fhb1* Encodes a Chimeric Lectin with Agglutinin Domains and a Pore-Forming Toxin-Like Domain Conferring Resistance to Fusarium Head Blight. *Nat. Genet.* **2016**, *48* (12), 1576–1580.
- Raza, A.; Razzaq, A.; Mehmood, S. S.; Zou, X.; Zhang, X.; Lv, Y.; Xu, J. Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. *Plants* **2019**, *8* (2), 34.
- Razzaq, A.; Sadia, B.; Raza, A.; Khalid Hameed, M.; Saleem, F. Metabolomics: A Way Forward for Crop Improvement. *Metabolites* **2019a**, *9* (12), 303.
- Razzaq, A.; Saleem, F.; Kanwal, M.; Mustafa, G.; Yousaf, S.; Imran Arshad, H. M.; Hameed, M. K.; Khan, M. S.; Joyia, F. A. Modern Trends in Plant Genome Editing: An Inclusive Review of the CRISPR/Cas9 Toolbox. *Int. J. Mol. Sci.* **2019b**, *20* (16), 4045.
- Reddy, T. V.; Dwivedi, S.; Sharma, N. K. Development of TILLING by Sequencing Platform Towards Enhanced Leaf Yield in Tobacco. *Ind. Crops Prod.* **2012**, *40*, 324–335.
- Rout, B. M. TILLING: A Novel Reverse Genetics Technique for Screening of Gene Mutants in Plants. *Biotica Res. Today* **2020**, *2* (5), 252–254.
- Schreiber, M.; Barakate, A.; Uzrek, N.; Macaulay, M.; Sourdille, A.; Morris, J.; Hedley, P. E.; Ramsay, L.; Waugh, R. A Highly Mutagenised Barley (cv. Golden Promise) TILLING Population Coupled With Strategies for Screening-By-Sequencing. *Plant Methods* **2019**, *15* (1), 99.
- Serrat, X.; Esteban, R.; Guibourt, N.; Moysset, L.; Nogués, S.; Lalanne, E. EMS Mutagenesis in Mature Seed-Derived Rice Calli as a New Method for Rapidly Obtaining TILLING Mutant Populations. *Plant Methods* **2014**, *10* (1), 1–14.
- Sestili, F.; Botticella, E.; Bedo, Z.; Phillips, A.; Lafiandra, D. Production of Novel Allelic Variation for Genes Involved in Starch Biosynthesis Through Mutagenesis. *Mol. Breed.* **2010**, *25* (1), 145.
- Sestili, F.; Garcia-Molina, M. D.; Gambacorta, G.; Beleggia, R.; Botticella, E.; De Vita, P.; Savatin, D. V.; Masci, S.; Lafiandra, D. Provitamin A Biofortification of Durum Wheat Through a TILLING Approach. *Int. J. Mol. Sci.* **2019**, *20* (22), 5703.
- Sestili, F.; Palombieri, S.; Botticella, E.; Mantovani, P.; Bovina, R.; Lafiandra, D. TILLING Mutants of Durum Wheat Result in a High Amylose Phenotype and Provide Information on Alternative Splicing Mechanisms. *Plant Sci.* **2015**, *233*, 127–133.
- Silletti, M. F.; Petrozza, A.; Stigliani, A. L.; Giorio, G.; Cellini, F.; D'Ambrosio, C.; Carriero, F. An Increase of Lycopene Content in Tomato Fruit Is Associated with a Novel *Cyc-B* Allele Isolated Through TILLING Technology. *Mol. Breed.* **2013**, *31* (3), 665–674.
- Simmonds, J.; Scott, P.; Brinton, J.; Mestre, T. C.; Bush, M.; Del Blanco, A.; Dubcovsky, J.; Uauy, C. A Splice Acceptor Site Mutation in TaGW2-A1 Increases Thousand Grain Weight in Tetraploid and Hexaploid Wheat Through Wider and Longer Grains. *Theor. App. Genet.* **2016**, *129* (6), 1099–1112.
- Slade, A. J.; McGuire, C.; Loeffler, D.; Mullenberg, J.; Skinner, W.; Fazio, G.; Holm, A.; Brandt, K. M.; Steine, M. N.; Goodstal, J. F. Development of High Amylose Wheat Through TILLING. *BMC Plant Biol.* **2012**, *12* (1), 69.
- Smýkal, P.; Nelson, M. N.; Berger, J. D.; Von Wettberg, E. J. The Impact of Genetic Changes During Crop Domestication. *Agronomy* **2018**, *8* (7), 119.
- Stemple, D. L. TILLING—A High-Throughput Harvest for Functional Genomics. *Nat. Rev. Genet.* **2004**, *5* (2), 145–150.

- Suzuki, T.; Eiguchi, M.; Kumamaru, T.; Satoh, H.; Matsusaka, H.; Moriguchi, K.; Nagato, Y.; Kurata, N. MNU-Induced Mutant Pools and High Performance TILLING Enable Finding of Any Gene Mutation in Rice. *Mol. Genet. Genom.* **2008**, *279* (3), 213–223.
- Tadele, Z. Mutagenesis and TILLING to Dissect Gene Function in Plants. *Curr. Genom.* **2016**, *17* (6), 499–508.
- Taheri, S.; Abdullah, T. L.; Jain, S. M.; Sahebi, M.; Azizi, P. TILLING, High-Resolution Melting (HRM), and Next-Generation Sequencing (NGS) Techniques in Plant Mutation Breeding. *Mol. Breed.* **2017**, *37* (3), 40.
- Taylor, N. E.; Greene, E. A. PARSESNP: A Tool for the Analysis of Nucleotide Polymorphisms. *Nucl. Acids Res.* **2003**, *31* (13), 3808–3811.
- Thapa, R.; Carrero-Colón, M.; Rainey, K. M.; Hudson, K. Tilling by Sequencing: A Successful Approach to Identify Rare Alleles in Soybean Populations. *Genes* **2019**, *10* (12), 1003.
- Till, B. J.; Burtner, C.; Comai, L.; Henikoff, S. Mismatch Cleavage by Single-Strand Specific Nucleases. *Nucl. acids Res.* **2004**, *32* (8), 2632–2641.
- Till, B. J.; Cooper, J.; Tai, T. H.; Colowit, P.; Greene, E. A.; Henikoff, S.; Comai, L. Discovery of Chemically Induced Mutations in Rice by TILLING. *BMC Plant Biol.* **2007**, *7* (1), 19.
- Till, B. J.; Reynolds, S. H.; Greene, E. A.; Codomo, C. A.; Enns, L. C.; Johnson, J. E.; Burtner, C.; Odden, A. R.; Young, K.; Taylor, N. E. Large-Scale Discovery of Induced Point Mutations with High-Throughput TILLING. *Genom. Res.* **2003**, *13* (3), 524–530.
- Till, B. J.; Zerr, T.; Comai, L.; Henikoff, S. A protocol for TILLING and Ecotilling in Plants and Animals. *Nat. Protoc.* **2006**, *1* (5), 2465–2477.
- Triques, K.; Sturbois, B.; Gallais, S.; Dalmais, M.; Chauvin, S.; Clepet, C.; Aubourg, S.; Rameau, C.; Caboche, M.; Bendahmane, A. Characterization of *Arabidopsis thaliana* Mismatch Specific Endonucleases: Application to Mutation Discovery by TILLING in Pea. *Plant J.* **2007**, *51* (6), 1116–1125.
- Uauy, C.; Paraiso, F.; Colasuonno, P.; Tran, R. K.; Tsai, H.; Berardi, S.; Comai, L.; Dubcovsky, J. A Modified TILLING Approach to Detect Induced Mutations in Tetraploid and Hexaploid Wheat. *BMC Plant Biol.* **2009**, *9* (1), 115.
- Varadaraju, A.; Ramadoss, B. R.; Gurunathan, S.; Balaram, R.; Ganesamurthy, K.; Ram, S. G. TILLING by Sequencing (TbS) Reveals Mutations in Flowering Control Genes That Are Associated with Altered Plant Architecture in Mungbean (*Vigna radiata* (L.) R. Wilczek). *Genet. Resour. Crop Ev.* **2020**, 1–16.
- Verma, P.; Tandon, R.; Yadav, G.; Gaur, V. Structural Aspects of DNA Repair and Recombination in Crop Improvement. *Front. Genet.* **2020**, *11*, 1103.
- Waltz, E., CRISPR-Edited Crops Free to Enter Market, Skip Regulation. Nature Publishing Group, 2016.
- Wienholds, E.; Van Eeden, F.; Kusters, M.; Mudde, J.; Plasterk, R. H.; Cuppen, E. Efficient Target-Selected Mutagenesis in Zebrafish. *Genom. Res.* **2003**, *13* (12), 2700–2707.
- Xin, Z.; Wang, M. L.; Barkley, N. A.; Burow, G.; Franks, C.; Pederson, G.; Burke, J. Applying Genotyping (TILLING) and Phenotyping Analyses to Elucidate Gene Function in a Chemically Induced Sorghum Mutant Population. *BMC Plant Biol.* **2008**, *8* (1), 103.
- Yano, R.; Hoshikawa, K.; Okabe, Y.; Wang, N.; Dung, P. T.; Imriani, P. S.; Shiba, H.; Ariizumi, T.; Ezura, H. Multiplex Exome Sequencing Reveals Genome-Wide Frequency and Distribution of Mutations in the ‘Micro-Tom’ Targeting Induced Local Lesions in Genomes (TILLING) Mutant Library. *Plant Biotechnol.* **2019**, *36* (4), 223–231.

- Zhang, H.; Mittal, N.; Leamy, L. J.; Barazani, O.; Song, B. H. Back Into the Wild—Apply Untapped Genetic Diversity of Wild Relatives for Crop Improvement. *Evolutionary App.* **2017**, *10* (1), 5–24.
- Zhong, R.; Wang, Y.; Gai, R.; Xi, D.; Mao, C.; Ming, F. Rice SnRK Protein Kinase *OsSAPK8* Acts as a Positive Regulator in Abiotic Stress Responses. *Plant Sci.* **2020**, *292*, 110373.
- Zhu, H.; Li, C.; Gao, C. Applications of CRISPR–Cas in Agriculture and Plant Biotechnology. *Nat. Rev. Mol. Cell Biol.* **2020**, 1–17.



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CHAPTER 11

T-DNA Insertion Mutagenesis

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ABSTRACT

In recent years insertion mutagenesis has established itself as an imperative tool for engendering mutant crop plant using T-DNA as biological mutagen. Some of the mutants developed have evinced to be a valuable source in plant breeding program. In this chapter, we have reviewed various techniques, advantages, and limitation of insertional mutagenesis.

11.1 INTRODUCTION

In the 19th century, the pioneering work done by Gregor Johann Mendel led to the invention of the law of heredity, which later became one of the paramount science in the field of plant breeding. Evolution is one of the cardinal phenomena and a practical breeding approach relies upon genetic variations, which is the driving force for evolution. Mostly these genetic variations are generated by recombination of genes within species population and interaction with environmental abiotic factors (Novak and Brunner, 1992).

In the prehistoric era, nomadic hunters collected their food from generously available natural resources like fruits, seeds, tubers, etc. but the advancement of civilization and the considerable increase in the human population led to the inauguration of large-scale food production system based on plant domestication. Newer plant varieties were cultivated for human usage and the approach is termed plant breeding. In the initial days, simple offspring selection with the desired traits was the prime technique that exploited the random occurrence of spontaneous mutation (Shu, 2012). With the progress of hybridization methods, further advancement in plant breeding techniques took place. Using hybridization methodology two expedient genes were combined and found in two disparate parents belonging to two or more distinctive varieties to produce progeny with superior traits than parental type (Novak and Brunner, 1992; Shu, 2012). Since then recombinant breeding, by crossing two different genotypes and selection of traits afterward became quotidian practice followed by plant breeders.

With the advent of induced mutation leading to genetic alteration after the experimental work done by Lewis John Stadler, a geneticist laid the foundation of further escalation in plant breeding and gave the concept of mutation breeding (Shu, 2012). Mutation created at gene level gets further amplified during meiosis where there is crossing over of alleles present on homologous chromosomes supervised by independent assortment during gametes formation. The variation produced as an effect of the mutation in crop plants serve as the base for natural selection and act as a compelling force for evolution (Parveen, 2006; Bhat, 2006a,c, 2007a, b, c; Khan, 2005).

11.2 MUTATION BREEDING

Induced mutation through chemical, physical, and biological agents brings about unforeseen heritable changes in the genome sequence of an organism (Parveen, 2007; Sharma, 2009; Gulfishan, 2010, 2011, 2012; Bhat, 2005b, 2007b; Roychowdhury and Tah, 2013). This heritable change exhibits a notable effect on phenotype (Gulfishan, 2015; Bhat, 2006b; Bhat and Wani (2017a, b); Bhat et al., 2017; Kharkwal, 2012) and is utilized in developing new plant varieties. It serves as the uttermost preeminent approaches of a plant breeding program (Fig. 11.1).

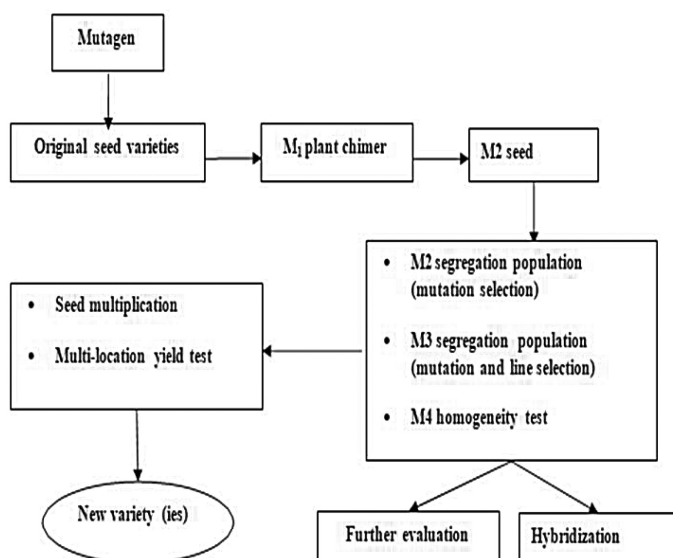


FIGURE 11.1 Representation of steps involved in mutation breeding.

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New cultivar of crops plants that are sterile and not able to produce viable seeds, for example, Banana and some seedless grapes can be developed by induced mutation (Ahloowalia and Maluszynski, 2001; Broerties and Van Harten, 1988; Chai, 2004). Induced mutation technique is also sometimes applied to bring out disparate color variants of ornamental plants (Broerties and Van Harten, 1988; Predieri, 2000; Chai, 2004; Kondo, 2009).

Mutation breeding techniques employ three types of mutagenesis, which can be caused by irradiation treatment by X-rays or gamma rays, by mutagenic chemicals, inventing mutation at predetermined sites in DNA molecule known as site-directed mutagenesis, and insertion of T-DNA by genetic transformation known as insertion mutagenesis (Kharkwal and Shu, 2009; Forster and Shu, 2012).

11.3 HISTORY

Mutation as a potent source for creating variation was first illustrated by Hugo de Vries in the late 19th century, while rediscovering the Mendelian law of inheritance (Kharkwal, 2012). According to him these changes noticed in the organism were heritable and brings about phenotypic changes. This variation

was distinct from that caused by segregation and recombination. He coined the term “Mutation” for this type of genetic variation.

However, the history of plant mutation dates back to 300 BC when some mutant crop varieties were noticed in China (Van Harten, 1998). After the discovery of mutagenic action of X- rays by Stadler on crops like maize, barley, and wheat use of radiation as a source of creating genetic variation in crop plants was popularized. The first commercial-induced mutant variety of tobacco was released in 1934. In the year 2006, Acquaah reported around 77 induced mutant cultivars, which increased in number, 484 by the year 1995 (Acquaah, 2006) including different types of plants like ornamental plants, fruit trees, crops, etc. bearing some beneficial modified agronomic traits like abiotic stress resistance, early maturity, and enhanced nutrient content. Since year 1950’s onwards induced mutation technique became one of the prevalent techniques in plant breeding program owing to the beneficial variation created in agronomic traits of allure. Disparate mutant plant varieties created with aid of induced mutation is enlisted in Table 11.1.

TABLE 11.1 Different Mutant Crop Varieties with Improved Agronomic Traits Created by Induced Mutagenesis.

Main agronomic trait	Crop
Biotic stress resistance	
Resistance to bacterial wilt (<i>Ralstonia solanacearum</i>)	Tomato
Resistance to stem rot (<i>Sclerotinia sclerotiorum</i>)	Rapeseed
Resistance to powdery mildew (<i>Podosphaera leucotricha</i>) and apple scab (<i>Venturia inaequalis</i>)	Apple
Resistance to <i>Ascochyta</i> blight and <i>Fusarium</i> wilt	Chickpea
Resistance to yellow mosaic virus	Mungbean
Resistance to black stem rust	Durum wheat
Resistance to stripe rust	Wheat
Resistance to blast, yellow mottle virus, bacterial leaf blight and bacterial leaf stripe	Rice
Resistance to <i>Myrothecium</i> leaf spot and yellow mosaic virus	Soybean
Resistance to bacterial blight, cotton leaf curl virus	Cotton
Resistance against pathogen striga (<i>Striga asiatica</i>)	Maize
Abiotic stress resistance	
Lodging resistance, acid sulphate soil tolerance	Rice
Semi-dwarf cultivar/dwarf	Rice
Early maturity	Rice
High fibre quality	Cotton

TABLE 11.1 (Continued)

Main agronomic trait	Crop
Acidity and drought tolerance	Lentil (<i>Lens culinaris</i> Medikus)
Tolerance to cold and high altitudes	Rice
Acidity and drought tolerance	Rice
Improvement of crop quality and nutrition	
Oil quality improvement	Soybean
	Canola
	Peanut
	Sunflower
Improvement of protein quality	Soybean
High-amylose content	Cassava
Oilseed meals low in phytic acid	Soybean
Phytate (anti-nutrient)	Barley
High-resistant starch	Rice
Dark green obovate leaf pod; increased seed size, higher yield, moderately resistant to diseases, increased oil and protein content	Groundnut

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11.4 MUTAGENIC AGENTS

The most preferred agents used for creating mutations are grouped in two broad categories of physical and chemical mutagen (Acquaah, 2006; Mba, 2010). To induce mutation in important crop plants planting material like seedlings, leaf, stem, in vitro cultured gametes and cells, or sometimes even whole plants are used. Vegetatively propagated plant forms like bulbs, tubers, and rhizome are also used because of their meristematic ability (Wani, 2014; Mba, 2013). When chemical mutagens are used generally mutation created are genetic point mutation and physical mutagens generate lesion in DNA and chromosomal aberration. The choice of mutagen is based on its effectiveness in creating mutation and its availability.

11.4.1 PHYSICAL MUTAGENESIS

It has been set forth that >60% of mutant varieties released till now were developed with help of physical mutagens (Mba, 2012, 2013). Under the category of physical mutagens, different types of radiations are grouped.

Radiation is the main form of energy traveling through a distance in form of waves. The first radiation to be used to induce mutation were X-rays. Various other particles emitted by unstable radioactive nuclei like neutrons, protons, β - particles, α -particles, and γ -rays are also widely used (Acquaah, 2006) owing to its high penetration power. Some common physical mutagen used in plant breeding are presented in Table 11.2. Physical mutagen generally causes a lesion in DNA double-strand or single strand. Gamma radiation has a short wavelength and exalted energy level than that of protons and X-rays and can penetrate easily deep into tissues (Amano, 2006). Neutrons are avowed to cause chromosomal aberrations but have lesser penetration power. They are used to induce mutation on explants like desiccated seeds (Acquaah, 2006) but their use is confined (Kharkwal, 2012). Other radiation that is nonionizing types are U.V rays discovered by Altenbung (1934) and have low penetration power but when cells are lay open to UV radiation it results in the formation of pyrimidine dimer (Kharkwal, 2012; Naito, 2005). Physical mutagens have an advantage over chemical mutagen in case of the degree of accuracy and uniform penetrating power in tissue especially gamma rays (Yamaguchi, 2003).

TABLE 11.2 Some of the Commonly Used Physical Mutagens.

Mutagen	Source	Characteristics	Hazard
X-rays	X-ray machine	Penetrates inside tissues from a few millimetres to many centimetres	Dangerous
Gamma rays	Radioisotopes and nuclear reaction	Very penetrating into tissues; sources are ^{60}Co (Cobalt-60) and ^{137}Cs (Caesium-137)	Dangerous, very penetrating
Neutrons	Nuclear reactors or accelerators	There are different types (fast, slow, thermal); produced in nuclear reactors; uncharged particles penetrate tissues to many centimetres; source is ^{235}U	Very hazardous
Beta particles	Radioactive isotopes or accelerators	Are electrons; ionize; shallowly penetrating; sources include ^{32}P and ^{14}C	May be dangerous
Alpha particles	Radioisotopes	Very shallowly penetrating	Very dangerous
Protons	Nuclear reactors or accelerators	Penetrate tissues up to several centimetres	Very dangerous

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11.4.2 CHEMICAL MUTAGENS

Chemical substances have a subtle mutagenic effect as compared with physical mutagens (Acquaah, 2006) but they do not require any special facilities center with sophisticated devices for its application as required in the case of radiation. Some chemical mutagens are carcinogenic by nature so extra precaution is needed at the time of its application. Generally, the plant material is soaked in a mutagen solution for a certain time to induce mutation. Many chemical mutagens have been reported by the time but in plant breeding program very few chemical mutagens have been tested (Wani, 2014) like alkylating agents, which have been utilized in inducing mutation in >75% of registered mutant plant varieties reported by International Atomic Energy Association. The most customarily used alkylating agents were EMS (Ethyl methane sulphonate), 1-methyl-1-nitrosourea, and 1-ethyl-1-nitrosourea (Wani, 2014; IAEA, 2015). These alkylating agents react with DNA molecules by alkylating the phosphate group present on DNA and also purine and pyrimidine bases (Acquaah, 2006). Another class of chemical mutagen extensively used to induce mutation are base analogues like 5-bromouracil and maleic hydrazide, which are structural isomers of nitrogenous bases of DNA. Some common chemical mutagens are enlisted in Table 11.3.

TABLE 11.3 Some of the Commonly Used Chemical Mutagens.

Mutagen	Example	Mode of action
Alkylating agents	1-methyl-1-nitrosourea (MNU); 1-ethyl-1-nitrosourea (ENU); methyl methanesulphonate (MMS); ethyl methanesulphonate (EMS); dimethyl sulphate (DMS); diethyl sulphate (DES); 1-methyl-2-nitro-1-nitrosoguanidine (MNNG); 1-ethyl-2-nitro-1-nitrosoguanidine (ENNG); N,N-dimethylnitrous amide (NDMA); N,N-diethylnitrous amide (NDEA)	Transfer alkyl group thereby creating mutation and mismatch pairing.
Azide	Sodium azide	React with bases and add atom
Hydroxylamine	Hydroxylamine	React with bases and add atom
Nitrous acid	Nitrous acid	A deaminating agent that causes replacement of cytosine by uracil, leading to mismatch pairing.

TABLE 11.3 (Continued)

Mutagen	Example	Mode of action
Acridines	Acridine orange	They are intercalating agents that slips between DNA bases causing a distortion of the DNA double helix
Base analogues	5-bromouracil (5-BU); maleic hydrazide; 5- bromodeoxyuridine; 2-aminopurine (2AP)	They get incorporated during DNA replication causing transitions (purine to purine or pyrimidine to pyrimidine) mutation

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The concentration of mutagen, duration, and temperature affect the mutagenesis caused by chemical mutagens.

11.5 T- DNA MUTAGENESIS

In the last two decades use of biological agents like transposable elements and T-DNA have paved the way to explore in detail various other aspects of gene and chromosome and their behavior (Feldmann, 1991; Konez, 1992; Walden, 1990). Mostly in the case of plants mutant varieties have been created by the use of physical or some chemical mutagen or by insertion of exogenous DNA segment in the plant genome. The segment of DNA inserted are either mobile genetic element known as transposons or T- DNA, a section of bacterial circular DNA. These segments of DNA are integrated in plant genome by the aid of *Agrobacterium tumefaciens*-mediated gene transfer or by direct transfer using gene bombardment process through gene gun, by electroporation, microinjection, or by use of certain chemicals like polyethylene glycol. T-DNA-induced mutant genes are tagged by T-DNA insertion and can be easily cloned. Several gene discovery projects are currently underway using T-DNA and transposon insertional mutagenesis method (Pan, 2003).

Transposable elements have been colossally utilized for gene cloning using sequences of transposons as molecular tags but its application is finite only to plant species having a well-defined transposon system as found in Maize and Antirrhinum.

The most proffer method for creating mutant plant varieties through insertional mutagenesis is grounded on the insertion of T-DNA isolated from a soil-borne bacterium *A. tumefaciens* (Feldmann, 1991; Radhamony, 2005).

11.6 T-DNA TRANSFER PROCESS

For the accomplishment of insertion mutagenesis favorably in plant vector-mediated T-DNA transfer is the most quotidian tool used by researchers. The gene of interest is inserted betwixt the left and right border of T-DNA and can be transferred into plant cells followed by its incorporation in the plant genome (Fig. 11.2). The integration process is comprised of many steps that involves various bacterial protein and plant enzymes. It has been reported by various researchers that *Agrobacterium* plasmid system has been successfully utilized to create various transformants of the model plant Arabidopsis (Pan, 2003). Figure 11.2 represents the scheme for creating T-DNA insertion mutagenesis in Arabidopsis to develop a transgenic plant.

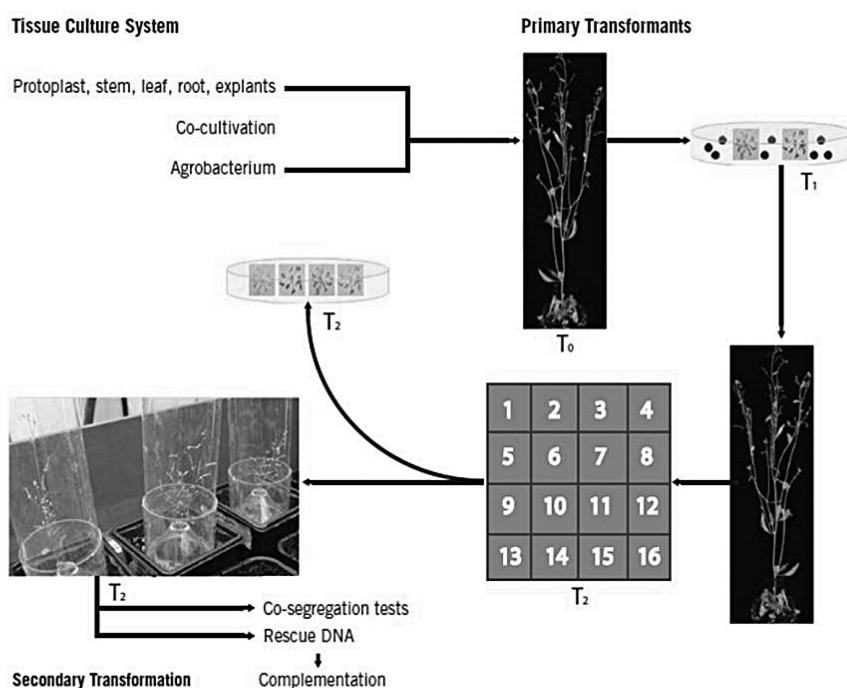


FIGURE 11.2 Steps for creating T- DNA insertion in *Arabidopsis* plant.

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11.7 T-DNA TAGGING VECTOR

Soil bacterium-derived Ti plasmid (tumor-inducing plasmid) has a special region flanked by 25 bp repeat sequence categorized as left and right border (LB and RB). Ti plasmid also carries another section, which mobilizes the transfer of T-DNA inside plant cell (Fig. 11.3). Prior to the selection of vector for T-DNA transfer some important points should be considered including the presence of resistance marker in bacteria (Kanamycin, streptomycin, tetracycline, etc) and plants, size of the vector, presence of unique restriction sites available on plasmid for cloning a gene of interest.

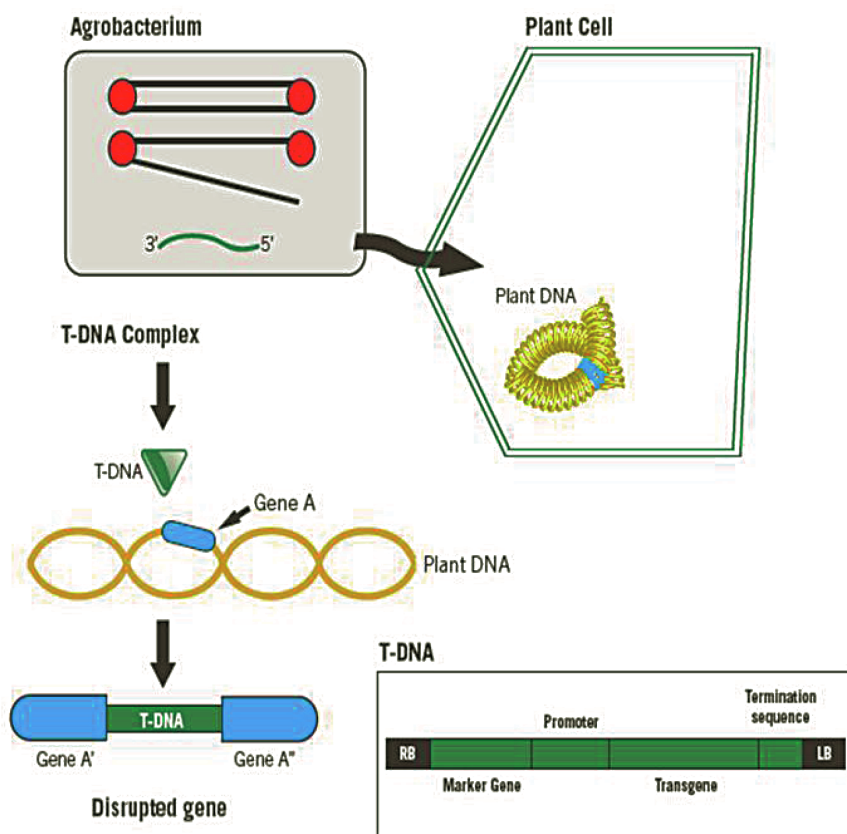


FIGURE 11.3 A sequential representation of T-DNA insertion and tagging system.

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11.8 AGROBACTERIUM-BASED VECTOR SYSTEM COMMONLY USED

Some of the commonly used vector systems are

11.8.1 COINTEGRATE VECTOR

This system is based on cointegration of two plasmids, intermediate and disarmed within *Agrobacterium*. In this system gene of interest to be transferred in plant, genome are initially copied inside *Escherichia coli* then transferred in *Agrobacterium*.

11.8.2 BINARY VECTORS

This system is a dual plasmid-based system most commonly used for plant transformation consisting of one plasmid having vir region necessary for the transfer of T-DNA and other the disarmed plasmid also known as mini or micro Ti having two origins of replication for both *E. coli* and *Agrobacterium* system. The gene of interest is inserted within T-DNA region of mini Ti and cloned in *E. coli*.

11.8.3 PASSIVE VECTOR

This system consist of marker gene lacking either the promoter sequence or sequence associated to “minimal promoter” missing, which is necessary to bring out the normal gene expression. Once upon their incorporation in plant genome they guide gene expression leading to marker gene expression with aid of screenable marker gene like NPTII, Lux, or GUS the promoters can be screened as transcriptional fusion.

11.8.4 ACTIVE VECTORS

This vector actively modifies the gene expression in either a positive or negative manner and T-DNA tags carrying transcriptional enhancer sequence can be used to cause this effect.

11.9 GENETIC TRANSFORMATION SYSTEM

The use of *Agrobacterium*-mediated gene transfer system has been used for transgenic plant development but reports of the occurrence of somaclonal variation along with the desired mutants plants is a matter of concern for plant breeders. Valveken and his group in 1988 reported that root transformation system was good means for T- DNA insertion mutagenesis in *Arabidopsis* but along with that of occurrence of somaclonal variants were also observe (Valvekens, 1988). In *Arabidopsis*, several other plant regeneration method using leaf, stem, protoplast, and cotyledons have been developed, however, a high frequency of somaclonal variation was also recorded in plant regenerates. The prime reason for the occurrence of somaclonal variation in the transgenic plant is due to the long duration of regeneration period required for transgenic plant development during which plant tissue remains exposed to exogenous phytohormones and other lab conditions. The occurrence of somaclonal variation makes it tedious to screen out the cells transformed by T- DNA insertion. To overcome this problem, several transformation system using *Agrobacterium*-mediated T-DNA transfer and insertion have been developed distinctively for plant system, which greatly lessens the chance of occurrence of somaclonal variation.

11.10 IN VITRO TRANSFORMATION SYSTEM

Agrobacterium-mediated gene transfer has proved a reliable medium for the headway of transgenic plants. Conventionally cocultivation method of explants like stem, leaf, and root tips with *Agrobacterium* containing media and then the transfer of the transformed cells to growth media is followed for transfer of T-DNA in the plant genome. It was delineated by researchers that the zygotic embryo serves as a righteous explant source with high regeneration ability and without any apparent cases of somaclonal variants as there are very few cell division after fertilization in embryonic initial stage. A reliable plant transformation system (Veyres, 2008) using zygotic embryo of *Arabidopsis* has been developed and depicted in Figure 11.4.

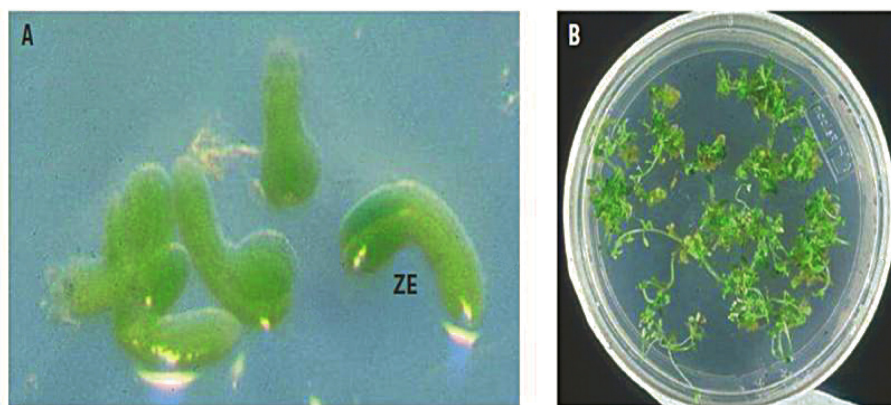


FIGURE 11.4 (A) Embryo transformation. (B) Regeneration of transformed plants in vitro. *Source:* Reproduced with permission of the Licensor through PLSclear from Sangwan, 2012. © CAB International.

11.11 *IN PLANTA* TRANSFORMATION SYSTEM

Since early 1987 various techniques for *in planta* transformation were available using *Agrobacterium* (Radhamony, 2005). In the model plant, *Arabidopsis* initially transformed of seeds with help of *Agrobacterium* was developed, which later proved to be an effective method for T-DNA insertional mutagenesis (Feldmann and Marks, 1987). Using this strategy, the large number of transformed plants can be developed and can be used for gene tagging. Vacuum infiltration techniques were developed for *Arabidopsis* genome transformation using *Agrobacterium* as they have the capability of uniformly transform diverse cell types (Bechtold, 1993; Clough and Bent, 1998). Many other refined methods for *in plant* transformation was developed like “floral dip” method in which floral tissues were immersed in *Agrobacterium*-containing solution (Clough and Bent, 1998). These *in plant* transformation techniques also guarantee a minimized rate of somaclonal variation occurrence, utilizing floral dip methods T-DNA transformed the population of *Arabidopsis* can be developed carrying T-DNA section inserted within any gene of interest.

11.12 INSERTION MUTATION FACILITATED GENE CLONING

T-DNA sequence inserted in genome not only beget the disruption in gene expression but also do the tagging of effected gene and it is easy to recover

the effected genes as T-DNA sequence inserted is known. Assorted techniques used for the recovery of the effected gene are based on cloning or PCR (Allen, 1994; Liu, 1995).

11.13 RECOVERY OF MUTATED PLANT GENE

The most customarily used technique for isolation of mutated gene is PCR based as they are easy to use, give rapid analysis report, and is inexpensive. Some of the methods utilized for gene isolation is described below

11.13.1 LAMBDA LIBRARY SCREENING

A genomic DNA library is constructed in bacteriophage λ vector followed by its screening using a probe, the T-DNA. Lambda library always generates a plant flanking DNA but this process is tedious and very time consuming.

11.13.2 PLASMID RESCUE

Quite a small amount of plant DNA containing T-DNA portion is restricted followed by allowing its self ligation and then transformation of the fragment into *E. coli*. Antibiotic-resistant colonies of *E. coli* are then selected and analysis of plasmid is done to figure out those plasmid containing plant DNA but this technique is not suitable for all T-DNA inserts.

11.13.3 INVERSE POLYMERASE CHAIN REACTION

This technique involves digestion of DNA into fragments containing known sequence followed by its intramolecular ligation (Figure 5). Circular DNA developed is then amplified by PCR using various primers specially designed to perfectly anneal within the region containing known sequence. The flanking sequence can be amplified using this method but is suitable only for DNA segment, which can be monomerically circularized.

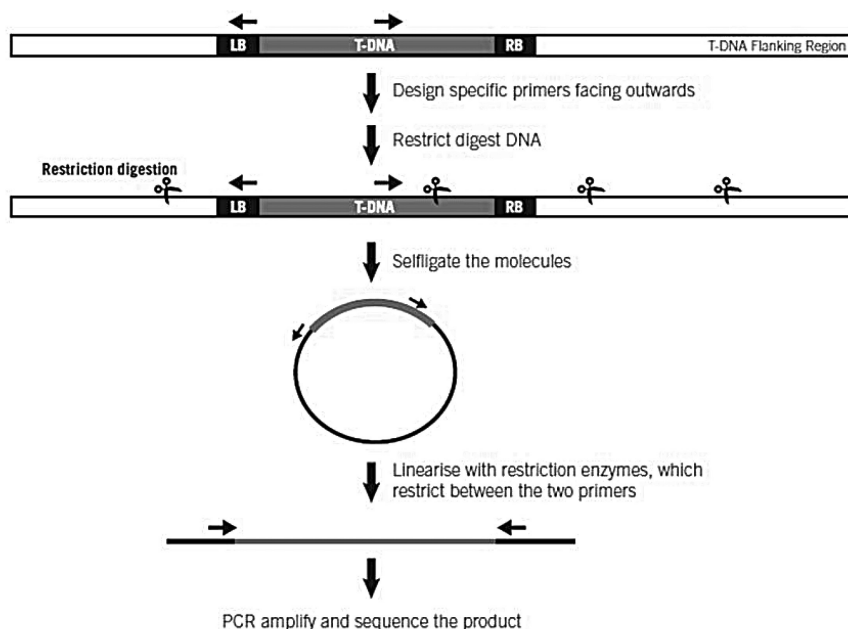


FIGURE 11.5 Schematic representation of inverse PCR.

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11.13.4 GENOME WALKING

In this technique, high-quality DNA is digested by using enzymes like *DraI*, *PvuII*, and *EcoRV*. Digested DNA fragments are ligated with adaptor referred to as “Genome Walker Libraries” at this stage. A pair of gene-specific primers (GSP) are designed of 26–30 nucleotide length and having high G/C content (40–60%), which is prime requirement for proper annealing of primer with DNA template. Primer PCR reaction is carried and the product obtained with adaptor primer (AP1) and GSP1 after a primary walk is diluted and further used in form of template for a secondary walk along with AP2 and GSP2. The product obtained is extracted by gel electrophoresis and then sequenced.

11.13.5 RAPID AMPLIFICATION OF GENOMIC DNA ENDS

In this technique digestion of genomic DNA with restriction endonuclease enzyme is carried and product obtained is ligated into plasmid vector. Special PCR primer is designed one homologous to known sequence in genomic

DNA and other homologous to specific sequence on plasmid. Ligated product is then transferred inside bacteria and plasmid DNA recovered is subjected to PCR for amplification.

11.14 T-DNA INSERTION MUTANTS RESOURCES

Till date *Agrobacterium*-mediated T-DNA insertional mutagenesis have been employed to develop various mutant varieties. A huge population of T-DNA inserted mutant *Arabidopsis* plants have been generated by various research group (Krysan, 1999; <http://signal.salk.edu/cgi-bin/tdnaexpress>; http://Arabidopsis.info/info/MASC_2007.pdf; <http://www.GABI-kat.de>). A number of gene involved in metabolic and development pathways of plants have been cloned from T-DNA tagged lines (Radhamony, 2005). Some of the genes cloned are genes involved in the ethylene signal transduction pathway, which can be used to develop plant more resistant to abiotic stress or with improved self-life of fruits and vegetables due to manipulated senescence cycle. It was demonstrated in year 2007 by Forster that use of T-DNA transformed mutants to elucidate phytochrome model of barley related to increasing yield (Forster, 2007).

T-DNA tagging has been reportedly utilized for the discovery of genes in rice. In other crop plant, like tomato functional analysis tool based on insertion mutation with Ac/Ds system for miniature variety, Micro-Tom development has been used. Modified T-DNA insertion mutation approaches are now being used for tagging genes in several economically important crop plants like Brassica, tomato, rice, medicago, and poplar.

11.15 ADVANTAGES AND LIMITATIONS OF T-DNA INSERTIONAL MUTAGENESIS

Some of the advantages using T-DNA insertion mutagenesis are T-DNA can be straightforwardly inserted into genomic DNA, insertion of T-DNA is random, and it can tag the mutated locus. There are also some stumbling block associated with T-DNA use and insertion are complex arrangement of T-DNA, insertion at multiple site, vector sequence insertion, DNA content per nucleus modification, and chromosomal aberration caused (Radhamony, 2005).

11.16 CONCLUSION

Naturally occurring mutation is an evolutionary process that led to recombination and formation of new improved genotypes. Induced mutation studies providing new combination of alleles by different induced mutation techniques have contributed significantly in recent years to increase genetic diversity and one such technique, insertional mutagenesis have evinced very useful for the development of mutant varieties of commercially important crop plant by insertion of biological agents like T-DNA.

KEYWORDS

- **insertion**
- **mutagenesis**
- **T-DNA**
- **plant breeding**
- **crop**

REFERENCES

- Acquaah, G. *Principles of Plant Genetics and Breeding*; Wiley-Blackwell: Chichester, 2006.
- Ahloowalia, B. S.; Maluszynski, M. Induced Mutations—A New Paradigm in Plant Breeding. *Euphytica* **2001**, *118* (2), 167–173.
- Allen, M. J.; Collick, A.; Jeffreys, A. J. Use of Vectorette and Subvectorette PCR to Isolate Transgene Flanking DNA. *PCR Methods Appl.* **1994**, *4*, 71–75.
- Altenbung, E. The Artificial Production of Mutations by Ultraviolet Light. *Am Naturalist* **1934**, *68*, 491–507.
- Amano, E. Use of Induced Mutants in Rice Breeding in Japan. *Plant Mutation Rep.* **2006**, *1* (1), 21–24.
- Bechtold, N.; Ellis, J.; Pelletier, G. In planta Agrobacterium-Mediated Gene Transfer by Infiltration of Adult *Arabidopsis thaliana* Plants. *CR Acad. Sci.: Life Sci.* **1993**, *316*, 1194–1199.
- Bhat, T. A.; Gulfishan, M.; Wani, A. A. Cytomixis: Causes and Consequences as a Case Study in *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Published by Springer Germany, 2017.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Comparative Analysis of Meiotic Abnormalities Induced by Gamma Rays, EMS and MMS in *Vicia faba* L. *J. Ind. Bot. Soc.* **2005a**, *84*, 45–48.

- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Clastogenic Effects of EMS and MMS in *Vicia faba* L. *J. Cyto. Genet.* **2005b**, *6*, 117–122.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by Gamma Rays and EMS in *Vicia faba* L. *Vegetos* **2005c**, *18* (1&2), 143–145.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Effect of Gamma Rays on Certain Cytomorphological Parameters in Two Varieties of *Vicia faba* L. *Adv. Plant Sci.* **2006a**, *19* (1), 227–232.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Wani, N.A Radiation Induced Pod and Seed Mutants and MMS Induced Closed Flower Mutant in Broad Bean (*Vicia faba* L.). *Indian J. Genet.* **2006b**, *66* (3), 249–250.
- Bhat, T. A.; Parveen, S.; Khan, A. H. MMS-Induced Cytomixis in Pollen Mother Cells of Broad Bean (*Vicia faba* L.). *Turk. J. Bot.* **2006c**, *30*, 273–279.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Studies on the Effect of EMS on Meiosis and Pollen Fertility in *Vicia faba* L. *Adv. Plant Sci.* **2006d**, *19* (1), 243–247.
- Bhat, T. A.; Khan, A.H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by MMS, Gamma Rays and Their Combination in Two Varieties of *Vicia faba* L. *Asian J. Plant Sci.* **2007a**, *6* (3), 558–561.
- Bhat, T. A.; Parveen, S.; Khan, A. H. Meiotic Studies in Two Varieties of *Vicia faba* L. (Fabaceae) After MMS Treatment. *Asian J. Plant Sci.* **2007b**, *61* (1), 51–55.
- Bhat, T. A.; Sharma, M.; Anis, M Comparative Analysis of Mitotic Aberrations Induced by Diethyl Sulphide (DES) and Sodium Azide (SA) in *Vicia faba* L. (Fabaceae). *Pak. J. Biol. Sci.* **2007c**, *10* (5), 783–787.
- Bhat, T. A.; Sharma, M.; Anis, M. Comparative Analysis of Meiotic Aberrations Induced by Diethylsulphate and Sodium Azide in Broad Bean (*Vicia faba* L.). *Asian j. Plant Sci.* **2007d**, *6* (7), 1051–1055.
- Bhat, T. A.; Wani, A. A. Mutagenic Effects on Meiosis in Legumes and a Practical Case Study of *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Published by Springer Germany, 2017.
- Broertjes, C.; Van Harten, A. M. Applied Mutation Breeding for Vegetatively Propagated Crops, Developments in Crop Science, Vol. 12; Elsevier Publ.: Amsterdam, 1988; pp 197–204.
- Chai, M.; Ho, Y. W.; Liew, K. W. et al. Biotechnology and in Vitro Mutagenesis for Banana Improvement. In *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations*; Jain, S. M., Swennen, R., Eds.; Science Publishers, Inc.: Enfield, NH, 2004; pp 59–77.
- Clough, S. J.; Bent, A. F. Floral Dip: A Simplified Method for Agrobacterium-Mediated Transformation of *Arabidopsis thaliana*. *Plant J.* **1998**, *16*, 735–743.
- Feldmann, K. A. T-DNA Insertion Mutagenesis in Arabidopsis Mutational Spectrum. *Plant J.* **1991**, *1*, 71–82.
- Feldmann, K. A.; Marks, M. D. Agrobacterium-Mediated Transformation of Germinating Seeds of *Arabidopsis thaliana*. *Mol. Gen. Genet.* **1987**, *208*, 1–9.
- Forster, B. P.; Franckowiak, J. D.; Lundqvist, U.; Lyon, J.; Pitkethly, I.; Thomas, W. B. The Barley Phytomer. *Anna. Bot.* **2007**, *100*, 725–733.
- Forster, B. P.; Shu, Q. Y. Plant Mutagenesis in Crop Improvement: Basic Terms and Applications. In *Plant Mutation Breeding and Biotechnology*; Shu, Q. Y., Forster, B. P., Nakagawa, H., Eds.; CABI: Wallingford, 2012; pp 9–20.
- GABI-Kat SimpleSearch (<http://www.GABI-Kat.de>) is a Flanking Sequence Tag (FST)-based database for T-DNA insertion mutants.

- Gulfishan, M.; Bhat, T. A.; Oves, M. Mutants as a Genetic Resource for Future Crop Improvement. *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools*; Al-Khayri, J. M. Eds.; Springer International Publishing: Switzerland, 2015.
- Gulfishan, M.; Jafri, I. F.; Khan, A. H.; Bhat, T. A. Methyl Methane Sulphonate Induced Desynaptic Mutants in *Capsicum annum* L. *Chrom. Bot.* **2013**, *8*, 59–62.
- Gulfishan, M.; Khan, A. H.; Bhat, T. A. Studies on Cytotoxicity Induced by DES and SA in *Vicia faba* L. var. Major. *Turk. J. Bot.* **2010**, *34*, 31–37.
- Ganai, F. A.; Khan, A. H.; Bhat T. A.; Parveen, S.; Wani, N. A. Cytogenetic Effects of Methyl Methane Sulphonate (MMS) on Varieties of Chickpea (*Cicerarietinum*L.) *J. Cytol. Genet.* **2005**, *6*, 97–102.
- Gulfishan, M.; Khan, A. H.; Gefferi, I. F.; Bhat, T. A. Assessment of mutagenicity induced by MMS and DES in *Capsicum annum* L. *Saudi J. Biol. Sci.* **2012**, *19*, 251–255.
- Gulfishan, M.; Khan, A. H.; Haneef, I.; Bhat, T. A. Genotoxic Effect of Diethyl Sulphate in Two Varieties of *Capsicum annum* L. *Nucleus* **2001**, *54* (2), 107–111.
- IAEA. IAEA Mutant Database; International Atomic Energy Agency: Vienna; 2015. <http://mvd.iaea.org/> (accessed July 2015).
- Khan, A. A.; Ansari, M. Y. K.; Shahab, D.; Bhat, T. A. Induced Variations for Quantitative Traits in *Vicia faba* L. *Vegetos* **2005a**, *18* (1&2), 83–86.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. DES Induced Variations for Economic Traits in Broad Bean (*Vicia faba* L.). *Biosci. Biotechnol. Res. Asia* **2005b**, *3* (2), 363–366.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. Clastogenic Effects of 8-Hydroxy Quinoline in *Vicia faba* L. *Adv. Plant Sci.* **2007a**, *20* (1), 85–87. ISSN 0970–3586.
- Khan, A. A.; Ansari, M. Y.K.; Shahab, D.; Bhat, T. A. Comparative Analysis of Meiotic Abnormalities Induced by Caffeine and DES in *Vicia faba* L. *Adv. Plant Sci.* **2007b**, *20* (1), 73–76.
- Kharkwal, M. C. A Brief History of Plant Mutagenesis. In *Plant Mutation Breeding and Biotechnology*; Shu, Q. Y., Forster, B. P., Nakagawa, H. Eds.; CABI: Wallingford, 2012; pp 21–30.
- Kharkwal, M. C.; Shu, Q. Y. The Role of Induced Mutations in World Food Security. In *Induced Plant Mutations in the Genomics Era*; Shu, Q. Y., Ed.; Food and Agriculture Organization of the United Nations: Rome, 2009; pp 33–38.
- Koncz, C.; Schell, J.; Rkdei, G. P. T-DNA Transformation and Insertion Mutagenesis. In *Methods in Arabidopsis Research*; Koncz, C., Chua N-H., Schell, J., Eds.; World Scientific: Singapore, 1992; pp 224–273.
- Kondo, E.; Nakayama, M.; Kameari, N. et al. Red–Purple Flower Due to Delphinidin 3,5-Diglucoside, a Novel Pigment for Cyclamen spp.; Generated by Ion-Beam Irradiation. *Plant Biotechnol.* **2009**, *26* (5), 565–569.
- Krysan, P. J.; Young, J. C.; Sussman, M. R. T-DNA as an Insertional Mutagen in Arabidopsis. *Plant Cell* **1999**, *11*, 2283–2290.
- Leitao, J. M. Chemical Mutagenesis. In *Plant Mutation Breeding and Biotechnology*; Shu, Q. Y., Forster, B. P., Nakagawa, H., Eds.; CABI: Wallingford, 2012; pp 135–158.
- Liu, Y. G.; Mitsukawa, N.; Oosumi, T.; Whittier, R. F. Efficient Isolation and Mapping of *Arabidopsis thaliana* T-DNA Insert Junctions by Thermal Asymmetric Interlaced PCR. *Plant J.* 1995, *8*, 457–463.
- Mba, C. Induced Mutations Unleash the Potentials of Plant Genetic Resources for Food and Agriculture. *Agronomy* **2013**, *3* (1), 200–231.

- Mba, C.; Afza, R.; Bado, S. et al. Induced Mutagenesis in Plants Using Physical and Chemical Agents. In *Plant Cell Culture: Essential Methods*; Davey, M. R., Anthony, P., Eds.; John Wiley & Sons, Ltd.: Chichester, 2010; pp 111–130.
- Mba, C.; Afza, R.; Shu, Q. Y. Mutagenic Radiations: X-Rays, Ionizing Particles and Ultraviolet. In *Plant Mutation Breeding and Biotechnology*; Shu, Q. Y., Forster, B. P., Nakagawa, H., Eds.; CABI: Wallingford, 2012.
- Naito, K.; Kusaba, M.; Shikazono, N. et al. Transmissible and Nontransmissible Mutations Induced by Irradiating *Arabidopsis thaliana* Pollen with g_e Rays and Carbon Ions. *Genetics*. **2005**, 169 (2), 881–889.
- Nakagawa, H. *Plant Mutation Breeding and Biotechnology*; CABI: Wallingford, 2012; pp 83–90.
- Nenchewa, D. In Vitro Propagation of Chrysanthemum. In *Protocols for In Vitro Propagation of Ornamental Plants*; Jain, S. M., Sergio, O., Eds.; Humana Press, 2010; pp 177–185.
- Novak, F. J.; Brunner, H. Plant Breeding: Induced Mutation Technology for Crop Improvement. *IAEA Bull.* **1992**, 4, 25–33.
- Oladosu, Y.; Rafii, M. Y.; Abdullah, N. Principle and Application of Plant Mutagenesis in Crop Improvement: A Review. *Biotechnol. Biotechnol. Equip.* **2016**, 30 (1), 1–16.
- Oldach, K. H. Mutagenesis. In *Biology and Breeding of Food Legumes*; Pratap, A., Kumar, J., Eds.; CABI: Wallingford, 2011; pp 208–219.
- Pan, X.; Liu, H.; Clarke, J.; Jones, J.; Bevan, M.; Stein, L. ATIDB: *Arabidopsis thaliana* Insertion Database. *Nucl. Acids Res* **2003**, 31, 1245–1251.
- Parveen, S.; Anis, M.; Bhat, T. A.; Khan, A. H. Cytological Effects of Physical and Chemical Mutagens in *Trigonella foenum-graecum* L. *Adv. Plant Sci.* **2007**, 20 (11), 633–636.
- Parveen, S.; Anis, M.; Bhat, T. A.; Khan, A. H. Efficiency and Effectiveness of Physical and Chemical Mutagens in M₂ Generation of *Trigonella foenum-graecum* L. *J. Cytol. Genet.* **2006**, 7, 1–4.
- Predieri, S. Mutation Induction and Tissue Culture in Improving Fruits. *Plant Cell Tissue Organ Culture* **2000**, 64 (2–3), 185–210.
- Radhamony, R. N.; Prasad, A. M.; Srinivasan, R. T-DNA Insertional Mutagenesis in *Arabidopsis*: A Tool for Functional Genomics. *E. J. Biotechnol.* **2005**, 8, 1–20.
- Roychowdhury, R.; Tah, J. Mutagenesis a Potential Approach for Crop Improvement. In *Crop Improvement: New Approaches and Modern Techniques*; Hakeem, K. R., Ahmad, P., Ozturk, M., Eds.; Springer: New York, 2013; pp 149–187.
- Sangwana, R. S.; Ochatt, S.; Nava-Saucedo, J. E.; Sangwan-Norreela, B. T-DNA Insertion Mutagenesis. In *Plant Mutation Breeding and Biotechnology*; Shu, Q. Y., Forster, B. P., Nakagawa, H., Eds.; CABI: Wallingford, 2012.
- Sharma, M.; Khan, A. H.; Bhat, T. A. Assessment of Mutagenicity of Individual and Combination Treatment of Gamma Rays and MMS in Broad Bean (*Vicia faba* L). *Cytologia*. **2009**, 74 (2), 235–241.
- Shu, Q. Y.; Forster, B. P.; Nakagawa, H. Principles and Applications of Plant Mutation Breeding. In *Plant Mutation Breeding and Biotechnology*; Shu, Q. Y., Forster, B. P., Nakagawa, H., Eds.; CABI: Wallingford, 2012; pp 301–325.
- T-DNA Express: *Arabidopsis* Gene Mapping Tool: <http://signal.salk.edu/cgi-bin/tdnaexpress>.
- The Multinational Coordinated *Arabidopsis thaliana* Functional Genomics Project. http://Arabidopsis.info/info/MASC_2007.pdf

- Valvekens, D.; Van Montagu, M.; Van Lijsebettens, M. A. Tumefaciens Mediated Transformation of *Arabidopsis thaliana* Root Explants by Using Kanamycin Selection. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 5536–5546.
- Van Harten, A. M. *Mutation Breeding: Theory and Practical Applications*; Cambridge University Press: Cambridge, 1998.
- Veyres, N.; Danon, A.; Aono, M.; Galliot, S.; Karibasappa, Y.; Diet, A.; Grandmottet, F.; Tamaoki, M.; Lesur, D.; Pilard, S.; Boitel-Conti, M.; Sangwan, B.; Sangwan, R. The Arabidopsis Sweetie Mutant Is Affected in Carbohydrate Metabolism and Defective in the Control of Growth, Development and Senescence. *Plant J.* **2008**, *55*, 665–686.
- Walden, R.; Koncz, C.; Schell, J. The Use of Gene Vectors in Plant Molecular Biology. *Meth. Mol. Cell Biol.* **1990**, *4*, 175–194.
- Wani, M. R.; Kozgar, M. I.; Tomlekova, N. et al. Mutation Breeding: A Novel Technique for Genetic Improvement of Pulse Crops Particularly Chickpea (*Cicerarietinum* L.). In *Improvement of Crops in the Era of Climatic Changes*; Parvaiz, A., Wani, M. R., Azooz, M. M., Lam-son, P. T., Eds.; Springer: New York, 2014; pp 217–248.
- Wani, N. A.; Bhat, T. A.; Khan, A. H.; Ansari, M. Y. K. Sodium Azide Induced Variability in Chickpea (*Cicerarietinum*L). *Vegetos* **2005**, *18* (1&2), 105–107.
- Wani, A. A.; Bhat, T. A. Asynapsis and Desynapsis in Plants: In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Yamaguchi, H.; Nagatomi, S.; Morishita, T. et al. Mutation Induced with Ion-Beam Irradiation in Rose. *Nucl. Instr. Meth. Phys. Res.* **2003**, *206*, 561–556.



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CHAPTER 12

Different Types of DNA Molecular Markers and Genotyping Applications for Mutation Breeding

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ABSTRACT

The molecular markers were developed in 1980s, these molecular markers have changed the fate of plant breeding. Since ancient times different types of molecular markers have been developed and have been used for plant breeding and crop improvement. Due to the sequencing technology, new molecular markers have been developed, which are also used for the crop improvement. In the present review, the main objective is to provide a brief insight into the different types of molecular markers and their application in crop improvement by using the breeding technology. From the literature, it is clear that for complex and unstructured population, associative mapping and genotyping by sequencing technologies have helped in mapping. Altogether, in the present chapter various types of molecular markers, their application in plant breeding and genotyping has been discussed.

12.1 INTRODUCTION

Molecular markers also known as the Genetic markers. Molecular marker is defined as the fragment of DNA that is associated with certain location

within the genome, thus we can say that molecular markers are the heritable differences in any of the two different individuals present in homologous chromosome at a specific position. These variations usually follow the Mendelian pattern. The molecular markers play a very important role in the biological sciences. The DNA-based molecular markers have played a very important tool in the fields of embryology, physiology, taxonomy, genetic engineering, etc (Schlotterer, 2004). Broadly molecular markers are classified into two main types: classical markers and DNA markers.

12.2 CLASSICAL MARKERS

Since ancient times classical markers have been used by the plant breeders to identify elite genotypes. The classical markers are mainly morphological marker, cytological marker, and biochemical/protein marker.

12.3 MORPHOLOGICAL MARKERS:

Morphological markers are those markers that can be easily identified by seeing the external morphology of any living organism thus these markers can be identified or detected by visual inspection or by eyes. The main examples of such type of marker in plants include differences in the plant weight, plant coloration, plant height, aroma yield, etc. Morphological molecular markers have been used from ancient time for the selection of the desirable trait. Morphological markers show certain disadvantages. These markers are usually small in number and are mostly affected by the plant growth stages and the environment (Eagles et al., 2001)

12.4 CYTOLOGICAL MARKERS

The markers in which the study of chromosomes is done are known as the cytological markers. Chromosome banding and the karyotype show the structural features of chromosomes. They can be detected by the banding patterns. Mostly these variations can be seen in color, width, order, and position of the chromosomes. The euchromatin and the heterochromatin are used to detect the differences between the mutated and the normal chromosome. Different bands are produced by different stains and these can be used to differentiate between normal and mutated chromosomes. The linkage groups and the physical mapping can be studied with the help of the cytological markers (Jiang, 2013).

12.5 BIOCHEMICAL/PROTEIN MARKERS

The other name for the biochemical markers is the protein markers. These are the markers in which the polymorphism is detected in protein profile. This polymorphism in a protein marker can be detected with the help of gel electrophoresis followed by staining of a particular protein with a specific dye. The biochemical markers have been used in crop improvement and diversity analysis. Polymorphism in protein profile in the protein marker is detected by electrophoresis and this polymorphism is due to the variations in the structural genes that encode the proteins; however, the post-translational modification in the proteins is also responsible for the variations. In protein markers, two classes of proteins are used. These are isoenzymes /isozymes and alloenzymes/allozymes.

Isozymes are the allelic variants of the same enzyme, these are usually encoded by different loci (Weeden and Wendel, 1989). These are also known as the multiple forms of a same enzyme. The allozymes are the form of enzyme that differs in the sequence of the amino acid and perform the same enzyme function. Among these two, the most widely used protein-based marker is isozymes. The term isozyme was introduced in 1959 by Markert and Moller. Isozymes are generally codominant in nature. According to Moller, the multiple forms of an enzyme in the same population of an individual is isozyme.

During isozyme analysis, protein is extracted from the tissue extract. Later this extract is separated by electrophoresis on the polyacrylamide or starch gel and the bands get separated due to difference in the charges and also due to difference in their sizes. The protein bands are stained with the help of a suitable dye the dye reacts with the protein and form the bands. The band formation occurs at the position of the migration of the enzyme, this position shows the relative activity of the enzyme. During the reaction multiple polymorphic and informative bands are formed and the number of bands indicate the number of loci, structural configuration of enzyme, and the state of enzyme (homozygosity/heterzygosity) in the individual. It has been documented that I most of the cultivated species the polymorphism of isoenzyme markers is less. So, due to this reason, this marker has not been widely used in most of the species.

One of the important objectives in the agriculture is to develop the better and improved cultivars. The common method for crop improvement is by conventional breeding in which two plants of desirable traits are crossed with one another. The method of the conventional breeding involves the crossing of whole genomes and the selection of the superior recombinants

among the several segregation products. The procedure of the conventional breeding is laborious, involved keen skills, many crosses, many generations and careful selection.

The problems of conventional breeding were overcome by the discovery of DNA marker technology. Different types of the molecular markers have been used for the identification of the different loci in plant species (Gupta and Varshney, 2004). Broadly saying three types of the molecular markers have been identified. These are markers based upon hybridization, based upon polymerase chain reaction (PCR) and markers based upon sequencing.

12.6 DNA MARKERS

The molecular markers or DNA-based molecular markers are polymorphic nucleotide sequences present between different individuals. These markers have been used for genotyping and diversity analysis (Staub and Gupta, 1996). Molecular markers can be defined as a fragment of DNA that reveals variations/mutations. These variations are also used to detect the polymorphism between different individuals. Usually, this polymorphism is associated with a particular position in the genotype. Thus we can say that in simple sense DNA marker is a small nucleotide sequence in DNA showing the polymorphism. The polymorphism may be either due to deletion, insertion, transversion, and substitution between different individuals. Commonly the following two methods are used to detect the polymorphism using these markers. These are Southern blotting (Southern, 1975), and PCR technique (Mullis, 1990). In addition to PCR and Southern blotting, new array chip technique and sequencing technique has also been developed and used for polymorphism detection.

The ideal DNA molecular marker should possess the following features.

- These molecular markers should be highly polymorphic.
- These markers should have the even distribution along the whole genome.
- These should be codominant.
- These markers should identify different loci easily.
- These should not show the pleiotropic effect
- These markers should be cost effective.
- These markers should be easily detected and should be easily automated.
- These markers should be reproducible.
- These markers should be specific to genome mainly of polyploids.

12.7 CLASSIFICATION OF MOLECULAR MARKERS

The DNA-based molecular markers have played a very important role in the improvement of crops and in molecular breeding. These markers have been classified into different groups on the following basis:

12.7.1 MODE OF GENE ACTION

On the basis of mode of action DNA molecular markers may be either codominant markers or the dominant markers.

12.7.2 METHOD OF DETECTION

On the basis of method of detection, these markers may be either PCR-based molecular markers or the hybridization-based molecular markers.

12.7.3 MODE OF TRANSMISSION

On the basis of mode of transmission, these markers may show either maternal organelle inheritance, maternal nuclear inheritance, paternal organelle inheritance, or both bi-parental nuclear inheritance (Semagn, 2006).

12.8 ADVANTAGES OF DNA-BASED MARKERS

DNA-based markers offer several advantages over the morphological markers. Some of the main advantages of these markers are as follows:

12.8.1 INCREASED RELIABILITY

DNA-based molecular markers are more reliable than the phenotypic assays as the phenotypic assays are mostly influenced by the environmental conditions.

12.8.2 INCREASED EFFICIENCY

DNA-based molecular markers can be easily detected at seedling stages so are more efficient than other markers. These markers save time.

12.8.3 REDUCED COST

Most of the DNA-based molecular markers especially PCR-based assays are cost-effective.

12.8.4 UNIQUENESS

Mostly, the DNA-based molecular markers are able to detect the mutations and are mostly unique for each allele of a gene.

Thus, seeing the above advantages of the molecular markers, these can be used to for genotyping and the gene pyramiding. For the development of the DNA-based molecular markers either genomic DNA libraries are used or random PCR amplification of genomic DNA is preferred. Sometimes these markers can be obtained by both PCR amplification or from genomic libraries. Large number of the DNA markers have been developed, these DNA markers have played a important role in crop improvement. Many physical and genetic maps have been prepared using the molecular markers. Molecular markers have also been used in marker-assisted selection (MAS).

DNA molecular markers have changed the mutation-based plant breeding. For example, sequencing has made possible the development of simple PCR-based markers for the genotyping and for the allele-specific amplification, cleaved amplified polymorphic sequences (CAPS), high-resolution melt analysis, and other assays. Due to the low cost of the molecular marker-based methods, these methods have become reliable for crop improvements. Similarly, as a result of high throughput sequencing, many thousand samples of DNA can be analyzed within a short period of time. This sequencing data will help in the mutation breeding technology. For the analysis, many methods have been described, but the new techniques have been developed due to direct DNA sequencing processes. Sequencing has nowadays, became the precise tool for mutation breeding, genotyping, and MAS.

To detect the mutation in the DNA sequence that code for a particular character, that is, a target gene, which code for a particular character is one of the main applications of the DNA markers and high-throughput DNA sequencing. The other main application is pyramiding or introgressing important mutant traits through marker-assisted backcrossing. The mutant lines and varieties can be identified using the DNA markers. Different types of molecular markers are used for the mutation breeding since ancient times. Some of the important molecular markers used nowadays are the amplified

fragment length polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), Simple Sequence Repeat (SSR), etc. In this chapter, we will have a brief description of some of the important molecular markers.

12.9 RFLP MOLECULAR MARKERS

RFLP is the first generation DNA molecular markers. These molecular markers are ancient and have been considered to be the very important tools for the plant genome mapping. RFLP molecular marker is based upon the Southern-Blotting. In this method, both restriction and hybridization with probe DNA is used. In RFLP molecular marker, DNA is restricted and then the probe is hybridized with the restricted DNA. RFLP markers are one of the important markers for plant genome mapping. RFLP was first developed in early 1980 (Botstein et al., 1980). RFLP markers are codominant. RFLP molecular marker has been used as very important markers tools for comparative and synteny mapping. The methodology of this technique is simple and no special equipments are required. This technique of genotyping is highly reproducible. In this process, the researcher only requires a genomic clone, which can be used for the hybridization so as to detect the polymorphism.

During the RFLP, the DNA is restricted with the restriction enzymes. Then the restricted DNA is separated by gel electrophoresis. The separated restricted fragments are later transferred to the Nylon membrane by the Southern blotting, after that they are hybridized with the labeled probes to detect the polymorphism. The main step of the RFLP is to select a suitable probe to detect the polymorphism. These probes are either taken from the cDNA clones and PstI-derived genomic clones. RFLP is a codominant molecular marker and is highly reproducible and reliable. The restriction sites at a particular allele can be detected by the presence of a band, whereas the absence of the band at the same position in other sample indicates the absence of that allele. RFLP technology is time-consuming and expensive but they have been successfully used in genotyping and in the genetic mapping (Miller and Tanksley, 1990), this marker is also used for diversity analysis (Desplanque et al., 1999). The main disadvantages of RFLP technique are that in absence of sequence data, it becomes problematic to interpret complex RFLP allelic systems. RFLP is laborious, expensive and time consuming (Mukesh et al., 2018). This technique also requires large quantity of highly purified DNA.

12.10 RAPD MOLECULAR MARKER

RAPD technique was developed by Williams et al. (1990). This marker was developed to overcome the economic and temporal losses of RFLP. RADP is a PCR-based molecular marker, in this technique random short synthetic oligonucleotide universal primers are used to amplify the particular DNA fragment. The primers used in the RAPD serve as both the forward and the reverse primers. The RAPD amplified DNA fragments usually vary in size and are in the range of 0.5–5 kb. The RAPD PCR amplified products are separated on agarose gel and are visualized by ethidium bromide. The gel is scored in the binary form 1 for presence of band and 0 for absence of the band. The polymorphism in RAPD is either due to the variation in the annealing site of the primer or due to the length differences in the amplified sequence between primer annealing sites (Williams et al., 1990). RAPD molecular markers are easy to use and cost effective. These markers does not require laborious technology like RFLP. This marker is also universal and does not require highly purified DNA. SCAR and single nucleotide polymorphism (SNP) molecular markers are also produced by sequencing the RAPD products of interest. This system also yields high levels of polymorphism. These markers are randomly distributed throughout genome, so these have very high genomic abundance. Various variants of the RADP technology have been developed and used for the breeding, genotyping, diversity analysis, and genetic mapping like Arbitrarily Primed-PCR, DNA Amplification Fingerprinting, Multiple Arbitrary Amplicon Profiling (Kesawat and Das, 2009). The main drawback of RAPD molecular markers is that it is not highly reproducible and it does not detect the allelic variations in the heterozygotes (Jiang, 2013).

12.11 INTER SIMPLE SEQUENCE REPEATS (ISSR) MOLECULAR MARKER

ISSR is stretches of DNA that are present between repeat motifs in DNA. For this technique, PCR primers are designed to anneal the repeat motifs and amplify the intervening genomic regions. Level of polymorphism will decide the number of PCR products and the method is often referred as DNA fingerprinting (Zietkiewicz et al., 1994). ISSR molecular marker is used to amplify the DNA segments in between two identical microsatellite repeat regions oriented in opposite directions. The primers that are used in

ISSR contain the microsatellite repeats, which may be either dinucleotide, tri-nucleotide, tetra-nucleotide, or pentanucleotide. The primers used in the ISSR technique may be either unanchored (Gupta et al., 1994; Meyer et al., 1993) or anchored (Zietkiewicz et al., 1994). The ISSR primers are longer than the RAPD primers. Here the primer maybe 15–30 m, while as in RAPD it is only 10 m. The PCR amplified product vary in the size from 200 to 2000 bp and these bands can be detected either by agarose or polyacrylamide gel electrophoresis.

For the ISSR molecular marker, no sequence information is required for the primer synthesis. ISSR molecular marker technique is easy, cost effective, simple, quick, time effective, and does not require radioactivity. These molecular markers usually show higher polymorphism due to their random distribution throughout the genome. ISSR molecular marker has been used in clone and strain identification, genetic diversity analysis and in gene mapping studies (Gupta et al., 1994; Zietkiewicz et al., 1994). This marker system has the main drawback of reproducibility.

12.12 AFLP MOLECULAR MARKERS

AFLPs are the combination of both PCR-based markers and restriction-based marker. This molecular marker was developed by Vos et al. (1995). This molecular marker has been used for genetic diversity analysis. This technique helps in high resolution genotyping as it produces many bands, these may be more than hundred in number. AFLP molecular marker is economic, efficient, and reproducible and shows high resolution, due to these features this marker is considered superior over the other markers like RAPDS, ISSR, and RFLP. AFLP markers have broad application in systematics, genetic diversity analysis, population genetics, and QTL mapping.

The first step in the AFLP technique is restriction of the template DNA. The DNA is restricted simultaneously with two enzymes, one of the enzyme is a rare cutter and other is a frequent cutter. The rare cutter is 6-bp recognition site enzyme and frequent cutter is 4-bp recognition site enzyme. After restriction digestion, the restricted product is ligated with the specific adaptors (Jiang, 2013). The adaptor-ligated product is selectively amplified by the PCR, using the specific primers. The primers used in PCR consist of three main regions, the core sequence, which is a part of the adaptor, a restriction enzyme-specific sequence, and one to five selective nucleotides. The banding profile is due to variations in the restriction sites or in the

intervening regions. The PCR amplified products are usually polymorphic and may range from 50 to 100 fragments per reaction, these fragments are separated by polyacrylamide gel electrophoresis and generally scored as dominant markers (Vos *et al.*, 1995).

Selectively Amplified Microsatellite Polymorphic Locus is a variation of the AFLP, in SAMPLE technique microsatellite loci are amplified using a single AFLP primer in combination with a primer complementary to compound microsatellite sequences (Morgante and Vogel, 1994). Selectively Amplified Microsatellite Polymorphic Locus molecular marker has been used in intraspecific than inter-specific diversity analysis studies. AFLP has many advantages then other markers like AFLP is highly reproducible, has high genomic abundance, generates many informative bands per reaction, and does not require any prior sequence information for the primer designing.

AFLP also has certain disadvantages like it require purified high molecular weight DNA. As we know that this technique produces high number of bands so, certain strict but subjectively determined criteria for acceptance of bands in the analysis should be adopted. It should also be kept in mind that these bands are not always independent (Kesawat and Das, 2009). AFLP molecular markers have been used in biodiversity analysis, mapping, genotyping, diversity analysis, and transcript profiling (Jiang, 2013).

12.13 SSR MOLECULAR MARKERS

SSRs, Microsatellites, Short Tandem Repeats, and Simple Sequence Length Polymorphisms are found both in prokaryotes as well as eukaryotes. They are present throughout the genome, especially in the euchromatin of eukaryotes, they are also present in both noncoding and coding nuclear and organellar DNA (Perez-Jimenez *et al.*, 2013; Phumichai *et al.*, 2015).

Microsatellite markers are SSRs and usually one to six nucleotide repeats are present. These are highly polymorphic. SSR molecular markers are highly polymorphic and informative so has been used since ancient times for the diversity analysis in many crop plants. This molecular marker is codominant (Oliveira *et al.*, 2006, Mason, 2015). SSR molecular markers are both useful for wild as well as cultivated species. In many species that grow wildly, SSR help in the studies of diversity measured on the basis of genetic distance, most commonly they are used for the inter and intra-specific genetic relations, whereas as SSR molecular marker is used in linkage mapping, QTL analysis MAS and DNA fingerprinting in cultivated species (Kalia *et al.*, 2011). In plant species in which full-sub families are used for constructing

linkage maps SSRs have been used for generating integrated maps (Pereira *et al.*, 2013), SSR has also been used in sequence-based mapping (Temnykh *et al.*, 2001), it is also used in linking the phenotypic and genotypic variation (Hayward *et al.*, 2015).

12.14 SNP MOLECULAR MARKERS

SNP marker is a single nucleotide base difference between two DNA sequences or individuals. SNPs provide the maximum markers because these are simplest and reliable form of molecular markers and can generate a single nucleotide base change. SNPs are present both in animals and in plants. It is documented that after every 100–300 bp in plants, there occurs a SNP (Xu, 2010). It has been observed that there is no fixed position for the presence of SNP, it may be present anywhere in the genome. RFLP (SNPRFLP) is the most common and the conventional method used to detect the SNPs. CAPS molecular marker is also used as method for the detection of the SNPs. Technique, sequencing is used to detect the SNPs (Vignala *et al.*, 2002). In addition to above-mentioned methods, various genotyping assays like primer extension, oligonucleotide ligation, and allele-specific hybridization are also used for the detection of the SNPs (Sobrinho *et al.*, 2005).

SNPs are codominant markers. This marker has become the potential marker in the genetic study. It is often linked to genes. SNPs can detect polymorphism very efficiently as they can be detected very fast and can also be efficiently automated. So, it is documented that SNPs have been used for many purposes like in breeding study, genotyping, MAS, QTL mapping, etc. These markers also possess certain drawbacks, like these markers are not cost-effective and these require highly purified DNA.

12.15 SINGLE-STRAND CONFORMATION POLYMORPHISM (SSCP) MOLECULAR MARKER

SSCP is a quick and easy molecular marker technique, which is most widely used for the detection of DNA polymorphism and single base pair change mutation. This technique has been used for the identification of heterozygotes. The amplified product of SSCP may vary from 200 to 850bp. These fragments are visualized by the gel electrophoresis and the nucleotide sequence variations can be detected easily (Vignala *et al.*, 2002). The basic principle behind the SSCP technique is that the conformation of the molecule is responsible for the electrophoretic mobility of single-strand

DNA. So, this technique can easily tell us the sequence variation without the sequencing process (Orita et al., 1989). In this technique, the PCR product is denatured and then the electrophoresis of the denatured PCR product is done upon nondenaturing gel. SSCP is based upon two techniques namely, Denaturing Gradient Gel Electrophoresis in which a double-stranded DNA gets converted to single-stranded DNA.

SSCP is co-dominant marker and requires low quantities of template DNA (10–100 ng per reaction), however, the sequence data is required to design PCR primers. SSCP is a useful and most powerful technique for genotyping and also for mutation detection. This technique is also used in the molecular breeding study as well.

12.16 SEQUENCE TAGGED SITE (STS) MOLECULAR MARKER

STS marker was first developed by Olsen et al. (1989). These are defined as the short nearly about 200–500 bp DNA sequence in that is having single occurrence in the genome with known location and base sequence. Mostly, any of the clone containing the unique sequence can be sequenced and used as STS. In plants, STS can be amplified by using PCR primer taken either from RFLP or AFLP fragments. The conversion of the AFLP fragments is a bit tedious in polyploids like wheat but has been successfully used in many crops (Guo et al., 2003). In literature, some authors have also documented the use of RAPD as STS marker, but actually, these are SCARs rather than STSs. STS marker is codominant molecular marker. It has been used in many studies like genetic and the physical mapping, the detect the polymorphism, they can be used in genetic diversity analysis, these can also detect the micro-deletions between different samples, these markers are highly reproducible, easy to use, economic, and cost effective.

12.17 MITOCHONDRIAL MICROSATELLITES

Plant mtDNA is large as compared with animals. The size of this is in the range of 200 and 2500 kb. The DNA of mitochondria contains both intros and the repeated sequences (Liu et al., 2011). Plant mitochondria shows the molecular heterogeneity. The plant mitochondria is usually, circular chromosome differing in size and in its abundance. It shows slow rate of evolution and due to this it cannot be used as a marker of choice in the population genetics and breeding technique (Zhang and Hewitt, 2003).

Microsatellite markers are costly and RAPD markers are cost effective, but microsatellites detect high polymorphism than RAPD. So, both these have limitations and to overcome these limitations, Wu et al. (1994) have developed randomly amplified microsatellite polymorphisms markers. This molecular marker exhibits greater level of polymorphism. In this molecular marker, the DNA is amplified by using SSR primers either alone or in the presence of the RAPD molecular marker (Salazar et al., 2014; Wu et al., 1994). Randomly amplified microsatellite polymorphism molecular marker is less costly, shows high level of polymorphism and are widely used in the genotyping analysis and breeding technique.

12.18 SEQUENCE-RELATED AMPLIFIED POLYMORPHISM (SRAP) MOLECULAR MARKER

Li and Quiros developed the SRAP in 2001. This method was developed to amplify the open reading frames. Two primers are used in this technique. These primers are usually 17–18 bp. These primers usually use the CCGG sequence in the forward primer and AATT in the reverse primer. The annealing temperature also varies, during the initial five cycles the annealing temperature is set at 35°C and the remaining cycles are run at 50°C. The PCR products can be visualized on agarose gel through autoradiography. SRAP is a dominant marker. It is a simple and efficient marker for the construction of genetic maps, genomic, and cDNA fingerprinting (Salazar et al., 2014).

12.19 RETROTRANSPOSONS

Transposons or transposable elements are the mobile genetic elements present in the genome of an organism. These are capable of changing their locations in the genome. These were first discovered in maize (Finnegan, 1989). Retrotransposons constitute of about 40–60% of the entire plant genome and are repetitive DNA (Grzebelus, 2006). These are mostly of two main types, i.e., Class I and class II.

Class I is also known as the retro-elements and are short sequences, which are interspersed in the genome. These are capable of forming their new copy after the process of transposition (Finnegan, 1989). Kalendar et al. (2004) classified retrotransposons into two subclasses LTRs and non-LTR. In plants usually LTR retrotransposons are widely distributed, nearly 40–70% of DNA of most of the crops contain LTR retrotransposons (Shirasu et al., 2000;

Pearce et al., 1996; Voytas and Boeke, 2002). On the basis of encoded gene order, LTR retrotransposons are further divided into Ty3/gypsy retrotransposons and Ty1/copia retrotransposons (Roy et al., 2015)

In Class II, DNA transposons usually, cut and paste method in the genome is used for transposition (Grzebelus, 2006). TIR and non-TIR are the two types of Class II transposons (Schulman and Wicker, 2013). Transposons act as an efficient molecular marker detection due to their wide distribution and abundance in the genome (Kalendar et al., 2011). Some important retrotransposon-based molecular markers are IRAP (Kalendar et al., 1999; Kalendar and Schulman, 2006; Poczaï et al., 2013; Fan et al., 2014), REMAP (Kalendar et al., 1999; Roy et al., 2015), RBIP (Flavell et al., 1998; Fan et al., 2014; Agarwal et al., 2008), and interprimer binding site (iPBS) (Kalendar et al., 2010, 2011; Baloch et al., 2015).

12.20 CAPS

CAPS markers involves the combination of both RFLP and PCR, due to this fact these were also named as PCR–RFLP (Maeda et al., 1990). this process involves both PCR and digestion process. During this process, the DNA is first amplified using PCR and then this product is digested with restriction enzymes (Jarvis et al., 1994; Michaels and Amasino, 1998). The CAPS products can be visualized both on the agarose and polyacrylamide gel. These molecular markers are codominant and have been used in genotyping, mutation detection, mapping, etc. (Spaniolas et al., 2006; Weiland and Yu, 2003).

12.21 SEQUENCE-BASED MARKERS

The process by which the nucleotide bases and its order in the DNA is identified is known as the sequencing, sequence-based markers are those molecular markers that can detect the particular sequence of DNA in unknown DNA pool. The sequence-based markers are nowadays widely used due to the less reliability and polymorphism of the other markers. Sequencing technology has helped in the field of both crop improvement and in genotyping. In plant breeding, mostly next-generation sequencing (NGS) and genotyping by sequencing have been used in the plant breeding (Franca et al., 2002; Davey et al., 2011). Heather and Chain (2016) have reviewed many sequencing technologies developed so far. However, some of the important sequencing methods are discussed below.

The first known sequencing method was Sanger sequencing and is also known as the plus-and-minus method. This method was given by Sanger and Coulson (1975). This method was based upon the principle that variations of a single nucleotide can be detected with the help of PAGE. Sanger sequencing method is also named as Sanger's dideoxy sequencing method (Heather and Chain, 2016). The DNA polymerase I of bacteria *Escherichia coli* and the T4 DNA polymerase were used in this method (Englund, 1971, 1972). The resultant polymerization products were observed by ionophoresis, loaded onto acrylamide gels. Due to certain limitations of this method, this is not used and Sanger and his coworkers gave other method of sequencing based upon the sequencing by polymerization by enzymes. This method helped in the detection of variations, it usually requires low-quality DNA and gave highly reproducible results. Although this method has many benefits but it is very costly, high time consuming, and covers low genome (Franca et al., 2002). Later, this limitation of the Sanger sequencing was overcome by the pyrosequencing.

Pyrosequencing is another sequencing technique and is based upon synthesis (Sanger et al., 1977) in this method, which phosphate is detected by synthesis of DNA (Ronaghi, 2001). In this method, a single-stranded biotin-labelled DNA template combined with specific enzymes is hybridized with the sequencing primer (Gharizadeh et al., 2006). There are two types of pyrosequencing techniques; solid phase sequencing and liquid phase sequencing (Ronaghi et al., 1998).

NGS is the new sequencing method and is very important and useful technique at a low cost. This technique leads to the synthesis of millions of sequences (Shendure and Ji, 2008). Nowadays many companies have developed NGS and they help in sequencing huge samples. Some common organizations are Illumina MiSeq and HiSeq 2500 (Bentley et al., 2008), Roche 454 FLX Titanium (Thudi et al., 2012), and Ion Torrent PGM (Rothberg et al., 2011). NGSs is cost-effective and covers whole genome more precisely (Deschamps et al., 2012; Metzker, 2010).

12.22 SOME ADVANTAGES OF NGS

Common advantages of the NGS are as follows:

1. This method is not costly.
2. NGS has been used for the whole genome sequencing and for the detection of the high number of the SNPs, this has also been used for the diversity analysis (Beissinger et al., 2013).

3. It is used in linkage map construction and whole genome studies (Elshire et al., 2011).
4. It is also used in sequencing of older DNA samples.
5. This technique has also helped in the field of metagenomics (Mardis, 2008).
6. Sequencing is also used for the genotyping (Poland and Rife, 2012).

It has been reported in literature that now many sequence-based markers have been developed like SNP, CAPS SNP-RFLP, and diversity array technology (DArT Seq) (Jaccoud et al., 2001; Wenzl et al., 2004). It requires very little amount of DNA, which is about 50–100 ng.

Table 12.1 given below shows the various advantages and disadvantages of different genetic markers

TABLE 12.1 Advantages and disadvantages of different genetic markers.

S.N.	Markers	Advantages	Disadvantages
1	Morphological	This is cheap and easy to use. Characterized	It is influenced by the environmental factors and plant growth stage; this also detect low polymorphism.
2	Isozymes	Co-dominant marker and does not require any specific instrument. It is easy to use.	It is influenced by the environmental factors and this also detect low polymorphism.
3	RFLPs	This doesn't require sequence information and is Co-dominant.	It is time consuming and is expensive, it also requires high quantity of DNA.
4	RAPD	It is easy, rapid and requires low quantity of DNA. It is a dominant marker.	It has low reproducibility and requires purified DNA.
5	AFLP	It is a dominant marker, it is more informative, reliable and highly reproducible.	It requires highly purified DNA.
6	SSRs	It is a Co-dominant marker. It is highly reproducible and requires less quantity of DNA.	It is not cost effective. It shows presence of more null alleles and the occurrence of homoplasy
7	ISSR	It is easy, rapid and requires low quantity of DNA.	It requires pure DNA and are less reproducible.
8	SRAP	It is easy, cost effective and realible. Bands can be easily isolated.	Moderate–high throughput ratio.

TABLE 12.1 (Continued)

S.N.	Markers	Advantages	Disadvantages
9	Retrotransposons	This method does not require information of sequences and shows high polymorphism. It is highly reproducible.	Dominant marker.
10	SNP	Most widely distributed. It does not require prior sequence information.	It is a Co-dominant marker and has very high developmental cost.
11	DArT	Highly polymorphic and does not require prior sequence information it is highly reproducible.	Dominant marker and it has high developmental cost.

Molecular markers have been used in the plant sciences since ancient times. It has been used both for diversity analysis as well as for improvements. Many genetic maps have been created by using different types of the markers. Initially, certain morphological changes among the organisms and geographical changes were used for the study of evolution but now molecular markers have been used for this purpose. With the use of molecular markers, many new varieties of important crops have been developed. Molecular markers have also help in the study of phylogeny and evolution, it has also helped in study of genetic structure of a species. It depends upon the choice of the plant breeder, which type of molecular marker should be used for the breeding program. During the selection of the marker, breeders consider many factors into consideration (Semagn et al., 2006). The choice of the marker usually depends upon the reliability, cost, reproducibility, and many other conditions and available resources for breeding program.

12.23 DNA MOLECULAR MARKER AND GENOTYPING APPLICATIONS FOR MUTATION BREEDING

Genetic markers have been used as indicators in plant breeding since earlier times, but it was only after 1980s that abundant molecular markers became available for reliable selection of agronomically important traits in breeding programmes. After 1980s, molecular markers have been efficiently used in the field of plant breeding. Nowadays, many molecular markers have been developed, which have helped in the breeding program. Now many new sequences based molecular markers have also been developed. These sequence-based markers have revolutionized the breeding program, it has

made our work fast and easy. Since then, molecular marker has significantly increased the efficiency and speed of plant breeding.

In addition to breeding these new techniques also helps in pyramiding of genes, diversity, detection of mutation, QTL analysis, and MAS (Vignal et al., 2002). The reverse genetics techniques have provided new insights into the landscape of induced mutations in plants, enabling a more rational choice and dosage of the mutagenic agent (Vignal et al., 2002). With the help of the genetic marker the mutations at any of the agronomically important loci can be identified with high speed and accuracy (Bhat et al., 2005a, b, 2006b, 2007b, 2017; Gulfishan et al., 2012; Gulfishan et al., 2015; Bhat and Wani, 2017; Wani and Bhat, 2017). The mutant traits can be isolated with the help of molecular markers; thus, it helps in the MAS of mutant traits. Molecular markers can also help in the direct screening of the induced mutations in known target genes, this is very helpful in plant breeding programs as it helps in the selection of any of the mutant during the early stages of the breeding program. It helps significantly in the mutant selection and is helpful for those plants with long juvenile stage (Vignal et al., 2002).

12.24 ADVANTAGES AND USE OF MOLECULAR TECHNIQUES FOR PLANT MUTATION BREEDING

As we have discussed earlier that molecular markers have been most widely used for mutation breeding, gene pyramiding, QTL analysis, molecular mapping, genotyping, and diversity analysis (Staub and Gupta, 1996). These molecular markers have many advantages than the morphological markers. Some of the important advantages of molecular markers are. Increased reliability, increased efficiency, reduced cost, and uniqueness. Thus, multiple genes or alleles can be screened simultaneously by the use of molecular markers or genotyping assays. It also helps in the pyramiding of the genes. The molecular markers have revolutionized the field of diversity analysis and breeding program. Molecular markers have been efficiently used for crop improvement. It is also cost-effective method, therefore is used nowadays for improvement (Parveen et al., 2006; Bhat et al., 2006a, c, 2007a, b, c; Khan et al., 2005a, b). Nowadays, many new sequencing methods have been developed for the mutation breeding and sequencing, by using these methods many thousands of samples can be analyzed in very short period of time (Parveen et al., 2007; Sharma et al., 2009; Gulfishan et al., 2010;

Bhat et al., 2007d; Gulfishan et al., 2011). The direct sequencing method is the most efficient and popular method for the development of new marker approaches that can help in the plant breeding.

Direct sequencing has thus emerged as new and precise tools for both MAS and mutation detection (Spencer-Lopes et al., 2018). One can use the sequencing and the DNA marker techniques at different stages to facilitate some of the investigations or accelerate specific steps in the mutation breeding programme. The molecular marker shows complete linkage with the mutant allele as compared with functional marker (Andersen and Lubberstedt, 2003). With respect to plant mutation breeding, two important applications of DNA markers and high-throughput DNA sequencing are:

1. Both these processes have helped in the genotypic screening at the early stages of a mutation breeding program as, both these can help in the detection of mutations in target genes of known sequence that control important traits.
2. It also helps in the gene introgressing or pyramiding important mutant traits.

Additional general applications of DNA markers is that it reduces the undesirable mutations and also these markers help to maintain the elite genetic background.

12.25 MARKER-ASSISTED BACKCROSSING

MAS also known as marker-aided selection is a technique in which the trait is selected on the basis of the marker. It is also known as an indirect selection process. The marker in the MAS can be biochemical marker, DNA marker or morphological marker, which can be linked to any useful trait like growth, yield, resistance to both biotic and abiotic stress etc. Thus we can say it helps in the selection of those plants that carry the useful trait. Both genetic mappings and the molecular markers have helped in the MAS of agronomical trait loci. This technology has been most widely used for the breeding and helps in the gene pyramiding of the important agronomic trait (Das et al., 2017). Figure 1 exhibits the important step in MAS. New traits can be selected from the donor plants using tightly linked markers, linked to the gene of interest. This type of selection is called “foreground selection,” while in “background selection” markers are used to select against other DNA from donor.

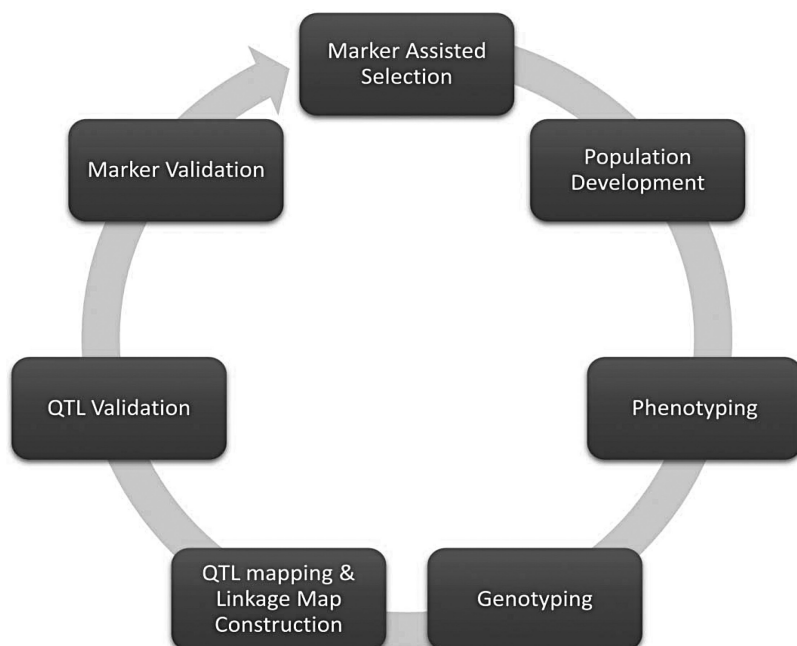


FIGURE 12.1 Some important steps involves in MAS.

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12.26 GENOTYPIC SELECTION

Selection of the mutant phenotype is the main step in the mutation breeding. This selection is not easy but it is time-consuming and requires the extensive resources. The desirable mutants can be selected by the phenotypic selection and afterward they are improved. The phenotypic selection of the mutant can also be done by the molecular screening for induced mutations in known target genes. Nowadays, the NGS platforms have also helped in the high-throughput discovery of induced mutations in plant genomes (Du et al., 2014; Li et al., 2016; Gupta et al., 2017; Datta et al., 2018), and can also help in the selection of the mutant. But to know the variation of any of the gene or locus the knowledge of the DNA sequences is required. Whole genome sequencing can be used to improve the DNA-based molecular marker (Abe et al., 2012; Schneeberger et al., 2009).

Similarly, the mutants can be identified and created by the reverse genetic methods. For detection of the mutation of large gene pools reverse genetics is used. In this method, both chemical mutagenesis and high throughput

sequencing of DNA fragments amplified through PCR are combined (Krasi-leva et al., 2017). So, by this method in a short period of time many of mutant populations comprising thousands of individual plants can be effectively screened for natural or induced sequence variations in a short time. For the process of the reverse genetics, the chemical mutagens have been most commonly used as reverse genetics can induce the single point mutations and these can be easily detected by the new techniques (Jankowicz-Cieslak and Till, 2015). Physical mutagenesis creates many lesions like SNPs, small and large insertions and deletions and genomic rearrangements, etc. so they are not used in the process of the reverse genetics (Datta et al., 2018).

Following are the three main factors that have been widely showing genotyping as the choice method for mutation detection:

1. The sequence data of large number of plants and crops species is available.
2. Use of the reverse genetic studies.
3. Cost effectiveness of the genotyping assays.

All these methods help to know the variation produced due to the mutations, and these also provide the sequences of target genes. When one wants to select a process for the mutant selection, one should consider the following factors like genotypic screen, practical concerns, and comparative cost-benefit analysis with direct (phenotypic) selection

12.27 CONCLUSION

In the present review, detailed study about various markers have been presented. It has also been documented that nowadays, many for genotyping, DNA fingerprinting, mutant selection, study of diversity, identification of a genotype many new strategies, and the techniques have been developed. It has been also documented that among different markers the molecular markers show high polymorphsim, are reproducible, can occur frequently in the genomes, are cost effective, fast, easy to score, and easy to assess. Most of the molecular markers show the codominant inheritance. But most of the molecular markers have one or the other limitations. It is further reported that no such molecular markers are available yet that fulfill all these requirements, so careful selection of molecular marker is required. A marker should be selected as such which can contain at least maximum desirable properties. So, RFLP, SSR, AFLP, and ISSR are some of the important markers that can be used for a wide range of applications in plants.

KEYWORDS

- **AFLP**
- **DArT**
- **ISSR**
- **polymerase chain reaction (PCR)**
- **RAPD**
- **RFLP**
- **SNP**
- **SSR molecular marker**
- **MAS**
- **functional marker**
- **genomic selection**

REFERENCES

- Abe, A.; Kosugi, S.; Yoshida, K.; Natsume, S.; Takagi, H.; Kanzaki, H.; Matsumura, H.; Mitsuoka, C.; Tamiru, M.; Innan, H.; Cano, L.; Kamoun, S.; Terauchi, R. Genome Sequencing Reveals Agronomically Important Loci in Rice Using Mut Map. *Nat. Biotechnol.* **2012**, *30*, 174–178.
- Agarwal, M.; Shrivastava, N.; Padh, H. Advances in Molecular Marker Techniques and Their Applications in Plant Sciences. *Plant Cell Rep.* **2008**, *27*, 617–631.
- Andersen, J. R.; Lubberstedt, T. Functional Markers in Plants. *Trends Plant Sci.* **2003**, *8*, 554–560.
- Baloch, F. S.; Alsaleh, A.; de Miera, L. E. DNA Based iPBS Retrotransposon Markers for Investigating the Population Structure of Pea (*Pisum sativum*) Germplasm from Turkey. *Biochem. Syst. Ecol.* **2015**, *61*, 244–252.
- Beissinger, T. M.; Hirsch, C. N.; Sekhon, R. S. et al., Marker Density and Read Depth for Genotyping Populations Using Genotyping-by-Sequencing. *Genetics* **2013**, *193*, 1073–1081.
- Bentley, D. R.; Balasubramanian, S.; Swerdlow, H. P. et al., Accurate Whole Human Genome Sequencing Using Reversible Terminator Chemistry. *Nature* **2008**, *456*, 53–59.
- Bhat, T. A.; Gulfishan, M.; Wani, A. A. Cytomixis: Causes and Consequences as a Case Study in *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A.; Wani, A. A., Eds.; Springer: Germany, 2017.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Comparative Analysis of Meiotic Abnormalities Induced by Gamma Rays, EMS and MMS in *Vicia faba* L. *J. Ind. Bot. Soc.* **2005a**, *84*, 45–48.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Ganai, F. A. Clastogenic Effects of EMS and MMS in *Vicia faba* L. *J. Cyto. Genet.* **2005b**, *6*, 117–122.

- Bhat, T. A.; Khan, A. H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by Gamma Rays and EMS in *Vicia faba* L. *Vegetos* **2005c**, *18* (1&2), 143–145.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Effect of Gamma Rays on Certain Cytomorphological Parameters in Two Varieties of *Vicia faba* L. *Adv. Plant Sci.* **2006a**, *19* (1), 227–232.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Wani, N. A. Radiation Induced Pod and Seed Mutants and MMS Induced Closed Flower Mutant in Broad Bean (*Vicia faba* L.). *Indian J. Genet.* **2006b**, *66* (3), 249–250.
- Bhat, T. A.; Parveen, S.; Khan, A. H. MMS-Induced Cytomixis in Pollen Mother Cells of Broad Bean (*Vicia faba* L.). *Turk. J. Bot.* **2006c**, *30*, 273–279.
- Bhat, T. A.; Khan, A. H.; Parveen, S.; Ganai, F. A. Studies on the Effect of EMS on Meiosis and Pollen Fertility in *Vicia faba* L. *Adv. Plant Sci.* **2006d**, *19* (1), 243–247.
- Bhat, T. A.; Khan, A. H.; Parveen, S. Spectrum and Frequency of Chlorophyll Mutations Induced by MMS, Gamma Rays and Their Combination in Two Varieties of *Vicia faba* L. *Asian J. Plant Sci.* **2007a**, *6* (3), 558–561.
- Bhat, T. A.; Parveen, S.; Khan, A. H. Meiotic Studies in Two Varieties of *Vicia faba* L. (fabaceae) After MMS Treatment. *Asian J. Plant Sci.* **2007b**, *61* (1), 51–55.
- Bhat, T. A.; Sharma, M.; Anis, M. Comparative Analysis of Mitotic Aberrations Induced by Diethyl Sulphide (DES) and Sodium Azide (SA) in *Vicia faba* L. (Fabaceae). *Pak. J. Biol. Sci.* **2007c**, *10* (5), 783–787.
- Bhat, T. A.; Sharma, M.; Anis, M. Comparative Analysis of Meiotic Aberrations Induced by Diethylsulphate and Sodium Azide in Broad Bean (*Vicia faba* L.). *Asian J. Plant Sci.* **2007d**, *6* (7), 1051–1055.
- Bhat, T. A.; Wani, A. A. Mutagenic Effects on Meiosis in Legumes and a Practical Case Study of *Vicia faba* L. In *Chromosome Structure and Aberrations*; Bhat, T. A.; Wani, A. A., Eds.; Springer: Germany, 2017.
- Botstein, D.; White, R. L.; Skolnick, M.; Davis, R. W. Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms. *Am. J. Hum. Genet.* **1980**, *32*, 314–331.
- Das, G.; Patra, J. K.; Baek, K. H. Corrigendum: Insight Into MAS: A Molecular Tool for Development of Stress Resistant and Quality of Rice Through Gene Stacking. *Front. Plant Sci.* **2017**, *8*, 1321.
- Datta, S.; Jankowicz-Cieslak, J.; Nielen, S.; Ingelbrecht, I.; Till, B. J. Induction and Recovery of Copy Number Variation in Banana Through Gamma Irradiation and Low Coverage Whole Genome Sequencing. *Plant Biotechnol. J.* **2018**. <https://doi.org/10.1111/pbi.12901>
- Davey, J. W.; Hohenlohe, P. A.; Etter, P. D. et al., Genome-Wide Genetic Marker Discovery and Genotyping Using Next Generation Sequencing. *Nat. Rev. Genet.* **2011**, *12*, 499–510.
- Deschamps, S.; Llaca, V.; May, G. D. Genotyping-by-Sequencing in Plants. *Biology* **2012**, *1*, 460–483.
- Desplanque, B.; Boudry, P.; Broomberg, K.; Saumitou-Laprade, P.; Cuguen, J.; van Dijk, H. Genetic Diversity and Gene Flow Between Wild, Cultivated and Weedy Forms of *Beta vulgaris* L. (Chenopodiaceae), Assessed by RFLP and Microsatellite Markers. *Theor. Appl. Genet.* **1999**, *98*, 1194–1201.
- Du, Y.; Li, W.; Yu, L.; Chen, G.; Liu, Q.; Luo, S.; Shu, Q.; Zhou, L. Mutagenic Effects of Carbon-Ion Irradiation on Dry *Arabidopsis thaliana* Seeds. *Mutat. Res.* **2014**, *759*, 28–36.
- Eagles, H. A.; Bariana, H. S.; Ogonnaya, F. C.; Rebetzke, G. J.; Hollamby, G. J.; Henry, R. J.; Henschke, P. H.; Carter, M. Implementation of Markers in Australian Wheat Breeding. *Crop Pasture Sci.* **2001**, *52*, 1349–1356.

- Elshire, R. J.; Glaubitz, J. C.; Sun, Q. et al., A Robust, Simple Genotyping-By-Sequencing (GBS) Approach for High Diversity Species. *PLoS One* **2011**, *6*, 19379.
- Englund, P. T. Analysis of Nucleotide Sequences at 3' termini of Duplex Deoxyribonucleic Acid with the Use of the T4 Deoxyribonucleic Acid Polymerase. *J. Biol. Chem.* **1971**, *246*, 3269–3276.
- Englund, P. T. The 30-Terminal Nucleotide Sequences of T7 DNA. *J. Mol. Biol.* **1972**, *66*, 209–224.
- Fan, F.; Cui, B.; Zhang, T.; Ding, G.; Wen, X. LTR-Retrotransposon Activation, IRAP Marker Development and Its Potential in Genetic Diversity Assessment of Masson Pine (*Pinus massoniana*). *Tree Genet. Genomes*. **2014**, *10*, 213–222.
- Finnegan, D. J. Eukaryotic Transposable Elements and Genome Evolution. *Trends Genet.* **1989**, *5*, 103–107.
- Flavell, A. J.; Knox, M. R.; Pearce, S. R. Retrotransposonbased Insertion Polymorphisms (RBIP) for High Throughput Marker Analysis. *Plant J.* **1998**, *16*, 643–650.
- Franca, L. T.; Carrilho, E.; Kist, T. B. A Review of DNA Sequencing Techniques. *Q. Rev. Biophys.* **2002**, *35*, 169–200.
- Ganai, F. A.; Khan, A. H.; Bhat, T. A.; Parveen, S.; Wani, N. A. Cytogenetic Effects of Methyl Methane Sulphonate (MMS) on Varieties of Chickpea (*Cicer arietinum* L.). *J. Cytol. Genet.* **2005**, *6*, 97–102.
- Gharizadeh, B.; Herman, Z. S.; Eason, R. G. et al., Large-Scale Pyrosequencing of Synthetic DNA: A Comparison with Results from Sanger Dideoxy Sequencing. *Electrophoresis* **2006**, *27*, 3042–3047.
- Grzebelus, D. Transposon Insertion Polymorphism as a New Source of Molecular Markers. *J. Fruit Ornament. Plant Res.* **2006**, *14*, 21–29.
- Gulfishan, M.; Bhat, T. A.; Oves, M. Mutants as a Genetic Resource for Future Crop Improvement. In *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools*; Al-Khayri, J. M. et al., Eds.; Springer International Publishing: Switzerland, 2015.
- Gulfishan, M.; Jafri, I. F.; Khan, A. H.; Bhat, T. A. Methyl Methane Sulphonate Induced Desynaptic Mutants in *Capsicum annum* L. *Chrom. Bot.* **2013**, *8*, 59–62.
- Gulfishan, M.; Khan, A. H.; Gefferi, I. F.; Bhat, T. A. Assessment of Mutagenicity Induced by MMS and DES in *Capsicum annum* L. *Saudi J. Biol. Sci.* **2012**, *19*, 251–255.
- Gulfishan, M.; Khan, A. H.; Bhat, T. A. Studies on Cytotoxicity Induced by DES and SA in *Vicia faba* L. var. Major. *Turk. J. Bot.* **2010**, *34*, 31–37.
- Gulfishan, M.; Khan, A. H.; Haneef, I.; Bhat, T. A. Genotoxic Effect of Diethyl Sulphate in Two Varieties of *Capsicum annum* L. *Nucleus* **2011**, *54* (2), 107–111.
- Guo, P. G.; Bai, G. H.; Shaner, G. E. AFLP and STS Tagging of a Major QTL for Fusarium Head Blight Resistance in Wheat. *Theor. Appl. Genet.* **2003**, *106*, 1011–1017.
- Gupta, P.; Reddaiah, B.; Salava, H.; Upadhyaya, P.; Tyagi, K.; Sarma, S.; Datta, S.; Malhotra, B.; Thomas, S.; Sunkum, A.; Devulapalli, S.; Till, B. J.; Sreelakshmi, Y.; Sharma, R. Next-Generation Sequencing (NGS)-Based Identification of Induced Mutations in a Doubly Mutagenized Tomato (*Solanum lycopersicum*) Population. *Plant J. For Cell Mol. Bio.* **2017**, *92*, 495–508.
- Gupta, P. K.; Varshney, R. K. Cereal Genomics: An Overview In *Cereal Genomics*; Gupta, P. K., Varshney, R. K., Eds.; Kluwer Academic Press: Dordrecht, The Netherlands, 2004; p 639.
- Heather, J. M.; Chain, B. The Sequence of Sequencers: The History of Sequencing DNA. *Genomics* **2016**, *107*, 1–8.
- Hyman, E. D. A New Method of Sequencing DNA. *Anal Biochem.* **1988**, *174*, 423–436.

- Jaccoud, D.; Peng, K.; Feinstein, D. et al., Diversity Arrays: A Solid State Technology for Sequence Information Independent Genotyping. *Nucl. Acids Res.* **2001**, *29*, E25.
- Jarvis, P.; Lister, C.; Szabo, V. Integration of CAPS Markers Into the RFLP Map Generated Using Recombinant Inbred Lines of *Arabidopsis thaliana*. *Plant Mol. Biol.* **1994**, *24*, 685–687.
- Jiang, G. L. Molecular Markers and Marker-Assisted Breeding in Plants. In *Plant Breeding from Laboratories to Fields*; Andersen, S. B., Ed.; InTech: Rijeka, 2013; pp 45–83.
- Jing, R.; Bolshakov, V.; Flavell, A. J. The Tagged Microarray Marker (TAM) Method for High-Throughput Detection of Single Nucleotide and Indel Polymorphisms. *Nat. Protoc.* **2007**, *2*, 168–177.
- Kalendar, R.; Antonius, K.; Smykal, P.; Schulman, A. H. iPBS: A Universal Method for DNA Fingerprinting and Retrotransposon Isolation. *Theor Appl Genet.* **2010**, *121*, 1419–1430.
- Kalendar, R.; Flavell, A. J.; Ellis, T. H. N.; Sjakste, T.; Moisy, C.; Schulman, A. H. Analysis of Plant Diversity with Retrotransposon-Based Molecular Markers. *Heredity* **2011**, *106*, 520–530.
- Kalendar, R.; Grob, T.; Regina, M.; Suoniemi, A.; Schulman, A. IRAP and REMAP: Two New Retrotransposon-Based DNA Fingerprinting Techniques. *Theor Appl Genet.* **1999**, *98*, 704–711.
- Kalendar, R.; Schulman, A. H. IRAP and REMAP for Retrotransposon- Based Genotyping and Fingerprinting. *Nat. Protoc.* **2006**, *1*, 2478–2484.
- Kalendar, R.; Vicient, C. M.; Peleg, O.; Ananthawat-Jonsson, K.; Kalendar, R.; Vicient, C. M.; Peleg, O.; Bolshoy, A.; Schulman, A. H. Large Retrotransposon Derivatives: Abundant, Conserved But Nonautonomous Retroelements of Barley and Related Genomes. *Genetics.* **2004**, *166*, 1437–1450.
- Kalia, R. K.; Rai, M. K.; Kalia, S.; Singh, R.; Dhawan, A. K. Microsatellite Markers: An Overview of the Recent Progress in Plants. *Euphytica* **2011**, *177*, 309–334.
- Kesawat, M. S.; Das, B. S. Molecular Markers: It's Application in Crop Improvement. *J. Crop Sci. Biotech.* **2009**, *12*, 168–178.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. DES Induced Variations for Economic Traits in Broad Bean (*Vicia faba* L.). *Biosci. Biotechnol. Res. Asia* **2005b**, *3* (2), 363–366.
- Khan, A. A.; Ansari, M. Y. K.; Bhat, T. A.; Shahab, D. Clastogenic effects of 8-hydroxy Quinoline in *Vicia faba* L. *Adv. plant sciences.* **2007a**, *20* (1), 85–87. ISSN 0970–3586.
- Khan, A. A.; Ansari, M. Y. K.; Shahab, D.; Bhat, T. A. Induced Variations for Quantitative Traits in *Vicia faba* L. *Vegetos* **2005a**, *18* (1&2), 83–86.
- Khan, A. A.; Ansari, M. Y. K.; Shahab, D.; Bhat, T. A. Comparative Analysis of Meiotic Abnormalities Induced by Caffeine and DES in *Vicia faba* L. *Adv. Plant Sci.* **2007b**, *20* (1), 73–76.
- Krasileva, K. V.; Vasquez-Gross, H. A.; Howell, T.; Bailey, P.; Paraiso, F.; Clissold, L.; Simmonds, J.; Ramirez-Gonzalez, R. H.; Wang, X.; Borrill, P.; Fosker, C.; Ayling, S.; Phillips, A. L.; Uauy, C.; Dubcovsky, J. Uncovering Hidden Variation in Polyploidy Wheat. *Proc. Nat. Acad. Sci.* **2017**, *114*, E913–E921.
- Kumar, A.; Bennetzen, J. L. Plant Retrotransposons. *Annu. Rev. Genet.* **1999**, *33*, 479–532.
- Li, G.; Quiros, C. F. Sequence-Related Amplified Polymorphism (SRAP), a New Marker System Based on a Simple PCR Reaction: Its Application to Mapping and Gene Tagging in Brassica. *Theor. Appl. Genet.* **2001**, *103*, 455–461.
- Li, S.; Zheng, Y.; Cui, H.; Fu, H.; Shu, Q.; Huang, J. Frequency and Type of Inheritable Mutations Induced by γ Rays in Rice as Revealed by Whole Genome Sequencing. *J. Zhejiang Univ. Sci. B.* **2016**, *17*, 905–915.

- Liu, Y.; Xue, J. Y.; Wang, B.; Li, L.; Qiu, Y. L. The Mitochondrial Genomes of the Early Land Plants *Treubia lacunosa* and *Anomodon rugelii*: Dynamic and Conservative Evolution. *PLoS One*. **2011**, *6*, 25836.
- Maeda, M.; Uryu, N.; Murayama, N.; Ishii, H.; Ota, M. T.; Suji, K.; Inoko, H. A Simple and Rapid Method for HLA-DP Genotyping by Digestion of PCR Amplified DNA with Allele-Specific Restriction Endonucleases. *Hum. Immunol.* **1990**, *27*, 111–121.
- Mardis, E. R. Next-Generation DNA Sequencing Methods. *Annu Rev Genom. Hum. Genet.* **2008**, *9*, 387–402.
- Markert, C. L.; Moller, F. Multiple Forms of Enzymes: Tissue, Ontogenetic, and Species Specific Patterns. *Proc. Natl. Acad. Sci. USA* **1959**, *45*, 753–763.
- Mason, A. S. SSR Genotyping. In *Plant Genotyping*; Batley, J., Ed.; Springer: New York, 2015; pp 77–89.
- Metzker, M. L. Sequencing Technologies-the Next Generation. *Nat. Rev. Genet.* **2010**, *11*, 31–46.
- Michaels, S. D.; Amasino, R. M. A Robust Method for Detecting Single-Nucleotide Changes as Polymorphic Markers by PCR. *Plant J.* **1998**, *14*, 381–385.
- Miller, J. C.; Tanksley, S. D. RFLP Analysis of Phylogenetic Relationships and Genetic Variation in the Genus *Lycopersicon*. *Theor. Appl. Genet.* **1990**, *80*, 437–448.
- Morgante, M.; Vogel, J. Compound Microsatellite Primers for the Detection of Genetic Polymorphisms. *US Patent Application No.* **1994**, *8*, 326–456.
- Mukesh, K.; Veena, C.; Ujjwal, S.; Manoj, K. S.; Sunil, M.; Naresh, R. K. Biochemical and Molecular Markers for Characterization of Chrysanthemum Germplasm: A Review. *J. Pharmacogn. Phytochem.* **2018**, *7*, 2641–2652.
- Mullis, K. B. The Unusual Origin of the Polymerase Chain Reaction. *Scientific J.* 1990, *262*, 56–65.
- Nadeem, M. A.; Nawaz, M. A.; Shahid, M. Q.; Dogan, Y.; Comertpay, G.; Yıldız, M.; Hatipoğlu, R.; Ahmad, F.; Alsaleh, A.; Labhane, N.; Özkan, H.; Chung, G.; Baloch, F. S. DNA Molecular Markers in Plant Breeding: Current Status and Recent Advancements in Genomic Selection and Genome Editing. *Biotechnol. Biotechnol. Equip.* **2018**, *32*, 261–285.
- Oliveira, E. J.; Pádua, J. G.; Zucchi, M. I.; Vencovsky, R.; Vieira, M. L. C. Origin, Evolution and Genome Distribution of Microsatellites. *Genet. Mol. Biol.* **2006**, *29*, 294–307.
- Olsen, M.; Hood, L.; Cantor, C.; Botstein, D. A Common Language for Physical Mapping of the Human Genome. *Science* **1989**, *245*, 1434–1435.
- Orita, M.; Suzuki, Y.; Sekiya, T.; Hayashi, K. Rapid and Sensitive Detection of Point Mutations and DNA Polymorphisms Using Polymerase Chain Reaction. *Genomics* **1989**, *5*, 874–879.
- Parveen, S.; Anis, M.; Bhat, T. A.; Khan, A. H. Cytological Effects of Physical and Chemical Mutagens in *Trigonella foenum-graecum* L. *Adv. Plant Sci.* **2007**, *20* (11), 633–636.
- Parveen, S.; Anis, M.; Bhat, T. A.; Khan, A. H. Efficiency and Effectiveness of Physical and Chemical Mutagens in M2 Generation of *Trigonella foenum-graecum* L. *J. Cytol. Genet.* **2006**, *7*, 1–4.
- Pearce, S. R.; Pich, U.; Harrison, G.; Flavell, A. J.; Heslop-Harrison, J. S.; Schubert, I.; Kumar, A.; TheTy1-Copia Group Retrotransposons of *Allium Cepa* Are Distributed Throughout the Chromosomes But Are Enriched in the Terminal Heterochromatin. *Chrom. Res.* **1996**, *4*, 357–364.
- Pereira, G. S.; Nunes, E. S.; Laperuta, L. D. C.; Braga, M. F.; Penha, H. A.; Diniz, A. L.; Munhoz, C. F.; Gazaffi, R.; Garcia, A. A. F.; Vieira, M. L. C. Molecular Polymorphism and Linkage Analysis in Sweet Passion Fruit, an Outcrossing Species: Molecular Map in Sweet Passion Fruit. *Ann. Appl. Biol.* **2013**, *162*, 347–361.

- Perez-Jimenez, M.; Besnard, G.; Dorado, G.; Hernandez, P. Varietal Tracing of Virgin Olive Oils Based on Plastid DNA Variation Profiling. *PLoS One*. **2013**, *8*, e70507.
- Phumichai, C.; Phumichai, T.; Wongkaew, A. Novel Chloroplast Microsatellite (cpSSR) Markers for Genetic Diversity Assessment of Cultivated and Wild *Hevea* Rubber. *Plant Mol. Biol. Report*. **2015**, *33*, 1486–1498.
- Poczai, P.; Varga, I.; Laos, M.; Cseh, A.; Bell, N.; Valkonen, J. P. T.; Hyvönen, J. Advances in Plant Genetargeted and Functional Markers: A Review. *Plant Methods*. **2013**, 9–6.
- Poland, J. A.; Rife, T. W. Genotyping-by-Sequencing for Plant Breeding and Genetics. *Plant Genome*. **2012**, *5*, 92–102.
- Ronaghi, M.; Pyrosequencing Sheds Light on DNA Sequencing. *Genome Res*. **2001**, *11*, 3–11.
- Ronaghi, M.; Uhl-en, M.; Nyren, P. A Sequencing Method Based on Real-Time Pyrophosphate. *Science* **1998**, *281*, 363–365.
- Rothberg, J. M.; Hinz, W.; Rearick, T. M. et al., An Integrated Semiconductor Device Enabling Non-Optical Genome Sequencing. *Nature*. **2011**, *475*, 348–352.
- Roy, N. S.; Choi, J. Y.; Lee, S. I.; Kim, N. S. Marker Utility of Transposable Elements for Plant Genetics, Breeding, and Ecology: A Review. *Genes Genom*. **2015**, *37*, 141–151.
- Saal, B.; Wricke, G. Clustering of Amplified Fragment Length Polymorphism Markers in a Linkage Map Of Rye. *Plant Breed*. **2002**, *121*, 117–123.
- Salazar, J. A.; Rasouli, M.; Moghaddam, R. F.; Zamani, Z.; Imani, A.; Martínez-Gómez, P. Low-Cost Strategies for Development of Molecular Markers Linked to Agronomic Traits in *Prunus*. *Agric Sci*. **2014**, *5*, 430–439.
- Sanger, F.; Coulson, A. R. A Rapid Method for Determining Sequences in DNA by Primed Synthesis with DNA Polymerase. *J. Mol. Biol*. **1975**, *94*, 441–448.
- Sanger, F.; Nicklen, S.; Coulson, A. R. DNA sequencing with Chain-Terminating Inhibitors. *Proc. Natl. Acad. Sci*. **1977**, *74*, 5463–5467.
- Schlotterer, C.; The Evolution of Molecular Markers-Just a Matter of Fashion? *Nat. Rev. Genet*. **2004**, *5*, 63–69.
- Schneeberger, K.; Ossowski, S.; Lanz, C.; Juul, T.; Petersen, A. H.; Nielsen, K. L.; Jorgensen, J. E.; Weigel, D.; Andersen, S. U. SHORE Map: Simultaneous Mapping and Mutation Identification by Deep Sequencing. *Nat. Methods*. **2009**, *6*, 550–551.
- Schulman, A. H.; Wicker, T. A Field Guide to Transposable Elements. In *Plant Transposons and Genome Dynamics in Evolution*; Fedoroff, N. V., Ed., Vol. 2; Wiley-Blackwell: Oxford, 2013; pp 15–40.
- Semagn, K.; Bjørnstad, A.; Ndjioudjop, M. N. An Overview of Molecular Marker Methods for Plants. *Afr. J. Biotechnol*. **2006**, *2540*, 25–68.
- Sharma, M.; Khan, A. H.; Bhat, T. A. Assessment of Mutagenicity of Individual and Combination Treatment of Gamma Rays and MMS in Broad Bean (*Vicia faba* L). *Cytologia* **2009**, *74* (2), 235–241.
- Shendure, J.; Ji, H.; Next-Generation DNA Sequencing. *Nat. Biotechnol*. **2008**, *26*, 1135–1145.
- Shirasu, K.; Schulman, A. H.; Lahaye, T.; Schulze-Lefert, P. A Contiguous 66- kb Barley DNA Sequence Provides Evidence for Reversible Genome Expansion. *Genome Res*. **2000**, *10*, 908–915.
- Sobrinho, B.; Briona, M.; Carracedoa, A. SNPs in Forensic Genetics: A Review on SNP Typing Methodologies. *Forensic Sci. Int*. **2005**, *154*, 181–194.
- Southern, E. M. Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis. *J. Mol. Biol*. **1975**, *98*, 503–517.

- Spaniolas, S.; May, S. T.; Bennett, M. J. Authentication of Coffee by Means of PCR-RFLP Analysis and Lab-on-a-Chip Capillary Electrophoresis. *J. Agric. Food Chem.* **2006**, *54*, 7466–7470.
- Spencer-Lopes, M. M.; Forster, B. P.; Jankuloski, L. Manual on Mutation Breeding Third Edition. Plant Breeding and Genetics Subprogramme Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture Organization of the United Nations. Rome, Italy, 2018; 301 pp.
- Staub, J. E.; Serquen, F. C.; Gupta, M. Genetic markers, Map Construction, and Their Application in Plant Breeding. *Hort. Sci.* **1996**, *31*, 729–741.
- Temnykh, S.; DeClerck, G.; Lukashova, A.; Lipovich, L.; Cartinhour, S.; McCouch, S.; Computational and Experimental Analysis of Microsatellites in Rice (*Oryza sativa* L.), Frequency, Length Variation, Transposon Associations, and Genetic Marker Potential. *Genome Res.* **2001**, *11*, 1441–52.
- Thudi, M.; Li, Y.; Jackson, S. A. et al. Current State-of-Art of Sequencing Technologies for Plant Genomics Research. *Brief Funct Genomics.* **2012**, *11*, 3–11.
- Vignala, A.; Milana, D. A Review on SNP and Other Types of Molecular Markers and Their Use in Animal Genetics. *Genet. Sel. Evol.* **2002**, *34*, 275–305.
- Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; Lee, T. V. D.; Hornes, M. et al., AFLP: A New Technique for DNA Fingerprinting. *Nucl. Acids Res.* **1995**, *23*, 4407–4414.
- Voytas, D. F.; Boeke, J. D. Ty1 and Ty5 of *Saccharomyces Cerevisiae*. In *Mobile DNA II American Society of Microbiology*; Craigie, R., Gellert, M., Lambowitz, A., Eds.; ASM Press: Washington, DC, 2002; pp 631–662.
- Wang, Y.; Shahid, M. Q.; Baloch, F. S. Phylogeographical Studies of Glycine Soja: Implicating the Refugium During the Quaternary Glacial Period and Large-Scale Expansion After the Last Glacial Maximum. *Turk. J. Agric. For.* **2016**, *40*, 825–838.
- Wani, N. A.; Bhat, T. A.; Khan, A. H.; Ansari, M. Y. K. Sodium Azide Induced Variability in Chickpea (*Cicer arietinum* L). *Vegetos* **2005**, *18* (1&2), 105–107.
- Wani, A. A.; Bhat, T. A. Asynapsis and Desynapsis in Plants. In *Chromosome Structure and Aberrations*; Bhat, T. A., Wani, A. A., Eds.; Springer: Germany, 2017.
- Weeden, N.; Wendel, J. Genetics of Plant Isozymes. In *Isozymes in Plant Biology*; Soltis, D., Soltis, P., Eds.; Dioscorides Press: Portland, OR, 1989; pp 46–72.
- Weiland, J. J.; Yu, M. H. A Cleaved Amplified Polymorphic Sequence (CAPS) Marker Associated with Root-Knot Nematode Resistance in Sugarbeet. *Crop Sci.* **2003**, *43*, 1814.
- Wenzl, P.; Carling, J.; Kudrna, D. et al., Diversity Arrays Technology (DArT) for Whole-Genome Profiling of Barley. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9915–9920.
- Williams, J. G. K.; Kubelik, A.; Livak, K. J.; Rafalski, J. A.; Tingey, S. V. DNA Polymorphisms Amplified by Arbitrary Primers Are Useful as Genetic Markers. *Nucl. Acids Res.* **1990**, *18*, 6531–6535.
- Wu, K. S.; Jones, R.; Danneberger, L.; Scolnik, P. A. Detection of Microsatellite Polymorphisms Without Cloning. *Nucl. Acids Res.* **1994**, *22*, 3257–3258.
- Zhang, D. X.; Hewitt, G. M. Nuclear DNA Analyses in Genetic Studies of Populations: Practice, Problems and Prospects. *Mol. Ecol.* **2003**, *12*, 563–584.
- Zietkiewicz, E.; Rafalski, A.; Labuda, D. Genome Fingerprinting by Simple Sequence Repeat (SSR)-Anchored Polymerase Chain Reaction Amplification. *Genomics.* **1994**, *20*, 176–183.

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