Amarjit S Tanda *Editor*

Molecular Advances in Insect Resistance of Field Crops

Modern and Applied Approaches

Springer

Molecular Advances in Insect Resistance of Field Crops

Amarjit S. Tanda
Editor

Molecular Advances in Insect Resistance of Field Crops

Modern and Applied Approaches

Springer

Editor
Amarjit S. Tanda
School of Horticulture
University of Western Sydney
Rosehill, NSW, Australia

ISBN 978-3-030-92151-4 ISBN 978-3-030-92152-1 (eBook)
https://doi.org/10.1007/978-3-030-92152-1

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Dedicated to my family
Mother Mrs Bhagwanti
Father S Tara Singh Tanda
My wife Mrs Paramjit Kaur Tanda
Geni Tanda daughter
Dr Gurminder Singh Bharee Son-in-Law
Mr Amargurjot Singh Tanda Son

Foreword

In the 1960s, there were large-scale concerns about the world's ability to feed itself. The "green revolution" technology resulted in major growth in food-grain yield. Between 1966 and 1990, the population of the densely populated low-income countries grew by 80%, but food production more than doubled. The advancement in biotechnology was the development of bioengineered, high-yielding cultivars of wheat and rice crops having multiple resistance to insects and diseases and crop production sustainability. Recent progress in functional genomics research and the genetic improvements of insect resistance in field crops is quite promising especially in cereals. The present tome "Molecular Advances in Insect Resistance of Field Crops-Modern and Applied Approaches," is of the latter kind. On this modern subject, Dr. Tanda has attempted to highlight the theme that plants have advanced in bewildering cluster of morphological and biochemical hindrances for security against insects and different herbivores. RNA interference (RNAi) could be used selectively to kill an insect pest species without adversely affecting nontarget species by targeting the genes essential for pest insect's growth, development, or reproduction. The mechanism of RNAi and steps involved in fruit host plant-induced RNAi for insect pest control followed by present status of RNAi-based insect pest control in fruit crops in which specific vital genes have been silenced and used in improving food security and livelihoods. The information on potential and

limitations of molecular markers to identify genes of interest and QTLs for marker-assisted development of crop cultivars with insect resistance will be beneficial for sustainable crop production. It is widely understood that the advancement of insect-resistant and safe cultivars of plants and progress in integrated pest management need a mind-boggling comprehension of insect host plant relationships. The chapter targets the strategies for RNA interference against different insect pests of fruit plants for enhanced fruit production. Double-stranded RNA (dsRNA) stability, dsRNA uptake mechanisms, dsRNA production cost, off-target effects, and RNAi resistance are discussed here to resolve practical field applications. It is vital to use the molecular markers in crop breeding and their potentials and limitations in insect resistance research. In order to overcome chemical defenses, many insects have developed a number of counteradaptations which function either before or after consumption of food. In the next chapter, an attempt has been made to describe glucosinolate-myrosinase based defenses and their role in specialist and generalist insect herbivores interactions. In current understanding of the plant defense mechanism and genetic resources, species-wise availability of genetic sources of aphid resistance in wheat and related species is summarized, including a review of aphid resistance gene identified in wheat and its progenitors in wheat research. The correlation of molecular profiles with biological features of whitefly populations pave the way for deciphering the underlying scrutiny in the whitefly species in different crop resistance and improvement.

Exploitation of plant R genes, lectin coding genes, ribosome-inactivating proteins, plant secondary metabolites, and RNAi can be of great importance in imparting resistance against turnip aphid. However, there are acceptance and biosafety issues related with the use of transgenics. Genetic engineering, wide hybridization, marker-assisted selection, gene pyramiding, RNAi, CRISPR-Cas system, proteomics, and metabolomics have been discussed for creating novel resistance against insect pest and for the development of insect natural enemies conferring beneficial traits in biotechnological interventions for creating novel resistance against major insect pests of rice chapter. The integration of genomics and proteomics with metabolomics could be a promising field of research for developing improved crops and providing sustainable biological elements for integrated pest management. With changing climatic conditions, developing resistant cultivars while taking care of changing populations of planthoppers and biotype development is a major challenge. Advances in next-generation sequencing, high-throughput genotyping, and genome editing technologies hold a great potential to tackle this challenge. Most of the genetically engineered (GE) cultivars are the main focus of today's biotechnological industries for sustainable agriculture. These crops that provide protection against insects and diseases are important tools that complement an integrated pest management technology (IPMT) strategy. Genetically engineered cultivars which are developed by gene editing biotechnology may provide a preventive defense against the insect pests and plant diseases, a suitable alternative crop system for blending in IPMT program, in the future agro industry. In the gene editing, CRISPR (Clustered consistently interspaced short palindromic rehashes) and the Cas9 (CRISPR-related quality) are a multifaceted tool in modern crop

improvement programs. The concept of CRISPR-CAS9 system and its application on insect genome has been utilized in plants and animals, including arthropods which are important in forestry, agriculture, horticulture, fisheries, and public health. A multi-omic approach is required to understand everything from genes to ecology. In, multi-omic approaches in insect pest interactions against resistance, current omics technologies, tools and methods of multi-omics, as well as approaches undertaken by researchers to comprehend host plant resistance, and their use in insect pest management technology. Recent approaches of miRNA-mediated genetic regulation offer a conceptual model depicting the mechanism of miRNA-mediated plant–insect interaction. The potential biotechnological applications of miRNA in insect resistance and crop improvement have been well depicted. Lastly, various approaches utilized by cultivars in response to insect herbivory with genomic, molecular procedures and exploring of molecular strategies to understand host plant–insect interaction in designing novel insect-resistant crop cultivars have been highlighted.

This is, indeed, an advance awesome book and an uncommon blend of pure and applied science presented in the broader human social context. I would like to congratulate Dr. Amarjit Tanda, the editor of this book, for bringing out a valuable collection of chapters concerning the most important aspects of molecular advances in insect resistance research. This book will serve as a vital reference tool of benefit to scientists, students, policymakers, and other researchers in academia and agro industry.

University of California
Davis, CA, USA

Gurdev S. Khush

Plant Breeding Genetics and Biotechnology
IRRI, Los Baños, Philippines

Preface

By the year 2050, US farmers will need to reach an impressive level of food production to help feed a growing world population. They will have to operate multifaceted businesses with stunning new biotechnology to enhance efficiency on farms. The world's population is expected to reach 9.1 billion people in 2050. Growers globally must enhance food production 70% to meet the needs of the larger population, according to a report from the Food and Agriculture Organization of the United Nations. However, present pest management practices, based chiefly on the intensive use of toxic farm chemicals, are unsuccessful to minimize the crop damages by insect pests, which still destroy an estimated one-fifth of the global agricultural production of important crops. Pesticidal interventions in the agroecosystem have created human health hazards, lowered environmental quality, and disrupted natural control of insect pests. Therefore, there is an urgent need to strengthen nonchemical approaches for reducing pest damages, which should be safe, economical, and durable. Modern agro bio-techniques have empowered us to reach the rising demand for food, feed, and fiber for the growing human population globally through improved production of major field crops. Insect-resistant cultivars represent one of the most environmentally benevolent, economically viable, and ecologically sustainable options for utilization in integrated pest management programs. Pioneer research work on insect plant resistance was carried out by Prof R. H. Painter and his faculty members in IPRI, Kansas State, in 1926. Later on, he published a book *Insect Resistance in Crop Plants*. He developed hundreds of insect-resistant cultivars of rice, wheat, maize, sorghum, cotton, sugarcane, and other crops which were adopted extensively for enhancing crop yield globally. Prof G. S. Khush and his colleagues working at IRRI, Los Banos, Philippines, developed insect pest- and disease-resistant rice cultivars which are grown worldwide boosting the rice productivity. In agro industry, the annual economic value of insect resistance genes implemented is more than US$2 billion as per recent estimates. In spite of breathtaking successes and substantial contributions in insect resistance in field crops, only a few books have been published using biotechnology.

Recent developments in molecular genomic technology, RNA interference, genetic diversity analysis, and high-throughput phenotyping techniques have empowered us to design breeding products in short time and efficiently as compared to conventional breeding methods. New crop breeding strategies involving genomic selection and accelerated breeding tools are being accepted globally. This new book, Molecular Advances in Insect Resistance of Field Crops: Modern and Applied Approaches, emphasizes the recent developments in host-plant resistance to insects, which have enhanced our capability to develop insect pest-resistant cultivars for improving crop quality along with agriculture sustainability. In the introductory chapter, the editor has attempted to highlight the theme that plants have advanced in bewildering cluster of morphological and biochemical hindrances for security against insects. The investigation of these interrelationships is of incredible importance for designing the future insect-resistant cultivars. RNA interference (RNAi) could be used selectively to kill insect pest without adversely affecting pollinators and predators by targeting the genes essential for pest insect's growth, development, or reproduction. The mechanism of RNAi and steps involved in fruit host plants-induced RNAi for insect pest control followed by present status of RNAi-based insect pest control in fruit crops in which specific vital genes have been silenced have been described in Chap. 2.

Marker-assisted selection has been found to be effective to select cultivars for insect resistance. The information on potential and limitations of molecular markers to identify genes of interest and QTLs for marker-assisted development of crop cultivars with insect resistance for food security have been elaborated in Chap. 3. Glucosinolate hydrolysis products may either directly protect the plant by having an effect on the insect biology or behavior, or indirectly by attracting the pest's predators. To overcome chemical defenses, many insects have developed many counter-adaptations which act either before or after consumption of food. In Chap. 4, these glucosinolate-myrosinase based defenses and their role in insect–plant interactions are reviewed in. Integration of resistant varieties into IPMT processes can be achieved only through interdisciplinary collaboration by plant breeders and entomologists involved in the development of insect-resistant cultivars using molecular genetic techniques. Chapter 5 enumerated to discuss the recently available molecular tools like QTL, marker-assisted selection, genetic engineering including transgenics, genes and proteins to explore in insect resistance of cereal crops. Plants respond to herbivores rapidly in response to damage by the insects by producing a variety of plant secondary traits to minimize the damage and colonization by the herbivores. The importance of constitutive and induced resistance for pest management and sustainable crop production is summarized in Chap. 6. Plant biotechnology is encircled by a multitude of scientific tools for screening and genetic manipulation of plants to develop and select new desired characteristics to insect resistance for crop improvement. In Chap. 7, genetic engineering, wide hybridization, marker-assisted selection, gene pyramiding, RNAi, CRISPR-Cas system, proteomics, and metabolomics have been debated for creating novel resistance against insect pest conferring beneficial traits. The integration of genomics and proteomics with metabolomics will enrich comprehension of the gene–function relationship

that can be utilized in achieving crop productivity. The proficiency of these methods could be a promising field of research for developing improved crops and providing sustainable biological elements for integrated pest management technology (IPMT) as we have proposed. Wild relatives like *Brassica fruticulosa*, *B. montana,* and *Rorippa indica* are reported to be resistant to aphid and have potential to be utilized in aphid breeding systems. However, conventional breeding methods are tedious and time consuming and several biotechnological tools can complement the conventional breeding procedures for developing aphid-resistant cultivars. Exploitation of plant R genes, lectin coding genes, ribosome-inactivating proteins, plant secondary metabolites, and RNAi can be of great importance in imparting resistance against turnip aphid. However, there are acceptance and biosafety issues related with the use of transgenics. The need to have a better understanding of the plant–aphid interactions to develop an aphid-resistant cultivar which is reliable, safe, and acceptable is illustrated in Chap. 8. With changing climatic conditions, developing resistant cultivars while taking care of changing populations of plant hoppers and biotype development is the major challenge in rice. However, advances in next-generation sequencing, high-throughput genotyping, and genome editing technologies hold a great potential to tackle this challenge. Future research priorities should concentrate on high-throughput screening of germplasm and utilization of genomic approaches for identifying and transferring novel genes of resistance to hoppers from different sources besides identifying durable combination of genes for marker-assisted pyramiding. Genome editing approaches, such as CRISPR/Cas9, used to identify novel alleles for resistance and developing cultivars resistant to hoppers for sustainable rice production are outlined in Chap. 9. Presently grown crop varieties have many disadvantages, such as losses in yield being susceptible to pests, overuse of pesticides, and pollution of soil, water, and environment. Chapter 10 describes that genetically engineered cultivars which are developed by gene editing molecular biotechnology may provide a preventive defense against the insect pests as a suitable alternative crop system for blending in IPMT program in the future agro industry. Generally, *T. boeoticum, Ae. tauschii,* and *T. araraticum* had the greater levels of antibiosis to BCOA, whereas *Ae. tauschii* and *T. turgidum* had the higher levels of overall resistance to GB, while *T. araraticum* and *T. dicoccoides* presented the higher levels of overall resistance to EGA. In Chap. 11, a summarized picture of species-wise availability of genetic sources of aphid resistance in wheat and related species is highlighted. Innovative molecular technology has been used in assortment of plant and animal species, including different insect pests in forestry, agriculture, horticulture, fisheries, and public health. The most recent exploration propels regarding novel CRISPR/Cas frameworks (CRISPR/Cpf1 and CRISPR/C2c2) and their possibilities for becoming significant innovation in insect needs are discussed in Chap. 12 in its effective application in the alteration of both insect and noninsect arthropod genomic approaches. Omics technology is beneficial in identifying novel chemical compounds with the potential to be resistant to insect pests. A multi-omics strategy is needed to comprehend from genes to ecological systems. A quick overview of the topic is narrated in Chap. 13, like current omics tools and procedures of multi-omics, as well as strategies used by different research scientists

to understand host plant resistance, its governing factors, and their utility in insect pest management technology. Recent approaches of miRNA-mediated genetic regulation offer a conceptual model depicting the mechanism of miRNA-mediated plant–insect interaction. The potential biotechnological applications of miRNA in insect resistance and crop improvement have been well illustrated in Chap. 14. In the last chapter (Chap. 15), various approaches utilized by cultivars in response to herbivory with genomic molecular procedures attempting insect resistance programs and in future exploring molecular strategies to comprehend host plant–insect interaction in designing novel insect-resistant crop cultivars have been narrated.

I am greatly thankful to all the contributors for the meticulous work they have done in compiling their great and magnificent chapters. Without their determined efforts, this book project would not have been possible. I would like to kindly thank Dr. Kenneth K. Teng, Publishing Editor, Life Sciences Springer, for providing support for this book. I also acknowledge all the reviewers who helped to improve the chapters. I would like to thank Ms. Kritheka Elango, Project Coordinator (Books), Springer Nature, and its editorial staff for timely completing the production process for this book.

I am indebted to Dr. Gurdev S. Khush, former Principal Plant Breeder and Head, Division of Plant Breeding Genetics and Biochemistry, International Rice Research Institute, Los Baños, The Philippines, and presently Adjunct Professor, University of California, Davis, USA, for showering his blessings on me by writing Foreword of this book.

I must make mention of my immediate family and friends, without whose involvement and support completion of this book would not have been possible. No one can do any creative work, such as the writing of a scientific book, without the cooperation of his/her spouse. My wife Paramjit showed utmost patience and cooperation during the preparation of this book. My son Amargurjot made sure that I was kept away from tedious household duties. My daughter Geni and son-in-law Dr. Gurminder frequently motivated me by checking up on the progress of the book.

Rosehill, NSW, Australia Amarjit S. Tanda
10 September, 2021

Contents

Editor and Contributors

About the Editor

Amarjit S. Tanda obtained BSc (Agri) (1974), MSc (1976), and PhD (1980) degrees in Entomology from Punjab Agricultural University, Ludhiana. He specializes in entpollinatology (or insect pollination), gnotobiology, insect pest and nematode management of crops, biological control, and integrated pest management technology (IPMT). He is a peer reviewer of **Springer Nature** books and referee journals of National and International repute. Dr. Tanda established role of honeybees in Asiatic cotton hybrid seed production. He proposed "Entpollinatology" for insect pollination. He designed new and simple technique for root exudates collection in vitro. He standardized the gnotobiotechnique for root culture screening and testing nematicides. He crafted insect damage indexing technique and investigated vacuuming and brushing methodology for IPMT. He has served at his alma mater for 15 years. He has worked at School of Horticulture, University of Western Sydney, Richmond, Australia, and CSIRO, North Ryde, NSW, Australia. He was awarded *Charted Biologist* degree *by the Institute of Biology*, *UK*, and Merit Scholarship, Fellowship for B.Sc., M.Sc., and Ph.D. He was presented with Guest Speaker Certification Award by Norwegian Plant Protection Institute, Ås, Norway. He was the recipient of Best Poem Award, Bhasa Vibhag Punjab, and PAU Best Poet Award. He was presented with Life Achievements Award, Punjabi

Association, Brisbane, Australia. He has published 9 books in literature and co-edited *Bibliography of Entomology*. He has published 135 research papers, 7 book reviews, 9 review articles, and 117 science articles. He was elected as a Fellow of The Linnean Society, London. He was an honorary member of the American Association of Professional Apiculturists, Australian Society of Plant Scientists, and Australian Native Bee Association Inc. He was elected as life fellow of Entomological Society of India and was a member of Insect Science Society of India, Ecological Society of India, and Plant Pathological Society of India.

Contributors

S. Aarthi Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

Ankita Babbar School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Suhas Sutar Bharat School of Applied Sciences, Centurion University of Technology and Management, Bhubaneswar, Odisha, India

Umesh Bharti Department of Zoology, Post Graduate Govt. College for Girls, Chandigarh, India

Dharminder Bhatia Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

Parveen Chhuneja School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Mukesh K. Dhillon Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Barkat Hussain Division of Entomology, SKUAST-K, Srinagar, Jammu and Kashmir, India

Raj Kumar Joshi Department of Biotechnology, Rama Devi Women's University, Bhubaneswar, Odisha, India

Somala Karthik Department of Entomology, PG College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Samastipur, Bihar, India

Maninder Kaur School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Pavneet Kaur School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Ravneet Kaur Department of Zoology, Post Graduate Govt. College for Girls, Chandigarh, India

Simranpreet Kaur Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India

Renu Khanna Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

Sarwan Kumar Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

Bijayalaxmi Mahanty Department of Biotechnology, Rama Devi Women's University, Bhubaneswar, Odisha, India

Pooja Manchanda School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Gurbachan S. Miglani School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Rukmini Mishra School of Applied Sciences, Centurion University of Technology and Management, Bhubaneswar, Odisha, India

Jatindra Nath Mohanty Centre for Biomedical Informatics, Siksha O Anusandhan University, Bhubaneswar, Odisha, India

Satyabrata Nanda MS Swaminathan School of Agriculture, Centurion University of Technology and Management, Bhubaneswar, Odisha, India

Deepika Narang School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Kumari Neelam School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Neha Panwar Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India

Battu Jabez Raju Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

M. Revathi Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

Morthala Shankara Sai Reddy Department of Entomology, PG College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Samastipur, Bihar, India

Preetinder Singh Sarao Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

T. Sathya Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India

V. Selvanarayanan Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

Hari C. Sharma International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Dr YS Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India

Beant Singh Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

Sandeep Singh Department of Fruit Science, Punjab Agricultural University, Ludhiana, Punjab, India

Gaurav Kumar Taggar Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

Amarjit S. Tanda School of Horticulture, University of Western Sydney, Richmond, NSW, Australia

Yogesh Vikal School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Abdul Rasheed War Crop Health Sciences, Jubilee Hills, Hyderabad, Telangana, India

Mohd Yousuf War Department of Botany, Degree College, Sopore, India

Gummudala Yashaswini Department of Entomology, PG College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Samastipur, Bihar, India

List of Figures

List of Tables

Abbreviations

aChE	Acetylcholinesterase
aChE-like-1	Acetylcholinesterase-like-1
aChE-like-2	Acetylcholinesterase-like-2
ago11	Argonaute1
al	Early
alpha COP	Alpha coatomer protein
aqp	Aquaporin water channel
Cas	CRISPR-associated (Cas) proteins
cathepsin D	cathepsin D
chE-2-like	Cholinesterase 2-like
chs	Chitin synthases
chs1	Chitin synthase1
cp	Cuticular protein
cp19	Cuticle protein 19
cpr	NADPH-cytochrome P450 reductase
CRISPR	Clustered regularly interspaced short palindromic repeats
csp	Candidate chemosensory protein
cxe1	Carboxylesterase gene
dsx	Doublesex
ecr	Ecdysone receptor
eG1a	Endoglucanase 1a
eG1b	Endoglucanase 1b
eG2	Endoglucanase 2
eG3	Endoglucanase 3
eG4	Endoglucanase 4
fbp1	Fat body protein1
gstd1	Glutathione *S*-transferase1
gste2	Glutathione *S*-transferase2
gus	Gustavus
hr3	Hormonal receptor 3
iap	Inhibitor of apoptosis
lola	Longitudinals lacking

magu	magu
na_v	Voltage-gated sodium channel
noa	Fatty acid elongase
nuc	Gut nuclease
obp21	Odorant binding protein21
pbp1	Pheromone binding protein gene
per	Period
rab11	Small GTPase
rac	rac
rh6	Opsin gene
rho	rho
rpl13	Ribosomal protein 13
rpl19	Ribosomal protein 19
SgRNA	Single guide RNA
shrb	Shrub
spr	Sex-peptide receptor
suc	Sucrase
suh	Sucrose hydrolase
TALEN	Transcription activator-like effector nucleases
tektin1	tektin1
topi	Matotopetli
tps	Trehalose-6-phosphate synthase
tra	Transformer
tra2	Transformer2
troponin C	Troponin C
trxt	Theoredoxin T
tssk1	Testis-specific serine/threonine kinases1
upd	Unpaired
v-ATPase D	Vacuolar-type ATPase subunit D
v-ATPase	Vacuolar-type ATPase
vg	Vitellogenin
vgR	Vitellogenin receptor
vha26	v-Type proton ATPase subunit E
wg	Wingless
yellow	Melanin synthesis gene
ZFN	Zinc finger nucleases

Chapter 1
Mutualistic Plant Associations Related to Insect Resistance

Amarjit S. Tanda

1.1 Introduction

The evolutionary interactions between plants and insect pests are asymmetric: the biochemical and structural assortment of the angiosperms offers a profusion of niches for the evolutionary radiation (cladogenesis) of insects, while insects do not affect plant evolution or may create anagenic adaptations within the plants. So, plants have not developed resistance to insects, but the insects have, or were, evolved ever since their emergence, especially in food selection. Host plant selection is usually a behavioural procedure which is managed basically by chemoreception system. Consequently, the emergence of specific insect/host plant relations mostly is the consequence of evolutionary conversations in the insects' chemosensory processes. The adaptations to the food preference of the new host plant may be a minor procedure. The 'plant realm' and also the 'class Insecta' are addressed as the two prevailing gatherings of living organic entities, as far as the bounty of species just as in the measure of biomass (Schoonhoven et al. 2005). Thus, a lion's share of 300,000 plant species needs bee pollinators for multiplication (Tanda 2019a, b, c, 2020, 2021a, b, c, d, e, f, g). Vivid, scented blossoms and flower nectarines were created by plants for alluring the animal pollinators. The blossom life structures guaranteed that while taking care of, the pollinators additionally got the pollen dust (Kearns et al. 1998). Therefore, to forestall over-abuse, plants have likewise developed a structural and biochemical hindrances for assurance against insects and different herbivores. While a portion of these boundaries are combined by plants paying little heed to the presence of constitutive protection, numerous others are created uniquely because of induced defences in the plant system. Just those insect species, which can beat these impediments at least one plant animal categories by shirking, detoxification, and so

A. S. Tanda (✉)
School of Horticulture, University of Western Sydney, Richmond, NSW, Australia

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_1

forth, can get to that plant species as food. Insects which harm the monetarily significant plants have been named as insect pests. The significant mutualistic and opposing communications among plants and insects are presented hereunder.

1.2 Insect Pollinators and Angiosperm Mutualism

The most clear and broad material illustration of mutualism is between bee-pollinated blossoming plants and their pollinators (Tanda 2019a, b, c, 2020, 2021a, b, c, d, e, f, g). Almost 80% of all blooming plants are bisexual and bear blossoms with stamen and pistils in a same bloom. This advances self-fertilization and subsequently inbreeding occurs. The plants avoid self-fertilization either by separating the sexes in time and space (differences in the timing of maturation) or by self-incompatibility. Both mechanisms promote cross-pollination, which is assisted by various agencies, e.g. wind, water, animals, etc. More than three-fourths of all blooming plants are completely or mostly pollinated by insects (Faegri and Pijl 1971; Tanda 2019a, b, c). The monetary estimation of bug pollinators is colossal. Most of the important plants such as oilseeds, pulses, fruits, vegetables, nuts, spices, and ornamentals (Hill 1997; Atwal 2000) show improved yields with animal pollination (Klein et al. 2007). It has been estimated that animal pollination has an economic value of €153 billion annually, which is nearly one-tenth of the global agricultural production (Galai et al. 2009). Some of the widely accepted estimates of the number of angiosperms pollinated by animals vary from 67 to 96% of all angiosperm species (Axelrod 1960; Nabhan and Buchmann 1997). Ollerton et al. (2011) observed that these estimates are not based on firm data. They compiled data on published and unpublished community-level surveys of plant-pollinator interactions and concluded that proportion of animal-pollinated species was 78% in temperate-zone communities and 94% in tropical communities, with a global mean of 87.5% of all flowering plants. The pollinators benefit from rewards in the form of nectar and pollen. Both are nutrient-rich foods with nectar containing 50% sugars and pollen 15–60% proteins and other essential elements (Proctor et al. 1996; Roulston et al. 2000). Together, they provide nourishment for the bees, which are the most important among insect pollinators (Schoonhoven et al. 2005). The entomophilous flowering plants and the pollinating insects constitute an example par excellence of mutualism. However, the degree of mutualism varies among various plant-pollinator combinations (Schoonhoven et al. 2005). In some cases, there is an obligate mutualism, and a species of plant can only be pollinated by a single species of pollinator, which depends on it for food (Tanda 2019a, b, c, 2020, 2021a, b, c, d, e, f). Figs (*Ficus* spp.) are reliant upon the fig wasps for pollination (Wiebes 1979). Different species of fig are cross-pollinated by a particular species of wasp, as the pollination in *Ficus carica* Linnaeus is carried out by the fig wasp, *Blastophaga psenes* (Linnaeus) (Ramirez 1970). Similarly an obligate relationship is found in yucca moths (Prodoxidae) and yucca plants (Agavaceae). The yucca moths are the alone pollinators of yucca plants and lay eggs in the locule of the ovary so that the hatching caterpillars can start feeding on the seeds (Pellmyr and Krenn 2002).

Darwin described that the Christmas orchid, *Angraecum sesquipedale* Thouars, had a long green nectary and forecasted that there must be a gigantic moth species with long proboscis to suck the nectar from the long flower nectary. Later on, Rothshild and Jordan observed the Morgan's sphinx moth, *Xanthopan morganii* Walker, with an enlarged proboscis as the only pollinator of *A. sesquipedale*, native to Madagascar (Kritsky 2001). This type of mutualistic relationship in plant pollinators is not very common. Burkle and Alarcon (2011) reported that plant-pollinator associations are largely distributed with a high degree of annual turnover of pollinator populations and the significance of an insect pollinator may differ for pollination services for the same plant under dynamic climatic conditions.

Doubtlessly, various insect pollinators have served the development of angiosperms differently, and fossil reports describe that pollination mechanism was found around 250 Myr ago (Labandeira 2013). The old flowering plants might be cross-pollinated by the wind birds or other animals. Due to the benefits of insect pollinators, its significance enhanced in the future (Cox 1991; Crepet et al. 1991). Entomophilic plants possess various flower sizes, shapes, colours, and fragrances which may have been resolved by the needs of flower pollinator species. The pollen grains may have a shape or sticky materials which help to adhere to the body of the insect. The hairy body parts of the insects also assist in the spread of the pollen. Foxglove, (*Digitalis purpurea* Linnaeus), flowers cross-pollinated by the bumblebees look like a bell; however, the flowers of *Calopheria* spp. fertilized by the butterfly have tubular corolla, adapted for the enlarged proboscis (Schoonhoven et al. 2005). Additionally, the later consists of maximum amino acids than blossoms nourished on by flies (Baker and Baker 1986). Few brightly coloured flowers develop sterile 'reward anthers' to allure pollinators (Nepi et al. 2003). The flowers of orchid Mirror of Venus, *Ophrys speculum* Link, mimic the virgin female wasps of their pollinator, *Dasyscolia ciliata* (Fabricius), by discharging female sex pheromone to attract the male wasps. The male wasps attempt to mate with the blooms and operate as pollination agent (Ayasse et al. 2003). At present, hymenopterans are the predominating class working in plant pollination; however, other insect groups are too crucial in the history of pollination. Basically the beetles and flies pollinate the basal flowers (Thien et al. 2000). Honeybees have adapted themselves to a flower nutrition (Atwal 2000) and like pollen grains in spite of impermeable cuticle (Velthius 1992). Honeybees show flower constancy by foraging the blooms of a single plant. It enhances the effectiveness of a pollinator and assists in plant reproductive isolation. The insects' ability to recall amalgamations of flower odours and colours contributes a crucial role in flower fidelity. Honeybees have been observed to have the capacity to differentiate 700 various floral fragrances (Schoonhoven et al. 2005).

1.3 Antagonistic Plant-Pest Relationships

Insects are the most assorted and massively effective living beings on this globe and attack plants for food. Species in Lepidoptera, Orthoptera, Phasmida, or predominantly Hemiptera and Thysanoptera are herbivorous; however, Coleoptera,

Hymenoptera, and Diptera are partially herbivorous but mostly carnivorous (Schoonhoven et al. 2005). Several insects live on all parts of plants; however, solid feeders are defoliators or borers, and others suck the sap (aphids, jassids), lessen plant vigour, and act as vectors such as whitefly. Mostly insects are specific in their food plant choice. Monophagous insects live on a single or a few related plant species, but oligophagous insects feed on a number of plants. Polyphagous species feed on various plants belonging to many families for survival (Panda and Khush 1995). Studies on herbivorous insects have demonstrated that one-tenth of these insects are capable of living on plants of more than three families. Each insect host range is determined by structural, biochemical, and ecological elements. Excluding Orthoptera, all other herbivores are highly consisted of specific species living on specialized species of plant (Schoonhoven et al. 2005). Bruce (2015) described that the herbivores get converted to specialize over time; however, few of polyphages carry on as crop pests. Insects can identify and respond to host signals for nourishing and egg-laying. In spite of this, antagonistic connections between plants and phytophagous insects continue to work, as herbivory has been seen to enhance the plant development and strength in few instances (Owen 1980; Vail 1994; Sadras and Felton 2010). Production minimizes due to insects; however, there are instances of enhanced yield reported in insect-attacked in comparison to insect-unattacked crop plants (Harris 1974). The automatic reply to damage may in few plants more than counterbalance the damage done. It is based on how plants answer to damage by insects or other herbivores. Sesame tissues cultured alone or with okra suppressed egg hatch and penetration of roots by juveniles, delayed adult development, and encouraged development of males in *Meloidogyne incognita*. Gall formation was inhibited on excised roots of okra by co-culturing with sesame. Sesame callus reduced penetration, discouraged nematode build-up in okra, and caused an increase in numbers of males showing antagonism of sesame to root-knot nematode on okra (Tanda and Atwal 1988; Tanda et al. 1988, 1989).

1.3.1 Plant Defence Mechanisms

Plants are motionless and have to protect themselves against herbivores. Many plants in natural environments exhibit small or no evident attack despite of large populations of plant feeders. Insects feed about 10% of all plant biomass annually (Barbosa and Schulz 1987; Arora and Sandhu 2017). Plants have developed a large range of structural and biochemical attributes to save from herbivores. Contrastingly, insect injury is more in cropping area as many of these attributes have been strayed while breeding more palatable and tasty plants and outyielding the crop genotypes used traditionally. There is a demand to investigate such plant defence mechanisms to manipulate them in agro-industry (see Chap. 10).

1.3.2 Plant Structural Defence Mechanisms

1.3.2.1 Epicuticular Waxes

The epicuticle protects the plant surface by waxes against desiccation, herbivore, and disease attack. Thickness, structure, and wax coating number may be different in plants giving rise to variations in the total plant dry weight. These wax layers work as resistance to the insect pest attack (Jeffree 1986), and the mechanoreceptor and chemoreceptor present on the tarsi of insects and mouth parts get negative tactile and chemical stimuli. In Brassicaceae, leaf epicuticular wax ensues in non-preference for ingesting by the flea beetle, *Phyllotreta cruciferae* (Goeze) (Bodnaryk 1992). However, wax coating may also have adverse influence by liking few insects. Plants with glossy leaf surfaces have also been reported to be resistant or non-preference to insect pests in many cases (Eigenbrode and Espelie 1995). Indirectly crystals of wax and waxy flowers may also damage the sticking, mobility, and efficiency of predators eventuating in higher herbivore abundance (Eigenbrode et al. 1999).

1.3.2.2 Hairy Structures

In plant mostly, the epidermal surface is protected with hairlike structures, which vary in form, size, position, and their role (Werker 2000). Generally, the hairs on the aerial parts of a plant are called as trichomes; however, the pubescence is mentioned when plant surface is protected by the collective trichomes. These trichomes vary in size from a few microns to several centimetres, and the form differs largely in various plants. They are glandular and non-glandular (Payne 1978). Non-glandular trichomes may work as fence for the attack of insects on the surface of plants or stop the herbivores' feeding on the plant tissues, thus preventing the plants from any damage (Ram et al. 2004). Glandular trichome structures are developed to produce a number of chemical substances (Fahn 2000), which perform as crucial chemical barricades against insect pests and diseases (Glas et al. 2012). In black bean, *Phaseolus vulgaris* Linnaeus, curved trichomes were observed to stick the aphid, *Aphis craccivora* Koch (Johanson 1953), and the leafhopper, *Empoasca fabae* (Harris), resulting in damage and death (Pillemer and Tingey 1978). In few instances, the density of trichomes has been found to be persuading interestingly, in response to insect nourishing. When plants were attacked by the cabbage white butterfly, *Pieris rapae* (Linnaeus), and the cabbage looper, *Trichoplusia ni* (Hubner), on young black mustard, *Brassica nigra* (Linnaeus) W. D. J. Koch, plants led to enhanced trichome density on new foliage (Traw and Dawson 2002). Few insect pests have also been observed to have evolved morphological and biochemical modifications to counteract the effect of hairy growth. These trichomes may also have role in plant resistance indirectly, by restricting the searching efficiency of predators of herbivores. On glabrous varieties, than on hairy leaf surfaces, the parasitic wasp, *Encarsia formosa* Gahan, is greatly more effective in searching the whitefly nymphs (van Lenteren et al. 1995).

1.3.2.3 Leaf Surface Rigidity

In a lowland tropical forest, plant toughness was reported as the best forecaster of interspecific differences in herbivory ranking (Coley 1983). By overthrow of cellulose, lignin, suberin, and callose with sclerenchymatous fibres, plant cell walls become stronger and resistant to piercing and sucking by insect mouth parts and ovipositors of adult females and chewing by insects using mandibles. Solid-stemmed cultivars of wheat with toughness were found to be resistant to stem sawfly, *Cephus cinctus* Norton (Platt and Farstad 1946). Rind hardness was a significant element in sugarcane, in minimizing the internode borer *Diatraea saccharalis* (Fabricius) attack (Martin et al. 1975). In alfalfa, seed losses due to the seed chalcid *Bruchophagus roddi* (Gussakovsky) were lower in genotypes possessing more lignified pod walls (Springer et al. 1990).

1.3.2.4 Design and Plant Size

The preference of a plant cultivar to be a host for insect pests may differ with plant design, architecture, and size. The spacing of plant canopy, variations in stem, leaf and bud shapes and their sizes, and angles of branches may impact insect liking and survivance. From monocots through herbs and bushes and trees, the increase in size and design of plants is related with the enhancement of diversity of the connected insect life (Lawton 1983). The indirect impacts of plant structures on herbivory are also arbitrated through their effect on the parasites and predators. As compared to normal leaf varieties, okra-leaved cultivars in cotton are less attacked by bollworms, whitefly, and boll weevil (Ram et al. 2004). Varieties with little cotyledons and unifoliated leaves in soybean were tolerant to the legume seedling fly, *Ophiomyia phaseoli* (Tryon), and these are the sites where the female insects oviposits (Talekar and Tengkano 1993).

1.3.3 Biochemical Productions as Barriers

Plants have developed a number of chemical structures to avoid attack by insects and other herbivores. As few chemicals are associated with basic metabolism, several other compounds have been reported to deter, repel, kill, or stop insects and other herbivores from feeding on some plants as their food (Chapman 1974; Harborne 1993; Mithofer and Boland 2012). As plant feeding insects have evolved the capacity to manipulate their hosts, the plants have acknowledged by developing defending biochemical secretions to prevent herbivore damage (Johnson 2011). These biochemicals secreted by plants may be grouped into holistic nutrition and allelochemicals.

1.3.3.1 Holistic Nutrition

Crop plant suitability as a host for one or more insect pests is reliant on its capacity to offer holistic nutrition for growth and multiplication of insects. Plants generally provide nutrients at suboptimal ratios, from an insect's outlook, which are amalgamated with indigestible structural mixtures of cellulose and lignins, and a diversity of allelochemicals (Schoonhoven et al. 2005). The biochemicals apply a large number of behavioural, physiological, and growth-impeding impacts, some of which may even result in to insect killing. Many insects have the same needs for food, comprising of carbohydrates, amino acids, fatty acids, sterols, and some micronutrients; however, plants are often nutritionally inferior in itself. The key classes of basic plant amino acids, carbohydrates, and lipids intricated in the physiological plant procedures act as important nutrients for herbivores. So, alterations in basic plant metabolism and nutrients highly impact the living and reproduction of plant feeders (Berenbaum 1995). Mainly, nitrogen is crucial as insects are incompetent to utilize plants organic and inorganic nitrogen as it is suboptimal for the insect needs (Schoonhoven et al. 2005). This may work as a main obstacle for the full utilization of plants by a large number of insects. Appealingly, the herbivores consist of about 50% of the total arthropods in less than one-third of insect fauna, showing that once the nitrogen insufficiency is broken, these insects are capable to reach a sufficient nutrition supplies (Strong et al. 1984).

1.3.3.2 Crop Plant Nutrient Deficiency: A Resistance Mechanism Against Insects

Crop host plant, having insufficient one or more important elements of nutrition needed by the herbivores, may demonstrate insect tolerance through antibiotic and antixenotic impacts on the insect development and similarly may also form disparity of accessible essential nutrients (Arora and Dhaliwal 2004).

Cotton Genotypes Few cotton cultivars with built-in protection depending on essential nutrients have been developed for the leafhopper, *Amrasca biguttula* (Ishida); whitefly, *Bemisia tabaci* (Gennadius); stem weevil, *Pempherulus affinis* (Faust); and thrips complex (Uthamasamy 1996). The whitefly *B. tabaci*, with genetically resistant genotypes, exhibited more amounts of K, P, and Mg and small of N and Fe in comparison to susceptible cultivars. However, sugars, proteins, Ca, and Cu did not exhibit important correlation with whitefly population multiplication. Another report mentioned that total contents of sugar in some cotton genotypes were positively associated with whitefly attack at the vegetative stages but negatively connected with it after crop flowering period (Rao et al. 1990). For the leafhopper, *A. biguttula*, more prone cultivars, Acala 4–42, had large contents of reducing sugars (2.55%), proteins (18.49%), and free amino acids (10.15 mg/g) in comparison to highly tolerant BJR 741 holding 1.63% reducing sugar, 13.45% proteins, and 6 mg/g free amino acids (Singh and Agarwal 1988).

Rice Genotypes Resistant rice cultivars to thrips, *Stenchaetothrips biformis* (Bagnall), contained notably low reducing sugars and free amino acids as compared to the prone cultivars (Thayumanavan et al. 1990). Contents of asparagine in small amounts in rice cultivar 'Mudgo' were contemplated to be the fundamental reason of tolerance to brown plant hopper, *Nilaparvata lugens* (Stal). Brown plant hopper confined with Mudgo variety showed underdeveloped ovaries with few eggs; however, those kept with susceptible cultivars developed normal ovaries with maximum eggs (Sogawa and Pathak 1970). The gall midge *Orseolia oryzae* (Wood-Mason)-resistant cultivars PTB 18, PTB 21, and Leuang 152 had greater amounts of free amino acids and low sugars in their shoot apices than non-tolerant cultivars Jaya and IR8. Stems of resistant (TKM6) and moderately resistant (Ratna) cultivars had low amino acids and sugars than susceptible varieties (IR8) against the stem borer, *Scirpophaga incertulas* (Walker), (Vidyachandra et al. 1981).

Legume Contents Auclair (1963) demonstrated the significance of amino acid contents in the pea plant on the susceptibility to aphid, *Acyrthosiphon pisum* (Harris). He reported that the amounts of amino acids in the susceptible cultivars were significantly greater than those in the resistant varieties. High proportions of non-reducing sugars and little percentage of starch in the seeds of chickpea GL 645 might be accountable for the less attack of the pod borer *H. armigera* in the cultivar under trial in comparison to the infestor (Chhabra et al. 1990). In pigeon pea, genomics against pod borers, small amino acid, protein and sugar amounts, and high phenol contents developed resistance. Sugar amounts were more in seeds (3.64–4.82%) and in the pod coat (3.66–4.92%) of susceptible genotypes (ICPLI, ICPLS7, and UP AS20). Total sugar proportions in the resistant varieties varied between 2.86 (ICPLS3024) and 3.51% (HS9–2) in the seeds and 2.91 (ICPLS3024) and 3.44% (HS9–2) in the pod coat. The amino acid concentrations were small in the pod coating (1.40–1.52 mg/g) and seed (1.39–1.55 mg/g) of resistant pigeon pea genotypes assessed in comparison to the susceptible varieties (1.89–2.57 mg/g in pod coat, 2.04–2.62 mg/g in seed). Extremely significant positive correlation found between amino acid present and infestation of borers helped the potential contribution of amino acids in providing resistance to the pod borers (Sahoo and Patnaik 2003).

1.3.3.3 Phytochemicals

The allelochemicals or phytochemicals secreted by plants are mostly minor metabolites which do not contribute largely in the basic plant pathways of metabolism. As the basic metabolic pathways are the same in almost all angiosperms, these secondary materials differ largely in various crop plants (Schoonhoven et al. 2005). Fraenkel (1959) established that these metabolites serve to repel many herbivores. It has been reported that the plant develop a number of minor substances, and more than 200,000 of these have been recognized (Dixon and Strack 2003). Further, the allelochemicals have been categorized into two classes such as allomones, which

help the host plant, and kairomones, which assist the herbivores. In different types of insect-plant associations, the action of allelochemicals can decide the ranking of a plant either as a kairomone host and non-host or as allomone-resistant host plant and allomone-susceptible host (Panda and Khush 1995). Allomone plants are contemplated as a big element accountable for plant defence mechanism against herbivores, and these have been utilized to enhance levels of resistance in many field crops (Green and Hedin 1986). The different secondary plant metabolites used in plant defence mechanisms against insects have been described shortly (Rosenthal and Berenbaum 1991; Arora and Dhaliwal 2004; Schoonhoven et al. 2005; Arora and Sandhu 2017).

Unusual Amino Acids In many unrelated higher and some lower plants, nonprotein or unusual amino acids are ubiquitous. About 600 amino acids have been described from different legumes. Nonprotein amino acids may provide defence against natural enemies and diseases due to their constructional analogy to the usually important amino acids in nutrition. The biological impacts on herbivores are partially due to the correlated molecules which get involved wrongly into the insect protein synthesis system or through stopping of biosynthetic tracks (Rosenthal 1991; Huang et al. 2011; Yan et al. 2015). In creating insect development disturbance, canavanine, azetidine-2-carboxylic acid, 2,4-diaminobutyric acid, mimosine, 3-hydroxyproline, 5-hydroxynorvaline, β-cyanoalanine, and pipecolic acid are important (Parmar and Walia 2001; Yan et al. 2015). Root exudates and extracts from in vitro grown seedlings of sesame showed an inhibitory effect on egg hatch and juvenile penetration by root-knot nematode, *Meloidogyne incognita*. Analysis of root exudates of sesame showed seven free amino acids, i.e. aspartic acid, glutamic acid, valine, proline, serine, glycine, and leucine, whereas the exudates of okra had ten free amino acids, viz. glycine, serine, leucine, isoleucine, alanine, arginine, glutamic acid, glutamine, lysine, and cystine. Three sugars, viz. fructose, glucose, and sucrose, were found in sesame root exudates and extracts. Commercial amino acids both singly and in combination inhibited egg-hatching (Tanda et al. 1989).

Organic Compounds Terpenoids are the biggest and greatest diverse group of organic compounds observed in crop plants. They show gigantic chemical variations and complexness; however, all are established by blend of five-carbon isopentane, and many of them are lipophilic compounds (Ruzicka 1953). Terpenoids attain their highest structural and functional variety in the plant flora. About 30,000 terpenoids are found in plant systems, and a large number of them act as protection against insect pests and diseases or as allures for crop pollinators and fruit scattering organisms. Gershenzon and Croteau (1991) reported that the terpenoids are composed of two or more than five carbon units in their forms: monoterpenoids (2×C5), sesquiterpenoids (3×C5), diterpenoids (4×C5), triterpenoids (6×C5), tetraterpenoids (8×C5), and polyterpenoids [(C5) n where $n > 8$].

Monoterpenoids have been established to act as toxins and restraints for feeding and laying eggs against many herbivores. Among monoterpenoids, the important example of insect toxin is pyrethrum, working as a botanical insecticide, reported in

the leaves and flowers of *Chrysanthemum* spp. In pyrethrum, the active ingredient is a combination of monoterpene esters commonly called as pyrethroids (Casida 1973).

Cotton and other plants belonging to Malvaceae have pigmented spherical glands found in their foliage, blossoms, and branches of plants. The pigments of these glands, in addition to anthocyanin, possess more amounts of a variety of monoterpenoids and sesquiterpenoids particularly gossypol. Gossypol is a phenolic compound, sesquiterpene dimer with two aldehyde remainders. Gossypol is poisonous to a number of insect pests, resulting in big decline in the survivance, multiplication, and development of numerous major lepidopterous and coleopterous herbivores. The contagion of gossypol to insects is assumed to arise from its irrevocable to proteins in the gastrointestinal area, bringing about a decrease in the digestion of proteins. In the gastrointestinal region, the proteins may be the eaten dietary proteins or the digestive enzymes developed by the herbivore (Meisner et al. 1977). A key secondary metabolite of the common dandelion, *Taraxacum officinale* G. H. Weber ex Wiggers, the sesquiterpene lactone, beta-D-glucopyranosyl ester (TA-G), saves the plant against its main native root feeders, the common European cockchafer, *Melolontha melolontha* Linnaeus, by discouraging larval infestation (Huber et al. 2016).

In terpenoids, triterpenoids (C30) are the biggest with six C5 isoprene units. The three main classes of triterpenes are the cucurbitacins, limonoids, and saponins which have important contributions in plant-insect herbivore interplays. In the Cucurbitaceae, cucurbitacins are a class of approximately 20 very bitter and toxic tetracyclic triterpenes, cramped chiefly to the host plants. These substances act as poisons and restraints for feeding against a large number of herbivores (Tallamy et al. 1997). Few specific insects attacking cucurbits are capable to absorb or prevent these poisons and even utilize cucurbitacins as host identifying signals (Abe and Matsuda 2000).

With a fundamental structure of 26 carbon atoms, the limonoids are a big class of very oxygenated compounds and are reported in Rutaceae, Meliaceae, and Cneoraceae. These limonoids are very strong feeding deterrents against many herbivores. More than 100 triterpenoids have been detected from the neem (*Azadirachta indica* A. Juss.) seeds, and a many of them are working as deterrents and antifeedants against crop insect pests. Azadirachtin is the chief among these triterpenoids, which is effectual at doses as minimum as 50 parts per billion. Over 400 insects have been found to be susceptible to neem compositions at different concentrations. With antifeedant actions, neem is demonstrated to influence the living, growing, multiplication, vigour, and egg-laying capacity of herbivores (Schumutterer 1995; Dhaliwal and Arora 2001).

In many crop plants, saponins are most common and made up of a sugar part (glycoside) associated with a hydrophobic aglycone, which may be a triterpene or a steroid, both of which develop from the C30 precursor, squalene. In soybeans, beans, peas, tea, spinach, sugar beet, and quinoa, triterpenoid saponins have been isolated. In oats, capsicum, peppers, aubergine, tomato seed, allium, and asparagus, steroidal saponins are detected (Francis et al. 2002). Saponins apply a powerful insecticidal reaction against many insect groups resulting in enhanced kill, decreased food intake, weight loss, growth lagging, and moulting faults (Geyter et al. 2007).

Natural Substances The alkaloids are a diverse group of natural substances that found in all groups of living life; however, they are typical in plant systems. They mostly comprise primary products that have one or more nitrogen atoms, mostly in amalgamation as component of a cyclic system. Many of them are products of usual amino acids, for instance, as lysine, tyrosine, tryptophan, histidine, and ornithine (Facchini 2001). They occur in about 20% of the angiosperms. Mostly, each species carrying alkaloid exhibits its own distinctive, alkaloid shape explained genetically. Many alkaloids have been described to be poisonous or repellent to herbivores. Due to their nature containing nitrogen, several alkaloids impede with the major elements of acetylcholine transference in the nervous system. Nicotine and nornicotine obtained from tobacco were important as botanical insecticides before the invention of organic insecticides made synthetically (Dhaliwal and Arora 2001). Pyrrolizidines, quinolizidines, indole alkaloids, benzylisoquinolines, steroid alkaloids, and methylxanthines are many classes of alkaloids at dietary concentrations over 0.1% and act as insect's deterrents and to other herbivores (Schoonhoven et al. 2005).

Glucosinolates About 100 sulphur or nitrogen carrying unique minor compounds such as glucosinolates comprise a little class of which work as harbingers of oils in mustard. Together with the family Brassicaceae, glucosinolates are found generally in the Brassicales order. Glucosinolates seem to work as successful chemical protections against a number of non-adapted herbivores (Fahey et al. 2001). Heynhold genome, at a minimum 52 genes, is intricated in glucosinolate biosynthesis in the thale cress *Arabidopsis thaliana* (Linnaeus) (Arabidopsis Genome initiative 2000; Halkier and Gershenzon 2006). When insects infest crop plants, glucosinolates are broken down by myrosinase enzyme into many metabolites acting as deterrents against insects (Hopkins et al. 2009). On the flip side, a small group of *Brassica* feeders are capable to use glucosinolates in searching and identifying the host. Glucosinolates and their evaporative compounds formed by hydrolysis are also utilized as signals by predators of *Brassica* feeding herbivores (Louda and Mole 1991).

Juvenoids and Ecdysteroids For the growth, development, multiplication, and survival of herbivores, the endocrine system is crucial. Though several insect hormones are demonstrated, the juvenile hormone (JH) and the ecdysone or moulting hormone (MH), two strong hormones are established to contribute in these procedures. Juvenoids and ecdysteroids are match of these hormones. It is assumed that plants may have evolved juvenoids and ecdysteroids as fine defence mechanisms against herbivores. Crops possessing more ecdysteroid amounts, i.e. >1000 ppm, are prevented by insect pests. There are few main juvenoids derived from plants such as farnesol, sesamin, juvabione, sterculic acid, bakuchiol, and thujic acid which are familiar to disorder metamorphosis, moulting, and multiplication in herbivores.

Crop Proteinase Inhibitors In some crop plants, protease inhibitors (PIs) comprise a plentiful and significant group of substances which have a defending mechanism against insect pests (Dunaevsky et al. 2005). New reports utilizing microarrays and proteomic proposals have disclosed that the plant defence mechanisms relying on proteins contribute more significantly against insects than perceived before

(Felton 2005; Zhu-Salzman et al. 2008). Arginases, polyphenol oxidases, and peroxidases, the defence proteins, may have properties against microbes, and others, for example, chitinases, cysteine proteases, lectins, and leucine amino peptidases, may also be poisonous (Zhu-Salzman et al. 2008). Nevertheless, the anti-insect action of plant proteins is easily disabled by proteases, and proteolysis-susceptible proteins can be avoided with PIs (Mithofer and Boland 2012). Serine, cysteine, and aspartate proteinases and metallocarboxypeptidases stop the actions of different enzymes in herbivores mostly insect peptidases which are intricated in the development and multiplication of insects. The PIs also minimize the food digesting capability of the herbivores, thus resulting in to the scarcity of amino acids, the major components of food finally decelerating the growth and affecting the starvation. Numerous PIs have been found in plant system (De Leo et al. 2002), which are successful against a number of lepidopteran and hemipteran herbivores (War and Sharma 2014). PIs were positively assessed for their trypsin and *H. armigera* gut proteinase inhibitory action in various parts of the tomato plants (Damle et al. 2005).

Phytohaemagglutinins Lectins or phytohaemagglutinins are proteins with a capability to reversibly attach to the carbohydrate moieties of complicated carbohydrates without changing the covalent shape of any of the identified glycosyl myths. Lectins are found globally all over the plant kingdom, where they comprise 6–11% of the total plant proteins. Mostly the legume seeds cotyledons are abundant in lectins. Lectins are connected with the defence mechanisms of crops against insect pests and diseases (Liener 1991). *Arisaema helleborifolium* Schott lectin showed anti-insect action towards the melon fruit fly second instar larvae, *Bactrocera cucurbitae* (Coquillett) (Kaur et al. 2006).

Plant Phenolics In plants phenolics are very common and are fragrant compounds with one or more hydroxyl groups (Harborne 1994). Hydroxybenzoic acids like vanillic acid, hydroxycinnamic acids like caffeic acid, and coumarins are comparatively simple phenolics (Schoonhoven et al. 2005). Coumarins comprise a 5,6-benz-2-pyrone skeleton and may be differently hydroxylated, alkylated, alkoxylated, or acylated. Coumarins can discourage eating and impede with growth of herbivores. Coumarin, bergamottin, is capable of killing eggs of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), though mammein is insecticidal to the beetles attacking mustard. Coumarins seem to work as kairomones for some insects that are particularly eating on coumarin carrying plants (Berenbaum 1991b). Flavonoids are established mostly in all higher plants in the phenolics, and many plants exhibit their own distinguishing flavonoid contour. To make a water-soluble glycoside, flavonoids have a basic C6-C3-C6 structure, which is related to a sugar moiety. Flavonoids derived from plants are catechin, a botanical insecticide rotenone, and phaseolin, all of them operate as impediments against insect pests (Schoonhoven et al. 2005).

Tannins are polyphenolic combinations generally available in higher plants. The phenolic hydroxyl groups of tannins tie to nearly all soluble proteins, developing

insoluble copolymers. Proteins joined to tannins are indigestible and thus reduce the nutritious worth of plant parts (Schoonhoven et al. 2005).

Latex in Laticifers Latex is found in special tissues known as laticifers, having chemically unspecified milky suspensions or emulsions in aqueous fluids (Agrawal and Konno 2009), and as a defence mechanism. Tiny insects get ensnared in latex physically or their mouthparts may stick together, and chemical constituents in latex including proteins and toxins adversely affect the insect growth (Dussourd 1995). Injuring of laticifers by insects leads to leakage at injury site (Mithofer and Boland 2012). In the milkweed, *Hoodia gordonii* (Masson) Sweet ex Decne, feeding by larvae and oviposition by *T. ni* adults, was discouraged when latex was mixed in artificial diet or applied on the leaves of the host plants (Chow et al. 2005).

1.3.3.4 Allelochemicals as Host Plant Defences

Allelochemicals in Maize Maize is damaged by a variety of insect pests, and anti-herbivore defences in maize comprise small molecules known as benzoxazinoids (Frey et al. 2009), chlorogenic acid (Cortes-Cruz et al. 2003), and maysin (Rector et al. 2003) besides defence-linked proteins (Chuang et al. 2014). Xie et al. (1992) examined for hydroxamic acid many lines of maize resistant to western corn rootworm, *Diabrotica virgifera* Le Conte. Root extracts in all tests were having four main hydroxamic acids such as 2,4-dihydroxy-7methoxy-l,4-benzoxazin-3-(4H)-one (DIMBOA), 2,4-dihydroxy7,8dimethoxy-l,4-benzoxazin-3(4H)-one (DIM2BOA), 2-hydroxy,7-methoxy,1,4-benzoxazin-3(4H)-one (HMBOA), and 6-methoxy-benzoxazolinone (MBOA). These hydroxamic acids retarded the growth and development and loss in weight and also reduced the head capsule width of rootworm larvae. Wiseman et al. (1992) described a greatly significant negative link between weight variations in corn earworm, *Helicoverpa zea* (Boddie), and in the fall armyworm, *Spodoptera frugiperda* J.E. Smith, larvae and maysin contents in the silks of many corn lines.

Allelochemicals in Cotton In cotton, the allelochemicals such as gossypol, gossypurin, heliocides, hemigossypolone, tannins, anthocyanins, flavonoids, and phenolics have been found to affect negatively on infesting herbivores. Gossypol was described to show resistance to cotton bollworm *Heliothis zea* (Bottger et al. 1964). Generally, many cotton varieties grown commercially have a gossypol concentration of about 0.5% in squares. With more gossypol cotton varieties (No. 16482, 6501, and Termez-14) had harmful effects such as prolonging incubation period, higher kill in young larvae, and reducing the weight of larvae in comparison to the cultivars having less gossypol contents (Vilkova et al. 1988). They also reported that antibiotic effect of high level of gossypol contents decreased the fecundity (more than 50%) of *H. armigera*. Gossypol is also described to influence the boll nutritional value adversely by developing complex compounds joining with amino acids, proteins, and enzymes. Mohan et al. (1994) reported that genotypes with maximum

gossypol glands on the surface of the ovary in Asiatic cotton *Gossypium arboreum* Linnaeus decreased the attack of bollworm complexes in *H. armigera*, *Earias vittella* (Fabricius), and *Pectinophora gossypiella* (Saunders). When healthy cotton plants were analysed, for the cotton stem weevil, *P. affinis*, the amount of tannins was minimum in susceptible MCU5 and more in the resistant successions. The contents grew in the gall region when the plants were attacked, and the concentration was high in resistant accessions in comparison to the susceptible genotype MCU5. No difference was reported in the total phenolic amounts in the resistant and susceptible accessions of healthy stems. The amount of total phenolics, however, enhanced in the gall regions significantly when attacked, even the concentration rose more in resistant accessions. It can be established that more development in tannin and phenolic amounts might offer a defensive tool against the stem weevil infestations (Uthamasamy 1996).

Allelochemicals in Vegetable Crops In *Solanum* species against the Colorado potato beetle (CPB), *L. decemlineata*, and the potato leafhopper, *E. fabae*, glycoalkaloids in potatoes perform as natural resistance mechanisms. Many wild *Solanum* species have exhibited a positive relationship between total leaf glycoalkaloid concentration and resistance to *Leptinotarsa* species. Completely stopping eating, leptin is a very successful antifeedant, whereas tomatine and demissine are halfway in action, followed by the effectiveness of solanine and chaconine (Tingey 1984). Resistance of tetraploid potato (*Solanum tuberosum* L.) selection ND 2858-1 in the field and its backcross progeny against the Colorado potato beetle is developed by antibiosis mechanism. In detached leaf tests on resistant cultivars, neonates of CPB evolved slowly, and weight gain in larvae after 4 days was discouraged by 75% in proportionate to larval growth and weight gain on susceptible accessions. Foliar glycoalkaloid assays showed low levels of leptins I and II in resistant genotypes (Lorenzen et al. 2001). Against the tomato fruit borer *H. zea*, the wild species of tomato, *Lycopersicon hirsutum* and *L. hirsutum f. glabratum*, exhibited the phenomenon of antibiosis. For the antibiosis process, the chemicals involved were L-tomatine, 2-tridecanone, phenolics, and elements including iron zinc (Ferry and Cuthbert Jr 1975; Dimock and Kennedy 1983; Kashyap 1983). The toxicity tested of allelochemical 2-tridecanone was maximum against *H. zea*, *Manduca sexta* Linnaeus, and *L. decemlineata*. Maximum phenolic amounts have been reported to conclude resistance to the *H. armigera* species (Banerjee and Kalloo 1989), whereas high contents of tomatine are harmful to the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Steehius and van Gelder 1985). In tomato, the protease inhibitor and chlorogenic acid were involved in resistance against aphid (Felton et al. 1989). In glandular trichomes of *Lycopersicon hirsutum f. typicum* accession (LA) 1777, the sesquiterpene carboxylic acids (SCA), (+) E-α-santalen-12-oic, (−)-E-endo-α-bergamoten-12-oic, and (+)-E endo-β-berqamoten-12-ion acids were developed which is more resistant to herbivores generally attacking tomato, *L. esculentum*, grown commercially. The larvae of tomato fruitworm, *H. zea*, and the beet armyworm, *Spodoptera exigua* (Hubner), showed reduction in eating, growth, and survival in the presence of such chemical compounds. In diet found lethal to the

larvae, at concentrations as low as 2 mg SCA/g of diet and a concentration of 60 mg SCA/g, the sublethal effects were reported (Frelichowski Jr and Juvik 2001).

Root exudates and extracts from in vitro grown seedlings of sesame showed an inhibitory effect on egg hatch and juvenile penetration by root-knot nematode, *Meloidogyne incognita*. Analysis of root exudates of sesame showed seven free amino acids, i.e. aspartic acid, glutamic acid, valine, proline, serine, glycine, and leucine, whereas the exudates of okra had ten free amino acids, viz. glycine, serine, leucine, isoleucine, alanine, arginine, glutamic acid, glutamine, lysine, and cystine (Tanda et al. 1989).

1.3.4 Various Plant Defence Mechanisms

Plant defence mechanisms may be categorized into basic, which are found in the host plants disregarding the occurrence of insect or non-insect pests, and incited, which are developed in response to different abiotic and biotic stressors.

1.3.4.1 Basic Plant Defence Mechanisms

Plants have developed an abundance of structural and chemical defence mechanisms that are incorporated into their tissues disregarding the presence or absence of insects. These basic plant defence mechanisms can repel, deter, inebriate, derange, or disrupt the feeding on plant tissues, growth, and development of herbivores (Arora and Dhaliwal 2004; Ram et al. 2004; Mithofer and Boland 2012). These phytotechnological protection systems comprise of the following:

(a) The texture and composition of the plant covers (Johnson 1975)
(b) Existence of anatomical structures, for example, thin veins, thorns, silica, trichomes, or resins (Hanover 1975)
(c) Lack of essential nutrients (House 1961)
(d) Existence of substances similar to hormones which inhibit the growth of insects (Williams 1970)
(e) Inappropriate pH or osmotic pressure (Beck 1965)
(f) Accretion of secondary metabolites (Chapman 1974)

There are a large number of secondary metabolites comprising amino acids to alkaloids, terpenes, phenolics, steroid, cyanogen, and glycosides in mustard oil (Mithofer and Boland 2012). Additionally, plant systems may also transform nitrogen into compounds which are not accessible to herbivores (White 1978). The benefits of similar basic defences to insects are that these are developed during the time of high metabolic activities and can be used over an increased time period. These physiological devices act against a large number of generalist insects; however, regular subjection to such chemicals develops powerful critical pressure on the plant feeders, which may lead in the development of specialist herbivores.

1.3.4.2 Incited Plant Defence Mechanisms

To deter feeding by insect pests and stop colonization, incited plant defence mechanism is operated in the existence of insects and allows the plant (Sadras and Felton 2010). The insect feeding processes switch on many protection signals, resulting in to acceptable defence reactions (Wu and Baldwin 2010; Hogenhout and Bos 2011; Bruce 2015). Various plant species have also been described to acknowledge to insect females for depositing eggs in the same way (Hilker and Meiners 2006). Volatile organic compounds (VOCs) liberated by plants have been reported to allure predators of herbivores (Tamiru et al. 2011; Fatouros et al. 2012) or incite direct protections so that the rate of insect development is decreased on plants nursing eggs (Gieselhardt et al. 2013). Crop plants react to elicitors produced from mouth secretions of herbivores, mechanical injury, and exogenous inducer application. The mouth secretions/regurgitants of herbivores consist of many plant defence extrinsic molecules of elicitors; the crucial ones are conjugates of fatty acids (FACs). FACs are comprised of two related groups or moieties such as a fatty acid or an amino acid. It has been found that the fatty acid and amino acid develop from the plant and the insect, respectively, and are made in the midgut of herbivores. FACs not only act as main elicitors for plants to show the unique insect-plant interplay to discern insect infestation but also are intricated in insect nitrogen metabolism process. The first FAC separated from mouth secretion of the beet armyworm *S. exigua* larvae was *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin), and it excites maize plant tissues to develop volatiles, which allure natural enemies of the insect pest (Alborn et al. 1997). In tobacco plants, regurgitation of the tobacco hornworm, *M. sexta*, has *N*-linolenoyl-glu, a potential elicitor of volatile emissions. Additionally, few FACs stimulate mitogen-activated protein kinase (MAPK) pathway, developing many plant defence chemicals playing a part in signalling transduction regarding different stresses such as drought, diseases, and insect infestations.

MAPK signal pathway in eukaryotes and its important contribution in plant gesturing particularly for pathogen stresses are well demonstrated. The chief role of MAPK in governing plant transcriptomes has been described (Wu and Baldwin 2010). In tobacco plants, few FACs stimulate accretion of 7-epi-jasmonic acid, which operates as insect defence genes. Moreover, FACs also encourage nicotine and proteinase inhibitors (PI) in the coyote tobacco, *Nicotiana attenuata* (Torr. ex S. Watson) (Wu and Baldwin 2010; War and Sharma 2014). The plant plasma membrane is open to the environment and stimulates a large number of events following identification of pest injury. Alterations in cell membrane potential (Vm) actuated by insects are followed by fast electrical cues, which are functioning systematically in nature. Calcium ions (Ca+2) act as a second messenger signal pathways in many crop plants. After the insect attack, the signal may be seen a few seconds as a single transient oscillation or duplicate spikes with particular subcellular localization lag time, amplitude, and frequency. The Ca+2 cues stimulate calmodulin and other calcium-sensing proteins. This encourages a cascade of downstream impacts, like changed protein phosphorylation and gene expression structures (Furstenberg-Hagg et al. 2013). Herbivory results in the gathering of plant hormones; the main are

salicylic acid (SA), jasmonic acid (JA), and ethylene. They arbitrate different signal transduction pathways found in plant defence devices against different biotic and abiotic strains. The important transduction pathways associated with plant protection against insects are phenylpropanoid and octadecanoid pathways arbitrated by SA and JA, respectively. All these pathways result in the synthesis and gathering of toxins at the site of feeding or in other plant parts, which are then carried to the location of feeding. Besides, antioxidative enzymes engaged in plant defence get gathered in plant tissues damaging site (Wu and Baldwin 2010). Yan et al. (2015) described the amassing of nonprotein amino acid 5-hydroxynorvaline in leaves of maize inbred line B73 following herbivory by the corn leaf aphid *Rhopalosiphum maidis* (Fitch) and the beet armyworm *S. exigua* and in response to application with methyl jasmonate, salicylic acid, and abscisic acid. Basic and actuated defences can either be direct or indirect. Direct defences spot the herbivores, while indirect defences work through the engagement of predators of herbivores in the support of plants. Some volatile organic compounds (VOCs), having terpenoids, fatty acid derivatives, and a few aromatic compounds, act as attractants to the natural enemies of insect pests (Mithofer and Boland 2012).

1.3.5 Insect Defences Encountering Plant Defence Mechanisms

Through an abundance of structural and chemical defence mechanisms, plants avoid themselves from insect attack. These defences may have applied heavy selection pressure on the herbivores leading to the development of adaptations in insects. The insect modifications to plant defences can be physical, behavioural, or biochemical and consist of different devices, for example, penetration barricades, toxic excretions, sequestrations, temporary attaching with carrier proteins and stocking of toxins in adipose plant tissues, enzymatic detoxifications, and feeding site mutations. It is crucial to know about these insect modifications to plant defences to reduce their impacts on the steadiness of resistance in plants to insects. The major counteracting defence insect strategies to plant defences (War and Sharma 2014; Bruce 2015) are briefly presented below.

1.3.5.1 Insect Processes to Plant Defence Mechanisms

Several herbivores have evolved special tools to get the better of the slippery waxy cover which gives a big hurdle to the walking and motion of insects on plant surfaces. For good bonding to the slippery cover, the minute setae on tarsal pulvilli of few chrysomelids expel an adhesive substance (Gorb and Gorb 2002). Leafhoppers of *Empoasca* species can utilize their tarsal pulvilli as suction cupping device (Lee et al. 1986), though several lepidopteran caterpillars have adhesive silken thread as

a rope ladder to the leaf cover to act as a foot clasping instrument (Eigenbrode 2004). To control the complication of trichomes on the plant layers, the aphid *Myzocallis schreiberi* Hille Ris Lambers and Stroyan possesses a special device in the shape of claws and flexible empodia that function to have a fine grasp on the short woolly trichomes on the host plant surface, the Holm oak, *Quercus ilex* Linnaeus (Kennedy 1986). The hardness of leaves has been reported to minimize the attack of plant feeders. As a modification to the plant toughness, in caterpillars of *Pseudaletia unipuncta* Haworth, the head and chewing musculature are double the size when eat on tough grasses as compared to soft artificial nourishment; nevertheless, body mass is alike (Bernays 1986). Water lily beetles *Galerucella nymphaeae* (Linnaeus) consuming the tough water lily have excessively larger mandibles than conspecifics eating on the great water dock grin, *Rumex hydrolapathum* Huds., second host with mushy leaves (Pappers et al. 2001).

1.3.5.2 Insect Modifications Against Protease Preclusions

In a few host plants when the insect attack, they make protease inhibitors for protection. Insect attack on *N. attenuata* immediately produces and gathers trypsin PIs; *M. sexta* and *S. exigua* larvae accomplished better on trypsin PI-lacking plants in comparison to alike plants making PIs (Zavala et al. 2004; Steppuhn and Baldwin 2007). Still, several insects have modified to host plant PIs, which enhances the attack to the host crop plants. This defensive response to PIs by herbivores is a key barricade to the exploitation and use of PIs for a firm plant protection permitting the devices by which insects prevent the PI-based plant defence mechanism. In crop insect pests, two kinds of resistance or adaptation strategies to protease inhibitors have been established. One of them is based on the different proteases which are contrary to PIs (Parde et al. 2010). These unfeeling proteases can be found constitutively in the plant system and/or are actuated when the other proteases are forbidden to recompense their damages (Jongsma et al. 1995; Parde et al. 2012). *S. exigua* has been described to modify to potato proteinase inhibitor II by the gut proteinase actions, which is not developed by the PIs. Additionally, when tested on the soybean proteinase inhibitor (SPI) diet, insensitivity to the inhibitor for larval proteases was observed (Brioschi et al. 2007). Trypsin insensitivity to host plant PIs has been delineated from *Agrotis ipsilon* (Hufnagel), *T. ni*, and *H. zea* (Volpicella et al. 2003). Other resistance mechanisms to PIs in herbivores require the synthesis of particular proteases, which are capable to break down the protease inhibitors so as to decrease their inhibitory action. Proteolytic inactivation modification is a crucial mechanism evolved by herbivores to resist the proteolytic inhibition by PIs. When feeding on artificial diet with soybean PIs, a trypsin-like enzyme is manufactured newly by *S. frugiperda* (J.E. Smith) larvae (Brioschi et al. 2007). The larvae of diamondback moth, *Plutella xylostella* Linnaeus, have been observed to be insensitive to mustard trypsin inhibitor 2 (MTI2). Such insensitiveness has been ascribed to the deterioration of MTI2 by the pest, thus preventing the impact of the PI (Yang et al. 2009).

1.3.5.3 Conversions to the Mustard Oil Bomb

The 'mustard oil bomb', also called as the glucosinolate-myrosinase system, found in Brassicales (Brassicaceae, Capparidaceae, Tropaeolaceae), composes the most efficient and well-researched plant defence process against herbivores. Glucosinolates are categorized and are secured from thioglucosidase—myrosinase—their hydrolysing enzyme under usual environmental conditions. Although the glucosinolates are found in several plant cell systems, the myrosinase is only centralized in dispersed plant tissue cells. The myrosinase and glucosinolate meet together developing the unstable aglycones on tissue feeding, which automatically adjust into different active substances, mostly nitriles and isothiocyanates (Li et al. 2000; Hopkins et al. 2009). It has been described that more glucosinolate- and myrosinase-rich lines of *Brassica juncea* (Linnaeus) Czern. are more resistant to larvae *of Spodoptera eridania* (Cramer) than those with little contents of these inhibiting chemicals (Li et al. 2000). The larvae of *T.ni* prevented *A. thaliana* ecotypes that developed isothiocyanates on hydrolysis of glucosinolate and rather attacked on ecotypes that caused nitriles (Lambrix et al. 2001). Additionally, some parasitoids utilize glucosinolates that are produced by feeding herbivores to trace their host insects. In these instances, glucosinolates have a double function for the damaged plant, in direct as well as in indirect protection (Hopkins et al. 2009). Even few insects utilize glucosinolates for their own shield. *Myzus persicae* (Sulzer), *Athalia rosae* (Linnaeus), and *P. rapae* isolate glucosinolates into their haemolymph and body cells (Muller and Brakefield 2003; Kazana et al. 2007; Bridges et al. 2002). Upon the attack of natural enemies, the haemolymph releases glucosinolates that discourage the ants and the predatory wasps (Muller and Brakefield 2003). Few aphids particularly Brevicoryne *brassicae* (Linnaeus) and *Lipaphis erysimi* (Kaltenbach) seclude glucosinolates from the sap of phloem (Kazana et al. 2007; Bridges et al. 2002). Moreover, caterpillars of *P. rapae* clean the glucosinolates from plants by altering them contrary to toxic products to inert metabolites using a process of nitrile-specifier protein (NSP). The NSP procedure in the gut of *P. rapae* regulates the glucosinolate hydrolysis resulting into nitrile formation rather than toxic isothiocyanates (Wittstock et al. 2004).

1.3.5.4 Modifications Against Tannins

With the protein amino groups, tannins make hydrogen or covalent bonds, which result in the precipitation of proteins and the digestive enzymes of insect pests. Additionally, the chelation process of metal ions in the insect body by tannins minimizes their accessibility to the insects, thus influencing their development and multiplication. Tannins have also been described to stop feeding on plants and develop midgut lesions and pharmacological toxicity in insects (Bernays and Chamberlain 1980). Nevertheless, insects have evolved many adaptations to prevent the tannin poisoning. The important modified procedures insects utilize to keep away from the toxicity of tannins is the pH of the gut in alkaline form, assimilation of tannin using peritrophic membrane, polymerization, and discharging of the polyphenols after concentrating them (War and Sharma 2014). The surfactants created by lipid

digestion in the gut lumen avoid protein precipitation (Martin et al. 1987). The degree of oxygen in the foregut also contributes in the tannin toxicity. When the pH is more, oxygen levels are short; it minimizes the tannin autoxidation process, resulting in reduced toxicity. In lowering the toxicity of tannin, the antioxidative structure of insects also contributes significantly. Ascorbate lowers the tannin oxidation and result in reactive oxygen species (ROS) in the insect stomach (Krishnan and Sehnal 2006). Grasshoppers acquire a powerful midgut antioxidative defence mechanism, which empowers them to resist tannins. This antioxidative defence system mostly contains glutathione, α-tocopherol, and ascorbate. In *S. gregaria*, the resistance to tannins and its relation with peritrophic membrane have been ascribed to the ultrafiltration of tannins. In few insects such as *Melanoplus sanguinipes* (Fabricius), tannic acid does not tie with the peritrophic membrane. Additionally, peritrophic membrane saves the insect epithelium against lesions and any harm by ROS by assimilating extremely reactive ferrous ions (Barbehenn 2003).

1.3.5.5 Phytochemicals and Their Enzymatic Detoxification

To conquer plant chemical protections, enzymatic detoxification of toxic chemicals arbitrates the modification of herbivores to plant allelochemicals and thus assists the insects. Herbivores respond completely to the toxic allelochemicals, when offered with the natural host plant diet or merged in the artificial diet, by enhancing the metabolic processes that lead to the making of detoxifying enzymes, for example, monooxygenases and glutathione-*S*-transferases (GST) (Nitao 1989; Wadleigh and Yu 1988). The procedures of detoxification that work in insects rely on the chemistry of plant, and its degrees are mostly affected by the concentration of allelochemicals in the host plant (War and Sharma 2014). Insects use different enzymes for the detoxification of insecticides and allelochemicals of plants, and few strategies are universal (Francis et al. 2005; Scott et al. 2010). The most important is the process of polysubstrate monooxygenases which is also known as mixed-function oxidases. The constituent at the end of this structure is cytochrome P450, which is known as it assimilates maximum light around 450 nm when combined with carbon monoxide. Cytochrome P450 amalgamates even with the toxic substrate and with molecular oxygen, accelerating the oxidation process of the substrate. Cytochrome can merge with various lipophilic substrates and occurs as many isozymes that differ in their substrate explicitness (Feyereisen 2006). The P450s are considered as one of the main operators in insect-plant coexistence, as these are utilized by the host plants to release toxins and by the herbivores for plant chemical detoxification (Schuler 1996). *Drosophila mettleri* Heed living in desert area feed on cactus having toxic allelochemicals contain adaptable quantities of P450 associated with the metabolism of such toxins (Danielson et al. 1997). The metabolism of isothiocyanates, for example, 2-phyenylethylisothiocyanate, indole-3-carbinol, and indole-3-acetonitrile, in *S. frugiperda* midgut microsomes is Cyt P450-reliant (Yu 2000). Conversion of lepidopterans to minor metabolites of plants, for example, furanocoumarins, has been ascribed to P450s. Black swallowtail, *Papilio polyxenes* Fabricius, living on plants having furanocoumarins in diet allows up to 0.1%

xanthotoxin (Berenbaum 1991a), which is cleaned by P450 monooxygenases (Bull et al. 1986). An apparent concept of participation of P450 in detoxification of allelochemicals in plant system occurred after CYP6B1 sequencing from *P. polyxenes*, which instructs for P450s. Coding for P450s, expression of CYP6B161 and CYP6B162 is actuated in cell lines of lepidopterans, showing the participation of P450s in metabolism of linear furanocoumarins, for instance, xanthotoxin and bergapten (Ma et al. 1994). Several P450s found in phytochemical detoxification have been derived from insects, such as from parsnip webworm, *Depressaria pastinacella* Duponchel (Cianfrogna et al. 2002), *M. sexta* (Stevens et al. 2000), and *Helicoverpa* species. Moreover, the transformation of dihydrocamalexic acid to camalexin, which are the main *Arabidopsis* phytoalexins, is accelerated by cytochrome P450 PAD3 (Schuhegger et al. 2006). Resistance to glucosinolates in aphid is ascribed to the CYP81F2, which is a downriver bit of the indolic glucosinolate pathway mechanism (Pfalz et al. 2009). P450s have also been delineated from several other herbivores where they work to metabolize the phytochemicals. For instance, in *Musca domestica* Linnaeus, CYP6A1 detoxifies the terpenoids (Andersen et al. 1997). In *H. armigera*, P450 monooxygenase CYP6AE14 metabolizes gossypol (Mao et al. 2007); in *Anopheles gambiae* Giles, CYP6Z1 detoxifies xanthotoxin and bergapten, furanochromones, and natural myristicin, safrole, and isosafrole (Chiu et al. 2008). However, CYP6Z2 detoxifies xanthotoxin, lignin, piceatannol, and resveratrol (McLaughlin et al. 2008); and in *Diploptera punctata* Eschscholtz, CYP4C7 metabolizes sesquiterpenoids (Sutherland et al. 1998). In bark beetles, *Ips pini* Wood and Bright and *Ips paraconfusus* Lanier metabolize the monoterpenes, sesquiterpenes, and diterpenoid resin acids by using P450s (Seybold et al. 2006). Another enzyme system, the glutathione-*S*-transferase (GST) is occurring in insect resistance to host plants by metabolism of xenobiotics and catalyzation of the conjugation of electrophilic molecules using thiol category of lowered glutathione, leading in fast defecation and deterioration (Francis et al. 2005). This enzyme class has been incriminated in detoxification of insecticides that are neurotoxic and influence the development and multiplication of insects. Among them are spinosad, diazinon, DDT, nitenpyram, lufenuron, and dicyclanil (Sintim et al. 2009). Many reports have suggested the significance of GST in insect modification to phyto-glucosinolates, and minor plant metabolites added in the artificial diet of *S. frugiperda*, *S. litura*, *T. ni*, *M. persicae*, *Aulacorthum solani* (Kaltenbach), and *A. pisum* (Enayati et al. 2005). In *M. persicae*, more production of GST has been assigned to insect modification to glucosinolates and isothiocyanates in Brassicaceae, though there is no straight conflict of isothiocyanates, as aphids prick with their stylets right into the phloem tissues (Francis et al. 2005; Kim et al. 2008).

1.3.5.6 Counter-Defence Mechanisms in Herbivoral Gut Symbiontic Systems

In reply to the insect attack, the evocation of plant defences has been reported to be regulated by chat between jasmonic acid (JA) and salicylic acid (SA) pathways of signalling. Insects have various microbes in their gut area, and such symbionts can

adapt to plant-insect interplays (Hogenhout et al. 2009). In tomato, Chung et al. (2013) described that the grubs of Colorado potato beetle, *L. decemlineata*, utilized digestive track bacteria in their mouth secretions to control anti-herbivore defences. The antibiotic-untreated larvae reduced the development of JA and JA-responsive anti-herbivore defence mechanisms but enhanced SA gathering and SA-reactive gene expression. The reduction at the cellular level or plant defences led in increased larval development. In a study, the gut bacteria in three genera *Stenotrophomonas*, *Pseudomonas*, and *Enterobacter* were incriminated for defence subduing. Hammer and Bowers (2015) suggested the 'gut microbial facilitation hypothesis', saying that differences in insects in their ability to feed chemically protected plants can be due to the differences in their related microbial groups. Such reports have worn help from molecular research on gut bacteria. In Japanese common stink bug, *Megacopta punctatissima* (Montandon), the gut bacteria are competent of decarboxylating oxalate, a minor metabolite common in plants (Nikoh et al. 2011). Bacteria nursing in the gut of the mountain pine beetles is related with the terpene metabolism (Adams et al. 2013) and are efficient in detoxification of terpenes in vitro (Boone et al. 2013). From the midguts of gypsy moth larvae, the *Acinetobacter* species are able to detoxify the dietary phenolic glycosides (Mason et al. 2014). With the existence of gut bacteria largely in the insect mouth secretions, these may be linked with seizing of plant defence reactions in other instances of insect-plant interplay.

1.4 Insect Responses to Artificially Induced Plant Defence Mechanisms

With a contagious long history in the USA, the Hessian fly (HF), Mayetiola destructor (Say) (Cecidomyiidae: Diptera), is a major pest of wheat and spread in North Africa, Europe, West and Central Asia, North America, and New Zealand (Buntin and Chapin 1990). It has been effectively controlled using insect-resistant varieties bearing HF-specific R-gene(s). Nevertheless, in 6–8 years, virulent biotypes of HF are competent of defeating its resistance (Chen et al. 2009; Stuart et al. 2012). After egg-hatching, the neonate HF larvae creep on the upper surface of leaves and go to the seedling, where it continues feeding in susceptible cultivars but flops to do so in resistant genotypes. HF carrying virulent biotypes on a susceptible variety lead to a suitable interaction obliging pest elite; however, a virulent biotype in 3–5 days on the resistant variety causes incompatible interplay and death of insect (Subramanyam et al. 2015).

About 35 resistance genes (H1–H3, h4, H5–H34, and Hdic) from wheat and associated crop plants have been distinguished and incorporated in wheat varieties grown commercially (Chen et al. 2006; Stuart et al. 2012). For study of gene-for-gene (GNG) interaction, the HF wheat system is regarded as a model process between host and the insect (Hatchett and Gallun 1970; Subramanyam et al. 2015). Plants react to the injury of HF larvae by gathering of reactive oxygen species (Liu et al. 2010) in resistant varieties having R genes and the making of inhibitor enzymes

(Wu et al. 2008), lectins (Williams et al. 2002; Subramanyam et al. 2008), and other minor metabolites (Liu et al. 2007). Contrary to this, the adaptable interactions are distinguished by enhanced nutrient accessibility at the place of injury along with the gathering of nitrogen-rich molecules (Liu et al. 2007; Williams et al. 2011). Reports mention that the HF is competent to control resistance via recessive mutations in similar avirulence (HFAvr) genes (Aggrawal et al. 2014). The HFAvr genes code for proteins known as effectors that are inserted with the saliva into the plant tissues during the injury (Hogenhout et al. 2009). Plants bearing R genes can identify such secretions and excite the defensive routes (Chisholm et al. 2006). In virulent HF biotypes, the Avr proteins are adapted to either prevent discovery by the plant or fail to activate the defending mechanism (Chen et al. 2016).

Darwin in his magnum opus *On the Origin of Species* in 1859 mentioned that the 'Coadaptations of organic beings to each other…'. Organisms interact with each other with either the similar or another type of animal. Coevolution relates to genetic swap in two interacting animal species. Ehrlich and Raven (1964) reported the first interaction between Monarch butterfly and milkweed (a host plant). Neither any plant is susceptible to all the herbivores nor is any insect a pest of all plants it experience in environment. Additionally, less than one-third of all insects such as exclusively Lepidoptera, Orthoptera, and Phasmida; predominantly Hemiptera and Thysanoptera; or partially Coleoptera, Diptera, and Hymenoptera are plant pests; however, these belong to half of all herbivores. So mostly all crop plants have evolved having impressive structural and biochemical defensive mechanisms against the insect pests. However, those insects which are competent to break these barricades in one or more plants can reach these plants for feeding (Arora 2012). Insect pests continue evolving adaptations for detoxification or breaching such defensive systems. The results of a coevolutionary system since 400 My are the extant phytophages and their host plants (Labandeira 2013). For discerning their ecosystem, determining the sensory input, and reacting to it appropriately, insects have developed a sensitive biological system (Martin et al. 2011). Lucrative host locating and liking are basically governed by chemical signals. The insect reactions rely on host and environmental conditions (Riffell et al. 2009; Webster et al. 2010). Plants have also developed many structural and chemical defence mechanisms against insects. The insects one after another have developed to prevent these barricades, and many ideas have been put forward.

1.4.1 Coevolution

Theory of coevolution was detailed by Ehrlich and Raven (1964) and backed by Berenbaum (1983) later on. Many plants produce a prototypical phytochemicals according to this theory that is balmily poisonous to phytophages and active in the plant autecologically or physiologically. Few insect species attack on plants with mild phytochemicals, thus reducing plant strength. Due to plant mutations, their

recombinants produce novel, more noxious phytochemicals to occur in the plant system. Similar phytochemicals can occur independently in distantly associated plants. Insect attack is decreased because of toxic or repellent characteristics of the novel phytochemicals, so crop plants with higher potent defences are liked by the herbivores. *Phyllobrotica* species infest monogamously on *Scutellaria* species as reported by the cladograms (Farrell and Mitter 1990). Evidence is also available at the level of populations. Analysis reports of various populations of wild parsnip, *Pastinaca sativa* Linnaeus, and its pest the parsnip webworm, *Depressaria pastinacella* Duponchel, described trait matching between furanocoumarin-based chemical defence mechanisms in plants and cytochrome P450 monooxygenase-based insects' detoxification profiles (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003).

Coevolution is well defined in the brassicaceous plants and the pierid butterflies. The glucosinolate-myrosinase system developed in Brassicales 90 Myr before shows a major finding in anti-herbivore defence devices by plants. However, the Pierinae butterflies which used Fabales as host showed a metabolizing process as nitrile-specifier protein (NSP) and colonized the Brassicales. This resulted in increasing the species diversification rates in Pierinae as compared with that of their sister clade Coliadinae, whose members did not colonize Brassicales, thus lending strong support to the coevolutionary theory (Wheat et al. 2007; Edger et al. 2015).

1.4.2 Sequential Evolution Theory

The evolution of herbivores that comes after the evolution of plants, without affecting plant evolution significantly, is proposed by the theory of sequential evolution (Jermy 1976, 1984). So far reciprocal selective interactions between plants and insects have not been demonstrated. Insects select their hosts generally on the basis of chemical signals. Any alterations in chemical composition of plants or their chemosensory perception by herbivores may result in the development of new associations of insects and host plants. More evidence in support of the theory was presented by Labandeira (1998) and Janz et al. (2006), who showed that species richness in butterfly family Nymphalidae was strongly correlated with diversity of host use.

1.4.3 Diffuse Coevolution Theory

Diffuse coevolution or community coevolution theory suggests that in lieu of the pairwise reciprocal evolutionary interactions, coevolution must be regarded in a community context and not simply as a reciprocal interaction of two different species. Plant may be influenced by herbivore abundance, diseases, competing conspecifics, and plants of different species along with different host plants and insect pests and organisms at higher tropic levels (Fox 1988).

1.4.4 Geographic Mosaic Theory of Coevolution

This theory expresses that the coevolutionary system works at the level of populations rather than at a species level. Thompson (1994, 1999, 2005) stated that interspecific interactions frequently vary in biodiverse system. Additionally, populations vary in the extent to exhibit extreme specialization to one or more species. Gene flow among species, genetic drifting, novel trait selections, and extinction of some species reform the geographic mosaic of coevolution as the adaptations and specialization designs evolved locally, distributed to other population, or are finished. The result is a dynamic geographic pattern of coevolution between any two or more species. Across the Japanese islands, the coevolutionary relationship between the obligate seed predator; the camellia weevil, *Camellia japonica* Linnaeus; and its host plant, the Japanese camellia, *Camellia japonica* Linnaeus, serves as an interesting instance of geographic mosaic (Toju and Sota 2006; Toju et al. 2011). The thickness of camellia pericarp through which the female weevils inserted its ovipositors to deposit eggs into seeds linked with the length of the rostrum in female insects. In addition, the pericarp was significantly wider on islands abundant of weevils than on islands lacking weevils, and this characteristic was genetic.

1.5 Applied Aspects of Insect-Plant Correlations

For sustainable agriculture, the complex comprehension of insect-plant correlations has great applied importance. Growers desire to reduce crop damages brought about by insect pests and enhance crop yield. The fundamental strategies of insect-plant interplays are the main to obtain these aims as below:

1.5.1 Reproducing Resistant Cultivars Against Insect Pests

The most eco-friendly are the insect-resistant varieties which deliver economically practicable and ecologically durable choices for insect pest control. For more than a century, the research on breeding of insect-resistant crops has been carried out and bloomed as a new area of studies with the innovative research of Prof. R H Painter at Kansas State University, Manhattan, Kansas, USA, in the first half of the twentieth century (Painter 1951). An excellent early victory in using host plant resistance in pest control was achieved by grafting the European grapevines onto the resistant North American rootstocks (Painter 1951) against the grape phylloxera *Daktulosphaira vitifoliae* (Fitch) in France. In India, the research of Hussain and Lal (1940) found hairy cotton varieties resistant to jassid and resistant to cultivars, for instance, Punjab 4F, LSS, and 289 F/43, grown on large agriculture areas where jassid was a major pest by 1943. Reproducing stress-resistant plants has obtained

great significance over the past 70 years with the participation of national and international agricultural research institutes along with seed producers from private sector. Dozens of insect-resistant varieties have been evolved globally and are cultivated largely for crop production sustainability (Panda and Khush 1995). Economically, insect-resistant genomes used in cropping recently rescue us more than US$2 billion every year (Smith and Clement 2012). Isolation and cloning of genomes for wanted chemical traits have accelerated the breeding of insect-resistant varieties. An advance comprehension of plant resistance reactions to herbivory is also required for further manipulation of generated resistance and plant-emitted volatile substances for breeding of insect pest-resistant cultivars (Sandhu and Arora 2013). Utilization of insect-resistant genomes from unconnected microbes and their chartering into elite germplasm is another fruit-bearing proposal which has established largely in applied research. From *Bacillus thuringiensis*, 20 Bt genes revealing resistance to lepidopteran and coleopteran pests have been merged into cotton, corn, potato, soybean, and other crops (Shera and Arora 2015).

1.5.2 Insect Pest Management Using Cultural Practices

For suppressing insect pest attack, cropping pattern and insect control measures such as cultural practices play a crucial role. The knowledge of host plant-insect pest association is beneficial to alter the plant ecosystem against the insect pest or in favour of the parasites and predators. Early crop sowing, for example, in Northern India, has been observed to decrease the gall midge and leaf folder attack in rice, shoot fly and headbug losses in sorghum and millets, white grub injury in groundnut, and aphid destruction in crucifers (Dhaliwal and Arora 2006). Enhanced intrafield diversity via interculturing, sowing of trap crops, or hedging rows led to minimum losses by many insect pests. Tomato interculturing in cabbage has been described to decrease the destruction of diamondback moth. Intercropping with sesame resulted in decreased penetration of okra roots by *Meloidogyne incognita* second-stage juveniles (J2) and delayed nematode maturation; it favoured development of *M. incognita* males and increased yields of okra and chickpea in field tests (Tanda and Atwal 1988). The largest effect of intercropping sesame with okra was when they were 15–30 cm apart. In pot tests, most J2 penetrated okra roots in sandy loam soil and fewest in clay soil. Trap crop of African marigold decreases the damage of fruit borer *H. armigera* in tomato (Srinivasan 1994). Napier grass and Napier millet work as trap crops for minimizing the attack of stem borer *C. partellus* in maize and sorghum (Khan 1999; Dhaliwal and Arora 2006). The natural enemies of herbivores may achieve larger population densities in polycultures as compared to monocultures, because polycultures often provide extra food sources, for example, honeydew, nectar and, pollen, and more refuges where insects can protect in the shade (Coll 1998). Among 130 predators observed in polycultures, more than 50% achieved higher population densities, as compared to monocultures, whereas less than 10% of them attained poor population densities when surveyed (Andow 1991).

1.5.3 Botanical Insecticides

Several crop plants have evolved tracks to diverse groups of chemicals to avoid their utilization by insect pests since many centuries. These biochemicals put forth behavioural, physiological, and biochemical impacts on insects, and some of them may even kill the sensitive insects. Bioinsecticides have been used since ancient times by humans. In various parts of the globe for centuries, many plants and their products such as neem, pyrethrum, *Tephrosia*, tobacco, derris, *Ryania*, and sabadilla have been used to save the farm crops, grains, and other commodities from the devastations of insects and other types of pests (Dhaliwal and Arora 2001). Plant chemicals have also acted as prototypes for the evolution and synthesis of Nobel categories of insecticides. *Pyrethrum*, isolated from the dried flowers of *Chrysanthemum cinerariaefolium* Linnaeus, has been utilized as an important insecticide since prehistoric times. It is a powerful mephitic against insects and relatively harmless to mammals; however, it is very photolabile (Casida 1973). Consequently, the chemical structure of pyrethrum was explicated to synthesize its analogues with better photostability. During the 1980s, several chemicals such as fenvalerate, deltamethrin, fluvalinate, and cyfluthrin rose as famous insecticides (Dhaliwal and Arora 2006). Likewise, similar synthetic analogues of nicotine, an important bioinsecticide named as neonicotinoids, was developed from tobacco and now largely utilized against a variety of sucking insect pests and mites (Simon-Delso et al. 2015). Accordingly, bioinsecticides have not only demonstrated beneficial directly in pest management but have also worked as a model for the modern groups of synthetic insecticides. As plants possess tens of thousands of similar biochemicals, the compass of their utility in insect pest control is almost unlimited.

1.5.4 Insect Pest Biocontrol

For enhancing the effectiveness of natural biocontrol and integrated pest management technology (IPMT) as we proposed, the significance of research on tritrophic and multitrophic interplays can scarcely be aggravated. Volatile substances released by plants are demonstrated to allure predators of herbivores (Weseloh 1981). Ramachandran et al. (1991) described that the parasitoid *Microplitis demolitor* Wilkinson was captivated by the volatile 3-octanone liberated by soybean, a host plant of the soybean looper, *P. includens*. Parasitoid was distinctly more apprehended by the volatile guaiacol, which was located in its hosts' frass only; however, similar interactions may not benefit the predators. Hare (1992) observed a gamut of interactions between the predators and resistant plants such as synergistic to additive to none apparent and disruptive or antagonistic effects. A meta-analysis of 27 studies on interaction of resistant plant varieties and biocontrol of insect pests was carried out by Dhaliwal et al. (2004). About 29.6% showed antagonistic effect, whereas 25.9 and 33.3% were the cases of synergism and additive relationship, respectively. As understanding of multitrophic interactions enlarges, scientists and IPMT research workers should use it for insect pest control strategies (Verkerk 2004).

1.5.5 Use of Insect Behaviour in Control

In response to olfactory, visual, tactile, acoustic, and gustatory-sensory reaction from the host plant and ecosystem, insect behaviour is educed. For feeding and oviposition on host plants, cues are used by insects which can assist in the exploitation of similar behaviour, resulting in decreased crop losses (Foster and Harris 1997). In pest control, alluring and killing technique is the most famous behavioural manipulation manipulated. The attack of Japanese beetle *Popillia japonica* Newman is effectively controlled by a mixture of phenethyl propionate, eugenol, and geraniol, a female sex pheromone, and a food lure (Ladd et al. 1981). Foods baits have also been reported beneficial for monitoring and managing of tephritids. In the USA, protein hydrolysate-baited traps having insecticides have been proved effective against the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Chambers 1978). Attraction and annihilation, which are innovative techniques, have resulted successfully against the apple maggot fly, *Rhagoletis pomonella* (Walsh). Using olfactory and visual stimuli, the female flies search apple plants and acceptable oviposition locations. Wooden spheres in red colour and layered with a sticky material at one trap tree−1 provided a better control of fruits from *R. pomonella* (Aluja and Prokopy 1993; Foster and Harris 1997).

1.5.6 Insect Control by Push-Pull Mechanism

The push-pull IPMT or stimulo-deterrent proposal in pest control is a new manipulation of the behavioural technology for the use of pulling and pushing constituents in sequence to repel the insect away from the principal host plant and towards the crop used for trapping, from where they may be detached later on (Khan et al. 1997; Cook et al. 2007). This approach has been effectively practicable for the control of stem borers such as *C. partellus*, *Eldana saccharina* Walker, *Busseola fusca* Fuller, and *Sesamia inferens* Hampson attacking maize and sorghum in Eastern and Southern Africa. The egg-laying female borers are repulsed from the main crop by repellent non-host intercrops, especially molasses grass, silver leaf desmodium, or green leaf desmodium (push), and like to deposit eggs on alluring trap crop plants, basically Napier grass or Sudan grass (pull). Interculturing of molasses grass with maize enhanced parasitization by *Cotesia sesamiae* Cameron in addition to decreasing the damages of stem borer (Khan et al. 2011). Push-pull plans have also been efficiently used against *Helicoverpa* in cotton, *L. decemlineata* in potato, rapeseed pollen beetle *Brassicogethes aeneus* (Fabricius) in oilseed rape, onion maggot *Anthomyia antiqua* (Meigen) in onions, striped pea leaf weevil *Sitona lineatus* (Linnaeus) in beans, western flower thrips, *Frankliniella occidentalis* (Pergande) in chrysanthemum, and bark beetles (Scolitidae) in conifers, along with many veterinary and medical insect pests (Cook et al. 2007).

1.5.7 Insect Biotype Control

Insect biotypes are insects that can live on and damage cultivars that have resistant genes. The cultivation of insect-resistant varieties regularly applies selection pressure on the aimed pest, which reacts by evolving new physiological and behavioural tools for feeding and multiplication on the resistant varieties. In breeding schemes for cultivar resistance, biotype selection is one of the key pressures experienced. This idea concerns gene-for-gene connection between the host plant-resistant gene and insect pest virulent gene. Aphids have 18 species out of 39, in which 2 or more biotypes have been observed (Sandhu and Arora 2013). Brown plant hopper, *Nilaparvata lugens* Stal, on rice (Brar et al. 2015) and Hessian fly, *M. destructor*, on wheat are the key pests in which biotype evolution has resulted in to collapse of resistance in the field (Aggrawal et al. 2014; Subramanyam et al. 2015). Insect resistance can be enhanced by sequential release of varieties, gene pyramiding/stacking, and gene rotation (Sandhu and Arora 2013). A better advance knowledge of insect-plant interactions is important for effective control of insect biotypes for higher resistant genotype stability.

1.5.8 Biocontrol of Weeds

The damages created by weeds are more than those done by insect pests to field crops, and the herbicide utilization surpasses that of insecticides in plant protection programs, so there is an immediate demand to reinforce biological management of weeds. Exotic weeds may be effectively controlled by monophagous or oligophagous insects from the origin of plant place. Prominent successful weed control instances are of shellmound prickly pear, *Opuntia stricta* (Haworth) Haworth, in Australia using small Argentinian moth, *Cactoblastis cactorum* Berg (Dodd 1940), and of giant *Salvinia*, *Salvinia molesta* D. S. Mitchell, in Papua New Guinea by the release of weevil *Cyrtobagous salviniae* Calder & Sands imported from Brazil (Room 1990). Extensive schemes on biocontrol of weeds in Hawaii using herbivorous insects and pathogens have been carried out, leading in effective management of 7 out of 21 aimed weeds and significant partial management of another 3 species (Gardner et al. 1995; McFadyen 2003). For the management of native weeds, native insects have also been artificially reared and released. Native coccids, *Austrotachardia* sp. and *Tachardia* sp., are released for the suppression of *Cassinia* sp., native woody shrubs in Australia (Holtkamp and Campbell 1995). For controlling the parasitic weeds, *Orobanche* spp. in the southern USSR, the stem-boring agromyzid *Phytomyza orobanchia* Kaltenbach has been conserved and released (Kroschel and Klein 1999).

1.5.9 Insect Pollinator Protection for Crop Production

Crop insect pollinators are crucial for effective pollination and reproduction by a large variety of angiosperms (Tanda 2019a, b, c, 2020, 2021a, b, c, d, e, f). Even self-pollinating cotton crop species may exhibit yield boosting close to an efficient pollinator and its ecosystem (Tanda 1983, 1984, 2020, 2021a, b, c, d, e, f). Coffee shrubs present great yield enhancement in areas with strong native or introduced bee pollinator abundance (Roubik 2002; Tanda 2021a, b). Several investigations on plant-pollinator processes have targeted on a single plant species and mostly one or a few closely related groups. However, new reports have demonstrated that pollinator networks are comparatively vague, due to spatiotemporal differences in foraging by pollinators (Herrera 1996; Waser 1998; Burkle and Alarcon 2011). It is crucial to comprehend the fundamentals of spatial and temporal difference in plant-pollinator interplay to reply questions in group structure and its functioning. It will also be beneficial in designing optimal pollinator's protection measures (Burkle and Alarcon 2011). Environment and habitat alteration may disorder the coevality between the flower development period of plants and the active time of pollinators. Lack of nectar and pollen in crucial time may result in a decrease in pollinator's abundance (Hoover et al. 2012; Sharma et al. 2014). An accurate knowledge of the flowering crop plant and insect pollinator interactions may be beneficial in avoiding pollinator reduction and sustainable agricultural production.

1.6 Conclusions

In the agricultural ecosystem, both insects and crop plants are ruling life forms and are engaged in complex interrelationships. For effective reproduction, a large number of flowering plants need the services of pollinators. The flower shape, size, colour, and scent all serve to allure bee pollinators, which generally feed on nectar and pollen developed by these crop plants. Additionally, about 50% of all insect populations are herbivorous and rely on green plants for food, shelter, and egg deposition. Accordingly, the plants have developed a surprising diversity of structural and biochemical barricades to save themselves from insect pests and plant diseases. The insects which are capable to manage these barriers via avoidance, detoxification, and sequestration can obtain sufficient food supplies with very small competition from other insect species. Since 400 million years ago, reciprocal modification and counterconversion between plants and insects have, thus, been the main procedure driving a steady enhancement in biodiversity of both these life forms. The investigations of such interrelationships between insects and flowering plants are of great practical significance for future sustainable agricultural productivity. Genomics, proteomics, and RNAi, advanced bio-techniques of molecular biology, provide exciting breaks for future exploration and accurate comprehension of insect-plant relationships. These biotechnological studies are important for the protection of ecosystem biodiversity and evolving insect-resistant cultivars for sustainable insect pests and weed control system.

References

Abe M, Matsuda K (2000) Feeding responses of four phytophagous lady beetle species (Coleoptera: Coccinellidae) to cucurbitacins and alkaloids. Appl Entomol Zool 35:257–264
Adams AS, Aylward FO, Adams SM et al (2013) Mountain pine beetles colonizing historical and native host trees are associated with a bacterial community highly enriched in genes contributing to terpene metabolism. Appl Environ Micobiol 79:3468–3475
Aggrawal R, Subramanyam S, Zhao C et al (2014) Avirulence effector discovery in a plant galling and plant parasitic arthropod, the Hessian fly (Mayetiola destructor). PLoS One 9(6):e100958
Agrawal AA, Konno K (2009) Latex: a model for understanding mechanisms, ecology, and evolution of plant defence against herbivory. Annu Rev Ecol Evol Syst 40:311–331
Alborn T, Turlings TCH, Jones TH et al (1997) An elicitor of plant volatiles from beet armyworm oral secretion. Science 276:945–949
Aluja M, Prokopy RJ (1993) Host odour and visual stimulation interaction during intratree host finding behaviour of Rhagoletis pomonella flies. J Chem Ecol 19:2671–2696
Andersen JF, Walding JK, Evans PH, Bowers WS, Feyereisen R (1997) Substrate specificity for the epoxidation of terpenoids and active site topology of house fly cytochrome P450 6A1. Chem Res Toxicol 10:156–164
Andow DA (1991) Vegetation diversity and arthropod population response. Annu Rev Entomol 36:561–586
Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408(6814):796–815
Arora R (2012) Co-evolution of insects and plants. In: Arora R, Singh B, Dhawan AK (eds) Theory and practice of integrated pest management. Scientific Publications, Jodhpur, pp 49–75
Arora R, Dhaliwal GS (2004) Biochemical bases of resistance in plants to insects. In: Dhaliwal GS, Singh R (eds) Host plant resistance to insects: concepts and applications. Panima Publications, New Delhi, pp 84–125
Arora R, Sandhu S (2017) Insect-plant interrelationships. In: Arora R, Sandhu S (eds) Breeding insect resistant crops for sustainable agriculture. Springer, Singapore, pp 1–44
Atwal AS (2000) Essentials of beekeeping and pollination. Kalyani Publications, New Delhi
Auclair JC (1963) Aphid feeding and nutrition. Annu Rev Entomol 8:439–490
Axelrod DI (1960) The evolution of flowering plants. In: Tax S (ed) Evolution after Darwin, vol I. The evolution of life. University of Chicago Press, Chicago, pp 227–305
Ayasse M, Schiesl FP, Paulus HF et al (2003) Pollinator attraction in a sexually deceptive orchid by means of unconventional chemicals. Proc R Soc Lond B 270:517–522
Baker HG, Baker I (1986) The occurrence and significance of amino acids in floral nectars. Plant Syst Evol 151:175–186
Banerjee MK, Kalloo G (1989) Role of phenols in resistance to tomato leaf curl virus, Fusarium wilt and fruit borer in Lycopersicon. Curr Sci 52:575–576
Barbehenn RV (2003) Antioxidants in grasshoppers: higher levels defend the midgut tissues of a polyphagous species than a graminivorous species. J Chem Ecol 29:683–702
Barbosa P, Schulz JC (1987) Insect outbreaks. Academic, San Diego
Beck SD (1965) Resistance of plants to insects. Annu Rev Entomol 10:207–232
Berenbaum MR (1983) Coumarins and caterpillars: a case for co-evolution. Evolution 37:163–179
Berenbaum MR (1991a) Comparative processing of allelochemicals in the papilionidae (Lepidoptera). Arch Insect Biochem Physiol 17:213–221
Berenbaum MR (1991b) Coumarins. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary plant metabolites. Academic, London, pp 221–250
Berenbaum MR (1995) Turnabout is fairplay: secondary roles for primary compounds. J Chem Ecol 21:925–940
Berenbaum MR, Zangerl AR (1998) Chemical phenotype matching between a plant and its insect herbivore. Proc Natl Acad Sci U S A 95:13743–13748
Bernays EA (1986) Diet-induced head allometry among foliage chewing insects and its importance for graminivores. Science 231:495–497

Bernays EA, Chamberlain DJ (1980) A study of tolerance of ingested tannin in Schistocerca gregaria. J Insect Physiol 26:415–420
Bodnaryk RP (1992) Leaf epicuticular wax, an antixenotic factor in Brassicaceae that affects the rate and pattern of feeding of flea beetles, Phyllotreta cruciferae Goeze. Can J Plant Sci 72:1295–1303
Boone CK, Keefover-Ring K, Mapes AC et al (2013) Bacteria associated with a tree-killing insect reduce concentrations of plant defence compounds. J Chem Ecol 39:1003–1006
Bottger GT, Sheechan ET, Lukefahr MJ (1964) Relation of gossypol of cotton plants to insect resistance. J Econ Entomol 57:283–285
Brar DS, Sarao PS, Singh KS, Jena KK, Fujita D (2015) Biotechnological approaches for enhancing resistance to planthoppers in rice. In: Singh B, Arora R, Gosal SS (eds) Biological and molecular approaches in pest management. Scientific Publications, Jodhpur, pp 13–38
Bridges M, Jones AME, Bones AM et al (2002) Spatial organization of the glucosinolate myrosinase system in brassica specialist aphids is similar to that of the host plant. Proc R Soc Lond B 269:187–191
Brioschi D, Nadalini LD, Bengtsonb MH et al (2007) General up regulation of Spodoptera frugiperda trypsins and chymotrypsins allows its adaptation to soybean proteinase inhibitor. Insect Biochem Mol Biol 37:1283–1240
Bruce TJA (2015) Interplay between insects and plants: dynamic and complex interactions that have coevolved over millions of years but act in milliseconds. J Exp Bot 66:455–465
Bull DL, Ivie GW, Beier RC et al (1986) In vitro metabolism of a linear furanocoumarin (8-methoxypsoralen, xanthotoxin) by mixed-function oxidases of larvae of black swallowtail butterfly and fall armyworm. J Chem Ecol 12:885–892
Buntin DG, Chapin JW (1990) Biology of Hessian fly (Diptera: Cecidomyiidae) in the Southeastern United States: geographic variation and temperature-dependent phenology. J Econ Entomol 83:1015–1024
Burkle LA, Alarcon R (2011) The future of plant-pollinator diversity: understanding interaction networks across time, space and global change. Am J Bot 98:528–538
Casida JE (ed) (1973) Pyrethrum: the natural insecticide. Academic, New York
Chambers DL (1978) Attractants for fruit fly survey and control. In: Shorey HH, Mckelvey JJ (eds) Chemical control of insect behavior: theory and application. Wiley, New York, pp 327–344
Chapman RF (1974) The chemical inhibition of feeding by phytophagous insects. Bull Entomol Res 64:339–363
Chen MS, Fellers JP, Zhu YC et al (2006) A super-family of genes coding for secreted salivary gland proteins from the Hessian fly, Mayetiola destructor. J Insect Sci 6:12
Chen MS, Echegaray E, Whitworth RJ et al (2009) Virulence analysis of Hessian fly populations from Texas, Oklahoma and Kansas. J Econ Entomol 102:774–780
Chen M-S, Liu S, Wang H et al (2016) Genes expressed differentially in Hussian fly larvae feeding in resistant and susceptible plants. Int J Mol Sci 14(8):1324. https://doi.org/10.3390/ijms17081324
Chhabra KS, Kooner BS, Sharma AK et al (1990) Sources of resistance in chickpea: role of biochemical components on incidence of gram pod borer, Helicoverpa armigera (Hubner). Indian J Entomol 52:423–430
Chisholm ST, Cooker G, Day B et al (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814
Chiu TL, Wen Z, Rupasinghe SG et al (2008) Comparative molecular modelling of an Anopheles gambiae CYP6Z1, a mosquito P450 capable of metabolizing DDT. Proc Natl Acad Sci U S A 105:8885–8860
Chow JK, Akhtar Y, Isman MB (2005) The effects of larval experience with a complex plant latex on subsequent feeding and oviposition by the cabbage looper moth: Trichoplusia ni (Lepidoptera: Noctuidae). Chemoecology 15:129–133
Chuang WP, Herde M, Ray S et al (2014) Caterpillar attack triggers accumulation of toxic maize protein RIP2. New Phytol 201:928–939

Chung SH, Rosa C, Scully ED et al (2013) Herbivore exploits orally secreted bacteria to suppress plant defences. Proc Natl Acad Sci U S A 110:15728–15733
Cianfrogna JA, Zangerl AR, Berenbaum MR (2002) Dietary and developmental influences on induced detoxification in an oligophage. J Chem Ecol 28:1349–1364
Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol Monogr 53:209–233
Coll M (1998) Parasitoid activity and plant species composition in intercropped systems. In: Pickett CH, Bugg RL (eds) Enhancing biological control: habitat management to promote natural enemies of agricultural pests. Univ California Press, Berkeley, pp 85–119
Cook SM, Khan ZR, Pickett JA (2007) The use of push-pull strategies in integrated pest management. Annu Rev Entomol 52:375–400
Cortes-Cruz M, Snook M, McMullen MD (2003) The genetic basis of C-glycosyl flavone B-ring modification in maize (Zea mays L.) silks. Genome 46:182–194
Cox PA (1991) Abiotic pollination: an evolutionary escape for animal-pollinated angiosperms. Philos Trans R Soc B 333:217–224
Crepet WL, Friis EM, Nixon KC (1991) Fossil evidence for the evolution of biotic pollination. Philos Trans R Soc B 333:187–195
Damle MS, Giri AP, Sainani MN et al (2005) Higher accumulation of proteinase inhibitors in flowers than leaves and fruits as a possible basis for differential feeding preference of Helicoverpa armigera on tomato (Lycopersicon esculentum Mill, Cv. Dhanashree). Phytochemistry 66:2659–2667
Danielson PB, Maclnytre RJ, Fogleman JC (1997) Molecular cloning of a family of xenobiotic inducible drosophilid cytochrome P450s: evidence for involvement in host-plant allelochemical resistance. Proc Natl Acad Sci U S A 94:10797–10802
De Leo F, Volpicella M, Licciulli F et al (2002) Plant-PIs: a database for plant protease inhibitors and their genes. Nucleic Acids Res 30:347–348
Dhaliwal GS, Arora R (2001) Role of phytochemicals in integrated pest management. In: Koul O, Dhaliwal GS (eds) Phytochemical biopesticides. Harwood, Amsterdam, pp 97–118
Dhaliwal GS, Arora R (2006) Integrated pest management: concept and approaches. Kalyani Publications, New Delhi
Dhaliwal GS, Singh R, Jindal V (2004) Host plant resistance and insect pest management: progress and potential. In: Dhaliwal GS, Singh R (eds) Host plant resistance to insects. Panima, New Delhi, pp 517–558
Dimock MH, Kennedy GG (1983) The role of glandular trichomes in the resistance of Lycopersicon hirsutum f. glabratum to Heliothis zea. Entomol Exp Appl 33:263–268
Dixon RA, Strack D (2003) Phytochemistry meets genome analysis, and beyond. Phytochemistry 62:815–816
Dodd AP (1940) The biological campaign against prickly-pear. Commonwealth Prickly Pear Board, Brisbane
Dunaevsky YE, Elpidina EN, Vinokurov KS et al (2005) Protease inhibitors in improvement of plant resistance to pathogens and insects. Mol Biol 39:702–708
Dussourd DE (1995) Entrapment of aphids and whiteflies in lettuce latex. Ann Entomol Soc Am 88(2):163–172
Edger PP, Heidel-Fischer HM, Bekaert M et al (2015) The butterfly plant arms-race by gene and genome duplications. Proc Natl Acad Sci U S A 112:8362–8366
Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in co-evolution. Evolution 18:586–608
Eigenbrode SD (2004) The effects of plant epicuticular waxy blooms on attachment and effectiveness of predatory insects. Arthropod Struct Dev 33:91–102
Eigenbrode SD, Espelie KE (1995) Effects of plant epicuticular lipids on insect herbivores. Annu Rev Entomol 40:171–194
Eigenbrode SD, Kabalo NN, Stoner KA (1999) Predation, behavior and attachment by Chrysoperla plarabunda larvae on Brassica oleracea with different surface waxblooms. Entomol Exp Appl 90:225–235

Enayati AA, Ranson H, Hemingway J (2005) Insect glutathione transferases and insecticides resistance. Insect Mol Biol 14:3–8
Facchini PJ (2001) Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation, and metabolic engineering applications. Annu Rev Plant Physiol 52:29–66
Faegri K, Pijl LV (1971) The principles of pollination ecology. Pergamon Press, New York
Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5–51
Fahn A (2000) Structure and function of secretory cells. In: Hallahan DL, Gray JC (eds) Plant trichomes. Academic, New York, p 37
Farrell B, Mitter C (1990) Phylogenesis of insect/plant interactions: have Phyllobrotica leaf beetles (chrysomelidae) and the lamiales diversified in parallel? Evolution 44:1389–1403
Fatouros NE, Broekgaarden C, Bukovinszkine'Kiss G et al (2012) Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels. PLoS One 7(8):e43607. https://doi.org/10.1371/journal.pone.0043607
Felton GW (2005) Indigestion is a plant's best defence. Proc Natl Acad Sci U S A 102:18771–18772
Felton GW, Broaduray RM, Duffey SS (1989) Inactivation of protease inhibitor activity by plant derived quinones, complications for host-plant resistance against noctoid herbivore. J Insect Physiol 35:981–990
Ferry RL, Cuthbert FP Jr (1975) A tomato fruit worm antibiosis in Lycopersicon. Hortic Sci 10:46
Feyereisen R (2006) Evolution of insect P450. Biochem Soc Trans 34:1252–1255
Foster SP, Harris MO (1997) Behavioral manipulation methods for insect pest-management. Annu Rev Entomol 42:123–146
Fox LR (1988) Diffuse co-evolution within complex communities. Ecology 69:906–907
Fraenkel GS (1959) The raison d'etre of secondary plant substances. Entomol Exp Appl 12:473–486
Francis G, Kerem Z, Makkar HPS et al (2002) The biological action of saponins in animal systems: a review. Br J Nutr 88:587–605
Francis F, Vanhaelen N, Haubruge E (2005) Glutathione S-transferases in the adaptation to plant secondary metabolites in the Myzus persicae aphid. Arch Insect Biochem Physiol 58:166–174
Frelichowski JE Jr, Juvik JA (2001) Sesquiterpene carboxylic acids from a wild tomato species affect larval feeding behavior and survival of Helicoverpa zea and Spodoptera exigua (Lepidoptera: Noctuidae). J Econ Entomol 94:1249–1259
Frey M, Schullehner K, Dick R et al (2009) Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants. Phytochemistry 70:1645–1651
Furstenberg-Hagg J, Zagrobelnby M, Bak S (2013) Plant defence against herbivores. Int J Mol Sci 14:10242–10297
Galai N, Salles J-M, Settele J et al (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 68:810–821
Gardner DE, Smith CW, Markin GP (1995) Biological control of alien plants in natural areas of Hawaii. In: Delfosse ES, Scott RR (eds) Proceedings of the 8th international symposium on biological control of weeds. CSIRO, Melbourne, pp 35–40
Gershenzon J, Croteau R (1991) Terpenoids. In: Rosenthal GS, Berenbaum MR (eds) Herbivores: their interaction with secondary plant metabolites. Academic, London, pp 165–220
Geyter ED, Lambert E, Geelen D et al (2007) Novel advances with plant saponins as natural insecticides to control pest insects. Pest Technol 1:96–105
Gieselhardt S, Yoneya K, Blenn B et al (2013) Egg laying of cabbage white butterfly (Pieris brassicae) on Arabidopsis thaliana affects subsequent performance of the larvae. PLoS One. https://doi.org/10.1371/journal.pone.0056991
Glas JJ, Schimmel BCJ, Alba JM et al (2012) Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int J Mol Sci 13:17077–17103
Gorb EV, Gorb SN (2002) Attachment ability of the beetle Chrysolina fastuosa on various plant surfaces. Entomol Exp Appl 105:13–28
Green MB, Hedin PA (1986) Natural resistance of plants to pests: role of allelochemicals. In: ACS Symp Ser 296, American Chemical Society, Washington, DC

Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 57:303–333
Hammer TJ, Bowers MD (2015) Gut microbes may facilitate insect herbivory of chemically defended plants. Oecologia 179:1–14
Hanover JW (1975) Physiology of tree resistance to insects. Annu Rev Entomol 20:75–95
Harborne JB (1993) Introduction to ecological biochemistry. Academic, London
Harborne JB (1994) Phenolics. In: Mann J, Davidson RS, Hobbs JB, Banthorpe DB, Harborne JB (eds) Natural products: their chemistry and biological significance. Longman, Harlow, pp 362–388
Hare DJ (1992) Effects of plant variation on herbivore-enemy interactions. In: Fritz RS, Simms EL (eds) Plant resistance to herbivores and pathogens. Univ of Chicago Press, Chicago, pp 278–298
Harris P (1974) A possible explanation of plant yield increases following insect damage. Agro Ecosyst 1:219–225
Hatchett JH, Gallun RL (1970) Genetics of the ability of the Hessian fly, Mayetiola destructor to survive on wheat having different genes for resistance. Ann Entomol Soc Am 63:1400–1407
Herrera CM (1996) Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In: Lloyd DG, Barrett SCH (eds) Floral biology: studies on floral evolution in animal pollinated plants. Chapman & Hall, New York, pp 65–87
Hilker M, Meiners T (2006) Early herbivore alert: insect eggs induce plant defence. J Chem Ecol 32:1379–1397
Hill DL (1997) The economic importance of insects. Chapman & Hall, London
Hogenhout SA, Bos JIB (2011) Effector proteins that modulate plant-insect interactions. Curr Opin Plant Biol 14:422–428
Hogenhout SA, Vabder Hoorn RAL, Terauchi R et al (2009) Emerging concepts in effector biology of plant-associated organisms. Mol Plant-Microbe Interact 22:115–122
Holtkamp RH, Campbell MH (1995) Biological control, of Cassinia spp. (Asteraceae). In: Delfosse ES, Scott RR (eds) Proceedings of the 8th international symposium on biological control of weeds. CSIRO, Melbourne, pp 447–450
Hoover SER, Ladly JJ, Shchepetkine AR et al (2012) Warming, CO2, and nitrogen deposition interactively affect a plant-pollinator mutualism. Ecol Lett 15:227–234
Hopkins RJ, Van Dam NM, Van Loon JJA (2009) Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annu Rev Entomol 54:57–83
House HL (1961) Insect nutrition. Annu Rev Entomol 6:13–26
Huang T, Jander G, De Vos M (2011) Non-protein amino acids in plant defence against insect herbivores: representative cases and opportunities for further functional analysis. Phytochemistry 72:1531–1537
Huber M, Epping J, Gronover CS et al (2016) A latex metabolite benefits plant fitness under root herbivore attack. PLoS Biol 14(1):e1002332. https://doi.org/10.1371/journal.pbio.1002332
Hussain MA, Lal KB (1940) The bionomics of Empoasca devastens (Distant) on some varieties of cotton in the Punjab. Indian J Entomol 2:123–136
Janz N, Nylin S, Wahlberg N (2006) Diversity begets diversity: host expansions and the diversification of plant-feeding insects. BMC Evol Biol 6:4. https://doi.org/10.1186/1471-2148-6-4
Jeffree CE (1986) The cuticle, epicuticular waxes and trichomes of plants, with reference to their structure, functions and evolution. In: Juniper BE, Southwood TRE (eds) Insects and the plant surface. E. Arnold, London, pp 23–64
Jermy T (1976) Insect-host plant relationship-coevolution or sequential evolution? Symp Biol Hung 16:109–113
Jermy T (1984) Evolution of insect/plant relationships. Am Nat 124:609–630
Johanson B (1953) The injurious effects of the hooked epidermal hairs of the French beans (Phaseolus vulgaris L.) on Aphis craccivora Koch. Bull Entomol Res 44:779–788
Johnson HB (1975) Plant pubescence: an ecological perspective. Bot Rev 41:233–258

Johnson MT (2011) Evolutionary ecology of plant defences against herbivores. Funct Ecol 25:305–311

Jongsma MA, Bakker PL, Peters J et al (1995) Adaptation of Spodoptera exigua larvae to plant proteinase-inhibitors by induction of gut proteinase activity insensitive to inhibition. Proc Natl Acad Sci U S A 92:8041–8045

Kashyap RK (1983) Studies on resistance behavior of tomato genotypes against fruit borer. Dissertation, Haryana Agricultural University, Hisar

Kaur M, Singh K, Rup PJ et al (2006) A tuber lectin from Arisaema helleborifolium Schott with anti-insect activity against melon fruit fly Bactrocera cucurbitae (Coquillett) and anti-cancer effect on human cancer cell lines. Arch Biochem Biophys 445:156–165

Kazana E, Pope TW, Tibbles L et al (2007) The cabbage aphid: a walking mustard oil bomb. Proc R Soc Lond B 274:2271–2277

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: the conservation of plant-pollinator interactions. Annu Rev Ecol Syst 29:83–112

Kennedy CEJ (1986) Attachment may be a basis for specialization in oak aphids. Ecol Entomol 11:291–300

Khan ZR (1999) Habitat management strategies for control of insect pests in Africa. In: Dhaliwal GS, Arora R, Dhawan AK (eds) Emerging trends in sustainable agriculture. Commonwealth Publications, New Delhi, pp 187–197

Khan ZR, Ampong-Nyarko K, Chiliswa P et al (1997) Inter-cropping increases parasitism of pests. Nature 388:631–632

Khan ZR, Midega C, Pittchar J et al (2011) Push-pull technology: a conservation agriculture approach for integrated management of insect pests, weeds and soil health in Africa. Int J Agric Sustain 9:162–170

Kim JH, Lee BW, Schroeder FC et al (2008) Identification of indole glucosinolate breakdown products with antifeedant effects of Myzus persicae (green peach aphid). Plant J 54:1015–1026

Klein AM, Vaissiere BE, Cane JH et al (2007) Importance of pollinators in changing landscapes for world crops. Proc R Soc Lond B 274:303–313

Krishnan N, Sehnal F (2006) Compartmentalization of oxidative stress and antioxidant defence in the larval gut of Spodoptera littoralis. Arch Insect Biochem Physiol 63:1–10

Kritsky G (2001) Darwin's Madagascan hawk moth prediction. Am Entomol 37:206–210

Kroschel J, Klein O (1999) Biological control of Orobranche spp. with Phytomyza orobranchia Kalt, a review. In: Kroschel J, Abderabihi M, Betz H (eds) Advances in parasitic weed control at on-farm level, vol 2. Joint action to control Orobranche in the WANA region. Mardarof Verlag, Weikersheim, pp 135–159

Labandeira CC (1998) Early history of arthropod and vascular plant associations. Annu Rev Earth Planet Sci 26:329–377

Labandeira CC (2013) A paleobiologic perspective on plant-insect interactions. Curr Opin Plant Biol 16:414–421

Ladd TL, Klein MG, Tumlison JH (1981) Phenethyl propionate+eugenol+geraniol (3: 7: 3) and Japonilure: a highly effective joint lure for Japanese beetles. J Econ Entomol 74:665–667

Lambrix V, Reichelt M, Mitchell-Olds T et al (2001) The Arabidopsis epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences Trichoplusia ni herbivory. Plant Cell 13:2793–2807

Lawton JH (1983) Plant architecture and the diversity of phytophagous insects. Annu Rev Entomol 28:23–39

Lee YL, Kogan M, Larsen JR (1986) Attachment of the potato leafhopper to soybean plant surfaces as affected by morphology of pretarsus. Entomol Exp Appl 42:101–108

Li Q, Eigenbrode SD, Stringam GR et al (2000) Feeding and growth of Plutella xylostella and Spodoptera eridania on Brassica juncea with varying glucosinolate concentrations and myrosinase activities. J Chem Ecol 26:401–2419

Liener IE (1991) Lectins. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary plant metabolites. Academic, London, pp 327–354

Liu X, Bai J, Li H et al (2007) Gene expression of different wheat genotypes during attack by virulent and avirulent Hessian fly (Mayetiola destructor) larvae. J Chem Ecol 33:2171–2194

Liu X, Williams CE, Nemacheck JA et al (2010) Reactive oxygen species are involved in plant defense against a gall midge. Plant Physiol 152:985–999
Lorenzen JH, Belbyshev NE, Lafta AM et al (2001) Resistant potato selections contain leptine and inhibit development of Colorado potato beetle (Coleoptera: Chrysomelidae). J Econ Entomol 94:1260–1267
Louda S, Mole S (1991) Glucosinolates: chemistry and ecology. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary plant metabolites. Academic, London, pp 124–164
Ma R, Cohen MB, Berenbaum MR et al (1994) Black swallowtail (Papilio polyxenes) alleles encode cytochrome P450s that selectively metabolize linear furanocoumarins. Arch Biochem Biophys 310:332–340
Mao YB, Cai WJ, Wang JW et al (2007) Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nat Biotechnol 25:1307–1313
Martin FA, Richard CA, Hensley SD (1975) Host resistance to Diatraea saccharalis (F) relationship of sugarcane internode hardness to larval damage. Environ Entomol 4:687–688
Martin JS, Martin MM, Bernays EA (1987) Failure of tannic acid to inhibit digestion or reduce digestibility of plant protein in gut fluids of insect herbivores: implications for theories of plant defence. J Chem Ecol 13:605–621
Martin JP, Beyerlein A, Dacks AM et al (2011) The neurobiology of insect olfaction: sensory processing in a comparative context. Prog Neurobiol 95:427–447
Mason CJ, Couture JJ, Raffa KF (2014) Plant associated bacteria degrade defence chemicals and reduce their adverse effects on an insect defoliator. Oecologia 175:901–910
McFadyen REC (2003) Biological control of weeds using exotic insects. In: Koul O, Dhaliwal GS (eds) Predators and parasitoids. Taylor & Francis, London, pp 163–183
McLaughlin LA, Niazi U, Bibby J et al (2008) Characterization of inhibitors and substrates of Anopheles gambiae CYP6Z2. Insect Mol Biol 17:125–135
Meisner J, Navon A, Zur M et al (1977) The response of Spodoptera littoralis larvae to gossypol incorporated in artificial diet. Environ Entomol 6:243–244
Mithofer A, Boland W (2012) Plant defence against herbivores: chemical aspects. Annu Rev Plant Biol 63:431–450
Mohan P, Singh R, Narayanan S et al (1994) Relation of gossypol-gland density with bollworm incidence and yield in tree cotton (Gossypium arboreum). Indian J Agric Sci 64:691–696
Muller C, Brakefield PM (2003) Analysis of a chemical defence in sawfly larvae: easy bleeding targets predatory wasps in late summer. J Chem Ecol 29:2683–2694
Nabhan GP, Buchmann SL (1997) Services provided by pollinators. In: Daily GC (ed) Nature's services: societal dependence on natural ecosystems. Island Press, Washington, DC, pp 133–150
Nepi M, Guarnieri M, Pacini E (2003) 'Real' and feed pollen of Lagerstroemia indica: ecophysiological differences. Plant Biol 5:311–314
Nikoh N, Hosokawa T, Oshima K et al (2011) Reductive evolution of bacterial genome in insect gut environment. Genome Biol Evol 3:702–714
Nitao JK (1989) Enzymatic adaptation in a specialist herbivore for feeding on furanocoumarin containing plants. Ecology 70:629–625
Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? Oikos 120:321–326
Owen DF (1980) How plants may benefit from the animals that eat them? Oikos 35:230–235
Painter RH (1951) Insect resistance in crop plants. University of Kansas Press, Lawrence
Panda N, Khush GS (1995) Host plant resistance to insects. CABI, Wallingford
Pappers SM, Van Dommelon H, Van der Velde G et al (2001) Differences in morphology and reproductive traits of Galerucella nymphaeae from four host plant species. Entomol Exp Appl 99:183–191
Parde VD, Sharma HC, Kachole MS (2010) In vivo inhibition of Helicoverpa armigera gut proproteinase activation by non host plant protease inhibitors. J Insect Physiol 56:1315–1324
Parde VD, Sharma HC, Kachole MS (2012) Potential of proteinase inhibitors in wild relatives of pigeonpea against cotton bollworm/legume pod borers, Helicoverpa armigera. Am J Plant Sci 3:627–635

Parmar BS, Walia S (2001) Prospects and problems of phytochemical biopesticides. In: Koul O, Dhaliwal GS (eds) Phytochemical biopesticides. Harwood, Amsterdam, pp 133–210
Payne WW (1978) A glossary of plant hair terminology. Brittonia 30:239–255
Pellmyr O, Krenn HW (2002) Origin of a complex key innovation in an obligate insect-plant mutualism. Proc Natl Acad Sci U S A 99:5498–5502
Pfalz M, Vogel H, Kroymann J (2009) The gene controlling the Indole Glucosinolate Modifier 1 quantitative trait locus alters indole glucosinolate structures and aphid resistance in Arabidopsis. Plant Cell 21:985–999
Pillemer EA, Tingey WM (1978) Hooked trichomes and resistance of Phaseolus vulgaris to Empoasca fabae (Harris). Entomol Exp Appl 24:83–94
Platt AW, Farstad CM (1946) The reaction of wheat varieties to wheat stem sawfly attack. Sci Agric 26:231–247
Proctor M, Yeo F, Lack A (1996) The natural history of pollination. Harper Collins, London
Ram P, Singh R, Dhaliwal GS (2004) Biophysical bases of resistance in plants to insects. In: Dhaliwal GS, Singh R (eds) Host plant resistance to insects: concepts and applications. Panima Publications, New Delhi, pp 42–83
Ramachandran R, Norris DM, Phillips JK et al (1991) Volatiles mediating plant-herbivore-natural enemy interactions: soybean looper frass volatiles, 3-octanone and guaiacol, as kairomones for the parasitoid, Microplitis demolitor. J Agric Food Chem 39:2310–2317
Ramirez BW (1970) Host specificity of fig wasps (Agaonidae). Evolution 24:681–691
Rao NV, Reddy AS, Ankaish R et al (1990) Incidence of whitefly (Bemisia tabaci) in relation to leaf characters of upland plant cotton (Gossypium hirsutum). Indian J Agric Sci 60:619–624
Rector BG, Liang GM, Guo Y (2003) Effect of maysin on wild–type, deltamethrin-resistant and Bt-resistant Helicoverpa armigera (Lepidoptera: Noctuidae). J Econ Entomol 96:909–913
Riffell JA, Lei H, Christensen TA et al (2009) Characterization and coding of behaviorally significant odor mixtures. Curr Biol 19:335–340
Room PM (1990) Ecology of a simple plant-herbivore system: biological control of Salvinia. Trends Ecol Evol 5:74–79
Rosenthal GA (1991) Nonprotein amino acids as protective phytochemicals. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary plant metabolites. Academic, London, pp 1–34
Rosenthal GA, Berenbaum MR (eds) (1991) Herbivores: their interactions with secondary plant metabolites. Academic, London
Roubik DW (2002) The value of bees to the coffee harvest. Nature 417:708
Roulston TAH, Cane JH, Buckmann SL (2000) What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? Ecol Monogr 70:617–643
Ruzicka L (1953) Isoprene rule and biogenesis of terpenic compounds. Experientia 9:357–367
Sadras VO, Felton GW (2010) Mechanism of cotton resistance to arthropod herbivory. In: Stewart JM, Oosterhius D, Heitholt JJ et al (eds) Physiology of cotton. Springer, London, pp 213–228
Sahoo BK, Patnaik MP (2003) Effect of biochemicals on the incidence of pigeonpea pod borers. Indian J Plant Prot 31:105–108
Sandhu SK, Arora R (2013) Breeding for insect resistance in crop plants. In: Dhawan AK, Singh B, Bhullar MB, Arora R (eds) Integrated pest management. Scientific Publications, Jodhpur, pp 267–300
Schoonhoven LM, van Loon JJA, Dicke M (2005) Insect-plant biology. Oxford University Press, Oxford
Schuhegger R, Nafisi M, Mansourova M et al (2006) CYP71B15 (PAD3) catalyzes the final step in camalexin biosynthesis. Plant Physiol 141:1248–1254
Schuler M (1996) The role of cytochrome P450 monooxygenases in plant-insect interactions. Plant Physiol 112:1411–1419
Schumutterer H (ed) (1995) The neem tree, Azadirachta indica A. Juss. and other meliaceous plants: source of unique products for integrated pest management, medicine, industry and other purposes. VCH, Weinheim

Scott MI, Thaler SJ, Scott GF (2010) Response of a generalist herbivore Trichoplusia ni to jasmonate-mediated induced defence in tomato. J Chem Ecol 36:490–499
Seybold SJ, Huber DPW, Lee JC et al (2006) Pine monoterpenes and pine bark beetles: a marriage of convenience for defence and chemical communication. Phytochem Rev 5:143–178
Sharma S, Arora R, Singh B (2014) Impact of climate change on agriculturally important insects. J Insect Sci 27:159–188
Shera PS, Arora R (2015) Biointensive integrated pest management for sustainable agriculture. In: Singh B, Arora R, Gosal SS (eds) Biological and molecular approaches in pest management. Scientific Publications, Jodhpur, pp 373–429
Simon-Delso N, Amaral-Rogers X, Belzunces LP et al (2015) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ Sci Pollut Res 22:5–34
Singh R, Agarwal RA (1988) Role of biochemical components of resistant and susceptible cotton and okra in ovipositional preference of cotton leafhopper. Proc Indian Acad Sci (Anim Sci) 97:545–550
Sintim HO, Tashiro T, Motoyama N (2009) Response of the cutworm Spodoptera litura to sesame leaves or crude extracts in diet. J Insect Sci 9:52
Smith CM, Clement SL (2012) Molecular basis of plant resistance to arthropods. Annu Rev Entomol 57:309–328
Sogawa K, Pathak MD (1970) Mechanisms of brown planthopper (Hemiptera: Delphacidae) resistance of Mudgo variety of rice. Appl Entomol Zool 5:145–148
Springer TL, Kindler SD, Sorenson EL (1990) Comparison of pod-wall characteristics with seed damage and resistance to alfalfa seed chalcid (Hymenoptera: Eurytomidae) in Medicago species. Environ Entomol 19:1614–1617
Srinivasan K (1994) Recent trends in insect pest management in vegetable crops. In: Dhaliwal GS, Arora R (eds) Trends in agricultural insect pest management. Commonwealth Publications, New Delhi, pp 345–372
Steehius NM, van Gelder WMJ (1985) Tomato with whitefly resistance is nutritionally safe. Zaasbelangen 39:191–192
Steppuhn A, Baldwin IT (2007) Resistance management in a native plant: nicotine prevents herbivores from compensating for plant protease inhibitors. Ecol Lett 10:499–511
Stevens JL, Snyder MJ, Koener JF et al (2000) Inducible P450s of the CYP9 family from larval Manduca sexta midgut. Insect Biochem Mol Biol 30:559–568
Strong DR, Lawton JH, Southwood TRE (1984) Insects on plants: community patterns and mechanisms. Blackwell, London
Stuart JJ, Chen MS, Shukle R et al (2012) Gall midges (Hessian flies) as plant pathogens. Annu Rev Phytopathol 50:339–357
Subramanyam S, Smith DF, Clemens JC et al (2008) Functional characterization of HFR1, a high mannose N-glycan-specific wheat lectin induced by hessian fly larvae. Plant Physiol 147:412–426
Subramanyam S, Sardesai N, Minocha SC et al (2015) Hessian fly larval feeding triggers enhanced polyamine levels in susceptible but not resistant wheat. BMC Plant Biol 15:3. https://doi.org/10.1186/s12870-014-0396-y
Sutherland TD, Unnithan GC, Anderson JF et al (1998) Cytochrome P450 terpenoid hydroxylase linked to the suppression of insect juvenile hormone synthesis. Proc Natl Acad Sci U S A 95:12884–12889
Talekar NS, Tengkano W (1993) Mechanism of resistance to bean fly (Diptera: Agromyzidae) in soybean. J Econ Entomol 86:981–985
Tallamy DW, Stull J, Ehresman NP et al (1997) Cucurbitacins as feeding and oviposition deterrents to insects. Environ Entomol 26:678–683
Tamiru A, Bruce TJA, Woodcock CM et al (2011) Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. Ecol Lett 14:1075–1083
Tanda AS (1983) Assessing the role of honey bees in a field of Asiatic cotton (Gossypium arboreum L.). Am Bee J 123:593–594
Tanda AS (1984) Bee pollination increases yield of 2 interplanted varieties of Asiatic cotton (Gossypium arboretum L.). Am Bee J 124(7):539–540

Tanda AS (2019a) Entomophilous crops get better fruit quality and yield: an appraisal. Indian J Entomol 81(2):227–234
Tanda AS (2019b) Floral biology, foraging behavior and efficiency of European honey bee (Apis mellifera) in bitter gourd (*Momordica charantia* L.) pollination at Sydney Australia. Bee World. Submitted
Tanda AS (2019c) Entomofaunal effect enhances the quality and quantity in okra (*Abelmoschus esculenlum* L.) plantation. Indian J Entomol 81(1):16–17
Tanda AS (2020) Entpollinatology—a strong relationship between plants and insects for crop improvement. In: 6th Edition of Global conference on plant sciences and molecular biology (GPMB 2020) to be held on September 10-12, 2020, at Paris, France (Accepted, May 26, 2020)
Tanda AS (2021a) Why insect pollinators important in crop improvement?. Indian J Entomol (Accepted)
Tanda AS (2021b) Insect pollinators matter in sustainable world food production. Indian J Entomol (Accepted)
Tanda AS (2021c) Urbanization and its impact on native pollinators. In: The 1st international electronic conference on entomology will be held on 1st–15th July 2021 virtually
Tanda AS (2021d) Native bees are important and need immediate conservation measures: a review. In: The 1st international electronic conference on entomology will be held on 1st–15th July 2021 published in the Proceedings 1 July 2021, 68, x. https://sciforum.net/manuscripts/10523/manuscript.pdf
Tanda AS (2021e) Wild bees and their conservation. Indian J Entomol (Accepted)
Tanda AS (2021f) Biofloral phenology, Foraging Behaviour and entpollinatological effect of honey bees in Pomegranate (*Punica granatum*) fruit quality and yield. J Hortic 08:2
Tanda AS (2021g) Insect resistance and host plant relations: a milestone in sustainable crop production. Indian J Entomol (Accepted)
Tanda AS, Atwal AS (1988) Effect of sesame intercropping against the root-knot nematode (*Meloidogyne Incognita*) in okra. Nematologica 34(4):484–492
Tanda AS, Atwal AS, Bajaj YPS (1988) Antagonism of sesame to the root-knot nematode (*Meloidogyne Incognita*) on okra in tissue culture. Nematologica 34(1):78–87
Tanda AS, Atwal AS, Bajaj YPS (1989) In vitro inhibition of root-knot nematode *Meloidogyne incognita* by sesame root exudate and its amino acids. Nematologica 35:115–124
Thayumanavan B, Velusamy R, Sadasivam S et al (1990) Phenolic compounds, reducing sugars and free amino acids in rice leaves of varieties resistant to rice thrips. Int Rice Res Newsl 15:14–15
Thien LB, Azuma H, Kawano S (2000) New perspectives on the pollination biology of basal angiosperms. Int J Plant Sci 161:S225–S235
Thompson JN (1994) The co-evolutionary process. Chicago University Press, Chicago
Thompson JN (1999) Specific hypotheses on the geographic mosaic of co-evolution. Am Nat 153:S1–S14
Thompson JN (2005) Co-evolution: the geographic mosaic of co-evolutionary arms race. Curr Biol 15(24):R992–R994
Tingey WM (1984) Glycoalkaloids as pest resistance factors. Am Potato J 61:157–167
Toju H, Sota T (2006) Imbalance of predator and prey armament; Geographic clines in phenotypic interface and natural selection. Am Nat 167:105–117
Toju H, Abe H, Ueno S et al (2011) Climatic gradients of arms race coevolution. Am Nat 177:562–573
Traw MB, Dawson TE (2002) Differential induction of trichomes by three herbivores of black mustard. Oecologia 131:526–532
Uthamasamy S (1996) Biochemical basis of resistance to insects in cotton, Gossypium spp. In: Ananthakrishnan TN (ed) Proceedings of national symposium on biochemical bases of host plant resistance to insects. National Academy of Agricultural Sciences, New Delhi, pp 15–37
Vail SG (1994) Overcompensation, plant-herbivore mutualism, and mutualistic co-evolution—a reply to Mathews. Am Nat 144:534–536
Van Lenteren JC, Hua LZ, Kamerman JW et al (1995) The parasite host relationship between Encarsia Formosa (Hym., Aphelinidae) and Trialeurodes vaporariorum (Hom., Aleyrodidae).

XXVI. Leaf hairs reduce the capacity of Encarsia to control greenhouse whitefly on cucumber. J Appl Entomol 119:553–559
Velthius HWW (1992) Pollen digestion and the evolution of sociality in bees. Bee World 127:1383–1389
Verkerk RHJ (2004) Manipulation of tritrophic interactions for IPM. In: Koul O, Dhaliwal GS, Cuperus GW (eds) Integrated pest management: potential, constraints and challenges. CABI, Wallingford, pp 55–72
Vidyachandra B, Roy JK, Bhaskar D (1981) Chemical difference in rice varieties susceptible or resistant to gall midges and stem borers. Int Rice Res Newsl 6(2):7–8
Vilkova NA, Kunzetsova TL, Ismailov AL et al (1988) Effect of cotton cultivars with high content of gossypol on development of cotton bollworm Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae). Entomol Obozr 4:689–698
Volpicella M, Ceci LR, Cordewener J et al (2003) Properties of purified gut trypsin from Helicoverpa zea adapted to proteinase inhibitors. Eur J Biochem 270:10–19
Wadleigh RW, Yu SJ (1988) Detoxification of isothiocyanate allelochemicals by glutathione-S transferases in three lepidopterous species. J Chem Ecol 14:1279–1288
War AR, Sharma HC (2014) Induced resistance in plants and counter-adaptation by insect pests. In: Chandrasekar R, Tyagi BK, Guri ZZ, Reeck GR (eds) Short views on insect biochemistry and molecular biology, vol 2. International Book Mission, South India, pp 533–547
Waser NM (1998) Pollination, angiosperm speciation and the nature of species boundaries. Oikos 82:198–201
Webster B, Bruce T, Pickett J et al (2010) Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. Anim Behav 79:451–457
Werker E (2000) Trichome density and development. Adv Bot Res 31:1–36
Weseloh RM (1981) Host location by parasitoids. In: Nordland DA, Jones RJ, Lewis WJ (eds) Semiochemicals: their role in pest control. Wiley, New York, pp 79–95
Wheat CW, Vogel H, Wittstock U et al (2007) The genetic basis of plant-insect coevolutionary key innovation. Proc Natl Acad Sci U S A 104(51):201427–220431
White TCR (1978) The importance of relative food shortage in animal ecology. Oecologia 33:71–86
Wiebes JT (1979) Co-evolution of figs and their insect pollinators. Annu Rev Ecol Syst 10:1–12
Williams CM (1970) Hormonal interactions between plants and insects. In: Sondheimer E, Simeone JB (eds) Chemical ecology. Academic, New York, pp 103–132
Williams CE, Collier CC, Nemcheck JA et al (2002) A lectin-like wheat gene responds systemically to attempted feeding by avirulent first-instar Hessian fly larvae. J Chem Ecol 28:1411–1428
Williams CE, Nemacheck JA, Shukle JT et al (2011) Induced epidermal permeability modulates resistance and susceptibility of wheat seedlings to herbivory be Hessian fly larvae. J Exp Bot 62:4521–4531
Wiseman BR, Snook ME, Isenhour DJ et al (1992) Relationship between growth of corn earworm and fall armyworm larvae (Lepidoptera: Noctuidae) and maysin concentration in corn silks. J Econ Entomol 85:2473–2477
Wittstock U, Agerbirk N, Stauber EJ et al (2004) Successful herbivore attack due to metabolic diversion of a plant chemical defence. Proc Natl Acad Sci U S A 101:4859–4864
Wu JR, Baldwin IT (2010) New insights into plant responses to the attack from insect herbivores. Annu Rev Genet 44:1–24
Wu J, Liu X, Zhang X et al (2008) Differential responses of wheat inhibitor-like genes to Hessian fly, Mayetiola destructor, attacks during compatible and incompatible interactions. J Chem Ecol 34:1005–1012
Xie Y, Arnason JT, Philogene BJR et al (1992) Variation of hydroxamic acid content in maize roots in relation to geographic origin of maize germplasm and resistance to Western corn rootworm (Coleoptera: Chrysomelidae). J Econ Entomol 85:2478–2485
Yan J, Lipka AE, Schmelz EA, Buckler ES, Jander G (2015) Accumulation of 5-hydroxynorvaline in maize (Zea mays) leaves is induced by insect feeding and abiotic stress. J Exp Bot 66:593–602

Yang L, Fang Z, Dicke M et al (2009) The diamondback moth, Plutella xylostella, specifically inactivates Mustard Trypsin Inhibitor 2 (MTI2) to overcome host plant defence. Insect Biochem Mol Biol 33:55–61

Yu SJ (2000) Allelochemical induction of hormone-metabolizing microsomal monoxygenases in the Fall armyworm. Zool Stud 39:243–249

Zangerl AR, Berenbaum MR (2003) Phenotype matching in the wild parsnip and parsnip webworms: causes and consequences. Evolution 57:806–815

Zavala JA, Patankar AG, Gase K et al (2004) Manipulation of endogenous trypsin proteinase inhibitor production in Nicotiana attenuata demonstrates their function as antiherbivore defences. Plant Physiol 134:1181–1190

Zhu-Salzman K, Luthe DS, Felton GW (2008) Arthropod-inducible proteins: broad spectrum defences against multiple herbivores. Plant Physiol 146:852–858

Chapter 2
Current Scenario of RNA Interference-Based Control of Insect and Mite Pests of Fruit Crops

Gurbachan S. Miglani, Simranpreet Kaur, Sandeep Singh, and Maninder Kaur

2.1 Introduction

Agriculture has to constantly become accustomed to growing concerns about environment in concurrence with meeting the rising demand of consumers. The human population growing rapidly generates the prerequisite for the sustainable agricultural intensification throughout the world. Most recent prognoses suggest that by the year 2050, we will need to increase the food production by more than 50% to feed the ever-growing human population that will reach nearly 10 billion people (Searchinger et al. 2018). It can be fulfilled by adoption of latest technologies and mechanization to minimize yield gaps while curtailing environmental impacts. The major limitation in accomplishing the global food demands is control of insects and mites. Crop pests and pathogens cause nearly 300 billion USD of damage every year to plant-based food supplies around the world (Gautham and Bhardwaj 2020). These insects and mites cause loss of potential yields of all agricultural crops significantly by direct or indirect effects. The direct damage consists of necrosis or deformation of plant parts and propagation of plant pathogens. However, indirect damage comprises the quality loss of harvest produce in terms of damaged fruits and increase in overall production cost of fruit crops.

In the past few decades, control of the insect and mite pests has been accompanied by the excessive use of chemical pesticides. An average of 1 billion pounds of

G. S. Miglani (✉) · M. Kaur
School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

S. Kaur
Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India

S. Singh
Department of Fruit Science, Punjab Agricultural University, Ludhiana, Punjab, India
e-mail: sandeep_pau.1974@pau.edu

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_2

active ingredient of pesticides is applied worldwide to control these insect pests on crops like cotton, corn, fruits, and vegetables (Atwood and Paisley-Jones 2017). Although pesticides are highly efficient and have low cost, yet their indiscriminate use resulted in intensifying complications like development of insect resistance against the pesticides along with secondary pest outbreaks and persistence of pesticide residue in crop products (Wytinck et al. 2020a). However, within last years, advancement in biotechnological approaches like development of transgenic crops that express *Bacillus thuringiensis* (*Bt*) Cry toxin proteins reduced utilization of pesticides in various key crops such as cotton and maize, along with economic and environmental paybacks. But nowadays resistance in pest populations has also arisen against the *Bt* toxins, and outbreaks of nontarget pests have arisen along with the shift in the host crops of pests. The management strategy comprising biological, physical, and chemical methods is usually used to manage pests of fruit crops. The excessive use of pesticides to manage the pest population results in ecological backlashes; moreover, the cost of biological and physical control approaches is comparatively high (Hakeem et al. 2016).

The consumer's growing interest in pesticide free fruits has turned the attention of scientists toward alternative eco-friendly management strategies. In order to achieve the goal of residue-free fruit production, it becomes prudent to increase the level of fruit production both qualitatively and quantitatively by mitigating biotic and abiotic constraints to the maximum possible extent. The innovative technology which has the potential of a reduced risk approach to insect pest control is RNA interference (RNAi). There is an urgent need to include novel approaches like the utilization of double-stranded ribonucleic acid (dsRNA) as a mean of gene silencing for managing pests of fruit crops which will further reinforce the integrated pest management module for the concerned pests. RNAi as an efficient gene silencing technique has been used for the management of various insect pests by several researchers (Baum et al. 2007; Bachman et al. 2013; Zheng et al. 2015; Dong et al. 2016a). Utilization of RNAi approach successfully for pest control primarily depends on an effective target gene selection. It is of great importance to find the potential target genes for the progress of this new approach in pests of fruit crops.

RNAi is a technique which is based on the sequence-specific method of suppressing the expression of the targeted gene(s) (Mohanpuria et al. 2010). Each insect species has its unique gene sequences. To alter the target insect sequence, RNAi can potentially be designed in a species-specific manner. RNAi could be used selectively to kill pest insects without adversely affecting nontarget species by targeting the genes essential for pest insect's growth, development, and reproduction (Whyard et al. 2009). RNAi as an entomological research tool has been used to elucidate genes involved in physiological processes, embryogenesis, reproduction, and behavior in model and non-model insects (Belles 2010). RNAi or gene silencing is a posttranscriptional, RNA-dependent, sequence-specific process of turning down or shutting off the expression of certain genes, which ultimately will suppress the production of a specific protein in various organisms including insect pests (Yu et al. 2016). RNAi is a biological defense response which is conserved in nature and facilitates resistance to both endogenous parasitic and exogenous pathogenic nucleic

acids in a sequence-specific manner (Hannon 2002). In eukaryotic organisms, mechanism of RNAi involves exposure to molecules of dsRNA causing posttranscriptional degradation of homologous messenger RNA (mRNA) resulting in equivalent loss of function (Mello and Conte 2004). RNAi-based silencing of target gene can be achieved by various means such as direct feeding of dsRNA to an organism, or by engineering plants, or by the use of engineered bacteria to produce dsRNA, and all these strategies are operationally feasible for the basic investigation and practical application (Zhu et al. 2016). According to the central dogma of life, RNA was simply considered as a tool for bypassing genetic information from DNA to protein. But when "antisense-mediated silencing" of homologous genes was discovered, the role of RNA in regulation of gene expression was very well recognized (Nellen and Lichtenstein 1993).

In 1998, evidence was provided that dsRNA can cause prominent gene silencing in comparison with sense or antisense RNA in nematode *Caenorhabditis elegans* (Maupus) (Fire et al. 1998). The discovery of this technology earned them Nobel Prize in 2006 and led to the formation of an expeditiously growing field of biological science, termed as RNAi. Though, irrespective of its ultimate use, implementation of sequence-specific approach of RNAi always needs the screening of vital genes to target for the management of insect pests. There are various advantages of utilizing RNAi such as highly conserved system, specificity to target the candidate gene, stability, targeting multi-genes, wide adaptability, systemic nature, and heritability which make it a preferred technique for utilization in pest management. RNAi-associated gene silencing can result in death, molting deformity, and reduced reproduction rate in some insects (Yang et al. 2013). Here, we review the mechanism of RNAi and focus on the published work on RNAi technique used against various insect and mite pests of the fruit crops.

2.2 Mechanism of RNA Interference

The mechanism of RNAi involves two highly conserved enzyme systems: one is Dicer in animals including insects and Dicer-like (DCL) elements in plants, and the second one is the RNA-induced silencing complex (RISC). Dicer family members are large, multidomain proteins that contain a putative RNA helicase domain, PAZ (Piwi/Argonaute/Zwille) protein-protein interaction domain, two-tandem ribonuclease III (RNase III), and one or two dsRNA-binding domain(s). RISC consists of RNA and a major protein component, i.e., Argonaute, which is responsible for small interfering RNA (siRNA) binding as well as ribonuclease activity to cut the target mRNA. In RNAi mechanism, as shown in Fig. 2.1, dsRNA is processed by Dicer to convert it into 21–25 nt siRNA molecules which are incorporated into RISC. RISC utilizes only antisense strand of these small RNA molecules as a guide to search for its complementary base pairing with the target mRNA which finally results in cleavage of target mRNA or translation repression (Scott et al. 2013; Miglani 2015).

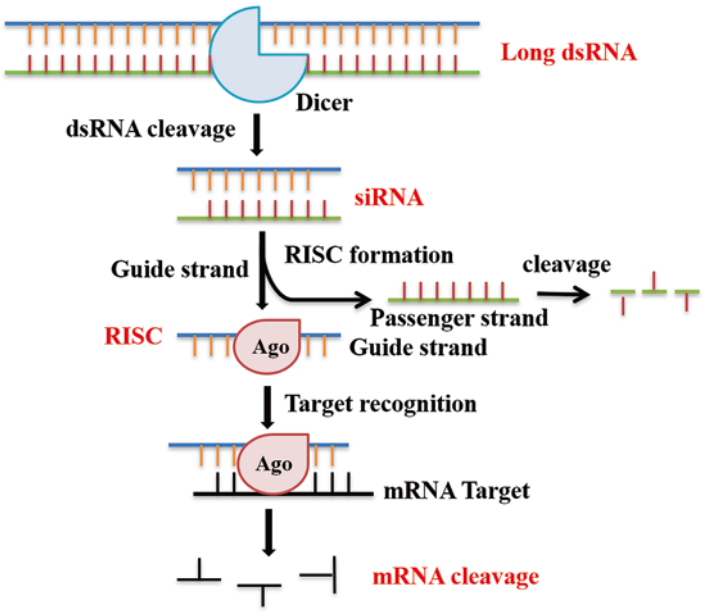

Fig. 2.1 Mechanism of RNA interference

To induce RNAi in a system requires the introduction of homologous dsRNA corresponding to a vital gene(s) especially in the case of insects or intron-containing hairpin RNA (ihpRNA) which increases the gene silencing by 90–100% (Smith et al. 2000; Wesley et al. 2001). RNAi is used in reverse genetics as a powerful tool in many insect pests and pathogens to study the functions of important genes. RNAi is a specific, stable, and efficient technique for the control of insect pests (Gordon and Waterhouse 2007; Price and Gatehouse 2008; Zotti and Smagghe 2015).

RNAi signals in the insect body have been found to be transferred by cell-autonomous and non-cell-autonomous ways. Cell-autonomous refers to innate RNAi present inside the cell, i.e., location of introduction or production of dsRNA and its silencing effects are the same, and the non-cell-autonomous refers to RNAi caused by the uptake of silencing signals from the environment and its transport from one cell to another. The non-cell-autonomous RNAi can be environmental when an insect takes dsRNA from the outside environment, or it can be systemic when dsRNA is taken from one cell or tissues and transferred to some other part(s) of the body (Huvenne and Smagghe 2010). In insects, very high silencing responses are achieved only when both environmental and systemic RNAi occur together (Price and Gatehouse 2008; Tomoyasu et al. 2008; Prentice et al. 2015). But as far as systemic RNAi has the capability to spread or the spread of RNAi signals are concerned, only plants and nematode worms have the advantage of RNA-dependent RNA polymerase (RdRP) feature (Pak and Fire 2007; Siomi and Siomi 2009). The systemic mechanism of RNAi involves the amplification of initial dsRNA signals

and transferring this signal to other tissues of the organism or even to the next generation (Huvenne and Smagghe 2010; Schott et al. 2014; Matsumoto and Hattori 2016). This systemic RNAi mechanism is not similar in all the organisms, for example, in nematodes. RNAi has the capability to spread its signals throughout the organism (Fire et al. 1998; Newmark et al. 2003; Katoch and Thakur 2013). But in insects and humans, this mechanism was found to be absent (Gordon and Waterhouse 2007). Instead in insects, the transmembrane channel-mediated uptake mechanism and endocytosis-mediated device of uptake for systemic RNAi have been reported so far (Huvenne and Smagghe 2010; Joga et al. 2016).

The transmembrane channel-mediated uptake mechanism was also observed in *C. elegans*. In this mechanism, the systemic RNAi was performed by *SID-1* (systemic RNAi defective) and its analogs *SID-2*, a transmembrane protein which expresses on the cell surface, thus enabling the RNAi signal to transfer between different cells, and is also responsible for the uptake of dsRNA from external environment to inside (Winston et al. 2002; Katoch and Thakur 2013). *SID-2* involves in uptake of dsRNA and it is present in the intestine of *C. elegans* (Cappelle et al. 2016). Both *SID-1* and *SID-2* are essential for non-autonomous RNAi function, *SID-2* involves in uptake, and then *SID-1* transfers the dsRNA into the cytoplasm (Jose et al. 2009; Cappelle et al. 2016). *SID-1* homologs have been found in several insects like domestic silkworm *Bombyx mori* (Linnaeus), red rust flour beetle *Tribolium castaneum* (Herbst), and European honey bee *Apis mellifera* (Linnaeus) (Xu and Han 2008; Katoch and Thakur 2013) and in aphid spp. (Huvenne and Smagghe 2010). But in the case of *Drosophila* spp., *SID-1* analogs were not found (Roignant et al. 2003). Therefore, *SID-1* is not essential for silencing in insects. In fruit fly and other insects which lack homologs of *SID-1*, an active receptor-mediated endocytosis is responsible for systemic RNAi and dsRNA uptake (Saleh et al. 2006; Whyard et al. 2009; Katoch and Thakur 2013). In this approach, uptake of dsRNA from the environment and then the signals for silencing are transported through vesicle-mediated intracellular trafficking (Saleh et al. 2006; Tomoyasu et al. 2008). If a mutation is introduced into endocytosis-mediated genes in *C. elegans*, it leads to the nullification of RNAi response. This proves that endocytosis-mediated RNAi mechanism is highly conserved in evolution (Saleh et al. 2006). But the mechanism of transfer of dsRNA to a suitable site in the cell by this method is not clearly understood.

In insects, the uptake of dsRNA by midgut epithelial cells is very much important for introducing RNAi response. The insect midgut in general is responsible for uptake of vitamins and minerals. Sometimes, peritrophic matrix and perimicrovillar membranes of the midgut in certain species of Coleoptera, Lepidoptera, and Hemiptera act as physical barriers in absorbing dsRNA (Hegedus et al. 2009). After absorption of dsRNA by midgut cells, transfer of these signals to intracellular machinery of RNAi is also of utmost importance. The uptake of dsRNA follows pathway of endocytosis and thus enables its transfer to the cytoplasm through the endosomal discharge (Varkouhi et al. 2011). RNAi efficacy is highly variable depending upon the insect species, the method of RNAi delivery, and the gene targeted through this approach.

2.3 Host-Induced RNAi-Based Pest Control

The RNAi technique can be successfully utilized as a control tactic against insect and mite pests (Gordon and Waterhouse 2007; Huvenne and Smagghe 2010; Joga et al. 2016; Li et al. 2020). In most of the functional genomic studies, microinjection or feeding of bacteria expressing dsRNA was used as a method of delivery to silence different target genes. Gene silencing caused distressing effects on the growth, development, and survival of insects (Xu et al. 2016). These investigations suggested the prospect of employment of approach based on feeding bioassay for insect pest control through RNAi. In host-induced RNAi (HI-RNAi) or plant-mediated approach, a crop plant is engineered with hairpin RNAi (hpRNAi) vector for the production of dsRNA against the target gene of insect pest. The dsRNA passes through the insect gut during feeding on plant parts leading to the induction of RNAi machinery and then resulting in silencing of the target gene in concerned insect pest (Xu et al. 2021).

The first demonstration of the success of HI-RNAi was on Western corn rootworm *Diabrotica virgifera virgifera* (LeConte) (Baum et al. 2007) and cotton bollworm *Helicoverpa armigera* (Hŏbner) (Mao et al. 2007). The dsRNA was expressed in tobacco, *Nicotiana tabacum*, against *cytochrome P450* (*CYP6AE14*) gene in *H. armigera* (Mao et al. 2007). Later, HI-RNAi was employed for silencing of various insect vital genes for the control of different insects (Zhu et al. 2012; Xiong et al. 2013; Thakur et al. 2014; Jin et al. 2015; Mamta et al. 2016). The plant-mediated or HI-RNAi was also combined with other transgenic approaches in order to boost resistance against pests. This strategy also provided considerable advantage in controlling sap-sucking insect pests. These insect pests were found resilient toward *Bt* toxin. They pose significant hazard to agriculture by acting as a vector for many virus-borne diseases and can be easily controlled by this approach in fruit crops against various insect pests.

There is no data available on plant-mediated or HI-RNAi in fruit crops against insect pests till date. But scientists are working toward it, and in the near future RNAi-mediated insect resistance crops will be released in the market. A schematic diagram of plant-mediated or HI-RNAi in fruit crops against insect pests represents an approach (Fig. 2.2) which involves various steps. Plant-mediated or HI-RNAi approach involves six steps: (a) identification of the vital target gene of target insect pest followed by, (b) hpRNAi vector construction containing target gene of interest, (c) target crop plant engineering with hpRNAi vector to produce the dsRNA against target gene of target insect pest, (d) insect feeding on RNAi-based insect resistance crop cause dsRNA enter into gut of insect, (e) leading to induction of RNAi machinery, and lastly (f) silencing of target gene in concerned insect pest cause death of insect by feeding on RNAi mediated insect resistance crop.

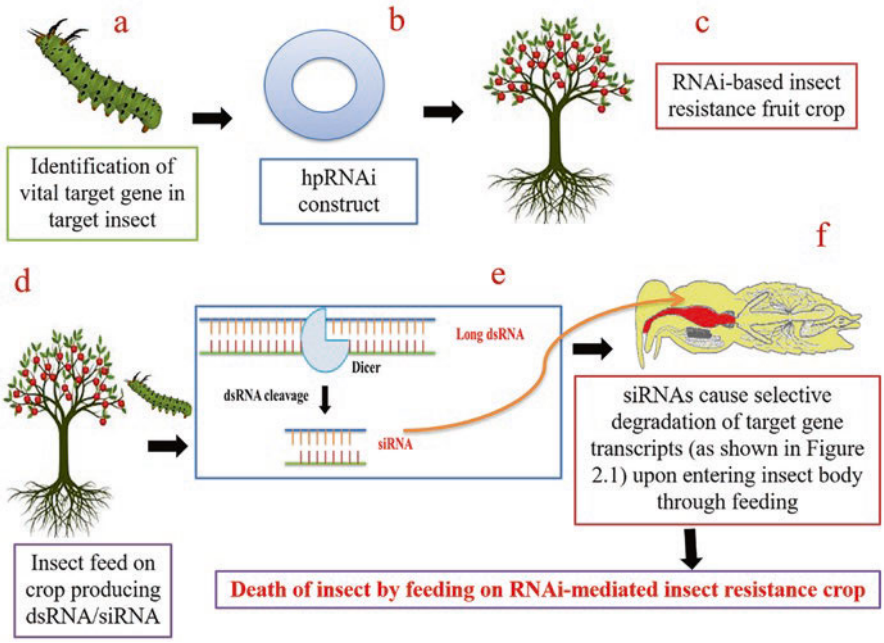

Fig. 2.2 Steps involved in host-induced RNA interference for insect pest control. *Abbreviation*: *hpRNAi* hairpin RNAi

2.4 RNA Interference-Mediated Pest Control in Fruit Crops

RNAi is a revolutionary technique that has been now established itself as a powerful means for studying function. It has shown an immense potential in insect pest control by silencing vital genes of target pests in recent years. To date, several researchers have validated successful RNAi experiments in the laboratory with various insect species, comprising economically important pests of fruit crops. Table 2.1 provides an overview of RNAi experiments performed in pests that causes significant yield losses in fruit crops so far and provides information about the delivery routes used for the concerned experiments and phenotypic effects that were observed in the insect pests as well as the knockdown levels of the transcripts. The efficiency of RNAi experiments in insect species varies as ranging from very low to almost complete knockdown of the concerned transcripts. Phenotypic effects are variable as well, depending on the target gene of the concerned insect and mite pests. Here we present an account of the research work done, on insects belonging to four orders, namely, Diptera, Hemiptera, Lepidoptera, and Blattodea, and two mite species belonging to order Acari, so far by the molecular biologists to control insect pests using RNAi technology.

Table 2.1 Examples of the use of RNA interference against different insect and mite pests of fruit crops

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
Diptera	*Anastrepha fraterculus* (Wiedemann) (South American fruit fly)	*Annona cherimola* (Miller) [Cherimoya], *Annona reticulata* (L.) [custard apple], *Artocarpus heterophyllus* (Lamarc) [jackfruit], *Citrullus lanatus* (Matsumura and Nakai) [watermelon], *Citrus* spp. (L.) [citrus], *Ficus carica* (L.) [common fig], *Mangifera indica* (L.) [mango], *Morus* spp. (L.) [mulberry]), *Musa* spp. (L.) [banana], *Olea europaea* (L.) [European olive], *Passiflora edulis* (Sims) [passion fruit], *Prunus* spp. (L.) [stone fruits], *Psidium guajava* (L.) [guava], *Punica granatum* (L.) [pomegranate]), *Pyrus communis* (L.) [pear], *Ziziphus jujuba* (Miller) [ber]	*v-ATPase*	*v-ATPase* uses the energy produced from *ATP* hydrolysis to transport protons across intracellular and plasma membranes of eukaryotic cells	Larva	Soaking	Resulted in 40% mortality and demonstrated the existence of a functional RNAi machinery	Dias et al. (2019)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
	Bactrocera dorsalis (Hendel) (oriental fruit fly)	*Annona cherimola* (Miller) [cherimoya], *Annona reticulata* (L.) [custard apple], *Artocarpus heterophyllus* (Lamarc) [jackfruit], *Citrullus lanatus* (Matsumura and Nakai) [watermelon], *Citrus* spp. (L.) [citrus], *Ficus carica* (L.) [common fig], *Mangifera indica* (L.) [mango], *Morus* spp. (L.) [mulberry], *Musa* spp. (L.) [banana], *Passiflora edulis* (Sims) [passion fruit], *Prunus* spp. (L.) [stone fruits], *Psidium guajava* (L.) [guava], *Punica granatum* (L.) [pomegranate], *Pyrus communis* (L.) [pear], *Ziziphus jujube* (Miller) [ber]	*magu, lola, topi, aly, rac, rho, upd, per*	Involved in spermatogenesis	Adult	Oral administration	Significantly affected the oviposition and reduced egg-hatching rate	Dong et al. (2016b)

(continued)

Table 2.1 (continued)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
			ecr, *rpl19*	Coordinates the molting and metamorphosis in insects through ecdysteroid hormone, and *rpl19* encodes a ribosomal protein	Larva	Feeding with artificial diet	Severe deformities of maggots, pupae and incomplete eclosion, absence of wings, abnormal abdomen of adults	Mohanpuria et al. (2021)
			rpl19, *v-ATPase D*, *noa*, *rab11*	*rpl19* encodes a ribosomal protein; *v-ATPase-D* maintains energy production; *noa* functions in the nervous system, the imaginal discs, the fat body, and the gonads of both sexes; and *rab11* regulates vesicular trafficking, essential for fertility, and regulates various cellular events	Adult	dsRNA feeding	Maximum downregulation of each gene varied from 35 to 100%. RNAi could be observed not only in the midgut but also in the ovary, nervous system, and fat body	Li et al. (2011)
			tra, *tra-2*	*tra* acts as the genetic switch that promotes female development by interaction with the *tra-2*	Egg	Microinjection	Sex reversion induced by either *Bdtra* or *Bdtra-2* in *B. dorsalis* was highly efficient	Liu et al. (2015)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
			tssk1, *tektin1*	Important for male fertility	Adult	dsRNA feeding	Knockdown of *Tssk1* and *Tektin1* caused male sterility	Sohail et al. (2019)
			wg	Involved in wing development	Larva	Artificial baits with nano-carrier dsRNA	Deformity of wings along with body distortion	Guo et al. (2020)
			spr	Regulates the suppression of female receptivity and induction of oviposition	Adult	Feeding with artificial diet	High mortality, decreased egg production, and profound impact on the eclosion rate of offsprings	Zheng et al. (2015)
			dsx	Acts as a linker between the upstream sexual determination hierarchy and downstream genes that perform various sex-specific functions	Adult	Microinjection	Inhibited the expression of yolk protein gene (*Bdyp1*) and delayed ovary development	Chen et al. (2008)
			ago-1	Responsible for patterning and morphogenesis	Adult	Microinjection	*ago-1* is indispensable for normal ovarian development	Yang et al. (2021)

(continued)

Table 2.1 (continued)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
			fbp1	Synthesizes proteins for insect development and reproduction	Larva	Topical application and feeding	Significantly reduced the eclosion rate and act as a storage protein	Yu et al. (2021)
	Bactrocera minax (Enderlein) (citrus fruit fly)	*Citrus* spp. (L.) [citrus]	*tps*	Instant source of energy and the initial substrate for chitin biosynthesis	Larva	Microinjection	Inhibited the expression of three key genes in the chitin biosynthesis pathway and showed *tps* is indispensable for larval-pupal metamorphosis	Xiong et al. (2016)
			rh6	Responsible for green spectral sensitivity	Adult	Microinjection	Eliminated the preference for green and significantly decrease oviposition in green unripe citrus	Wang et al. (2019a)
			obp21, *csp*	Transports incoming odorants to corresponding receptors	Adult	Microinjection	Affected antennal responses to D-limonene which activates antennal responsiveness for oviposition or host location	Xu et al. (2019)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
	Bactrocera oleae (Rossi) (olive fruit fly)	*Olea europaea* (L.) [European olive]	*yellow, troponin C*	Involved in male and female reproductive system	Adult	Microinjection	Significantly reduced oviposition rate	Gregoriou et al. (2021)
	Bactrocera tau (Walker) (pumpkin fruit fly)	*Artocarpus heterophyllus* (Lamarc) [jackfruit], *Citrullus lanatus* (Matsumura and Nakai) [watermelon], *Ficus carica* (L.) [common fig], *Mangifera indica* (L.) [mango], *Morus* spp. (L.) [mulberry], *Passiflora edulis* [passion fruit], *Prunus* spp. (L.) [stone fruits], *Psidium guajava* (L.) [guava], *Pyrus communis* (L.) [pear]	*tra2*	Cooperates with the sex-determining gene *transformer* (*tra*) to direct female differentiation	Adult	Microinjection	Produced a male-biased sex ratio, and some intersexes along with males harbor the testes having some defects in their external morphologies	Thongsaiklaing et al. (2018)

(continued)

Table 2.1 (continued)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
	Bactrocera tryoni (Froggatt) (Queensland fruit fly)	*Annona cherimola* (Miller) [cherimoya], *Musa* spp. (L.) [banana], *Annona reticulata* (L.) [custard apple], *Artocarpus heterophyllus* (Lamarc) [jackfruit], *Citrullus lanatus* (Matsumura and Nakai) [watermelon], *Ficus carica* (L.) [common fig], *Mangifera indica* (L.) [mango], *Morus* spp. (L.) [mulberry], *Passiflora edulis* (Sims) [passion fruit], *Prunus* spp. (L.) [atone fruits], *Psidium guajava* (L.) [guava], *Punica granatum* (L.) [pomegranate], *Pyrus communis* (L.) [pear], *Ziziphus jujube* (Miller) [ber]	*tssk1*, *topi*, and *trxt*	Responsible for male fertility	Adult	Microinjection and feeding	Treated males produced 75% fewer viable offspring	Cruz et al. (2018)
			dsRNase1, *dsRNase2* and *yellow*	*yellow* gene is responsible for melanin production	Adult	Microinjection and feeding with liposomes	Resulted in almost complete (99%) knockdown of *yellow* transcripts	Tayler et al. (2019)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
	Drosophila suzukii (Matsumura) (spotted wing *Drosophila*)	*Annona cherimola* (Miller) [cherimoya], *Annona reticulata* (L.) [custard apple], *Artocarpus heterophyllus* (Lamarc) [jackfruit], *Citrullus lanatus* (Matsumura and Nakai) [watermelon], *Citrus* spp. (L.) [citrus], *Ficus carica* (L.) [common fig], *Morus* spp. (L.) [mulberry], *Musa* spp. (L.) [banana], *Passiflora edulis* (Sims) [passion fruit], *Prunus* spp. (L.) [stone fruits], *Punica granatum* (L.) [pomegranate], *Pyrus communis* (L.) [pear], *Ziziphus jujube* (Miller) [ber],	*alpha COP, shrb, rpl13, vha26*	*alpha COP* gene mediates protein transport. *shrb* involved in the trafficking of transmembrane proteins, *rpl13* encodes ribosomal protein, and *vha26* maintains energy production	Larva and adult	Microinjection and feeding with artificial diet	Resulted in mortality in all stages of *D. suzukii*	Taning et al. (2016)

(continued)

Table 2.1 (continued)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
Hemiptera	*Bemisia tabaci* (Gennadius) (Whitefly)	*Citrullus lanatus* (Matsumura and Nakai) [watermelon], *Citrus* spp. (L.) [citrus], *Eriobotrya japonica* (Lindley) [loquat], *Ficus carica* (L.) [common fig], *Fragaria* spp. (Duchesne) [strawberry], *Morus* spp. (L.) [mulberry], *Passiflora edulis* (Sims) [passion fruit], *Psidium guajava* (L.) [guava], *Ziziphus jujube* (Miller) [ber]	*aChE, ecr*	*aChE* acts as a neurotransmitter, and *ecr* coordinates the molting and metamorphosis in insects through ecdysteroid hormone	Adult	Recombinant plant *Nicotiana tabacum* expressing dsRNA	More than 30% mortality was observed after 1 day of feeding exposure to the *aChE* and *ecr* transgenic lines	Malik et al. (2016)
	Diaphorina citri (Kuwayama) (Asian citrus psyllid)	*Citrus* spp. (L.) [citrus], *Ficus carica* (L.) [common fig]	*suh*	Responsible for osmotic homeostasis	Nymph and adult	Topical feeding	Nymph mortality and reduced adult lifespan	Santos-Ortega and Killiny (2018)
			gste2, gstd1	Involved in detoxification of insecticides	Adult	Oral feeding	Plays unique role in detoxification of the thiamethoxam and fenpropathrin	Yu and Killiny (2018)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
			cathepsin D, chs, iap	*cathepsin D* is responsible for protein digestion and involved in metamorphic events, *chs* involved in the production of the cuticular lining, and *iap* regulates apoptotic machinery	Nymph and adult	Feeding with artificial diet and *Murraya paniculata* leaves placed in dsRNA solutions	Significantly induced mortality in *D. citri*	Galdeano et al. (2017)
			aChE-like-1, aChE-like-2, chE-2-like	Acetylcholinesterase catalyzes the hydrolysis of the neurotransmitting agent, *acetylcholine* (a*Ch*), to choline and acetic acid	Nymph	Topical feeding	Induced susceptibility to carbamate and organophosphate insecticides	Kishk et al. (2017)

(continued)

Table 2.1 (continued)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
	Myzus persicae (Sulzer) (Green peach aphid)	*Citrullus lanatus* (Matsumura and Nakai) [watermelon], *Citrus* spp. (L.) [citrus], *Fragaria* spp. (Duchesne) [strawberry], *Malus domestica* (Borkh) [apple], *Passiflora edulis* (Sims) [passion fruit], *Persea americana* (Miller) [avocado], *Prunus* spp. (L.) [stone fruits], *Psidium guajava* (L.) [guava], *Punica granatum* (L.) [pomegranate]	Na_v	Play an essential role in neuronal signaling	Nymph	Oral feeding	High mortality, reduced fecundity and longevity	Tariq et al. (2019)
			cp	*cp* in conjunction with chitin constitute the cuticle, cross-linked during cuticle sclerotization, and are vital for adult molt	Nymph	Transgenic plant (*Arabidopsis*) expressing dsRNA	Significantly impaired fecundity	Bhatia and Bhattacharya (2018)
			cp19	*cp19* in conjunction with chitin constitute the cuticle, vital for adult molt	Adult	Feeding with artificial diet	Resulted in 43.3–50.8% mortality	Shang et al. (2020)
			gus	Acts as a positive regulator of insect spawning	Adult	Feeding with artificial diet	Significant reduction in the embryos and newborn nymphs	Gao et al. (2021)
	Planococcus citri (Risso) (Citrus mealybug)	*Citrus* spp. (L.) [citrus], *Ficus carica* (L.) [common fig], *Musa* spp. (L.) [banana], *Punica granatum* (L.) [pomegranate], *Vitis vinifera* (L.) [grapes]	*chs1*, *v-ATPase*	*chs1* involved in the production of the cuticular lining and *v-ATPase* maintains energy production	Nymph	Microinjection and recombinant TMV-infected plants	Lower fecundity and prominent death of crawlers	Khan et al. (2013)
	Pseudococcus maritimus (Ehrhorn) (Grape mealybug)	*Vitis vinifera* (L.) [grapes]	*aqp*, *suc*, *nuc*	Required for osmoregulation in phloem sap-feeding hemipteran insects	Nymph	Artificial liquid diet sandwiched between two layers of parafilm	Significantly increased insect mortality over 3 days	Arora et al. (2020)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
Lepidoptera	*Epiphyas postvittana* (Walker) (Light brown apple moth)	*Actinidia deliciosa* (Ferguson) [kiwifruit], *Citrus* spp. (L.) [citrus], *Fragaria* spp. (Duchesne) [strawberry], *Malus domestica* (Borkh) [apple], *Persea Americana* (Miller) [avocado], *Prunus* spp. (L.) [stone fruits], *Pyrus communis* (L.) [pear], *Vaccinium* spp. [blueberry],	*cxe1*, *pbp1*	*cxe1* degrades the sex pheromones and host plant volatiles, and *pbp1* is involved in the reception of the adult sex pheromone	Larva	Oral feeding	Reduction in only transcript levels of genes	Turner et al. (2006)
Blattodea	*Coptotermes formosanus* (Shiraki) (Formosan subterranean termite)	*Citrus* spp. (L.) [citrus]	*eG1a*, *eG1b*, *eG2*, *eG3*, *eG4*	*eG* catalyzes the first step in cellulose digestion	Worker	Microinjection and oral delivery	Reduced enzyme activity, mortality, and reduced weight of workers	Wu et al. (2018)

(continued)

Table 2.1 (continued)

Order	Insect	Scientific name [common name] of fruit crop(s) damaged	Insect gene(s) targeted	Function(s) of the gene(s)	Stage of insect	Strategy	Salient observation(s)	Reference
Acari	*Panonychus citri* (McGregor) (Citrus red mite)	*Citrus* spp. (L.) [citrus]	*hr3*	Plays an important role in molting and metamorphosis	Deutonymphs	Leaf disc-based delivery of dsRNA	Retarded development, high mortality, and disrupted molting process	Li et al. (2020)
			vg, vgR	*vg* is the precursor of *vitellin* (*Vn*) responsible for proper embryo development	Adult	Leaf disc-based delivery of dsRNA	Reduced oviposition	Ali et al. (2017)
	Tetranychus urticae (Koch) (Two-spotted spider mite)	*Annona cherimola* (Miller) [cherimoya], *Annona reticulata* (L.) [custard apple], *Artocarpus heterophyllus* (Lamarc) [jackfruit], *Citrullus lanatus* (Matsumura and Nakai) [watermelon], *Citrus* spp. (L.) [citrus], *Ficus carica* (L.) [common fig], *Morus* spp. (L.) [mulberry], *Musa* spp. (L.) [banana], *Passiflora edulis* (Sims) [passion fruit], *Prunus* spp. (L.) [stone fruits], *Punica granatum* (L.) [pomegranate], *Pyrus communis* (L.) [pear], *Ziziphus jujube* (Miller) [ber]	*cpr*	*cpr* are frequently the primary detoxification agents in the metabolism of toxic pesticides and plant allelochemicals	Adult	Leaf disc-based delivery of dsRNA	Significant reduction of resistance to multiple acaricides such as abamectin, bifenthrin, and fenpyroximate	Adesanya et al. (2020)

Abbreviations: *aChE-like-1* acetylcholinesterase-like-1, *aChE-like-2* acetylcholinesterase-like-2, *aChE* acetylcholinesterase, *ago11* argonaute1, *alpha COP* alpha coatomer protein, *al* early, *aqp* aquaporin water channel, *cathepsin D* cathepsin D, *chE-2-like* cholinesterase 2-like, *chs* chitin synthases, *chs1* chitin synthase1, *cp* cuticular protein, *cp19* cuticle protein 19, *cpr* NADPH-cytochrome P450 reductase, *csp* candidate chemosensory protein, *cxe1* carboxylesterase gene, *dsx* doublesex, *ecr* ecdysone receptor, *eG1a* endoglucanase 1a, *eG1b* endoglucanase 1b, *eG2* endoglucanase 2, *eG3* endoglucanase 3, *eG4* endoglucanase 4, *fbp1* fat body protein 1, *gstd1* glutathione *S*-transferase 1, *gste2* glutathione *S*-transferase 2, *gus* Gustavus, *hr3* hormonal receptor 3, *iap* inhibitor of apoptosis, *lola* longitudinals lacking, *magu* magu, na_v voltage-gated sodium channel, *noa* fatty acid elongase, *nuc* gut nuclease, *obp21* odorant binding protein 21, *pbp1* pheromone binding protein gene, *per* period, *rab11* small GTPase, *rac* rac, *rh6* opsin gene, *rho* rho, *rpl13* ribosomal protein 13, *rpl19* ribosomal protein 19, *shrb* shrub, *spr* sex peptide receptor, *suc* sucrase, *suh* sucrose hydrolase, *tektin1* tektin1, *topi* matotopetli, *tps* trehalose-6-phosphate synthase, *tra* transformer, *tra2* transformer 2, *troponin C* troponin C, *trxt* thioredoxin T, *tssk1* testis-specific serine/threonine kinases1, *upd* unpaired, *v-ATPase* vacuolar-type ATPase, *v-ATPase D* vacuolar-type ATPase subunit D, *vg* vitellogenin, *vgR* vitellogenin receptor, *vha26* v-type proton ATPase subunit E, *wg* wingless, *yellow* melanin synthesis gene

2.4.1 Diptera

2.4.1.1 *Anastrepha fraterculus* (Wiedemann)

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) is a major pest of several fruit crops (Table 2.1). This polyphagous fruit fly species occurs in the USA, Mexico, and Argentina and is associated with 116 plant species in Brazil alone (Zucchi 2008). This insect pest causes the damage by larval feeding and oviposition that hastens ripening and premature dropping of fruit. Notably, its presence hinders marketing of fruits because of quarantine limitations imposed by fruit fly-free countries. This pest causes global loss of range 2 billion USD every year, and in Brazil, the economic losses are up to 200 million USD annually (Macedo et al. 2017).

To reduce the pesticide load used for control of this pest, RNAi-based investigation was conducted by Dias et al. (2019) to analyze the efficiency of RNAi machinery and to assess the sensitivity for the uptake of dsRNA to generate RNAi response in *A. fraterculus* (Table 2.1). The delivery of dsRNA was performed by soaking the larvae to assess the silencing of the target gene, *v-ATPase*. The larvae of fruit fly soaked in ds-*v-ATPase* solution resulted in 85% *v-ATPase* knockdown within 48 h which ultimately reached cent per cent after 48 h. The *Dicer-2* and *Argonaute-2* expression was also increased with increase in exposure to dsRNA. The effect of silencing in the treated larvae was remained up to 72 h. This is the only study that provides an evidence of existence of functional machinery of siRNA in this pest and also showed that larval soaking in ds-*v-ATPase* led to efficient gene silencing along with high mortality in the *A. fraterculus*.

2.4.1.2 *Bactrocera dorsalis* (Hendel)

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is a key polyphagous pest throughout the world which causes significant losses in the production of fruit and vegetable crops due to its high reproductive potential, wide host range, adaptability to climate, overlapping generations, and invasiveness (White and Elson-Harris 1992; Clarke et al. 2005; Kaur et al. 2021) (Table 2.1). *Bactrocera dorsalis* was found to be distributed in 75 countries in Asia, Africa, South America, North America, and Oceania up to 2017. Asia and Africa became the most signified areas constituting about 86.3% of the total countries (Zeng et al. 2018). *Bactrocera dorsalis* has been reported to invade many new continents in the past years (Pieterse et al. 2017). The major incidence of *B. dorsalis* for the first time was reported in 1794 from India (Fabricius 1794; Clarke et al. 2019). It has gained a status of serious pest of a various fruit crops in the Indian subcontinent (Kamala Jayanthi et al. 2011). Around 50–60% annual yield losses were reported due to infestation of fruit flies on different hosts in India (Narayanan and Batra 1960). This pest has been reported to cause up to 100% fruit damage in rainy season on guava in India (Singh

and Sharma 2013). In addition to direct damage to fruits, an indirect loss is also related to quarantine limitations due to incursion of fruit flies in fruits and occasionally the meager occurrence of the fruit flies in a particular country (Ole-MoiYoi and Lux 2004).

Bactrocera dorsalis feeds and breeds on several fruit crops throughout the world. The adult females of fruit flies lay eggs inside fruits, particularly during the color break stage. Management of *B. dorsalis* is a challenging task as they are extremely polyphagous, multivoltine, mobile, and fertile, and all the life stages are unexposed except adults (Singh and Kaur 2016; Singh 2020) (Table 2.1). Thus, there is an urgent need to include novel approaches like the utilization of double-stranded ribonucleic acid (dsRNA) as a mean of gene silencing for managing the *B. dorsalis* which will further strengthen the integrated pest management module for this pest. Various researchers have successfully conducted the RNAi experiments for the control of this pest which provide the basis for further in-depth research. Sterile insect technique (SIT) is also used to control notorious insect pests; however, it has many limitations. But the SIT of males through RNAi would have numerous advantages over the radiation-based sterilization.

To accomplish RNAi-based sterilization, the appropriate target genes must be identified first. For the accomplishment of this goal, Dong et al. (2016b) selected eight candidate genes related to spermatogenesis (*magu*, *lola*, *topi*, *aly*, *rac*, *rho*, *upd*, *per*) for cloning and testing their potential activity in *B. dorsalis*. The oral delivery of dsRNAs resulted in the knockdown of the candidate genes and significantly reduced daily average number of eggs laid by the female flies along with decreased egg-hatching rate (Table 2.1). It negatively affected the quantity and quality of the spermatozoa. The length and number of spermatozoa in female spermatheca were significantly declined as compared to *gfp*-silenced control group. They conducted greenhouse trial and showed significant reduction in number of damaged oranges and larvae of *B. dorsalis* in ds-*rho* treated group as compared to control. This study provided strong evidence for the utilization of RNAi-based pest management, especially for the enhancement of SIT against *B. dorsalis* and other polyphagous species.

Another study was conducted by Mohanpuria et al. (2021) to investigate the potential of RNAi in *B. dorsalis* control by targeting its two vital genes, *ecr* and *rpl19* (Table 2.1). Feeding of *Bdecr* and *Bdrpl19* was done through artificial diet to maggots of *B. dorsalis*, and the effects of the silencing of the target genes resulted in high mortality along with severe deformities in treated maggots, emerged pupae, and adults.

In another experiment, Li et al. (2011) conducted RNAi strategy for the control of *B. dorsalis*. *Escherichia coli* strain HT115 was genetically engineered to express dsRNA targeting genes *rpl19*, *v-ATPase D*, *noa*, and *rab11* in *B. dorsalis*. There was lot of variation observed in the downregulation of each gene and RNAi was observed in different parts of the body (Table 2.1). Silencing of *rab11* through ingestion of dsRNA killed 20% of adult flies. Egg production was also affected through feeding ds-*noa* and ds-*rab11* compared to ds-*egfp* group. This study reported about the presence of RNAi in various tissues in addition to the midgut of the insect pests.

There are some genes which control gender development in the insects. The *tra* gene acts as a genetic switch which stimulates development of female by interaction with the *tra-2* gene in some dipteran species. In this context, Liu et al. (2015) conducted investigation on isolation, expression, and function of two genes, *tra* and *tra-2*, in *B. dorsalis* (Table 2.1). The results of function analyses of *Bdtra* and *Bdtra-2* showed that both genes were indispensable for development of female, as almost 100% males were obtained by using embryonic RNAi against either *Bdtra* or *Bdtra-2*. These RNAi males were further tested for fertility and more than 80% of them could mate. Although mated females could lay eggs, only 40–48.6% males gave rise to progeny. These results shed light on the development of a genetic sexing system with male-only release for this notorious agricultural pest (Liu et al. 2015).

RNAi-based SIT has a potential to control the economically important pest species, and functional characterization of genes responsible for male fertility can also boost the genetic SIT approach. *tssk1* has been involved to govern male fertility in both mammals and insects. Furthermore, *tektin1* has also been discovered to regulate male fertility in both human and mammals. Based on these observations, it has been suggested that *tssk1* and *tektin1* identified from *B. dorsalis* could be vital for male fertility in this pest. An investigation was conducted by Sohail et al. (2019) to analyze the expression profiles of *tssk1* and *tektin1* at different stages of development and in different tissues of *B. dorsalis* adult males. They found that both of these genes were highly expressed in the testis of adult males of *B. dorsalis*. RNAi results revealed that *tssk1* and *tektin1* knockdown caused male sterility and also significantly reduced the total numbers of spermatozoa (Table 2.1). Overall, this study demonstrates that *tssk1* and *tektin1* are novel genes that could be utilized for boosting RNAi-based SIT or their dsRNAs can be used as biopesticide to control *B. dorsalis*.

Many researchers are working toward the control of *B. dorsalis* through RNAi approach because this pest becomes nuisance due to its capacity to fly, polyphagous nature, and overlapping generations. The wings of *B. dorsalis*, an essential organ of flight, are the key reason for its extensive occurrence. RNAi-based investigation was conducted by Guo et al. (2020) to analyze the wing structure of *B. dorsalis*. Their results indicated that genes responsible for wing development were significantly upregulated in pupal stage of *B. dorsalis*. A key gene, *wg*, was selected through RNAi and used to enhance field control of *B. dorsalis*. Fruit damaging rate and offspring population decreased significantly by targeting this gene (Table 2.1). This study offers initial support for the application of pest control by regulation of wing development gene and suggests novel idea to control widespread *B. dorsalis*.

The genes responsible for oviposition play significant role in the population buildup of the insect pests. The *spr* is a key gene which regulates the female receptivity and oviposition. Zheng et al. (2015) conducted RNAi experiments to study the expression level of the *spr* gene in *B. dorsalis*. The RNAi effects of continuously feeding ds-*spr* to adults led to high mortality, decreased egg production capacity, and profoundly impacted the eclosion rate of the offspring of *B. dorsalis* (Table 2.1). The results demonstrated that feeding dsRNA-based RNAi could be used for

efficient control of insect pests. Furthermore, this research provides a potential target gene for RNAi-based control of *B. dorsalis*.

In another investigation, Chen et al. (2008) conducted RNAi experiments to evaluate the silencing effect of target gene *dsx* through microinjection in the abdomen of the adults in *B. dorsalis*. Results indicated that female-specific *dsx* dsRNAs reduce specifically its own transcript, inhibit selectively expression of the *Bdyp1*, and delay ovary development (Table 2.1). The number of matured eggs was significantly reduced, and female progeny showed deformed ovipositor after RNAi treatment. The silencing of *dsx* gene offers a promising and novel RNAi-based control approach for *B. dorsalis* in the near future.

Yang et al. (2021) conducted investigation on the spatiotemporal expression profile which showed relatively high transcriptional level of *ago-1* in the ovarian tissues of adult female of *B. dorsalis* during the sexual maturation period. RNAi-mediated silencing of *ago-1* led to a reduced ovary surface area (Table 2.1). The results indicate that *ago-1* is indispensable for normal ovarian development in *B. dorsalis* and this gene could be very useful for control of this pest.

The fat body, an essential tissue for insect metabolism, provides energy and synthesizes proteins for insect development and reproduction. As the developmental stages change, the function of the fat body changes accordingly. At the pupal stage, insects require large amounts of energy and materials for adult development. Because pupae do not ingest any food, all substances for adult development are stored during the larval stages, especially the late larval stage. Yu et al. (2021) targeted the *fbp1* gene through RNAi in *B. dorsalis*. Their results showed *Bdfbp1* expression significantly higher in the late third instar larvae as compared to first, second, and early third instar larvae (Table 2.1). Moreover, this gene was the highest expressed in newly formed pupae and also in fat body of female adults and newly emerged individuals. RNAi reduced *Bdfbp1* expression level which significantly decreased the rate of eclosion. Their results present that *Bdfbp1* may act as a storage protein and be linked to adult eclosion. Targeting of this gene will provide a control of this pest by interfering with the eclosion of *B. dorsalis* adults.

2.4.1.3 *Bactrocera minax* (Enderlein)

The citrus fruit fly, *Bactrocera minax* (Enderlein) (Diptera: Tephritidae), has been documented as one of the most distressing pests of citrus in the regions of Nepal, India, Bhutan, and China which is univoltine and specifically damages wild and cultivated citrus species (Wang and Lou 1995) (Table 2.1). Many researchers have conducted experiments based on RNAi approach for the control of this pest. The chief blood sugar in insects, trehalose, plays a vital role as an immediate energy source and the starting substrate for chitin biosynthesis. In insects, it is produced by catalysis of an essential enzyme, *trehalose-6-phosphate synthase* (*TPS*). Xiong et al. (2016) targeted *tps* gene in *B. minax*. They cloned and detected *tps* in all stages of development in *B. minax* but significantly expressed in the final (third) larval instar. Studies on expression patterns of tissue-specific *Bmtps* revealed that it was

primarily expressed in the fat body of this pest. Moreover, they found that injection of dsRNA into third larval instar effectively silenced *Bmtps* transcription in *B. minax* and thereby reduced the *tps* activity and trehalose content. Moreover, *tps* silencing inhibited the vital gene expression in chitin biosynthesis pathway along with abnormal phenotypes and high mortality. These outcomes demonstrated that *tps* gene was obligatory for the larval-pupal metamorphosis. In addition, RNAi experimental system in *B. minax* can provide a solid basis for further molecular biology and physiology investigation of this pest.

In another study, Wang et al. (2019a) targeted host-specific *B. minax*, which oviposit only into immature green fruits of citrus, and they carried out RNAi research to find out the molecular basis for recognition of host fruit color. They found that adults prefer green color over others and this color preference increased significantly in sexually mature over immature flies in laboratory and field assays. Moreover, they found that *rh6* gene has elevated expression in mature flies and this gene is responsible for green spectral sensitivity. Suppression of *rh6* through RNAi eliminated the green color preference, causing significant decrease in oviposition by *B. minax* in unripe green fruits of citrus (Table 2.1). These outcomes reveal that *rh6* gene controls the visual mechanism of utilization of host in *B. minax*, providing a genetic basis for locating visual host in a non-model insect herbivore.

Highly developed olfactory systems are present in insects which play vital roles in its host plant location, ecological adaptations, and oviposition behavior. *B. minax* is an oligophagous pest and mainly depends on the perception of chemical cues for host selection and oviposition behavior. Only few reports are there related to molecular components of the olfactory system of *B. minax*. Therefore, Xu et al. (2019) targeted the olfactory genes *csp* and *obp21* and found that ds-*obp21*- and ds-*csp*-treated *B. minax* showed poor electrophysiological response to an attractant, D-limonene. They suggested possible involvement of *csp* and *obp21* in olfactory perceptions of this fly (Table 2.1). Moreover, this investigation established the role of olfaction molecular basis, tributary for advance functional analyses of chemosensory processes in *B. minax* control.

2.4.1.4 *Bactrocera oleae* (Rossi)

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is an oligophagous pest and considered as the most serious pest of olives which significantly affect both the amount and quality of production in most olive-growing areas in the world (Table 2.1). For RNAi-based control of this pest, Gregoriou et al. (2021) developed an integrated approach for elucidation of reproductive and mating system of *B. oleae*. Initially, they performed RNA-seq analysis in reproductive tissues of virgin and mated insects. Transcriptome comparison resulted in the identification of the genes that expressed differentially after mating. Functional analysis of the genes showed variations in the catalytic, metabolic, and cellular processes after mating of *B. oleae*. Moreover, silencing of two differentially expressed genes, *yellow-g* and *troponin C*, through RNAi resulted in significant reduction in oviposition rate of

females. This study lays a foundation for further research into reproductive biology of the olive fruit fly for its control.

2.4.1.5 *Bactrocera tau* (Walker)

The pumpkin fruit fly, *Bactrocera tau* (Walker) (Diptera: Tephritidae), is an invasive agricultural pest with polyphagous nature which causes damage to many fruit crops and considered as an economically important agricultural pest. In Asian countries, huge loss of agricultural products occurred annually due to infestation of *B. tau*. To manage this notorious pest, Thongsaiklaing et al. (2018) conducted RNAi experiments by targeting the insect *tra2* gene which has a prevalent role in cooperating with the sex-determining gene *tra* to direct female differentiation. Knockdown of *Btau-tra2* produced a male-biased population and some intersexes (Table 2.1). They obtained intersexual and male sterility phenotypic variants of the *tra* and *dsx* orthologs which indicated that *Btau-tra2* had conserved splicing regulatory function which acted together with/upstream of *tra* and *dsx*. Furthermore, some RNAi males were fertile, but their fertilities were tremendously reduced. Likewise, RNAi males harbor the testes having some defects in their external morphologies. Also, a few surviving RNAi flies had twofold to threefold increased body size as compared to control. Their findings put forward that *tra2* gene is associated with male fertility and may also have an unprecedented role in the control of body size besides its conserved role in sex determination in *B. tau*.

2.4.1.6 *Bactrocera tryoni* (Froggatt)

The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), is a major pest of fruit crops in Australia and has known to infest more than hundred host plants (Dominiak et al. 2015). Its polyphagous nature, the climatic suitability, and the expansion of cultivated fruit crops have all contributed toward the success of this species. The SIT has been used since the 1960s for the management of *B. tryoni* in Australia. High-density mass-rearing conditions have contributed to reduce insect fitness, and sterilization method also led to compromise insect performance. RNAi approach provides an alternative sterilization approach for the species which showed negative impacts from radiation treatments. In this context, RNAi technique was investigated by Cruz et al. (2018) to check its potential for male sterilization in *B. tryoni* without adversely affecting mating efficiency. Adults were microinjected and fed with dsRNAs to target spermatogenesis genes (*tssk1*, *topi*, and *trxt*). Results showed significant gene knockdown for *tssk1* and *trxt* after 3 days of feeding, but interestingly *trxt* and *topi* produced an excess of transcripts after feeding for 10 days. But all the three dsRNAs reduced the fecundity of treated males (Table 2.1), and dsRNA-treated males actively competed with untreated males. These findings suggested that RNAi could serve as a means of sterilizing these insects in SIT program as an alternative to radiation.

In some insect like beetles, ingestion of small quantities of dsRNA is able to knockdown the expression of targeted genes. But, in other species, the presence of nucleases within the insect gut, which destroy dsRNA before it reaches target cells, makes ingestion of dsRNA ineffective. In another study, Tayler et al. (2019) observed that nucleases within the gut of *B. tryoni*-degraded dsRNA rapidly led to reduce RNAi efficacy. By incorporation of dsRNA with liposomes within the diet of adult insects, knockdown of *yellow* gene through RNAi was improved significantly, causing strong RNAi phenotypes. The co-feeding of both *yellow* and *dsRNase*-specific dsRNAs resulted in complete knockdown of yellow transcripts (Table 2.1). These findings suggest that the utilization of liposomes or co-delivery of nuclease-specific dsRNAs significantly advances inhibition of gene expression in *B. tryoni* through RNAi and could be an advantageous strategy to expand RNAi-based control in other insect species as well.

2.4.1.7 *Drosophila suzukii* (Matsumura)

The spotted wing Drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a serious polyphagous and invasive pest of soft and stone fruits and is difficult to control (Singh et al. 2020). RNAi holds great promise for pest control and is rapidly becoming a widely used functional genomics tool in insects. Taning et al. (2016) conducted research to evaluate whether RNAi is functional in *D. suzukii* and dsRNA oral delivery can bring about gene silencing and insecticidal activity (Table 2.1). Firstly, dsRNA targeting two vital genes, *alpha COP* and *shrb*, was microinjected into the haemolymph of adult flies, and results confirmed that RNAi was functional and gene silencing resulted in mortality of *D. suzukii*. Secondly, dsRNA targeting *alpha-COP* and two other important genes, *rpl13* and *vha26*, was mixed with artificial diet and fed to the larvae and adults of *D. suzukii*. They concluded that RNAi was functional in this pest and that RNAi can be induced either through microinjection or feeding of dsRNA to *D. suzukii*. Moreover, *vha26* is a potential target gene for further progress in development of RNAi-based insecticide, though a large-scale screening could potentially provide better target genes. Their investigation thus presents RNAi as a gifted approach for screening of potential target genes for control of *D. suzukii*.

2.4.2 Hemiptera

2.4.2.1 *Bemisia tabaci* (Gennadius)

Among sucking insect pests of agricultural importance, the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most damaging, causing losses in agronomic and horticultural crops, nearly worldwide. This pest has a broad host range comprising of nearly 500 plant species throughout the world. It is a vector of

plant viruses and causes yield losses by feeding damage and through the transmission of plant viruses that undermine plant growth and productivity. Using RNAi for knocking down vital genes of *B. tabaci* by expressing their homologous dsRNAs in plants has immense potential for management of this pest to reduce spread of plant virus diseases. Malik et al. (2016) used tobacco rattle virus-derived plasmid for *in planta* transient expression of the dsRNA homologous to the *aChE* and *ecr* genes of *B. tabaci*. They showed substantial mortality in adults of *B. tabaci*. *N. tabacum* plants expressing the homologous dsRNA to *B. tabaci* (*aChE* and *ecr*) were produced by fusing sequences derived from both genes. Mortality in adults was recorded after feeding on dsRNA producing *N. tabacum* plants (Table 2.1). Their investigation indicated that knockdown of vital genes involved in neuronal transmission and transcriptional activation has a great potential as a biopesticide to reduce whitefly population size and thereby decrease the spread of virus.

2.4.2.2 *Diaphorina citri* (Kuwayama)

The Asian citrus psyllid, *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae), is an economically important agricultural pest which damages the number of fruit crops and causes severe damage. The sucking insects mainly feed on phloem sap containing high sucrose content. To enhance the sucrose absorption from the midgut, sucrose hydrolase changes sucrose into glucose and fructose. RNAi approach as an alternate to pesticides was used to control this pest to reduce pesticide applications in the environment. Santos-Ortega and Killiny (2018) targeted *suh* gene of *D. citri*, the Huanglongbing (HLB) vector through RNAi (Table 2.1). The maximum gene expression of *Dcsuh* was observed in fourth and fifth instar nymphs. RNAi of *suh* was achieved through topical feeding, and results showed that application of 100 ng dsRNA-*DcSuh* was sufficient for reducing expression of targeted gene and caused high nymph mortality and reduced adult lifespan. Interestingly, some adults emerged from treated nymphs showed a swollen abdomen indicating that these insects were under osmotic stress. The metabolomic analyses showed accumulation of sucrose and reduced fructose, glucose, and trehalose in treated nymphs confirming the inhibition of activity of sucrose hydrolase. Moreover, secondary metabolites were reduced in treated nymphs, demonstrating reduction in the biological activities in *D. citri* under stress conditions. These findings thus provide basis for using *suh* as a potential target gene for effective RNAi-based control of *D. citri*.

To control *D. citri* populations, citrus growers use insecticides. However, the indiscriminate and continuous use of these insecticides can lead to pest resistance, which invariably leads to increased costs in production. Another investigation was conducted by Yu and Killiny (2018) for the RNAi-based control of *D. citri* by targeting *gst* genes. Knockdown of *Dcgste2* and *Dcgstd1* through RNAi followed by insecticide bioassay increased the mortalities of psyllids treated with thiamethoxam and fenpropathrin. Further, feeding with dsRNA interfusion (*dsDcgste2-d1*) co-silenced the expression of *DcGSTe2* and *DcGSTd1* in *D. citri* and led to increased susceptibility to both thiamethoxam and fenpropathrin (Table 2.1).

In another experiment, Galdeano et al. (2017) targeted the *cathepsin D*, *chs*, and *iap* genes of nymphs and adults of *D. citri* through feeding artificial diets containing dsRNAs and *Murraya paniculata* leaves placed in dsRNA solutions, respectively (Table 2.1). Adult mortality was amplified with the increase in amount of dsRNA for treatment. Both nymphs and adults fed with dsRNAs demonstrated significantly improved mortality over time as compared to the controls. These results showed that RNAi is a powerful tool for gene function studies and control of *D. citri*.

For the control of this pest, Kishk et al. (2017) conducted RNAi experiments in *D. citri* by targeting the *aChE* gene and c*hE-2-like* genes (Table 2.1). The dsRNA-*aChE* increased mortality in both nymphs and adults of *D. citri*. The highest mortality (>60%) was observed at the highest applied concentration of dsRNA (125 ng/μL). Silencing of *aChE* and *chE* in *D. citri* nymphs also increased the susceptibility of emerged adults to carbamate and organophosphate insecticides. These observations suggested that silencing of *aChE* and c*hE* genes in *D. citri* could be a promising tool to increase its susceptibility to insecticides for the control of this important vector.

2.4.2.3 *Myzus persicae* (Sulzer)

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is a major polyphagous sap-sucking pest that infests more than 400 species. Due to its feeding and transmission of more than 100 plant viruses, it is considered as one of the most damaging agricultural pests throughout the world. Its polyphagous behavior, cyclic parthenogenesis, and overlapping generations make it a highly successful insect pest (Hogenhout et al. 2008). Utilization of insecticides was earlier considered as the most effective way to combat *M. persicae*. Though, it has developed resistance against various insecticides resulting in control failures and losses in protected fruit crops. Thus, there is an urgent need to develop alternative (species-specific) control methods like RNAi for the control of this pest. Voltage-gated sodium channels (VGSC) are transmembrane proteins that initiate an action in excitable cells and play crucial role for neuronal signaling in insects. Since these channels play a vital role in nerve transmission, they have become primary targets for commercial insecticides. RNAi holds promising role for the control of agricultural insect pests. Tariq et al. (2019) targeted the VGSC ($MpNa_v$) gene in the *M. persicae* through oral feeding of artificial diets containing dsRNAs. Knockdown of $MpNa_v$ caused high mortality in third instar nymphs (Table 2.1). Moreover, significantly lower longevity and fecundity were observed in adults that had been fed with ds$MpNa_v$ solution at the nymphal stage. Gene expression studies revealed high aphid mortality along with lowered fecundity and longevity was traced to the downregulation of $MpNa_v$ by RNAi. These results showed that $MpNa_v$ is a viable target gene for RNAi-based biopesticide development.

In another RNAi-based approach, Bhatia and Bhattacharya (2018) targeted *cp* gene in *M. persicae* that senses seasonal photoperiodism and drives a shift from clonal to sexual generation in aphids (Table 2.1). Transgenic *Arabidopsis*

expressing dsRNA homologous to *cp* gene (*Mycp*) was developed. These results empirically demonstrated the suppression of fecundity in *cp*-downregulated aphids and consequent reduction in population size of aphid colonies on the transgenic lines.

The possibility of designing dsRNA effective for silencing the *cp19* gene in aphids but harmless to nontarget predator insects is very high. Shang et al. (2020) targeted *cp19* by ingesting species-specific dsRNAs to the aphid species, i.e., pea aphid, *Acyrthosiphon pisum* (Harris), and *M. persicae*, which produced 39.3–64.2% gene silencing and 45.8–55.8% mortality. Ingestion of non-species-specific dsRNA (ds-*cp19*) by *A. pisum* and *M. persicae* gave gene silencing levels ranging from 40.4 to 50.3% and 43.3 to 50.8% mortality (Table 2.1).

In recent years, many genes related to growth, development, and reproduction have been used as targets for pest control. These include *gus*, a highly conserved gene that has been reported to play an essential part in the genesis of germline cells and, hence, in fecundity in the model insect fruit fly, *Drosophila melanogaster* (Meigen). Gao et al. (2021) presented the first research experiment to target *gus* in *M. persicae*-designated *Mpgus* through RNAi and described its role in insect fecundity (Table 2.1). Results showed significant reduction in the number of embryos and newborn nymphs in the treated aphids as compared to control. Their investigation shows the significant role of *gus* gene in fecundity regulation in *M. persicae* which is a promising target gene for RNAi-based control of this pest.

2.4.2.4 *Planococcus citri* (Risso)

The citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), is a phloem feeder pest which causes loss of plant vigor and stunting on a range of fruit host plants. It also reduces quality of fruit and causes premature fruit drop leading to significant yield losses. Improved strategies are greatly needed for managing this pest. RNAi is a promising functional genomics tool and is being utilized as a practical tool for highly targeted insect control. Khan et al. (2013) investigated whether effects of RNAi can be induced by targeting *chs1* and *v-ATPase* gene in *P. citri* (Table 2.1). They used microinjection and recombinant tobacco mosaic virus (TMV) to express RNAi effects in tobacco, *Nicotiana benthamiana*. They reported that *P. citri* showed pronounced death of crawlers and lower fecundity after feeding on recombinant plants. This piece of research showed that *chs1* and *v-ATPase* were potential target genes for RNAi against *P. citri* and recombinant TMV was an efficient tool for assessing RNAi effectors in plants.

2.4.2.5 *Pseudococcus maritimus* (Ehrhorn)

The grape mealybug, *Pseudococcus maritimus* (Ehrhorn) (Hemiptera: Pseudococcidae), is an important pest and vector of plant diseases of grapevines (*Vitis* spp.). The lack of naturally occurring resistance traits in *Vitis* spp. hinders the management of this pest. Arora et al. (2020) reported that RNAi using dsRNA

against essential genes for feeding of sap can reduce insect survival. They targeted *aqp* and *suc* genes which are responsible for osmoregulation in related sap-sucking hemipteran insects (whiteflies and aphids) (Table 2.1). They also examined the *nuc* gene which reduce efficacy of RNAi by degrading administered dsRNA. The dsRNA delivery to the insects was done through artificial diet. Results showed significant increase in insect mortality over 3 days, compared to dsRNA-free controls. This research provides the foundation for development of plant RNAi against *P. maritimus* and related mealybug pests of grapevines.

2.4.3 Lepidoptera

2.4.3.1 *Epiphyas postvittana* (Walker)

The light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is a very serious pest of fruit crops. It is present in Australia, New Zealand, Hawaii, New Caledonia, and the UK and causes damage to apples or other plants (Wearing et al. 1991). It is an extremely polyphagous insect and considered to be a major pest of fruits (such as apples, strawberry, stone fruits, etc.). Turner et al. (2006) demonstrated that RNAi can be triggered by oral delivery of dsRNA to larvae in the horticultural pest, *E. postvittana*. They showed that larval gut gene (*cxe1*) transcript levels were reduced to less than half as compared to control within 2 days feeding *Eposcxe1* dsRNA (Table 2.1). Likewise, *pbp1* gene transcript levels were also reduced in antennae of adult by feeding *Epospbp1* dsRNA to larvae. This study provides basis for RNAi-based control of this pest.

2.4.4 Blattodea

2.4.4.1 *Coptotermes formosanus* (Shiraki)

Another important pest of fruit crops are the termites. The Formosan subterranean termite, *Coptotermes formosanus* (Shiraki) (Blattodea: Rhinotermitidae), causes serious damage to citrus, which consequently brings economic losses. These insect pests obtain nutrition and energy by feeding on wood and wood-related material with the help of endogenous and symbiotic cellulases (Bignell and Eggleton 2000). Endoglucanase is the significant cellulase in cellulose digestion. To manage this pest, Wu et al. (2018) investigated the effect of RNAi by targeting a conserved region of endoglucanase genes (*eG1a*, *eG1b*, *eG2*, *eG3*, eG4) in *C. formosanus* (*CfEGs*) (Table 2.1). Microinjection and oral delivery of dsRNA resulted in substantial silencing of target *CfEGs* and therefore led to reduced enzyme activity, reduced weight, and mortality as compared to control. They further combined *dsCfEG* with flufenoxuron for feeding workers which caused a lower enzymatic activity as compared to the *dsCfEG* or flufenoxuron-only treatment. Loss of weight and high

mortality were observed in the combined *dsCfEG* and flufenoxuron treatment. These results showed that *dsCfEGs* could be utilized in combination with other tactics to increase RNAi efficacy. This study thus provides basis for the use of RNAi in termite control.

2.4.5 Acari

2.4.5.1 *Panonychus citri* (McGregor)

The citrus red mite, *Panonychus citri* (McGregor) (Trombidiformes: Tetranychidae), is an important pest with extensive distribution throughout the world to cause considerable economic damage in citrus fruits. It feeds on leaves and fruit and occasionally on green twigs, causing a bronzing or silvering effect. To provide alternate to acaricides, Li et al. (2020) conducted RNAi experiments for the control of this pest by targeting the *hr3* gene which plays an important role in molting and metamorphosis of this pest (Table 2.1). Their result exhibited that *Pchr3* was primarily transcribed in the late deutonymph stage, the critical point at which the mites started molting, i.e., when the deutonymph stage was at least 24-h-old and immobile. Transcription reached the maximum level in 32-h-old deutonymphs and decreased by 36 h, where the mites remained in a motionless state. Additional silencing of *Pchr3* by delivery of dsRNA based on leaf disc method to 8-h-old deutonymph mites resulted in stunted development and high mortality of deutonymphs that suggested the role of *Pchr3* in controlling the molting in *P. citri*.

Management of this pest through pesticides led to enhanced pest resistance. In oviparous organisms, fabrication and development of yolk protein play a vital role in the reproduction. *Vitellin* (*vn*) is the source of egg storage that helps in proper functioning of *vitellogenin* (*vg*) and *vitellogenin receptor* (*vgR*). *VgR* is a very compulsory protein for the development of Vg into oocytes. Ali et al. (2017) targeted *vg* and *vgR* genes in *P. citri* and showed that females treated with *Pcvg* and *PcvgR* gene dsRNA exhibited reduced gene expression (Table 2.1). Knockdown of target genes significantly affected oviposition capacity up to 48% as compared to control. Synergistic effect of target gene dsRNA was also accessed that reduced oviposition by 60.42%. Moreover, combination of target dsRNA on protonymph and deutonymph also resulted in 67% and 70% reduction in eggs, respectively. This study suggested that abovementioned RNAi-based strategy controls *P. citri* population by reducing its reproduction.

2.4.5.2 *Tetranychus urticae* (Koch)

The two-spotted spider mite, *Tetranychus urticae* (Koch) (Trombidiformes: Tetranychidae), is a major polyphagous pest of 1100 host plant species including several highly valued economic crops (Migeon et al. 2010). The constant use of

acaricides leads to the evolution of acaricide resistance in this pest. The major reason for acaricide resistance is regulated by *Cytochrome P450*-mediated metabolic detoxification in *T. urticae*. *NADPH-cytochrome P450 reductase* (*cpr*) is a vital cofactor protein which donates electron(s) to microsomal *cytochrome P450s* to complete their catalytic cycle. Adesanya et al. (2020) investigated to understand the involvement of *cpr* in acaricide resistance in *T. urticae* (Table 2.1). They cloned and characterized full-length cDNA sequence of *Tucpr* that showed that *Tucpr* was transcribed universally in different life stages and the highest transcription was observed in the nymph and adult stages of *T. urticae*. *Tucpr* was significantly overexpressed in six acaricide-resistant populations as compared to susceptible one. Knockdown of *TuCPR* in *T. urticae* via RNAi led to decreased enzymatic activities of *Tucpr* and *cytochrome P450*, as well as a significant reduction in resistance to abamectin, bifenthrin, and fenpyroximate. This study thus highlighted *cpr* gene as a novel target for eco-friendly control of *T. urticae*.

2.5 Challenges in Using RNAi Technology

Utilization of RNAi appears to be promising for plant protection. However, various key concerns need to be solved before its proficient practical applications are realized in the field.

2.5.1 dsRNA Stability

The key concern for the use of RNAi-based biopesticides is regarding to its stability, particularly for the spray of dsRNA and application of siRNA. The various microorganisms have the ability for the degradation of dsRNA before its uptake by pathogens or pests. The quick dsRNA degradation could be due to the presence of nucleases in the gut lumen, saliva, and hemolymph of pests (Kennedy et al. 2004; Allen and Walker 2012; Katoch and Thakur 2013; Luo et al. 2013; Chung et al. 2018). The pH was found to vary from high to low range in the gut lumens of the insect pests depending upon the species. It can also decrease dsRNA stability either directly or indirectly by disturbing the activity of gut nucleases (Cooper et al. 2019). Environmental conditions may also exert various effects on dsRNA and siRNA stability. Studies also showed that degradation of dsRNA was also affected by water- and soil-type (Albright et al. 2017). Actin-dsRNA-derived Colorado potato beetle, *Leptinotarsa decemlineata* (Say), maintained its activity for 4 weeks after application to potato leaves. However, it inhibited weight gain of larva, delayed development, and increased mortality (San Miguel and Scott 2016). So, analyzing the process of degradation of dsRNA is helpful in assessing the possible effects of dsRNA in various environmental conditions and the target organisms.

2.5.2 dsRNA Uptake Mechanisms

Other key parameters, which denoted as most regulating factors at present, are the dsRNA uptake mechanisms into cells and, once entered, the recognition by target RNAi machinery toward the specific pattern or sequences. The uptake mechanism of dsRNAs was first described in *C. elegans*, along with explanation of systemic RNAi defective (SID) proteins. These proteins are involved in the dsRNA acquisition, transportation, and deriving siRNA along the body of nematode (Winston et al. 2002). Numerous SID-like proteins were described in insects without uniform results. In some insects, these proteins are found to be vital for stimulation of strong RNAi response; however, in other insects, they appear to be unnecessary (Wytinck et al. 2020a).

The other dsRNA mechanism, which has been newly proposed as one of the preferred routes for dsRNA entry, is the clathrin-mediated endocytosis. It has been recognized that endocytosis facilitated the dsRNA uptake in both insects and fungi (Wytinck et al. 2020b), but further investigations are required to elucidate the mechanism in more details. Evidence about adsorption and transportation of dsRNA is central to understand the evolution of resistance mechanisms in pest and pathogens, as already reported for *D. virgifera virgifera* (Khajuria et al. 2018).

Moreover, one of the utmost important, but poorly understood, factors is the capability of RNAi pathway to recognize the dsRNA of the target organism. In this context, conflicting results have been reported in insects. Although insects displayed varied responses irrespective of evolution, yet they tended to show differences among genera of the same family. For instance, as the recent study of coleopterans shows maximum susceptibility to RNAi, hemipterans and lepidopterans seem recalcitrant to RNAi treatments due either to reduced uptake of dsRNA or to the production of nucleases in their saliva (Dalakouras et al. 2016). Consequently, genetically modified organisms (GMOs) approaches relaying on the dsRNA expression in chloroplasts displayed a stronger efficacy as they do not process them into siRNA (Bally et al. 2018).

Presently, there is lack of information about identification of preferred nucleotide residues on dsRNA for their processing into siRNAs by Dicer-like enzymes apart from the preference of siRNAs or intact dsRNA delivery treatments. Particularly, evolutionary characteristics of Dicer-like enzyme sequence appear to be species-dependent in insects (Arraes et al. 2020). It can also lead to siRNA generation with species-dependent length distribution among different insects (Santos et al. 2019). Results suggested that achieving an optimal utilization of dsRNA as sustainable strategy for crop protection, formulation information (dsRNA size and concentration), uptake mechanisms, and features of RNAi machinery of target pests needed to be realized.

2.5.3 dsRNA Production Cost

For the implication of RNAi technique in field conditions, the key barrier is the production of an adequate amount of dsRNA. However, the traditional methods for production of dsRNA in the laboratory are costly and yield only a restricted amount of dsRNA which is not sufficient for large-scale applications (Ahn et al. 2019). Utilization of bacteria for the production of dsRNA *RNaseIII* deficiency appears to be a solution for this problem. However, only a limited work has demonstrated the production of dsRNA-based on microbes. Researchers also used an approach based on L1440-HT115 (DE3) system that has been efficaciously utilized for RNAi in oriental armyworm, *Mythimna separate* (Walker), and other insects as well (Das et al. 2015; Parsons et al. 2018). But this system is a bit costly and very tedious. The production efficiency of this system should be augmented with more research studies to meet market demands.

2.5.4 Off-Target Effects

RNAi is considered a sequence-specific mechanism, but a few investigations have shown that siRNA is not always specific and can have off-target effects which are challenging in RNAi-based pest management (Mamta and Rajam 2017). High conservation of some target genes between species upsurges the chances of off-targets among them. The *vATPase A* and *vATPase E* sequences from *L. decemlineata* shared nucleotide sequence identities about 83% and 79% to their counterparts in *D. virgifera virgifera*, respectively. The dsRNAs, i.e., *vATPase A* and *vATPase E* from *D. virgifera virgifera*, could reduce *L. decemlineata* fitness in a bioassay (Baum et al. 2007). Computational design program is required for the specific and systemic assessment of nontarget and off-target effects which should be further verified by additional bioassays. Moreover, feeding studies revealed that the length of dsRNAs at least 60 nucleotides (nt) is necessary for a proficient RNAi response in *D. virgifera virgifera* and *T. castaneum* (Wang et al. 2019a, b) and a minimum of 21-nt length is required for siRNA for efficient protection against *D. virgifera virgifera* (Bachman et al. 2013).

2.5.5 RNAi Resistance

The pathogenicity of pests and pathogens can result in development of resistance against RNAi-based products as they do for the conventional biopesticides through utilization of various mechanisms. But there could be fewer chances as compared to conventional commercialized transgenic crops expressing *Bt* toxins for management of pests (James 2010). A strategy based on RNAi induces downregulation of

the target gene by incomplete resistance in most of the cases which ultimately reduce the selection pressure that may contribute to long-lasting resistance. Genetic variation in an organism may also cause single-nucleotide polymorphisms (SNPs) in the target gene. Less complementarity between the target gene and dsRNA would reduce the RNAi efficiency. The difference between dsRNA and the original gene sequences reduces their complementarity resulting in reduced RNAi effect or RNAi resistance (Scott et al. 2013). Thus, the possibility of RNAi resistance should be taken into consideration while choosing it for practical applicability.

2.6 Future Perspectives

We have seen diverse applications of RNAi in crop protection approaches against pests in the past few years. Significant progress made in recent years is reviewed where RNAi technology has been applied to several crops and economic plants for protection against diseases like fungi, pests, and nematode (Liu et al. 2021). RNAi technology has ascended as a revolutionary strategy for qualitative and quantitative fruit production by controlling key pests of fruit crops. The wide use of host-induced gene silencing (HIGS) on a commercial scale appears possible in the near future. The key hurdles in HIGS strategy can become more efficient through optimal target and fragment selection methods, highly efficient transformation constructs, and stable transgenic systems. To this end, it is worthy to mention that the RNAi technology based on *v-ATPase* target gene has passed the genetically modified organisms (GMOs) safety evaluation in eight countries and regions including the USA, Brazil, and Japan. The US Environmental Protection Agency has also provided licenses for planting (Zotti et al. 2018), thus portraying an indefinable picture for the commercialization of RNAi technology. Technical hurdles are being overcome to allow a wide range of applications from laboratory to field. The technology of encapsulated dsRNA on leaves with spray-induced gene silencing (SIGS) will significantly promote dsRNA stability in the environment as well as during its uptake by pests enhancing plant protection. Cost-effective approaches for massive production of dsRNA (e.g., bacterial, plant, and synthetic production) are being optimized and will contribute to lowering costs of the technology. There is no doubt that a new era of RNAi-based pest control for fruit crop protection is right at the corner.

References

Adesanya AW, Cardenas A, Lavine MD, Walsh DB, Lavine LC, Zhu F (2020) RNA interference of NADPH-cytochrome P450 reductase increases susceptibilities to multiple acaricides in *Tetranychus urticae*. Pestic Biochem Physiol 165:104550. https://doi.org/10.1016/j.pestbp.2020.02.016

Ahn SJ, Donahue K, Koh Y, Martin RR, Choi MY (2019) Microbial based double-stranded RNA production to develop cost effective RNA interference application for insect pest management. Int J Insect Sci 11:1179543319840323. https://doi.org/10.1177/1179543319840323

Albright VC, Wong CR, Hellmich RL, Coats JR (2017) Dissipation of double-stranded RNA in aquatic microcosms. Environ Toxicol Chem 36(5):1249–1253. https://doi.org/10.1002/etc.3648

Ali MW, Zhang ZY, Xia S, Zhang H (2017) Biofunctional analysis of Vitellogenin and Vitellogenin receptor in citrus red mites, *Panonychus citri* by RNA interference. Sci Rep 7(1):16123. https://doi.org/10.1038/s41598-017-16331-3

Allen ML, Walker WB (2012) Saliva of *Lygus lineolaris* digests double stranded ribonucleic acids. J Insect Physiol 58(3):391–396. https://doi.org/10.1016/j.jinsPhys.2011.12.014

Arora AK, Clark N, Wentworth KS, Hesler S, Fuchs M, Loeb G, Douglas AE (2020) Evaluation of RNA interference for control of the grape mealybug, *Pseudococcus maritimus* (Hemiptera: Pseudococcidae). Insects 11(11):739. https://doi.org/10.3390/insects11110739

Arraes FBM, Martins-de-Sa D, Noriega Vasquez DD, Melo BP, Faheem M, de Macedo LLP, Morgante CV, Barbosa J, Togawa RC, Moreira VJV, Danchin EGJ, Grossi-de-Sa MF (2020) Dissecting protein domain variability in the core RNA interference machinery of five insect orders. RNA Biol 18(11):1653–1681. https://doi.org/10.1080/15476286.2020.1861816. Advance Access published 2020

Atwood D, Paisley-Jones C (2017) Pesticides industry sales and usage 2008–2012 market estimates. US Environmental Protection Agency, Washington, DC, pp 1–32

Bachman PM, Bolognesi R, Moar WJ, Mueller GM, Paradise MS, Ramaseshadri P, Tan J, Uffman JP, Warren J, Wiggins BE, Levine SL (2013) Characterization of the spectrum of insecticidal activity of a double-stranded RNA with targeted activity against Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte). Transgenic Res 22:1207–1222. https://doi.org/10.1007/s11248-013-9716-5

Bally J, Fishilevich E, Bowling AJ, Pence HE, Narva KE, Waterhouse PM (2018) Improved insect-proofing: expressing double-stranded RNA in chloroplasts. Pest Manag Sci 74:1751–1758. https://doi.org/10.1002/ps.4870

Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T, Roberts J (2007) Control of coleopteran insect pests through RNA interference. Nat Biotechnol 25:1322–1326. https://doi.org/10.1038/nbt1359

Belles X (2010) Beyond *Drosophila*: RNAi in vivo and functional genomics in insects. Annu Rev Entomol 55:111–128. https://doi.org/10.1146/annurev-ento-112408-085301

Bhatia V, Bhattacharya R (2018) Host-mediated RNA interference targeting a cuticular protein gene impaired fecundity in the green peach aphid *Myzus persicae*. Pest Manag Sci. https://doi.org/10.1002/ps.4900

Bignell DE, Eggleton P (2000) Termites in ecosystems, termites: evolution, sociality, symbioses, ecology. Springer, pp 363–387. https://doi.org/10.1007/978-94-017-3223-9_17

Cappelle K, de Oliveira CF, Van Eynde B, Christiaens O, Smagghe G (2016) The involvement of clathrin-mediated endocytosis and two Sid-1-like transmembrane proteins in double stranded RNA uptake in the Colorado potato beetle midgut. Insect Mol Biol 25:315–323. https://doi.org/10.1111/imb.12222

Chen S, Dai S, Lu K, Chang C (2008) Female-specific doublesex dsRNA interrupts yolk protein gene expression and reproductive ability in Oriental fruit fly, *Bactrocera dorsalis* (Hendel). Insect Biochem Mol Biol 38:155–165. https://doi.org/10.1016/j.ibmb.2007.10.003

Chung SH, Jing X, Luo Y, Douglas AE (2018) Targeting symbiosis related insect genes by RNAi in the pea aphid-Buchnera symbiosis. Insect Biochem Mol Biol 95:55–63. https://doi.org/10.1016/j.ibmb.2018.02.004

Clarke AR, Armstrong KF, Carmichael AE, Milne JR, Raghu S, Roderick GK, Yeates DK (2005) Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit fies. Annu Rev Entomol 50:293–319. https://doi.org/10.1146/annurev.ento.50.071803.130428

Clarke AR, Li ZH, Qin YJ, Zhao ZH, Liu LJ, Schutze MK (2019) *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is not invasive through Asia: it's been there all along. J Appl Entomol 143(8):797–801. https://doi.org/10.1111/jen.12649

Cooper AM, Silver K, Zhang J, Park Y, Zhu KY (2019) Molecular mechanisms influencing efficiency of RNA interference in insects. Pest Manag Sci 75:18–28. https://doi.org/10.1002/ps.5126

Cruz C, Tayler A, Whyard S (2018) RNA interference-mediated knockdown of male fertility genes in the Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae). Insects 9:96. https://doi.org/10.3390/insects9030096

Dalakouras A, Wassenegger M, McMillan JN, Cardoza V, Maegele I, Dadami E, Runne M, Krczal G, Wassenegger M (2016) Induction of silencing in plants by high pressure spraying of in vitro-synthesized small RNAs. Front Plant Sci 7:e163245. https://doi.org/10.3389/fpls.2016.01327

Das S, Debnath N, Cui Y, Unrine J, Palli SR (2015) Chitosan, carbon quantum dot, and silica nanoparticle mediated dsRNA delivery for gene silencing in *Aedes aegypti*: a comparative analysis. ACS Appl Mater Interfaces 7:19530–19535. https://doi.org/10.1021/acsami.5b05232

Dias N, Cagliari D, Kremer FS, Rickes LN, Nava DE, Smagghe G, Zotti M (2019) The South American fruit fly: an important pest insect with RNAi-sensitive larval stages. Front Physiol 10:794. https://doi.org/10.3389/fphys.2019.00794

Dominiak BC, Wiseman B, Anderson C, Walsh B, McMahon M, Duthie R (2015) Definition of and management strategies for areas of low pest prevalence for Queensland fruit fly *Bactrocera tryoni,* Froggatt. Crop Prot 72:41–46

Dong X, Li Q, Zhang H (2016a) The *noa* gene is functionally linked to the activation of the Toll/Imd signaling pathways in *Bactrocera dorsalis* (Hendel). Dev Comp Immunol 55:233–240. https://doi.org/10.1016/j.dci.2015.09.009

Dong YC, Wang ZJ, Chen ZZ, Clarke AR, Niu CY (2016b) *Bactrocera dorsalis* male sterilization by targeted RNA interference of spermatogenesis: empowering sterile insect technique programs. Sci Rep 6:35750. https://doi.org/10.1038/srep35750

Fabricius JC (1794) Entomologia systematica emendata et aucta, vol 4. Impensis Christ. Gottl. Proft., Copenhagen

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391:806–811. https://doi.org/10.1038/35888

Galdeano DM, Breton MC, Lopes JRS, Falk BW, Machado MA (2017) Oral delivery of double-stranded RNAs induces mortality in nymphs and adults of the Asian citrus psyllid, *Diaphorina citri*. PLoS One 12(3):e0171847. https://doi.org/10.1371/journal.pone.0171847

Gao Y, Ren R, Peng J, Wang D, Shi X, Zheng L, Zhang Z, Zhu C, Liu Y, Dai L, Zhang D (2021) The Gustavus gene can regulate the fecundity of the green peach aphid, *Myzus persicae* (Sulzer). Front Physiol 11:596392. https://doi.org/10.3389/fphys.2020.596392

Gautham HR, Bhardwaj ML (2020) International Year of Plant Health 2020 to focus on threat of pests on food security. Curr Sci 118:857

Gordon KH, Waterhouse PM (2007) RNAi for insect-proof plants. Nat Biotechnol 25:1231–1232. https://doi.org/10.1038/nbt1107-1231

Gregoriou ME, Reczko M, Kakani EG, Tsoumani KT, Mathiopoulos KD (2021) Decoding the reproductive system of the olive fruit fly, *Bactrocera oleae*. Genes 12:355. https://doi.org/10.3390/genes12030355

Guo S, Guo X, Zheng L, Zhao Z, Liu Z, Shen Z, Li Z (2020) A potential genetic control by suppression of the wing developmental gene wingless in a global invasive pest *Bactrocera dorsalis*. J Pest Sci 94(2):517–529. https://doi.org/10.1007/s10340-020-01263-1

Hakeem KR, Akhtar MS, Abdullah SNA (2016) Plant, soil and microbes, vol. 1: implications in crop science. Springer, New York, pp 1–366

Hannon GJ (2002) RNA interference. Nature 418:244–251. https://doi.org/10.1038/418244a

Hegedus D, Erlandson M, Gillott C, Toprak U (2009) New insights into peritrophic matrix synthesis, architecture, and function. Annu Rev Entomol 54:285–302. https://doi.org/10.1146/annurev.ento.54.110807.090559

Hogenhout SA, Ammar ED, Whitfeld AE, Redinbaugh MG (2008) Insect vector interactions with persistently transmitted viruses. Annu Rev Phytopathol 46:327–359. https://doi.org/10.1146/annurev.phyto.022508.092135

Huvenne H, Smagghe G (2010) Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: a review. J Insect Physiol 56:227–235. https://doi.org/10.1016/j.jinsphys.2009.10.004

James C (2010) A global overview of biotech (GM) crops: adoption, impact and future prospects. GM Crops 1:8–12. https://doi.org/10.4161/gmcr.1.1.9756

Jin S, Singh ND, Li L, Zhang X, Daniell H (2015) Engineered chloroplast dsRNA silences *cytochrome p450 monooxygenase*, *V-ATPase* and *chitin synthase* genes in the insect gut and disrupts *Helicoverpa armigera* larval development and pupation. Plant Biotechnol J 13:435–446. https://doi.org/10.1111/pbi.12355

Joga MR, Zotti MJ, Smagghe G, Christiaens O (2016) RNAi efficiency, systemic properties, and novel delivery methods for pest insect control: what we know so far. Front Physiol 7:553. https://doi.org/10.3389/fphys.2016.00553

Jose AM, Smith JJ, Hunter CP (2009) Export of RNA silencing from *C. elegans* tissues does not require the RNA channel *SID-1*. Proc Natl Acad Sci U S A 106(7):2283–2288. https://doi.org/10.1073/pnas.0809760106

Kamala Jayanthi PD, Verghese A, Sreekanth PD (2011) Predicting the Oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae) trap catch using artificial neural networks: a case study. Int J Trop Insect Sci 31:205–211. https://doi.org/10.1017/S1742758411000336

Katoch R, Thakur N (2013) Advances in RNA interference technology and its impact on nutritional improvement, disease and insect control in plants. Appl Biochem Biotechnol 169:1579–1605. https://doi.org/10.1007/s12010-012-0046-5

Kaur S, Singh S, Mohanpuria P, Li Z (2021) Successful rearing of fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) on a semi-solid artificial diet. Indian J Agric Sci 91(9):1342–1346

Kennedy S, Wang D, Ruvkun G (2004) A conserved siRNA degrading RNase negatively regulates RNA interference in *C. elegans*. Nature 427:645–649. https://doi.org/10.1038/nature02302

Khajuria C, Ivashuta S, Wiggins E, Flagel L, Moar W, Pleau M, Miller K, Zhang Y, Ramaseshadri P, Jiang C, Hodge T, Jensen P, Chen M, Gowda A, McNulty B, Vazquez C, Bolognesi R, Haas J, Head G, Clark T (2018) Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, *Diabrotica virgifera virgifera* LeConte. PLoS One 13:e0197059. https://doi.org/10.1371/journal.pone.0197059

Khan AM, Ashfaq M, Kiss Z, Khan AA, Mansoor S, Falk BW (2013) Use of recombinant tobacco mosaic virus to achieve RNA interference in plants against the citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae). PLoS One 8(9):e73657. https://doi.org/10.1371/journal.pone.0073657

Kishk A, Hizaz F, Anber H, Abdel TK, Abdel H, Sherbenib E, Hamed S, Killiny N (2017) RNA interference of acetylcholinesterase in the Asian citrus psyllid, *Diaphorina citri*, increases its susceptibility to carbamate and organophosphate insecticides. Pestic Biochem Physiol 9:4–10. https://doi.org/10.1016/j.pestbp.2017.09.004

Li X, Zhang M, Zhang H (2011) RNA interference of four genes in adult *Bactrocera dorsalis* by feeding their dsRNAs. PLoS One 6:e17788. https://doi.org/10.1371/journal.pone.0017788

Li G, Liu XY, Han X, Niu JZ, Wang JJ (2020) RNAi of the nuclear receptor HR3 suggests a role in the molting process of the spider mite *Panonychus citri*. Exp Appl Acarol 81(1):75–83. https://doi.org/10.1007/s10493-020-00486-2

Liu G, Wu Q, Li J, Zhang G, Wan F (2015) RNAi-mediated knock-down of *transformer* and *transformer 2* to generate male-only progeny in the Oriental fruit fly, *Bactrocera dorsalis* (Hendel). PLoS One 10(6):e0128892. https://doi.org/10.1371/journal.pone.0128892

Liu S, Geng S, Li A, Mao Y, Mao L (2021) RNAi technology for plant protection and its application in wheat. aBIOTECH. https://doi.org/10.1007/s42994-021-00036-3

Luo Y, Wang X, Wang X, Yu D, Chen B, Kang L (2013) Differential responses of migratory locusts to systemic RNA interference via double-stranded RNA injection and feeding. Insect Mol Biol 22:574–583. https://doi.org/10.1111/imb.12046

Macedo M, Avila S, Zucchi RA, Farias AF (2017) Mid-level image representation for fruit fly identification (Diptera: Tephritidae). In: Proceedings of the IEEE international conference on eScience, Auckland, pp 1–9

Malik H, Raza A, Amin I, Scheffler B, Brown JK, Mansoor S (2016) RNAi-mediated mortality of the whitefly through transgenic expression of double-stranded RNA homologous to acetylcholinesterase and ecdysone receptor in tobacco plants. Sci Rep 6:38469. https://doi.org/10.1038/srep38469

Mamta B, Rajam MV (2017) RNAi technology: a new platform for crop pest control. Physiol Mol Biol Plants 23:487–501. https://doi.org/10.1007/s12298-017-0443-x

Mamta B, Reddy KRK, Rajam MV (2016) Targeting chitinase gene of *Helicoverpa armigera* by host-induced RNA interference confers insect resistance in tobacco and tomato. Plant Mol Biol 90:281–292. https://doi.org/10.1007/s11103-015-0414-y

Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, Wang LJ, Huang YP, Chen X (2007) Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nat Biotechnol 25:1307–1313. https://doi.org/10.1038/nbt1352

Matsumoto Y, Hattori M (2016) Gene silencing by parental RNA interference in the green rice leafhopper, *Nephotettix cincticeps* (Hemiptera: Cicadellidae). Arch Insect Biochem Physiol 91(3):152–164. https://doi.org/10.1002/arch.21315

Mello CC, Conte D (2004) Revealing the world of RNA interference. Nature 431:338–342. https://doi.org/10.1038/nature02872

Migeon A, Nouguier E, Dorkeld F (2010) Spider Mites Web: a comprehensive database for the Tetranychidae, trends in acarology. Springer, pp 557–560. https://doi.org/10.1007/978-90-481-9837-5

Miglani GS (2015) Essentials of molecular genetics. Alpha Science International Ltd, Oxford

Mohanpuria P, Kumar V, Mahajan M, Mohammad H, Yadav SK (2010) Gene silencing: theory, techniques and applications. In: Catalano AJ (ed) Genetics-research and issues. Nova Science Publishers, Inc., New York, pp 321–334

Mohanpuria P, Govindaswamy M, Sidhu GS, Singh S, Kaur S, Chhuneja P (2021) Ingestion of bacteria expressing dsRNA to maggots produces severe mortality and deformities in fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Egypt J Biol Pest Control 31(1). https://doi.org/10.1186/s41938-020-00345-7

Narayanan ES, Batra HN (1960) Fruit flies and their control. ICAR, New Delhi

Nellen W, Lichtenstein C (1993) What makes an mRNA anti-sense-itive? Trends Biochem Sci 18:419–423. https://doi.org/10.1016/0968-0004(93)90137-c

Newmark PA, Reddien PW, Cebria F, Sanchez Alvarado A (2003) Ingestion of bacterially expressed double-stranded RNA inhibits gene expression in planarians. Proc Natl Acad Sci U S A 100(Suppl 1):11861–11865

Ole-MoiYoi OK, Lux SA (2004) Fruit flies in sub-Saharan Africa: a long-neglected problem devastating local fruit production and a threat to horticulture beyond Africa. In: Proc 6th int symp fruit flies econ imp, Ultra Litho, Johannesburg, South Africa, pp 5–10

Pak J, Fire A (2007) Distinct populations of primary and secondary effectors during RNAi in *C. elegans*. Science 315:241–244. https://doi.org/10.1126/science.1132839

Parsons KH, Mondal MH, McCormick CL, Flynt AS (2018) Guanidinium-functionalized interpolyelectrolyte complexes enabling RNAi in resistant insect pests. Biomacromolecules 19:1111–1117. https://doi.org/10.1021/acs.biomac.7b01717

Pieterse W, Terblanche JS, Addison P (2017) Do thermal tolerances and rapid thermal responses contribute to the invasion potential of *Bactrocera dorsalis* (Diptera: Tephritidae)? J Insect Physiol 98:1–6. https://doi.org/10.1016/j.jinsphys.2016.11.004

Prentice K, Pertry I, Christiaens O, Bauters L, Bailey A, Niblett C, Ghislain M, Gheysen G, Smagghe G (2015) Transcriptome analysis and systemic RNAi response in the African sweet potato weevil (*Cylas puncticollis*, Coleoptera: Brentidae). PLoS One 10:e0115336. https://doi.org/10.1371/journal.pone.0115336

Price DR, Gatehouse JA (2008) RNAi-mediated crop protection against insects. Trends Biotechnol 26:393–400. https://doi.org/10.1016/j.tibtech.2008.04.004

Roignant JY, Carre C, Mugat B, Szymczak D, Lepesant JA, Antoniewski C (2003) Absence of transitive and systemic pathways allows cell-specific and isoform-specific RNAi in *Drosophila*. RNA 9:299–308. https://doi.org/10.1261/rna.2154103

Saleh MC, van Rij RP, Hekele A, Gillis A, Foley E, O'Farrell PH, Andino R (2006) The endocytic pathway mediates cell entry of dsRNA to induce RNAi silencing. Nat Cell Biol 8:793–802. https://doi.org/10.1038/ncb1439

San Miguel K, Scott JG (2016) The next generation of insecticides: dsRNA is stable as a foliar-applied insecticide. Pest Manag Sci 72:801–809. https://doi.org/10.1002/ps.4056

Santos D, Mingels L, Vogel E, Wang L, Christiaens O, Cappelle K, Wynant N, Gansemans Y, Van Nieuwerburgh F, Smagghe G, Swevers L, Vanden-Broeck J (2019) Generation of virus-and dsRNA-derived siRNAs with species-dependent length in insects. Viruses 11:738. https://doi.org/10.3390/v11080738

Santos-Ortega Y, Killiny N (2018) Silencing of sucrose hydrolase causes nymph mortality and disturbs adult osmotic homeostasis in *Diaphorina citri* (Hemiptera: Liviidae). Insect Biochem Mol Biol 101:131–143. https://doi.org/10.1016/j.ibmb.2018.09.003

Schott D, Yanai I, Hunter CP (2014) Natural RNA interference directs a heritable response to the environment. Sci Rep 4:7387. https://doi.org/10.1038/srep07387

Scott JG, Michel K, Bartholomay LC, Siegfried BD, Hunter WB, Smagghe G, Zhu KY, Douglas AE (2013) Towards the elements of successful insect RNAi. J Insect Physiol 59:1212–1221. https://doi.org/10.1016/j.jinsphys.2013.08.014

Searchinger T, Waite R, Hanson C, Ranganathan J, Dumas P, Matthews E (2018) Creating a sustainable food future: synthesis report. World Resources Institute, Washington, DC, pp 1–96

Shang F, Ding BY, Ye C, Yang L, Chang TY, Xie J, Tang LD, Niu J, Wang JJ (2020) Evaluation of a cuticle protein gene as a potential RNAi target in aphids. Pest Manag Sci 76(1):134–140. https://doi.org/10.1002/ps.5599

Singh S (2020) Integrated pest management of fruit flies, *Bactrocera* spp. in rainy season guava in India. Agric Res J 57(4):541–547

Singh S, Kaur G (2016) Status of fruit flies on fruit crops in Punjab and their integrated management. Plenary lecture. In: Kamala Jayanthi PD, Chakravarthy AK, Raghava T, Kempraj V (eds) Tephritid seminar 2016-understanding Tephritids in Toto: taxonomy, ecology, quarantine and management. Indian Institute of Horticultural Research, Bengaluru, pp 51–52

Singh S, Sharma DR (2013) Management of fruit flies in rainy season guava through male annihilation technique using methyl eugenol-based traps. Indian J Hortic 70:512–518

Singh S, Huang J, Grieshop M (2020) The presence and accessibility of competitive resources affect trapping efficiency of Spotted-wing *Drosophila* (Diptera: Drosophilidae). J Econ Entomol 20(20):1–6. https://doi.org/10.1093/jee/toaa271

Siomi H, Siomi MC (2009) On the road to reading the RNA-interference code. Nature 457:396–404. https://doi.org/10.1038/nature07754

Smith NA, Singh SP, Wang MB, Stoutjesdijk PA, Green AG, Waterhouse PM (2000) Total silencing by intron-spliced hairpin RNAs. Nature 407:319–320. https://doi.org/10.1038/35030305

Sohail S, Tariq K, Zheng W, Waqar AM, Peng W, Raza MF, Zhang H (2019) RNAi-mediated knockdown of *tssk1* and *tektin1* genes impair male fertility in *Bactrocera dorsalis*. Insects 10(6):164. https://doi.org/10.3390/insects10060164

Taning CNT, Christiaens O, Berkvens N, Casteels H, Maes M, Smagghe G (2016) Oral RNAi to control *Drosophila suzukii*: laboratory testing against larval and adult stages. J Pest Sci 89:803–814. https://doi.org/10.1007/s10340-016-0736-9

Tariq K, Ali A, Davies TGE, Naz E, Naz L, Sohail S, Hou M, Ullah F (2019) RNA interference-mediated knockdown of voltage-gated sodium channel ($MpNa_v$) gene causes mortality in peach-potato aphid, *Myzus persicae*. Sci Rep 9(1):5291. https://doi.org/10.1038/s41598-019-41832-8

Tayler A, Heschuk D, Giesbrecht D, Park JY, Whyard S (2019) Efficiency of RNA interference is improved by knockdown of dsRNA nucleases in tephritid fruit flies. Open Biol 9(12):190198. https://doi.org/10.1098/rsob.190198

Thakur N, Upadhyay SK, Verma PC, Chandrashekar K, Tuli R, Singh PK (2014) Enhanced whitefly resistance in transgenic tobacco plants expressing double stranded RNA of *v-ATPase A* gene. PLoS One 9:e87235. https://doi.org/10.1371/journal.pone.0087235

Thongsaiklaing T, Nipitwattanaphon M, Ngernsiri L (2018) The transformer2 gene of the pumpkin fruit fly, *Bactrocera tau* (Walker), functions in sex determination, male fertility and testis development. Insect Mol Biol 27(6):766–779. https://doi.org/10.1111/imb.12517

Tomoyasu Y, Miller SC, Tomita S, Schoppmeier M, Grossmann D, Bucher G (2008) Exploring systemic RNA interference in insects: a genome-wide survey for RNAi genes in *Tribolium*. Genome Biol 9:R10. https://doi.org/10.1186/gb-2008-9-1-r10

Turner CT, Davy MW, MacDiarmid RM, Plummer KM, Birch NP, Newcomb RD (2006) RNA interference in the light brown apple moth, *Epiphyas postvittana* (Walker) induced by double-stranded RNA feeding. Insect Mol Biol 15(3):383–391. https://doi.org/10.1111/j.1365-2583.2006.00656.x

Varkouhi AK, Scholte M, Storm G, Haisma HJ (2011) Endosomal escape pathways for delivery of biologicals. J Control Release 151:220–228. https://doi.org/10.1016/j.jconrel.2010.11.004

Wang X, Lou L (1995) Research progress in the Chinese citrus fruit fly. Entomol Knowl 32:310–315

Wang Y, Andongma AA, Dong Y, Chen Z, Xu P, Ren X, Krosch MN, Clarke A, Niu C (2019a) *Rh6* gene modulates the visual mechanism of host utilization in fruit fly *Bactrocera minax*. Pest Manag Sci 75(6):1621–1629. https://doi.org/10.1002/ps.5278

Wang K, Peng Y, Fu W, Shen Z, Han Z (2019b) Key factors determining variations in RNA interference efficacy mediated by different double-stranded RNA lengths in *Tribolium castaneum*. Insect Mol Biol 28:235–245. https://doi.org/10.1111/imb.12546

Wearing CH, Thomas WP, Dugdale JS, Danthanarayana W (1991) Tortricid pests of pome and stone fruits, Australian and New Zealand species. In: Van der Geest LPS, Evenhuis HH (eds) Tortricid pests, their biology, natural enemies and control. Elsevier, Amsterdam, pp 453–472

Wesley SV, Helliwell CA, Smith NA, Wang MB, Rouse DT, Liu Q, Gooding PS, Singh SP, Abbott D, Stoutjesdijk PA, Robinson SP, Gleave AP, Green AG, Waterhouse PM (2001) Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J 27:581–590. https://doi.org/10.1046/j.1365-313x.2001.01105.x

White IM, Elson-Harris MM (1992) Fruit flies of economic significance: their identification and bionomics. CAB International, Wallingford, p 601

Whyard S, Singh AD, Wong S (2009) Ingested double-stranded RNAs can act as species-specific insecticides. Insect Biochem Mol Biol 39:824–832. https://doi.org/10.1016/j.ibmb.2009.09.007

Winston WM, Molodowitch C, Hunter CP (2002) Systemic RNAi in *C. elegans* requires the putative transmembrane protein SID-1. Science 295:2456–2459. https://doi.org/10.1126/science.1068836

Wu W, Gu D, Yan S, Li Z (2018) RNA interference of endoglucanases in the Formosan subterranean termite *Coptotermes formosanus* Shiraki (Blattodea: Rhinotermitidae) by dsRNA injection or ingestion. J Insect Physiol 2:112. https://doi.org/10.1016/j.jinsphys.2018.11.007

Wytinck N, Manchur CL, Li VH, Whyard S, Belmonte MF (2020a) dsRNA uptake in plant pests and pathogens: insights into RNAi-based insect and fungal control technology. Plants 9:1780. https://doi.org/10.3390/plants9121780

Wytinck N, Sullivan DS, Biggar KT, Crisostomo L, Pelka P, Belmonte MF, Whyard S (2020b) Clathrin mediated endocytosis is involved in the uptake of exogenous double-stranded RNA in the white mold phytopathogen *Sclerotinia sclerotiorum*. Sci Rep 10:1–12. https://doi.org/10.1038/s41598-020-69771-9

Xiong Y, Zeng H, Zhang Y, Xu D, Qiu D (2013) Silencing the *HaHR3* gene by transgenic plant-mediated RNAi to disrupt *Helicoverpa armigera* development. Int J Biol Sci 9:370–381. https://doi.org/10.7150/ijbs.5929

Xiong KC, Wang J, Li JH, Deng YQ, Pu P, Fan H, Liu YH (2016) RNA interference of a trehalose-6-phosphate synthase gene reveals its roles during larval-pupal metamorphosis in *Bactrocera minax* (Diptera: Tephritidae). J Insect Physiol 91:84–92. https://doi.org/10.1016/j.jinsphys.2016.07.003

Xu W, Han Z (2008) Cloning and phylogenetic analysis of sid-1-like genes from aphids. J Insect Sci 8:1–6. https://doi.org/10.1673/031.008.3001

Xu J, Wang XF, Chen P, Liu FT, Zheng SC, Ye H, Mo MH (2016) RNA interference in moths: mechanisms, applications and progress. Genes 7:88. https://doi.org/10.3390/genes7100088

Xu P, Wang Y, Akami M, Niu CY (2019) Identification of olfactory genes and functional analysis of *BminCSP* and *BminOBP21* in *Bactrocera minax*. PLoS One 14(9):e0222193. https://doi.org/10.1371/journal.pone.0222193

Xu L, Xu S, Sun L, Zhang Y, Luo J, Bock R, Zhang J (2021) Synergistic action of the gut microbiota in environmental RNA interference in a leaf beetle. Microbiome 9(98). https://doi.org/10.1186/s40168-021-01066-1

Yang WJ, Xu KK, Cong L, Wang JJ (2013) Identification, mRNA expression, and functional analysis of *chitin synthase 1* gene and its two alternative splicing variants in Oriental fruit fly, *Bactrocera dorsalis*. Int J Biol Sci 9:331–342. https://doi.org/10.7150/ijbs.6022

Yang RL, Zhang Q, Fan JY, Yue Y, Chen EH, Yuan GR, Dou W, Wang JJ (2021) RNA interference of *Argonaute-1* delays ovarian development in the Oriental fruit fly, *Bactrocera dorsalis* (Hendel). Pest Manag Sci 77(9):3921–3933. https://doi.org/10.1002/ps.6419

Yu X, Killiny N (2018) RNA interference of two glutathione S-transferase genes, DcGSTe2 and DcGSTd1, increases the susceptibility of Asian citrus psyllid (Hemiptera: Liviidae) to the pesticides, fenpropathrin and thiamethoxam. Pest Manag Sci 74(3):638–647. https://doi.org/10.1002/ps.4747

Yu XD, Liu ZC, Huang SL, Chen ZQ, Sun YW, Duan PF, Ma YZ, Xia LQ (2016) RNAi-mediated plant protection against aphids. Pest Manag Sci 72:1090–1098. https://doi.org/10.1002/ps.4258

Yu YC, Lu H, Chiang YC, Tsai CL, Zuo YH, Chen ME (2021) Molecular characteristics of fat body protein 1 in the Oriental fruit fly, *Bactrocera dorsalis*. Insects 12:319. https://doi.org/10.3390/insects12040319

Zeng Y, Reddy GVP, Li Z, Qin Y, Wang Y, Pan X, Jiang F, Gao F, Zhao ZH (2018) Global distribution and invasion pattern of Oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). J Appl Entomol 0:1–12. https://doi.org/10.1111/jen.12582

Zheng W, Liu Y, Zheng W, Xiao Y, Zhang H (2015) Influence of silencing sex-peptide receptor on *Bactrocera dorsalis* adults and offspring by feeding ds-spr. J Asia Pac Entomol 18:477–481

Zhu JQ, Liu S, Ma Y, Zhang JQ, Qi HS, Wei ZJ, Yao Q, Zhang WQ, Li S (2012) Improvement of pest resistance in transgenic tobacco plants expressing dsRNA of an insect associated gene *EcR*. PLoS One 7:e38572. https://doi.org/10.1371/journal.pone.0038572

Zhu J, Dong YC, Li P, Niu CY (2016) The effect of silencing 20E biosynthesis relative genes by feeding bacterially expressed dsRNA on the larval development of *Chilo suppressalis*. Sci Rep 6:28697. https://doi.org/10.1038/srep28697

Zotti MJ, Smagghe G (2015) RNAi technology for insect management and protection of beneficial insects from diseases: lessons, challenges and risk assessments. Neotrop Entomol 44:197–213. https://doi.org/10.1007/s13744-015-0291-8

Zotti M, Dos Santos EA, Cagliari D, Christiaens O, Taning CNT, Smagghe G (2018) RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. Pest Manag Sci 74:1239–1250. https://doi.org/10.1002/ps.4813

Zucchi RA (2008) Fruit flies in Brazil. University São Paulo, Brazil, São Paulo

Chapter 3
Molecular Markers for Insect Resistance: Potential and Limitations

Hari C. Sharma, Mukesh K. Dhillon, Pooja Manchanda, Gaurav Kumar Taggar, and Preetinder Singh Sarao

3.1 Introduction

The last two decades have seen rapid progress in molecular biology with whole genome sequencing of model organisms such as humans, *Saccharomyces*, *Arabidopsis*, and rice (Chalfie 1998; Sherman 1998; Palevitz 2000; Shoemaker et al. 2001; Piskur and Langkjaer 2004). Recombinant DNA technologies have the potential for identification of specific chromosomal regions carrying the genes associated with resistance to the target insect pests (Karp et al. 1997). There are many types of DNA markers, which have advantages for a particular application in linkage mapping and marker-assisted selection (MAS) for resistance to insect pests (Sharma 2009). Once genomic regions contributing to traits of interest have been identified, the alleles at each locus designated by molecular markers can be transferred into locally adapted high-yielding cultivars by crossing and tracked with the marker(s) in subsequent generations. Wild relatives of crops have useful alleles for insect resistance and can be utilized in crop breeding programs through a combination of conventional phenotyping and MAS (Xiao et al. 1996; Miflin 2000).

H. C. Sharma (✉)
International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Dr YS Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India

M. K. Dhillon
Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India

P. Manchanda
School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

G. K. Taggar · P. S. Sarao
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_3

Genetic maps based on recombination frequencies are important, but there can be discrepancies in physical and genetic maps. Therefore, it is important to correlate genetic and physical maps for fine-mapping and isolating the genes of interest. High-density genetic linkage maps have been developed for barley, *Hordeum vulgare*; maize, *Zea mays*; potato, *Solanum tuberosum*; rye, *Secale cereale*; sorghum, *Sorghum bicolor*; soybean, *Glycine max*; tomato, *Lycopersicon esculentum*; and wheat, *Triticum aestivum* (Paterson et al. 1991; Hernandez et al. 2001; Korzun et al. 2001; Boyko et al. 2002; Sharopova et al. 2002; Somers et al. 2004; Song et al. 2004). Molecular markers in some of these crops have been linked to genes expressing resistance to the target insect pests. The crossover between the gene of interest and the marker due to their location distance makes the identified marker in one cross to be irrelevant in another cross, unless the marker is linked to the resistance gene (Mohan et al. 1997). Once closely linked markers for insect resistance genes are identified, MAS can be practiced in early generations and at early stages of plant growth to speed up the selection process. The MAS can also be used for pyramiding resistance genes from diverse sources.

3.2 Mapping Populations

Identification of QTLs controlling insect resistance can be carried out by using different mapping populations. The parents used for the generation of mapping population should be polymorphic for the trait of interest. Before choosing the parent for the mapping population, a panel of genotypes should be screened for the extreme phenotypes to identify the genetically diverse parents for developing the mapping population. It takes five to six generations to transfer insect resistance traits into the high-yielding cultivars through conventional breeding. However, gene transfer from the wild relatives may take longer time due to the complexity of achieving interspecific hybrids on a sufficiently large scale to identify progeny with insect resistance and acceptable agronomic desirability. The improved lines with insect resistance, thus, developed need to be tested across seasons and locations, before a variety could be identified for cultivation by the farmers or for use in breeding programs. In marker-assisted selection programs, the elite breeding lines or cultivars can be crossed with the source of resistance and the F_1 hybrid backcrossed with the recurrent/elite parent (BC_1), and the gene transfer can be monitored through MAS until $BC_{3–5}$.

3.3 Molecular Markers

There are a number of techniques for detecting DNA sequence polymorphism (Paterson et al. 1991; Staub et al. 1996), wherein several types of molecular markers have been used to evaluate DNA polymorphism such as hybridization-based

markers (nucleic acid hybridization, Southern 1975), PCR-based markers (Mullis 1990), and sequence-based markers. The marker to be used in genomic studies should be (1) polymorphic in nature, (2) codominant in inheritance, (3) frequently occurred in the genome, (4) selectively neutral in behavior, (5) easy to access and fast assay, and (6) highly reproducible and should have (7) ease of exchange of data between laboratories, (8) clear distinct allelic features, and (9) no pleiotropic effects. The characterization of genetic diversity, genome fingerprinting, genome mapping, gene localization, genome evolution, population genetics, taxonomy, plant breeding, and diagnostics is based upon the combined use of several single locus detection systems for understanding various aspects of plant genome. Several types of molecular markers have been used for developing genetic linkage maps of different crops to identify quantitative trait loci (QTLs) associated with resistance to insects (Yencho et al. 2000; Smith 2005; Sharma 2009).

3.4 Identification of Molecular Markers for Insect Resistance in Field Crops

The linkage between QTL and molecular marker loci is determined the same way as phenotypic resistance is linked with the segregation of genes for resistance to insect pests. The QTL analyses help in identifying the loci from a group of polymorphic segregating molecular makers that contribute most significantly to explain the phenotypic variation for biological, morphological, and biochemical characters mediating insect resistance. A key component of QTL analysis is the calculation of a logarithm of the odds to base 10 (LOD) score, which is a statistical estimate of the likelihood of recombination between two loci due to chance alone. The LOD scores indicate whether the two loci are likely to be near one another on a chromosome and therefore likely to be inherited together. The progress in identifying genomic regions associated with resistance to insects in different crops is shown below.

*3.4.1 Rice (*Oryza sativa*)*

3.4.1.1 Rice Gall Midge (*Orseolia oryzae*)

An AFLP marker *SA598* linked to the gene *Gm7* (dominant gene nonallelic to *Gm2*), conferring resistance to rice gall midge biotypes 1, 2, and 4, has been identified (Sardesai et al. 2001). Biradar et al. (2004) tagged and mapped *Gm1* on chromosome 9 (using SSR markers *RM316*, *RM444*, and *RM219*). *Gm8* has been tagged and mapped on rice chromosome 8, wherein two fragments, *AR257* and *AS168*, have been linked to resistant and susceptible phenotypes, respectively (Jain et al. 2004). Another resistant phenotype-specific marker, *AP19*(*587*), was also identified using RAPDs. There is a tight linkage between the markers and the *Gm8* locus.

Himabindu et al. (2010) mapped the new resistance gene *Gm11* by using RIL mapping population, derived from the cross between TN1 and MR1523. The gene was mapped on chromosome 12 flanked by markers RM28574 and RM28706. Sama et al. (2014) identified a new gene, *Gm3*, that confers resistance to five of the seven Indian biotypes of the Asian rice gall midge. Li et al. (2019a, b) identified a new gene, *Gm6*, from a Kangwenqingzhan variety, derived from the gall midge-resistant landrace 'Daqiu', which was located on the long arm of chromosome 4 region flanked by markers YW91 and YW3–4. Later, Zhou et al. (2019) suggested that the resistance gene *Gm5* was located on the same region in chromosome 12 in three varieties near marker 12M22.6 flanked by markers *Z*57 and *Z*64. Recently, Leelagud et al. (2020) identified a new resistant gene, *Gm12*, in F_2 plants derived from a cross between KDML105 (susceptible) and MN62M (resistant). The locus was mapped between two flanking single-nucleotide polymorphism (SNP) markers, S2_76222 and S2_419160, on the short arm of rice chromosome 2.

3.4.1.2 Rice Stem Borer (*Tryporyza incertulas*)

Several RAPD markers linked to yellow stem borer resistance have been identified; however, the chromosome location of these genes is unknown (Selvi et al. 2002). Four phenotype-specific RAPD markers linked with resistance ($C1_{320}$ and $K6_{695}$) and susceptibility ($AH5_{660}$ and $C4_{1300}$) have been identified, of which $K6_{695}$ and $AH5_{660}$ were linked to the resistance gene(s) at distances of 12.8 cM and 14.9 cM, respectively. The reproducibility and association with the trait were confirmed with these markers in germplasm.

3.4.1.3 Rice Brown Plant Hopper (BPH) (*Nilaparvata lugens*)

The advances in molecular markers have led to identification of 40 major genes and QTLs designated from *Bph1* to *Bph40* for resistance to BPH from wild and cultivated rice germplasm. These genes are located on 7 (2, 3, 4, 6, 10, 11, and 12) of 12 rice chromosomes. Rice chromosome 12 contains eight genes, including *Bph1, bph2, Bph7, Bph9, Bph10, Bph18, Bph21,* and *Bph26*, followed by six genes, *Bph3, bph4, Bph22, Bph25, Bph29,* and *Bph32*, on chromosome 6. Nine genes, *Bph12, Bph15, Bph17, Bph20, Bph27, Bph33, Bph34, Bph35, and Bph36*, are located on chromosome 4. Four genes, *Bph11, Bph13, Bph14,* and *Bph19,* are located on chromosome 3 and two genes, *Bph37* and *Bph38*, on chromosome 1. One gene each, *Bph13, Bph30,* and *Bph28*, is located on chromosomes 2, 10, and 11, respectively. A number of BPH genes (*Bph1*, *bph2*, *Bph6*, *Bph7*, *Bph13*, *Bph15*, *Bph19*, *Bph20*, *Bph21*, *Bph25*, *Bph27*, and *Bph28*) have been fine mapped and a few genes cloned, which are suitable for marker-assisted selection for BPH resistance.

Hu et al. (2018) identified a new BPH-resistant gene, *Bph33*, in the $F_{2:3}$ populations and near-isogenic lines (NILs) derived from crosses between two BPH-resistant Sri Lankan rice cultivars (KOLAYAL and POLIYAL) and a BPH-susceptible

cultivar 9311, and two flanking InDel markers, H25 and D17, were developed. Kumar et al. (2018) mapped another BPH-resistant gene, *Bph34*, on the long arm of chromosome 4 by using F_2 population derived from a cross between susceptible *indica* cultivar PR122 and wild species *O. nivara* acc. IRGC104646. An introgression line RBPH660 derived from *Oryza rufipogon* led to the identification of a major BPH-resistant locus, *Bph35*, on chromosome 4 between InDel markers PSM16 and R4M13, which accounted for 51.27% of the phenotypic variation (Yuexiong et al. 2020). Li et al. (2019a, b) identified *Bph36* from wild rice GX2183-derived introgression lines (RBPH16 and RBPH17), mapped on the short arm of chromosome 4 flanked by InDel markers S13 and X48, and RM16766 and RM17033, respectively. Yang et al. (2019) identified *Bph37* in rice variety IR64, which flanked between markers RM302 and YM35 on chromosome 1. Another major BPH-resistant gene, *Bph38*, was identified on the long arm of chromosome 1 between SNP markers 693 and 369 (Balachiranjeevi et al. 2019). Two newly introgressed BPH resistance genes from *O. nivara* in the background of Swarna were designated as *Bph39(t)* and *Bph40(t)* (Akanksha et al. 2019). More recently, BPH-resistant QTLs were mapped using an RIL (F_7) population derived from the cross Swarna/PTB33, wherein four QTLs were found tightly linked with markers *QBph6.1* (RM7158-RM19606), *QBph6.3* (RM402-RM276), and *QBph12.1* (RM28378-RM28427) (Akula et al. 2020).

3.4.1.4 Rice Green Leafhopper (*Nephotettix cincticeps*)

The green rice leafhopper (GRH)-resistant gene, *Grh5*, located on the distal region of the long arm of chromosome 8, is tightly linked to markers *RM3754* and *RM3761* (Fujita et al. 2006). The QTLs for *N. virescens* resistance on chromosomes 3 and 11 are very near to *Grh2* and *Grh4*. The near-isogenic lines (NIL) containing both *Grh2* and *Grh4* express resistance to *N. virescens* (Wang et al. 2003, 2004). The *Grh3* was located on chromosome 6 by Saka et al. (2006) in cultivar 'Rantaj emas 2' to a 4.6-Mb interval between markers C288B and C133A. This locus has been further fine mapped to 435-kb region between RM20142 and RM20145 SSR markers (Hur et al. 2015). Phi et al. (2019) identified a new GRH-resistant gene, *Grh6*, between markers RM5414 and C60248. Later, Thein et al. (2019) identified four GRH-resistant QTLs, designated as *qGRH2, qGRH4, qGRH5,* and *qGRH11* in W1413 accession of African wild rice (*O. longistaminata*), wherein *qGRH2* was mapped on the long arm of chromosome 2, *qGRH4* on the short arm of chromosome 4, *qGRH5* on the short arm of chromosome 5, and *qGRH11* on the long arm of chromosome 11.

*3.4.2 Wheat (*Triticum *spp.)*

3.4.2.1 Hessian Fly (*Mayetiola destructor*)

Five SSR markers (*Xgwm136*, *Xcfa2153*, *Xpsp2999*, *Xgwm33*, and *Xbarc263*) linked to Hessian fly resistance gene, *Hdic*, are located on the short arm of chromosome 1A, in the same region as *H9*, *H10*, *H11*, and *H13* genes (Liu et al. 2005a, b, c, d). Sardesai et al. (2005) identified a new gene, *H32*, located on the long arm of chromosome 3D, which confers resistance to the highly pervasive biotype L of the Hessian fly. McDonald et al. (2014) identified a durum wheat line that confers resistance to Hessian fly populations from Maryland. Although the resistance of the durum donor appeared to involve more than one gene, one partially dominant but very effective gene, *H33*, was identified and successfully transferred in the hexaploid recipient. This gene was mapped to the short arm of wheat chromosome 3A, flanked by single sequence repeat markers *Xgwm218* and *Hbg284*. Li et al. (2013) identified a new gene, *H34*, that confers resistance to the biotype GP of the Hessian fly on chromosome 6B flanked by loci *Xsnp921* and *Xsnp2745*, explaining 37.2% of the phenotypic variation using a population of RILs derived from the cross 'Ning7840' and 'Clark' by single-seed descent method. Recently, Zhao et al. (2020) identified two novel QTLs for Hessian fly resistance from SD06165. The major QTL, designated as *H35,* was closely linked to SNP marker *SDOKSNP7679* on chromosome 3BS, and the minor QTL, designated as *H36*, was flanked by SNP markers *SDOKSNP1618* and *SDOKSNP8089* on chromosome 7AS.

3.4.2.2 Russian Wheat Aphid (RWA) (*Diaraphis noxia*)

A new biotype of Russian wheat aphid, appeared in 2003 and designated as RWA2, severely damaged the *Dn4* gene-resistant wheat in addition to other resistance genes *Dn1*, *Dn2, dn3, Dn5,* and *Dn6* in Colorado. To overcome this problem, Valdez et al. (2012) identified RWA2 resistance gene *Dn626580* in PI626580. Linkage mapping analysis showed three simple sequence repeat (SSR) markers, *Xbarc214, Xgwm473,* and *Xgwm437,* proximally linked to *Dn626580* near the centromere on the short arm of chromosome 7D at distances of 1.8, 5.0, and 8.2 cM, respectively. Fazel-Najafabadi et al. (2015) mapped *Dn2401* gene on the short arm of chromosome 7D in wheat line, CI2401. Recently, Li et al. (2018) showed that PI 682675 carries a dominant resistance gene, *Dn10*, flanked by simple sequence repeat markers *Xgwm437* and *Xwmc488* on chromosome 7DL.

3.4.2.3 Greenbug (*Schizaphis graminum*)

Several QTLs have been identified for resistance against greenbug, *S. graminum*, in wheat (Castro et al. 2004). Weng and Lazar (2002) used AFLP and SSR markers to tag a single dominant gene, *Gb3*, conferring resistance to greenbug (Zhu et al. 2005). *Aegilops tauschii*-derived greenbug resistance locus analysis in wheat revealed that microsatellite markers *Xwmc157* and *Xgdm150* flank *Gbx1* at 2.7 and 3.3 cM and *Xwmc671* is linked to *Gba*, *Gbb*, *Gbc*, and *Gbd* at 34.3, 5.4, 13.7, and 7.9 cM, while *Xgdm150* is distal to *Gbc* at 17.7 cM, wherein *Gbd* is different from *Gbx1* or *Gbz* and appears to be a new resistance gene (Weng et al. 2005). Genes *Gbx1*, *Gba*, *Gbc*, and *Gbd* are either allelic or linked to *Gb3*. *Gby* is another greenbug resistance gene in the wheat line 'Sando's selection 4040' and is inherited as a single semidominant gene located on wheat chromosome 7A (Boyko et al. 2004). The selection accuracy of RFLP markers *Xbcd98*, *Xpsrll9*, or *XZnfp* and *Pr1b* flanking *Gby* can be used to tag this gene with 99.78% efficacy and can be used in MAS (Boyko et al. 2004). Crespo-Herrera et al. (2014) remapped a previously reported gene for *S. graminum* resistance (putatively *Gba*) in 7DL and found a novel QTL associated with the number of aphids (*QGb.slu-2DL*) on chromosome 2DL.

3.4.2.4 Corn Leaf Aphid (*Rhopalosiphum padi*)

The first report on the genetic mapping of aphid resistance in wheat was mapped by Crespo-Herrera et al. (2014). A quantitative trait locus (QTL) for antibiosis (*QRp.slu.4BL*) that explained 10.2% of phenotypic variation was found in chromosome 4BL. Two QTLs were identified for corn leaf aphid tolerance (*QRp.slu.5AL* and *QRp.slu.5BL*) on chromosomes 5AL and 5BL, with an epistatic interaction between a locus on chromosome 3AL (*EnQRp.slu.5AL* and *QRp.slu.5AL*), explaining about 35% phenotypic variation (Crespo-Herrera et al. 2014).

*3.4.3 Maize (*Zea mays*)*

3.4.3.1 European Corn Borer (ECB) (*Ostrinia nubilalis*)

Seven QTLs have been identified for resistance against second-generation ECB (BNL5.62-UMCI57, UMC33-UMC128, NP1287-BNL, UMC137-UMC36, UMC175-BNL, BNL14.07-UMC59, and UMC64-NP1303) (Schon et al. 1993). Rind penetrometer resistance (RPR) has been found to be useful in enhancing stalk lodging resistance (Flint-Garcia et al. 2003). The molecular characterization of a population of testcrossed $F_{(2:3)}$ families of early-maturing maize germplasm resulted in identification of six QTLs for stalk damage rating, which explained 27.4% genotypic variance for ECB resistance (Papst et al. 2004). Of the 12 QTLs for neutral detergent fiber (NDF) and acid detergent fiber (ADF) in leaf sheath, 5 for each trait

were at or near QTL for European corn borer tunneling (Cardinal and Lee 2005). Four of the eight leaf sheath acid detergent lignin (ADL) QTLs were detected in the same genomic regions as ECB QTL. Therefore, resistance to ECB may be associated with a subset of the QTL observed for cell wall components and ADF and starch concentration in the stalk (Krakowsky et al. 2007).

3.4.3.2 Mediterranean Corn Borer (MCB) (*Sesamia nonagrioides*)

Resistance to MCB have been mapped in B73 × Mo17 cross (Ordas et al. 2009) and recombinant inbred maize populations (Ordas et al. 2010), each having two and three QTLs on chromosomes 1 and 9 and 1, 3, and 8, respectively. The results suggested the presence of pleiotropism or linkage between the genes controlling MCB resistance and agronomic traits and, thus, have less possibility of use in marker-assisted selection. Samayoa et al. (2014, 2015) reported QTLs for yield under *S. nonagrioides* infestation and identified six QTLs for resistance traits.

3.4.3.3 Southwestern Corn Borer (SWCB) (*Diatraea grandiosella*), African Stem Borer (*Busseola fusca*), and Spotted Stem Borer (*Chilo partellus*)

Eight QTLs linked to resistance against leaf feeding by SWCB were mapped in F_2 maize population, which explained 20% of the phenotypic variation (Brooks and Barfoot 2015). To co-localize genomic regions involved in hydroxycinnamate synthesis and resistance to corn borer in EP125 × PB130 population, seven QTLs were identified for p-coumarate, two for ferulate, and seven for total diferulates, explaining 81.7, 26.9, and 57.8% genotypic variance, respectively. These QTLs were mapped on chromosomes 1–7 and 9 (Santiago et al. 2016). In another study, a QTL for decreased tunneling by *B. fusca* was detected on chromosome 4, while a QTL for reduced tunneling and exit holes by *C. partellus* was detected on chromosomes 4 and 5, respectively (Munyiri and Mugo 2017). Meta-analyses of QTLs for response of the maize to stem borers and storage pests feeding on different plant parts of maize generated 24 leaf (LIR), 42 stem (SIR), and 20 kernel (KIR) insect resistance meta-QTLs (Badji et al. 2018).

3.4.3.4 Shoot Fly (*Atherigona* spp.)

Genotyping of F_2 population [CM143 (resistant) × CM144 (susceptible)] performed with 120 SSR markers mapped two major QTLs [*qDH9.1* (deadheart) and *qEC9.1* (oviposition)] on chromosome 9, explaining 15.03 and 18.89% phenotypic variance for resistance against shoot fly in maize, and suggested that shoot fly resistance is under polygenic control (Vikal et al. 2020).

3.4.3.5 Fall Armyworm (FAW) (*Spodoptera frugiperda*)

A multi-locus genome-wide association study (GWAS) detected 62 quantitative trait nucleotides (QTNs) related with FAW and maize weevil (MW) resistance traits on 10 maize chromosomes within or in close proximity to multiple insect resistance genomic regions concerning FAW, stem borer (SB), and MW (Badji et al. 2020).

*3.4.4 Sorghum (*Sorghum bicolor*)*

3.4.4.1 Sorghum Shoot Fly (*Atherigona soccata*)

Genetic linkage maps of two RIL mapping populations (BT × 623 × IS 15881-3 and 296B × IS 18551) identified polymorphic SSRs associated with resistance to shoot fly and/or phenotypic traits associated with resistance to this insect in sorghum (Folkertsma et al. 2003; Hash et al. 2003). Markers *Xtxp258* (bp 190/230) and *Xtxp289* (bp 270/294) are linked to trichome density; *Xgap1* (bp 180/254) and *Xtxp141* (bp 154/169) to deadheart incidence, leaf glossiness, and trichome density; *Xisp328* (bp 144/166) and *Xisp264* (bp 153/207) to leaf glossiness; and *Xisp258* (bp 170/193) and *Xtxp65* (bp 125/134) to deadheart incidence and leaf glossiness. Twenty-nine QTLs were detected by multiple QTL mapping (MQM) in the cross 296B (susceptible) × IS18551 (resistant), four each for leaf glossiness and seedling vigor, seven for oviposition nonpreference, six for deadhearts, two for adaxial trichome density, and six for abaxial trichome density. Seven markers, Xnhsbm1008, Xnhsbm1011, Xnhsbm1013, Xnhsbm1033, Xnhsbm1043, Xnhsbm1044, and Xnhsbm1048, linked to shoot fly resistance have been identified (Satish et al. 2009). Twenty-five QTLs (five each for leaf glossiness and seedling vigor, ten for deadhearts, two for adaxial trichome density, and three for abaxial trichome density) have been detected in individual and across environments. Xtxp278-Xisp10233, Xtxp320-Xcup16, and Xisep0625-Xgap1 were identified as putative candidate genes in the major QTL intervals for shoot fly resistance (Aruna et al. 2011). From a cross between IS18551 (resistant to shoot fly) and 296B (susceptible to shoot fly), a skeleton linkage map of 135 RILs (IS18551, shoot fly-resistant × 296B, shoot fly-susceptible) using SSR markers mapped 7 SSR markers each on linkage groups A and C, and favorable alleles for the QTLs (Xtxp248–Xtxp316, Xtxp248–Xtxp316, and Xtxp248–Xtxp316) were identified in the resistant parent IS18551 for shoot fly resistance (Apotikar et al. 2011). Introgression of shoot fly resistance QTLs into elite post-rainy season sorghum varieties using marker-assisted backcrossing led to the discovery of some novel molecular markers associated with the QTLs controlling resistance to shoot fly in sorghum (Gorthy et al. 2017).

3.4.4.2 Greenbug (*Schizaphis graminum*)

The 93 GBIK × Redlan-derived sorghum RILs mapped 12 linkage groups covering 1530 cM, wherein 4 SSRs (Sb5-214, Sb1-10, SbAGB03, and SbAGA01) and 1 RAPD (OPB12-795) markers were linked to QTLs associated with resistance to greenbug biotypes I and K (Agrama et al. 2002). Evaluation of 26 sorghum accessions from 12 countries for resistance to greenbug biotype I identified 26 AFLP primer combinations with 819 polymorphic fragments, suggesting relatively high level of polymorphism among the accessions (Wu et al. 2006). A set of sorghum RILs from the cross '96-4121' (greenbug-tolerant parent) × Redlan (greenbug-susceptible parent) evaluated with 60 SSR loci identified 3 closely linked markers (Xtxp12, Xcup20, and Sb1_10) mapped on LG 3 (Nagaraj et al. 2005). Two QTLs for resistance to greenbug on sorghum chromosome 9 (SBI-09) were closely flanked by the markers Xtxp358, Xtxp289, Xtxp67, and Xtxp230 (Wu and Huang 2008). The cDNA microarrays identified 21 sorghum genotypes as new sources of greenbug resistance having 1 major and a minor QTL on chromosome 9 (Huang 2011). To identify genomic regions contributing resistance to greenbug biotype I in a sorghum accession, PI 607900, a linkage map of 729.5 cM has also been constructed using 102 polymorphic SSR markers (69 genomic and 33 EST SSRs) (Punnuri et al. 2012). Further, the BT×623 (greenbug-susceptible line) × PI 607900 (greenbug-resistant line)-derived population revealed high phenotypic variation (72·9 to 80·9%) by the markers Starssbnm 93 and Starssbnm 102 on chromosome 9, and these can be used to breed for greenbug resistance in sorghum (Punnuri and Huang 2017).

3.4.4.3 Sorghum Midge (*Stenodiplosis sorghicola*)

Genetic regions located on two separate linkage groups were associated with the antixenosis mechanism of resistance to sorghum midge and explained 12 and 15% of the total variation in egg-laying, respectively, while one region was associated with antibiosis and explained 34.5% of the variation in egg and pupal counts (Tao et al. 2003).

3.4.4.4 Stem Borers (*Chilo partellus*, *Busseola fusca*, *Sesamia inferens*)

The QTL mapping for resistance to *B. fusca* and *C. partellus* with 4955 SNP markers using 243 $F_{9:10}$ sorghum RILs derived from ICSV 745 (S) × PB 15520-1 (R) revealed that 4 QTLs associated with *C. partellus* deadhearts, located on chromosomes 2, 6, and 9 (flanked by markers CS369_2, CS389_2, EF322_6, and BF152_9). Three QTLs for leaf feeding were located on chromosomes 2 and 6 (flanked by CS133_2, CS397_2, and EF184_6); seven QTLs for exit hole on chromosomes 2, 3, 4, 5, 6, and 7 (flanked by CS414_2, DB153_3, DB208_3, BC222_4, m05/015.6, EF255_6, and GH66_7); and three QTLs for stem tunneling on chromosomes 3 and

7 (flanked by markers DB152_3, GH70_7, and GH118_7). For *B. fusca*, ten QTLs were detected on chromosomes 1, 2, 3, 4, 8, and 5 (flanked by markers SB691_1, CS402_2, CS259_2, CS350_2, DB172_3, DB169_3, BC149_4, JK399_8, BF97_9, and BF106_9) for deadhearts and seven QTLs for leaf feeding on chromosomes 2, 3, 6, 8, and 10 (flanked by CS403_2, CS111_2, CS397_2, DB164_3, EF334_6, m08/014.9, and CPS158_10) (Muturi et al. 2021).

3.4.4.5 Head Bugs (*Calocoris angustatus*)

For mapping studies, A F_2 progeny derived from a cross between head bug-resistant Malisor 84-7 and susceptible S 34, two QTLs were detected on LG D, in the interval between markers RZ476 and SbRPG872, and on LG E, between markers SbRPG667 and CDO580 (Deu et al. 2004).

*3.4.5 Chickpea (*Cicer arietinum*)*

3.4.5.1 Pod Borer (*Helicoverpa armigera*)

Barmukh et al. (2020) undertook the development of a dense genetic map and QTL analysis with 3873 SNP markers, spanning a distance of 949.27 cM for pod borer resistance in chickpea. Comprehensive analyses identified 9 main effect QTLs and 955 epistatic QTLs, explaining up to 42.49 and 38.05% phenotypic variance, respectively, for resistance to *H. armigera*. One QTL cluster harboring main effect QTLs for three *H. armigera* resistance component traits, and explaining up to 42.49% of the phenotypic variance, was identified on CaLG03. However, further fine-mapping and functional characterization of these genes are required to pinpoint the candidate genes underlying the identified QTLs.

*3.4.6 Pigeon Pea (*Cajanus cajan*)*

3.4.6.1 Plume Moth (*Exelastis atomosa*)

Resistance to plume moth is dominantly controlled by a single locus or cluster of tightly linked alleles (Mishra et al. 2015). Bulked segregant analysis of 116 F_2 progenies identified a fragment OPA09910 linked to *PPM1* locus conferring resistance to plume moth. Further, the resistance-specific fragment OPA09910 was cloned, sequenced, and converted into a sequence characterized amplified region (SCAR) marker, SCOPA09942, which was also closely associated (10.3 cM) with the locus *PPM1*. BLAST analysis with pigeon pea genome sequence also confirmed its occurrence in CcLG02 (Scafseq.LG_V5.0fa) and contig 01597 (AFSP01.fsa1).

This SCAR marker showed reasonable screening efficiency in the F_2, F_3, and BC_1F_1 lines; thus, it can be used as genetic handle in marker-assisted introgression of the genomic fragment conferring plume moth resistance and screening of pigeon pea breeding lines (Mishra et al. 2015).

*3.4.7 Cowpea (*Vigna unguiculata*)*

3.4.7.1 Aphid (*Aphis craccivora*)

A cross between an aphid-resistant cultivated cowpea, IT 84S-2246-4, and aphid-susceptible wild cowpea, NI 963, evaluated for aphid resistance and RFLP marker segregation (Myers et al. 1996) revealed that one RFLP marker, *bg4D9b*, was tightly linked to aphid resistance gene (*Rac1*) and several flanking markers in the same linkage group (linkage group 1) were also identified. The close association of *Rac1* and *bg4D9b* presents an opportunity for cloning this insect resistance gene. A cowpea wild relative, TVNu-1158, has been successfully crossed with cowpea, and a set of RILs developed to generate a linkage map of cowpea (Souleymane et al. 2013). In addition, QTLs with effects on domestication-related traits have also been detected (Lo et al. 2018).

3.4.7.2 Thrips (*Thrips tabaci* and *Frankliniella schultzei*)

A cross between foliar thrips-susceptible IT93K503-1 and the resistant black-eyed cowpea cultivar ‘California Blackeye No. 46’ (CB46) identified three QTLs on linkage groups 5 and 7 (Muchero et al. 2010). These QTLs’ (*Thr-1*, *Thr-2*, and *Thr-3*) peaks were collocated with AFLP markers ACCCAT7, ACG-CTC5, and AGG-CAT1 and were linked with foliar damage caused by *T. tabaci* and *F. schultzei*.

3.4.7.3 Bruchid (*Callosobruchus maculatus*)

Genome-wide association study for bruchid resistance using 41,948 polymorphic SNP markers identified 11 SNPs linked to average number of eggs, bruchid holes, insect emergence, development period, and Dobie’s susceptibility index for bruchids (Miesho et al. 2019). Gene search via Phytozome identified six candidate genes (*Vigun08g132300, Vigun08g158000, Vigun06g053700, Vigun02g131000, Vigun01g234900*, and *Vigun01g201900*) to be associated with bruchid resistance traits, which could be incorporated into the farmers’ preferred cowpea cultivars.

*3.4.8 Common Bean (*Phaseolus vulgaris*)*

3.4.8.1 Leafhopper (*Emrasca* sp.)

Bulk segregant analysis and QTL analysis identified eight markers associated with resistance to potato leafhopper, *E. fabae*, and four markers were associated with resistance to *E. kraemeri* (Murray et al. 2004). Three markers were associated with resistance to both species. Composite interval mapping identified QTL for resistance to the leafhoppers on core-map linkage groups B1, B3, and B7.

3.4.8.2 Thrips (*Thrips palmi*)

Mesoamerican bean lines, BAT 881 and G 21212, showed transgressive segregation for resistance to thrips, *Thrips palmi*, in the field (Frei et al. 2005). A major QTL (*Tpr*6.1) for thrips resistance located on LG *b06* explained up to 26.8% variance, and the QTLs were mapped on LGs *b02*, *b03*, *b06*, and *b08*, some of which were located in regions containing genes encoding disease resistance.

3.4.8.3 Bruchid (*Zabrotes subfasciatus*)

Amkul et al. (2019) constructed a high-density linkage map to identify QTLs for resistance to bruchids in Zombi pea (*Vigna vexillata*). A linkage map based on F_2 population from a cross between 'TVNu 240' (resistant) and 'TVNu 1623' (susceptible) varieties has been used to construct a linkage map of 6529 single-nucleotide polymorphism markers generated from sequencing amplified fragments of specific loci. The map comprised 11 linkage groups, spanning 1740.9 cM, with an average of 593.5 markers per linkage group and an average distance of 0.27 cM between markers. One major and three minor QTLs for *C. chinensis* resistance and one major and one minor QTL for *C. maculatus* resistance were identified. The major QTLs for resistance to *C. chinensis* and *C. maculatus* appeared to be at the same locus.

*3.4.9 Mung Bean (*Vigna radiata*)*

3.4.9.1 Bruchids (*Callosobruchus* spp.)

Two markers, OPC-06 and STSbr2, are linked with the bruchid resistance locus *Br2* in a TC1966 × susceptible cross (Young et al. 1992). Chen et al. (2007) identified single dominant gene (*Br*) and the vignatic acid (*Va*) gene and identified eight RAPD markers linked to *Br* gene. Ten RAPD markers associated with bruchid resistance were identified in the progeny derived from the cross TC1966 × NM92 (mung bean yellow mosaic virus-resistant variety), of which four markers (OPW02,

UBC223, OPU11, and OPV02) were closely linked (Chen et al. 2007). The SSR markers SSRbr1, DMB-SSR158, and GBssr-MB87 have been reported for bruchid resistance in mung bean (Miyagi et al. 2004; Chotechung et al. 2011; Chen et al. 2013; Hong et al. 2015). In V2802 and TC 1966, chromosome 5 possesses the DMB-SSR 158 marker associated with *Vradi05g03940-VrPGIP1* and *Vradi05g03950-VrPGIP2* genes, which code for polygalacturonase inhibitor involved in bruchid resistance (Chen et al. 2013; Chotechung et al. 2016). The major QTL in TC1966 and DMB-SSr 158 marker are <0.1 cM away from the bruchid-resistant gene (Chen et al. 2013). Also, QTL *qBr* has been reported between markers VrBr-SSR013 and DMB-SSR158 at the same position. The sequence-changed protein genes (SCPs) and differentially expressed genes (DEGs) retain the transcript diversity and specificity of the *Br* genes (Liu et al. 2016), and the variations in DEGs promoter and of SCPs can be potential markers in breeding for resistance against bruchids. A major *Br* locus and a few minor loci with one or two genes might account for bruchid resistance in mung bean (Young et al. 1992; Chen et al. 2013). Two QTLs, *MB87* and *SOPU11*, have been reported to be associated with bruchid-resistant genes in the cross involving Sunhwa (susceptible) and Jangan (resistant variety developed from backcrossing with V2709) (Hong et al. 2015). Mei et al. (2009) reported a QTL in wild mung bean ACC41 that accounts for 98.5% of bruchid resistance. A mung bean population derived from TC1966 and V2802 carry a strong QTL locus on chromosome 5 for bruchid resistance, suggesting that they are co-segregating alleles (Schafleitner et al. 2016). VrPGIP2, which encodes a polygalacturonase-inhibiting protein (PGIP) in V2802 accession, is responsible for resistance to *C. chinensis* and *C. maculatus* (Kaewwongwal et al. 2017). One QTL, which controlled expression of resistance to both *C. chinensis* and *C. maculatus*, was located in a 237.35 Kb region of mung bean chromosome 5 that contained eight annotated genes, including *VrPGIP1* (LOC106760236) and VrPGIP2 (LOC106760237). Thus, tightly linked *VrPGIP1* and *VrPGIP2* are the likely genes at *Br* locus that confer bruchid resistance in mung bean 'V2709' (Kaewwongwal et al. 2020).

3.4.9.2 Bean Bug (*Riptortus clavatus*)

A 13.7-cM map genetic linkage of mung bean with six markers identified two QTLs for bruchid resistance and one QTL for bean bug resistance, which can be used for cloning of bruchid and bean bug resistance genes (Hong et al. 2015). The major constraint is the large distance between the markers and the gene/QTL controlling resistance to bean bug (Shi et al. 2009; Schafleitner et al. 2016).

*3.4.10 Soybean (*Glycine max*)*

3.4.10.1 Defoliators (*Helicoverpa zea* and *Pseudoplusia includens*)

A gene conferring resistance to corn ear worm, *Helicoverpa zea* from PI 229358, was mapped between 50 and 58 cM on the composite genetic map (Narvel et al. 2000). Mapping of QTLs associated with insect resistance from PI 229358 and PI 171451 identified a QTL on linkage group *D1b* (*SIR-D1b*) (Narvel et al. 2001): one major (RFLP marker *A584* on linkage group *M*) and two minor QTLs (RFLP markers *R249* on linkage group 'H' and *Bng047* on linkage group *D1*) for resistance to *H. zea* in soybean (Rector et al. 1998). Another RFLP map based on Cobb × PI 171451 and Cobb × PI 227687 revealed that a QTL on LG *H* was shared among all three resistant genotypes, and a major QTL on LG *M* was shared between PI 171451 and PI 229358, while a minor QTL on LG *C2* was unique to PI 227687, and a minor QTL on LG *D1* was unique to PI 229358 (Rector et al. 1999). An antibiosis and antixenosis QTL on linkage group *LG M* was detected in Cobb × PI 171451 and Cobb × PI 229358 populations for *H. zea* resistance (Rector et al. 2000). Resistance to a broad range of leaf-chewing insects has been found in PI 229358 and PI 227687, where resistance in PI 229358 is conferred by QTLs M, G, and H and in PI 227687 by QTL E. Pyramiding these QTLs with cry1Ac increased protection against Bt-tolerant pests and can effectively deploy Bt with plant resistance genes (Ortega et al. 2016).

3.4.10.2 Soybean Pod Borer (*Leguminivora glycinivorella*)

Zhao et al. (2015) identified four QTLs (ARC-Satt208-Sat292, Satt144-Sat074, Satt540-Sat244, and Satt345-Satt592) for soybean pod borer resistance, as well as for isoflavone content on chromosomes Gm7, Gm10, Gm13, and Gm17, which might be useful in MAS to breed soybean cultivars with pod borer resistance and high seed isoflavone content.

3.4.10.3 Soybean Aphid (*Aphis glycines*)

Resistance to soybean aphid is controlled by a single dominant gene, *Rag1*, in Dowling (Hill et al. 2006; and Jackson (Li et al. 2007). However, Zhang et al. (2009) identified two QTLs on linkage groups F and M in PI 567541B, conferring aphid-resistant alleles at both the loci, and hence could be useful in improving aphid resistance in soybean.

*3.4.11 Groundnut (*Arachis hypogea*)*

3.4.11.1 Aphid (*Aphis craccivora*)

Resistance to the aphid, *A. craccivora*, has been identified in the breeding line ICG 12991, which is controlled by a single recessive gene, mapped on linkage group 1 at 3.9 cM from a marker originating from the susceptible parent, explaining 76.1% of the phenotypic variation for aphid resistance (Herselman et al. 2004).

3.4.11.2 Groundnut Bruchid (*Caryedon serratus*)

QTL analysis for bruchid resistance in groundnut mapped two QTLs qTDP-b08 and qAE2010/11-a02 for total developmental period and adult emergence, respectively (Mondal et al. 2014). Additionally, three QTLs for TDP, adult emergence, and number of holes and one QTL for pod weight loss were identified, which explained 14–39% of the phenotypic variation.

*3.4.12 Barrel Medick (*Medicago truncatula*)*

3.4.12.1 Blue-Green Aphid (*Acyrthosiphon kondoi*) and Pea Aphid (*Acyrthosiphon pisum*)

A semidominant gene, *AIN* (*Acyrthosiphon*-induced necrosis) exhibiting hypersensitive reaction (HR) to blue-green aphid and pea aphid, has been identified in A17 and A20 genotypes of barrel medick, which presents a novel opportunity to use them as a model to study the role of the HR in defense responses to phloem-feeding insects (Klingler et al. 2009).

3.4.12.2 Aphid (*Aphis craccivora*)

QTL analysis using a F_2 population from a cross between barrel medick (*M. truncatula*) accession SA30199 and Borung revealed that resistance to *A. craccivora* is controlled in part by a major QTL on chromosome 2, explaining 39% of the antibiosis resistance (Kamphuis et al. 2012). The identified locus will facilitate marker-assisted breeding of *M. truncatula* for increased resistance to *A. craccivora* and other closely related *Medicago* species such as alfalfa.

3.5 Identification of Molecular Markers for Insect Resistance in Horticultural Crops

*3.5.1 Tomato (*Lycopersicon esculentum*)*

3.5.1.1 Aphid (*Macrosiphum euphorbiae*) and Whitefly (*Bemisia tabaci*)

A number of commercial cultivars of tomato contain the *Mi* gene, which provides resistance to three species of root knot nematodes (*Meloidogyne* spp.) (Roberts and Thomason 1986) and some populations of the potato aphid, *Macrosiphum euphorbiae* (Goggin et al. 2001; Rossi et al. 1998). Rossi et al. (1998) concluded that *Mi* and *Meu-1* are the same gene and *Mi* mediated resistance against both aphids and nematodes. The *Mi-1.2* gene is responsible for the resistance to both B and Q biotypes of silver leaf whitefly, *B. tabaci*, in transgenic tomato plants carrying this gene (Nombela et al. 2003).

*3.5.2 Melon (*Cucumis melo*)*

3.5.2.1 Melon Aphid (*Aphis gossypii*)

The genetic locus *Vat* (virus aphid transmission) controls antibiosis, antixenosis, and virus transmission resistance in PI 414723 (Pitrat and Lecoq 1982). Aphid resistance in the Korean accession, PI 161375, is also conditioned by *Vat*. Klingler et al. (2001) used 64 F2-derived F_3 families to map the aphid resistance locus, *Vat*, where RFLP markers NBS-2 and AC-39 flanked *Vat* at distances of 3.1 cM and 6.4 cM, respectively. NBS-2 is homologous to the nucleotide binding site-leucine-rich repeat (NBS-LRR) superfamily of plant resistance genes. Another homolog of this superfamily, NBS-5, was positioned ≈16.8 cM from *Vat*, raising the possibility that *Vat* resides in a cluster of NBS-LRR paralogs. RFLP marker AC-8 has similarity to plant lipoxygenases and positioned at ≈5.5 cM from *Vat*.

*3.5.3 Apple (*Malus *sp.)*

3.5.3.1 Rosy Leaf-Curling Aphid (*Dysaphis devecta*)

Alston and Briggs (1977) described three rosy leaf-curling aphid biotypes and four resistance genes. The gene for resistance to biotypes 1 and 2 from Cox's Orange Pippin was designated as *Sd*-1, while resistance to biotype 1 only, derived from Northern Spy, was designated as *Sd*-2. Another gene for resistance to biotype 3 was designated as *Sd*-3, which was derived from *Malus robusta* and *M. zumi*. The *Sd*-1 gene was fine mapped in aphid resistance region conferring resistance to biotypes 1

and 2 on linkage group 7 of var. Fiesta, wherein SdSSRa and 2B12a co-located with the RFLP marker MC064, which was tightly linked to *Sd*-1 and co-segregated with *Sd*-2 locus, suggesting that *Sd*-1 and *Sd*-2 are tightly linked, and probably allelic (Cevik and King 2002).

3.5.3.2 Rust Mite (*Aculus schlechtendali*)

The molecular map for *A. schlechtendali* resistance based on F_1 progenies the cultivars 'Fiesta' × 'Discovery' identified two QTLs on linkage group 7 of 'Fiesta'. The SSR marker Hi03a10 associated with one of the QTLs (AFLP marker E35M42-0146) was traced back in the 'Fiesta' pedigree to the apple cultivar 'Wagener', which may facilitate the breeding of rust mite-resistant apple cultivars (Stoeckli et al. 2009).

*3.5.4 Citrus (*Citrus *sp.)*

3.5.4.1 Citrus Leaf Miner (*Phyllocnistis citrella*)

Bernet et al. (2005) detected two antibiosis and six antixenosis putative QTLs controlling *P. citrella* resistance (trifoliate orange, *Poncirus trifoliate*, and sour orange, *Citrus aurantium*). One antibiosis QTL with marker CR7 on LG 7 of *P. trifoliata* (Pa) map; another antibiosis QTL with marker S2-AS4-800 on sour orange, *C. aurantium*, linkage map; and six antixenosis QTLs were mapped for *P. citrella* resistance in citrus, where the antibiosis QTL mapped with marker S2-AS4-800 in sour orange, which was similar to several nucleotide binding site-leucine-rich repeat-type resistance genes, and might be considered as a candidate gene for insect resistance in citrus.

*3.5.5 Black Currant (*Ribes nigrum*)*

3.5.5.1 Gall Mite (*Cecidophyopsis ribis*)

Resistance to *C. ribis* is available from other *Ribes* species, notably *R. grossularia* (Knight et al. 1974), where the resistance is controlled by a single gene, *Ce*. Using a bulked segregant analysis, Brennan et al. (2009) screened 90 AFLP primer combinations and constructed a linkage map around the resistance locus controlled by *Ce*. Subsequent testing identified *gmr* gene at 4.0 cM from an AFLP marker, E41M88-280, which is closely linked to gall mite resistance. Validation of this marker across a range of susceptible and resistant black currant germplasm with different genetic backgrounds confirmed its reliability for identification of mite-resistant germplasm containing gene *Ce*.

*3.5.6 Black Raspberry (*Rubus occidentalis*)*

3.5.6.1 Aphid (*Amphorophora agathonica*)

A locus for aphid, *A. agathonica*, resistance has been identified on a densely saturated genetic linkage map of black raspberry by using single-nucleotide polymorphism and transferable markers for F_1 population ORUS 4305, consisting of 115 progeny that segregated for aphid resistance (Bushakra et al. 2015). The linkage map of seven linkage groups representing the seven haploid chromosomes of black raspberry consisted of 274 markers on the maternal map and 292 markers on the paternal map including a morphological locus for aphid resistance. The aphid resistance gene, Ag_4, was mapped with SNP marker S99_32802. The phenotypic marker for aphid resistance, Ag4_AphidR, was located on RLG6 of the aphid-resistant parent ORUS 4153-1 and mapped to the same location as S99_32802.

3.6 Gene Pyramiding

Genetic engineering offers the advantage of rapid introgression of novel genes and traits into elite agronomic backgrounds (Mohan et al. 1997). Transgenic resistance to insects has been demonstrated in plants expressing insecticidal genes such as δ-endotoxins from *Bacillus thuringiensis*, protease inhibitors, enzymes, secondary plant metabolites, and plant lectins (Sharma et al. 2004; Sharma 2009). While transgenic plants with *Bt* genes have been deployed in several crops, the other genes have received less attention. The potential of some of the alternative genes can only be realized by deploying them in combination with conventional host plant resistance and *Bt* genes (Sharma et al. 2002; Sharma 2009). Many of the candidate genes used in genetic transformation of crops are quite specific. However, most of the crops are damaged by the large number of insect pests, and hence, there is a need for using the genes with different mechanisms to generate effective and sustainable seed-based technologies for pest management (Hadi et al. 1996; Karim et al. 1999), e.g., the activity of *Bt* genes in transgenic plants is enhanced by the serine protease inhibitors (MacIntosh et al. 1990; Zhao et al. 1997) and tannic acid (Gibson et al. 1995). However, this may have some metabolic cost to the plant in some cases, and different resistance gene products may also have deleterious or nullifying interactions.

Combining transgene- and QTL-mediated resistance can be used as a viable strategy for insect control. A QTL conditioning maize earworm resistance in soybean PI 229358 and the *cry1Ac* transgene from the recurrent parent Jack-*Bt* have been pyramided into BC_2F_3 plants by marker-assisted selection (Walker et al. 2002). Fewer larvae of corn earworm, *H. zea*, and soybean looper, *P. includens*, survived on leaves expressing the Cry1Ac protein. Weights of soybean looper larvae fed on foliage from transgenic plants with the PI-derived QTL were significantly lower than those fed transgenic tissue with the corresponding Jack chromosomal segment

(Walker et al. 2002). Therefore, combining transgene- and QTL-mediated resistance to lepidopteran insects may be a viable strategy for insect control.

3.7 Marker-Assisted Versus Phenotypic Selection

Expression of physicochemical traits associated with insect resistance is influenced by the environment and, thus, is less reliable than the molecular markers. Molecular markers are (1) unaffected by the environment, (2) are phenotype neutral, and (3) are detectable at all stages of the plant growth. A number of methods have been used for mapping QTLs associated with the traits of interest (Karp et al. 1997). However, it is important that the marker co-segregates with the gene and is closely linked (1 cM or less) with the trait of interest. Some molecular markers behave in a codominant manner to detect heterozygotes in segregating populations. The morphological markers typically behave in a dominant or recessive manner, and do not detect heterozygotes (Staub et al. 1996).

Theoretically, marker-assisted selection can be used to accelerate the pace and accuracy of transferring resistance genes into improved cultivars. The MAS takes 3–6 years, thus speeding up the pace of transferring the traits of interest into the improved varieties, and it does not require large-scale planting of the segregating progenies up to crop harvest, as only the plants with marker allele indicating the presence of the trait or QTL need to be maintained up to maturity. MAS in barley for resistance to cereal cyst nematode, *Heterodera avenae*, could be accomplished approximately 30 times faster and with 75% lower cost as compared to phenotypic selection (Kretschmer et al. 1997). Similar cost and labor savings have also been documented for microsatellite markers linked to cyst nematode, *Heterodera glycines*, resistance in soybean (Mudge et al. 1997). MAS is effective for scoring both resistance and susceptibility to European corn borer (Flint-Garcia et al. 2003). The two brown plant hopper resistance genes (*Bph14* and *Bph15*) have been introduced into three *japonica* rice varieties Shengdao 15, Shengdao 16, and Xudao 3 using marker-assisted backcross breeding (Xu 2013), thus overcoming the need for phenotypic selection (Breseghello 2013).

In contrast to the markers linked to resistance genes inherited as simple dominant traits, improvement of polygenic traits through MAS is difficult due to involvement of a number of genes and their interactions (epistatic effects). Under such conditions, MAS does not offer any advantage over the conventional phenotyping and often involves multiple field tests across environments. Several studies on QTLs linked to stem borer resistance in maize underscore the problems involved in using QTLs in MAS to breed for insect resistance. In many cases, the relative efficiency of phenotypic and MAS has been found to be similar (Groh et al. 1998a, b; Willcox et al. 2002). MAS and phenotypic selection for leaf feeding resistance to *D. grandiosella* and *D. saccharalis* improved the efficiency of selection by 4%, indicating that MAS is less efficient than phenotypic evaluation (Bohn et al. 2001).

Maximum progress has been made in breeding for insect resistance in common bean by using a combination of phenotypic selection and QTL-based index, followed by QTL-based index and conventional selection (Tar'an et al. 2003). Although the cost of MAS is approximately 90% less than the cost of conventional selection, accurate identification of QTL position and the cost to generate initial data for use in MAS makes conventional selection more reliable and cost-effective. Stromberg et al. (1994) did not get a better response to MAS than to conventional selection for resistance to southwestern corn borer. Three putative QTLs that accounted for 28% of the phenotypic variance did not exhibit any advantage over leaf damage ratings or larval weights.

The use of DNA-based markers for indirect selection is inefficient for quantitative traits with low heritability, as these are the most difficult characters to work with through conventional phenotypic selection as well. It is also difficult to develop effective markers for such traits. The expression of such traits is influenced by genotype × environment interaction and epistasis, which in addition to difficulties involved in accurately and precisely phenotyping such traits confounds the development of effective MAS systems. The quality of a MAS program can only be as good as the quality of the phenotypic data on which the development of that marker was based. Fine-mapping of such large QTLs by phenotypic screening of several hundred individuals exhibiting molecular marker evidence of genetic recombination is required to obtain tightly linked flanking markers that can be exploited in MAS. As a result, MAS has not been as effective to select for insect resistance as for plant disease resistance controlled by dominant genes.

3.8 Conclusion

A good beginning has been made in developing genetic linkage maps of many crops. However, the accuracy and precision of phenotyping for insect resistance remain a critical constraint to identify effective QTLs for insect resistance in many crops. There is a need for developing improved phenotyping to breed for resistance to insect pests. There are very few reports demonstrating the effective use of MAS for resistance to insect pests. There is a need to use marker-assisted selection to develop cultivars with diverse mechanisms of resistance (multiple genes) to insect pests and to strengthen *Bt* transgenic crops through introgression of such traits through MAS. Thus, there is not only a need for precise mapping of the QTLs associated with resistance to insects but also the development of new paradigms to make best use of molecular marker data. Equally important is the need to generate data on genetic diversity in insect populations across crops/regions. Only a combination of conventional and molecular approaches can be effective in developing cultivars with insect resistance for sustainable crop production and food security.

Acknowledgments We thank the managements of the International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru; ICAR-Indian Agricultural Research Institute, New Delhi; and Punjab Agricultural University, Ludhiana, for their encouragement and support.

References

Agrama H, Widle G, Reese J, Campbell L, Tuinstra M (2002) Genetic mapping of QTLs associated with greenbug resistance and tolerance in *Sorghum bicolor*. Theor Appl Genet 104(8):1373–1378

Akanksha S, Lakshmi VJ, Singh AK, Deepthi Y, Chirutkar PM, Ramdeen, Balakrishnan D, Sarla N, Mangrauthia SK, Ram T (2019) Genetics of novel brown planthopper *Nilaparvata lugens* (Stal) resistance genes in derived introgression lines from the interspecific cross *O. sativa* var. Swarna x *O. nivara*. J Genet 98:113

Akula SH, Dass MA, Surapaneni SK, Balaravi P (2020) Mapping of quantitative trait loci associated with resistance to brown planthopper in background of Swarna from a traditional variety PTB33. Euphytica 216:114

Alston FH, Briggs JB (1977) Resistance genes in apple and biotypes of *Dysaphis devecta*. Ann Appl Biol 87:75–81

Amkul K, Wang L, Somta P, Wang S, Cheng X (2019) Construction of a high density linkage map and genome dissection of bruchid resistance in zombi pea (*Vigna vexillata* (L.) A. Rich). Sci Rep 9:11719

Apotikar DB, Venkateswarlu D, Ghorade RB, Wadaskar RM, Patil JV, Kulwal PL (2011) Mapping of shoot fly tolerance loci in sorghum using SSR markers. J Genet 90(1):59–66

Aruna C, Bhagwat VR, Madhusudhana R, Sharma V, Hussain T, Ghorade RB, Khandalkar HG, Audilakshmi S, Seetharama N (2011) Identification and validation of genomic regions that affect shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 122:1617–1630

Badji A, Otim M, Machida L, Odong T, Kwemoi DB, Okii D, Agbahoungba S, Mwila N, Kumi F, Ibanda A (2018) Maize combined insect resistance genomic regions and their co-localization with cell wall constituents revealed by tissue-specific QTL meta-analyses. Front Plant Sci 9:1–17

Badji A, Kwemoi DB, Machida L, Okii D, Mwila N, Agbahoungba S, Kumi F, Rubaihayo P (2020) Genetic basis of maize resistance to multiple insect pests: integrated genome-wide comparative mapping and candidate gene prioritization. Genes 11:689

Balachiranjeevi CH, Prahalada GD, Mahender A, Jamaloddin A, Sevilla MA, Marfori-Nazarea CM, Vinarao R, Sushanto U, Baehaki CE, Li ZK, Ali J (2019) Identification of a novel locus, *BPH38(t)*, conferring resistance to brown planthopper (*Nilaparvata lugens* Stal.) using early backcross population in rice (*Oryza sativa* L.). Euphytica 215:185

Barmukh R, Roorkiwal M, Jaba J, Chitikineni A, Mishra SP, Sagurthi SR, Munghate R, Sharma HC, Varshney RK (2020) Development of a dense genetic map and QTL analysis for pod borer *Helicoverpa armigera* (Hübner) resistance component traits in chickpea (*Cicer arietinum L.*). Plant Genome 14:e20071. https://doi.org/10.1002/tpg2.20071

Bernet GP, Margaix C, Jacas J, Carbonell EA, Asins MJ (2005) Genetic analysis of citrus leafminer susceptibility. Theor Appl Genet 110:1393–1400

Biradar SK, Sundaram RM, Thirumurugan T, Bentur JS, Amudhan S, Shenoy VV, Mishra B, Bennet J, Sharma PN (2004) Identification of flanking SSR markers for a major rice gall midge resistance gene *Gm1* and their validation. Theor Appl Genet 109:1468–1473

Bohn M, Groh S, Khairallah MM, Hoisington DA, Utz HF, Melchinger AE (2001) Re-evaluation of the prospects of marker-assisted selection for improving insect resistance against *Diatraea*

spp. in tropical maize by cross validation and independent validation. Theor Appl Genet 103:1059–1067

Boyko EV, Kalendar R, Korzun V, Korol A, Schulman A, Gill BS (2002) A high density genetic map of *Aegilops tauschii* includes genes, retro-transposons and microsatellites which provide unique insight into cereal chromosome structure and function. Plant Mol Biol 48:767–790

Boyko EV, Starkey SR, Smith CM (2004) Molecular genetic mapping of *Gby,* a new greenbug resistance gene in bread wheat. Theor Appl Genet 109:1230–1236

Brennan R, Jorgensen L, Gordon S, Loades K, Hackett C, Russell J (2009) The development of a PCR-based marker linked to resistance to the blackcurrant gall mite (*Cecidophyopsis ribis* Acari: Eriophyidae). Theor Appl Genet 118:205–211

Breseghello F (2013) Traditional and modern plant breeding methods with examples in rice (*Oryza sativa* L.). J Agric Food Chem 61:8277–8286

Brooks S, Barfoot P (2015) GM crops: global socio-economic and environmental impacts 1996-2013. PG Economics Ltd, Dorchester

Bushakra JM, Bryant DW, Dossett M, Vining KJ, VanBuren R, Gilmore BS, Lee J, Mockler TC, Finn CE, Bassil NV (2015) A genetic linkage map of black raspberry (Rubus occidentalis) and the mapping of Ag_4 conferring resistance to the aphid, *Amphorophora agathonica*. Theor Appl Genet 128:1631–1646

Cardinal AJ, Lee M (2005) Genetic relationships between resistance to stalk-tunneling by the European corn borer and cell-wall components in maize population B73xB52. Theor Appl Genet 111:1–7

Castro AM, Vasicek A, Ellerbrook C, Gimenez DO, Tocho E, Tacaliti MS, Clua A, Snape JW (2004) Mapping quantitative trait loci in wheat for resistance against greenbug and Russian wheat aphid. Plant Breed 123:361–365

Cevik V, King G (2002) High-resolution genetic analysis of the Sd1 aphid resistance locus in *Malus* spp. Theor Appl Genet 105:346–354

Chalfie M (1998) Genome sequencing. The worm revealed. Nature 396:620–621

Chen HM, Liu CA, Kuo CG, Chien CM, Sun HC, Huang CC, Ku HM (2007) Development of a molecular marker for a bruchid (*Callosobruchus chinensis* L.) resistance gene in mungbean. Euphytica 157(1):113–122

Chen HM, Ku HM, Schafleitner R, Bains TS, Kuo CG, Liu CA, Nair RM (2013) The major quantitative trait locus for mungbean yellow mosaic Indian virus resistance is tightly linked in repulsion phase to the major bruchid resistance locus in a cross between mungbean [*Vigna radiata* (L.) Wilczek] and its wild relative *Vigna radiata* ssp. *sublobata*. Euphytica 192:205–216

Chotechung S, Somta P, Chankaew S, Srinives P, Somta P (2011) Identification of DNA markers associated with bruchid resistance in mungbean. Khon Khan Agric J 39:221–226

Chotechung S, Somta P, Chen J, Yimram T, Chen X, Srinives P (2016) A gene encoding a polygalacturonase-inhibiting protein (PGIP) is a candidate gene for bruchid (Coleoptera: Bruchidae) resistance in mungbean (*Vigna radiata*). Theor Appl Genet 129:1673–1683

Crespo-Herrera LA, Akhunov E, Garkava-Gustavsson L, Jordan KW, Smith CM, Singh RP, Ahman I (2014) Mapping resistance to the bird cherry-oat aphid and the greenbug in wheat using sequence-based genotyping. Theor Appl Genet 127:1963–1973

Deu M, Ratnadass A, Hamada MA, Noyer JL, Diabate M, Chantereau J (2004) Quantitative trait loci for head-bug resistance in sorghum. Afr J Biotechnol 4:247–250

Fazel-Najafabadi M, Peng J, Peairs FB, Simkova H, Kilian A, Lapitan NV (2015) Genetic mapping of resistance to *Diuraphis noxia* (Kurdjumov) biotype 2 in wheat (*Triticum aestivum L.*) accession CI2401. Euphytica 203:607–614

Flint-Garcia SA, Darrah LL, McMullen MD, Hibbard BE (2003) Phenotypic versus marker assisted selection for stalk strength and second generation European corn borer resistance in maize. Theor Appl Genet 107(7):1331–1336

Folkertsma RT, Sajjanar GM, Reddy BVS, Sharma HC, Hash CT (2003) Genetic mapping of QTL associated with sorghum shoot fly (*Atherigona soccata*) resistance in sorghum (*Sorghum bicolor*). Final Abstracts Guide, Plant & Animal Genome XI, p 42

Frei A, Blair MW, Cardona C, Beebe SE, Gu H, Dorn S (2005) QTL mapping of resistance to *Thrips palmi* Karny in common bean. Crop Sci 45(1):379–387

Fujita D, Doi K, Yoshimura A, Ysui H (2006) Molecular mapping of a novel gene, *Grh5*, conferring resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice, *Oryza sativa* L. Theor Appl Genet 113:567–573

Gibson DM, Gallo LG, Krasnoff SB, Ketchum REB (1995) Increased efficiency of *Bacillus thuringiensis* subsp. *kurstaki* in combination with tannic acid. J Econ Entomol 88:270–277

Goggin FL, Williamson VM, Ullman DE (2001) Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae) to the tomato resistance gene *Mi*. Environ Entomol 30:101–106

Gorthy S, Narasu L, Gaddameedi A, Sharma HC, Kotla A, Deshpande SP, Are AK (2017) Introgression of shoot fly (*Atherigona soccata* L. Moench) resistance QTLs into elite post-rainy season sorghum varieties using marker assisted backcrossing (MABC). Front Plant Sci 8:1494

Groh S, González-de-León D, Khairallah MM, Jiang C, Bergvinson D, Bohn M, Hoisington DA, Melchinger AE (1998a) QTL mapping in tropical maize: III. Genomic regions for resistance to *Diatraea* spp. and associated traits in two RIL populations. Crop Sci 38(4):1062–1072

Groh S, Khairallah MM, González-de-León D, Willcox M, Jiang C, Hoisington DA, Melchinger AE (1998b) Comparison of QTLs mapped in RILs and their test-cross progenies of tropical maize for insect resistance and agronomic traits. Plant Breed 117(3):193–202

Hadi MZ, McMullen MD, Finer JJ (1996) Transformation of 12 different plasmids into soybean via particle bombardment. Plant Cell Rep 15:500–505

Hash CT, Folkerstma RT, Ramu P, Reddy BVS, Mahalakshmi V, Sharma HC, Rattunde HFW, Weltzein ER, Haussmann BIG, Ferguson ME, Crouch JH (2003) Marker assisted breeding across ICRISAT for terminal drought tolerance and resistance to shoot fly and *Striga* in sorghum. In: Abstracts—in the wake of the Double Helix: from the green revolution to the gene revolution, 27–31 May 2003, University of Bologna. Bolona, Italy, p 82. http://www.double-helix.too.it

Hernandez P, Dorado G, Prieto P, Gimenez MJ, Ramirez MC, Laurie DA, Snape JW, Martin A (2001) A core genetic map of *Hordeum chilense* and comparisons with maps of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). Theor Appl Genet 102:1259–1264

Herselman L, Thwaites R, Kimmins FM, Courtois B, van der Merwe PJ, Seal SE (2004) Identification and mapping of AFLP markers linked to peanut (*Arachis hypogaea* L.) resistance to the aphid vector of groundnut rosette disease. Theor Appl Genet 109:1426–1433

Hill CB, Li Y, Hartman GL (2006) A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Sci 46:1601–1605

Himabindu K, Suneetha K, Sama VSAK, Bentur JS (2010) A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. Euphytica 174:179–187

Hong MG, Kim KH, Ku JH, Jeong JK, Seo MJ, Park CH, Kim YH, Kim HS, Kim YK, Baek SH, Kim DY, Park SK, Kim SL, Moon JK (2015) Inheritance and quantitative trait loci analysis of resistance genes to bruchid and bean bug in mungbean (*Vigna radiata* L. Wilczek). Plant Breed Biotechnol 3:39–46

Hu J, Chang X, Zou L, Tang W, Wu W (2018) Identification and fine mapping of *Bph33*, a new brown planthopper resistance gene in rice (*Oryza sativa* L.). Rice 11:55

Huang Y (2011) Improvement of crop protection against greenbug using the worldwide sorghum germplasm collection and genomics-based approaches. Plant Genet Res 9:317–320

Hur YJ, Cho JH, Lee JY et al (2015) Fine mapping of GRH3 conferring resistance to green rice leafhopper in rice (*Oryza sativa* L.). Mol Breed 35:89

Jain A, Ariyadasa R, Kumar A, Srivastava MN, Mohan M, Nair S (2004) Tagging and mapping of a rice gall midge resistance gene, Gm8, and development of SCARs for use in marker-aided selection and gene pyramiding. Theor Appl Genet 109:1377–1384

Kaewwongwal A, Chen J, Somta P, Kongjaimun A, Yimram T, Chen X, Srinives P (2017) Novel alleles of two tightly linked genes encoding polygalacturonase-inhibiting proteins (VrPGIP1

and VrPGIP2) associated with the *Br* locus that confer bruchid (*Callosobruchus* spp.) resistance to mungbean (*Vigna radiata*) accession V2709. Front Plant Sci 8:1692

Kaewwongwal A, Liu C, Somta P, Chen J, Tian J, Yuan X, Chen X (2020) A second *VrPGIP1* allele is associated with bruchid resistance (*Callosobruchus* spp.) in wild mungbean (*Vigna radiata* var. *sublobata*) accession ACC41. Mol Genet Genomics 295:275–286

Kamphuis L, Gao L, Singh K (2012) Identification and characterization of resistance to cowpea aphid (*Aphis craccivora* Koch) in *Medicago truncatula*. BMC Plant Biol 12:101

Karim S, Riazuddin S, Dean DH (1999) Interaction of *Bacillus thuringiensis* delta-endotoxins with midgut brush border membrane vesicles of *Helicoverpa armigera*. J Asia Pac Entomol 2:153–162

Karp A, Edwards KJ, Bruford M, Funk S, Vosman B, Morgante M, Seberg O, Kremer A, Boursot P, Arctander P, Tautz D, Hewitt GM (1997) Molecular technologies for biodiversity evaluation: opportunities and challenges. Nat Biotechnol 15:625–628

Klingler J, Kovalski I, Silberstein L, Thompson GA, Perl-Treves R (2001) Mapping of cotton-melon aphid resistance in melon. J Am Soc Hortic Sci 126(1):56–63

Klingler JP, Nair RM, Edwards OR, Singh KB (2009) A single gene, AIN, in *Medicago truncatula* mediates a hypersensitive response to both bluegreen aphid and pea aphid, but confers resistance only to blue green aphid. J Exp Bot 60:4115–4127

Knight RL, Keep E, Briggs JB, Parker JH (1974) Transference of resistance to blackcurrant gall mite, *Cecidophyopsis ribis*, from gooseberry to blackcurrant. Ann Appl Biol 76:123–130

Korzun V, Malyshev S, Voylokov AV, Bomer A (2001) A genetic map of rye (*Secale cereale* L.) combining RFLP, isozyme, protein, microsatellite and gene loci. Theor Appl Genet 102:709–717

Krakowsky MD, Lee M, Hollan JB (2007) Genotypic correlation and multivariate QTL Analyses for cell wall components and resistance to stalk tunneling by the European Corn Borer in Maize. Crop Sci 47(2):485–488. https://doi.org/10.2135/cropsci2006.05.0283

Kretschmer JM, Chalmers KJ, Manning S, Karakousis A, Bait AR, Islam MR, Logue SJ, Chac YW, Barker SJ, Lance RCM, Langridge P (1997) RFLP mapping of the *Ha2* cereal cyst nematode resistance gene in barley. Theor Appl Genet 94:1060–1064

Kumar K, Sarao PS, Bhatia D, Neelam K, Kaur A, Mangat GS, Brar DS, Singh K (2018) High resolution genetic mapping of a novel brown planthopper resistance locus, *Bph34* in *Oryza sativa* L. X *Oryza nivara* (Sharma & Shastry) derived interspecific F2 population. Theor Appl Genet 131:1163–1171

Leelagud P, Kongsila S, Vejchasarn P, Darwell K, Phansenee Y, Suthanthangjai A, Uparang C, Kawichai R, Yajai P, Boonsa-nga K, Chamarerk V, Jairin J (2020) Genetic diversity of Asian rice gall midge based on mtCOI gene sequences and identification of a novel resistance locus *gm12* in rice cultivar MN62M. Mol Biol Rep 47:4273–4283

Li Y, Hill CB, Carlson SR, Diers BW, Hartman GL (2007) Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group. Mol Breed 19:25–34

Li C, Chen M, Chao S, Yu J, Bai G (2013) Identification of a novel gene, *H34*, in wheat using recombinant inbred lines and single nucleotide polymorphism markers. Theor Appl Genet 126:2065–2071

Li G, Xu X, Carver BF, Guo P, Puterka G (2018) *Dn10*, a new gene conferring resistance to Russian wheat aphid biotype 2 in Iranian wheat landrace PI 682675. Crop Sci. https://doi.org/10.2135/cropsci2017.10.0649

Li Y, Li Z, Yang M, Tang L, Cheng L, Qiu Y (2019a) Characterization and application of a gall midge resistance gene (*Gm6*) from *Oryza sativa* 'Kangwenqingzhan'. Theor Appl Genet 133:579–591

Li Z, Xue Y, Zhou H, Li Y, Usman B, Jiao X, Wang X, Liu F, Qin B, Li R, Qiu Y (2019b) High-resolution mapping and breeding application of a novel brown planthopper resistance gene derived from wild rice (*Oryza rufipogon* Griff). Rice 12:41

Liu S, Brown-Guedira GL, Hatchett JH, Owuoche JO, Chen MS (2005a) Genetic characterization and molecular mapping of Hessian fly-resistance gene transferred from *T. turgidum* ssp. *dicoccum* to common wheat. Theor Appl Genet 111:1308–1315

Liu XM, Gill BS, Chen MS (2005b) Hessian fly resistance gene *H13* is mapped to a distal cluster of resistance genes in chromosome 6DS of wheat. Theor Appl Genet 111:243–249

Liu XM, Fritz AK, Reese JC, Wilde GE, Gill BS, Chen MS (2005c) *H9*, *H10*, and *H11* compose a cluster of Hessian fly-resistance genes in the distal gene-rich region of wheat chromosome 1AS. Theor Appl Genet 110:1473–1480

Liu XM, Smith CM, Gill BS (2005d) Allelic relationships among Russian wheat aphid resistance genes. Crop Sci 45:2273–2280

Liu MS, Kuo TCY, Ko CY, Wu DC, Li KY, Lin WJ, Lin CP, Wang YW, Schafleitner R, Lo HF, Chen CY, Chen LFO (2016) Genomic and transcriptomic comparison of nucleotide variations for insights into bruchid resistance of mungbean (*Vigna radiata* [L.] R. Wilczek). BMC Plant Biol 16:46

Lo S, Muñoz-Amatriaín M, Boukar O, Herniter I, Cisse N, Guo Y-N, Roberts PA, Xu S, Fatokun C, Close TJ (2018) Identification of QTL controlling domestication-related traits in cowpea (*Vigna unguiculata* L. Walp). Sci Rep 8:6261

MacIntosh SC, Kishore GM, Perlak FJ, Marrone PG, Stone TB, Sims SR, Fuchs RL (1990) Potentiation of *Bacillus thuringiensis* insecticidal activity by serine protease inhibitors. J Agric Food Chem 38:1145–1152

McDonald MJ, Ohm HW, Rinehart KD, Giovanini MP, Williams CE (2014) *H33*: a wheat gene providing hessian fly resistance for the Southeastern United States. Crop Sci 54:2046–2053

Mei L, Cheng XZ, Wang SH, Wang LX, Liu CY, Sun L, Xu N, Humphry ME, Lambrides CJ, Li HB, Liu CJ (2009) Relationship between bruchid resistance and seed mass in mungbean based on QTL analysis. Genome 52:589–596

Miesho B, Hailay M, Msiska U, Bruno A, Malinga GM, Ongom PO, Edema R, Gibson P, Rubaihayo P, Kyamanywa S (2019) Identification of candidate genes associated with resistance to bruchid (*Callosobruchus maculatus*) in cowpea. Plant Breed 138:605–613

Miflin B (2000) Crop improvement in the 21st century. J Exp Bot 51:1–8

Mishra RR, Nag A, Panigrahi J (2015) Development of dominant sequence characterized amplified region (SCAR) marker linked with plume moth (*Exelastis atomosa* Walsingham 1886) resistance in pigeonpea. Chilean J Agric Res 75(4):497–501

Miyagi M, Humphry ME, Ma ZY, Lambrides CJ, Bateson M, Liu CJ (2004) Construction of bacterial artificial chromosome libraries and their application in developing PCR-based markers closely linked to a major locus conditioning bruchid resistance in mungbean (*Vigna radiata* L. Wilczek). Theor Appl Genet 110:151–156

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol Breed 3:87–103

Mondal M, Ashok BH, Poonam AH, Anand MB (2014) Identification of quantitative trait loci for bruchid (*Caryedon serratus* Olivier) resistance components in cultivated groundnut (*Arachis hypogaea* L.). Mol Breed 33:961–973

Muchero W, Ehlers JD, Roberts PA (2010) QTL analysis for resistance to foliar damage caused by *Thrips tabaci* and *Frankliniella schultzei* (Thysanoptera: Thripidae) feeding in cowpea [*Vigna unguiculata* (L.) Walp.]. Mol Breed 25(1):47–56

Mudge J, Cregan PB, Kenworthy JP, Kenworthy WJ, Orf JH, Young ND (1997) Two microsatellite markers that flank the major soybean cyst nematode resistance locus. Crop Sci 37:1611–1615

Mullis KB (1990) The unusual origin of the polymerase chain reaction. Sci Am 262(4):56–65

Munyiri SW, Mugo SN (2017) Quantitative trait loci for resistance to spotted and African maize stem borers (*Chilo partellus* and *Busseola fusca*) in a tropical maize (*Zea mays* L.). Afr J Biotechnol 16(28):1579–1589

Murray JD, Michaels TE, Cardona C, Schaafsma AW, Pauls KP (2004) Quantitative trait loci for leafhopper (*Empoasca fabae* and *Empoasca kraemeri*) resistance and seed weight in the common bean. Plant Breed 123:474–479

Muturi PW, Mgonja M, Rubaihayo P, Mwololo JK (2021) QTL mapping of traits associated with dual resistance to the African stem borer (*Busseola fusca*) and spotted stem borer (*Chilo partellus*) in sorghum (*Sorghum bicolor*). Int J Genomics. https://doi.org/10.1016/j.fcr.2020.108029

Myers GO, Fatokun CA, Young ND (1996) RFLP mapping of an aphid resistance gene in cowpea (*Vigna unguiculata* (L.) Walp). Euphytica 91:181–187

Nagaraj N, Reese JC, Tuinstra MR, Smith CM, Amand P, Kirkham MB, Kofoid KD, Campbell LR, Wild GE (2005) Molecular mapping of sorghum genes expressing tolerance to damage by greenbug (Homoptera: Aphididae). J Econ Entomol 98:595–602

Narvel JM, Chu W, Fehr W, Cregan PB, Shoemaker RC (2000) Development of multiplex sets of simple sequence repeat DNA markers covering the soybean genome. Mol Breed 6:175–183

Narvel JM, Walker DR, Rector BG, All JN, Parrott WA, Boerma HR (2001) A retrospective DNA marker assessment of the development of insect resistant soybean. Crop Sci 41:1931–1939

Nombela G, Williamson VM, Muniz M (2003) The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. Mol Plant Microbe Interact 16:645–649

Ordas B, Malvar RA, Santiago R (2009) Mapping of QTL for resistance to the Mediterranean corn borer attack using the intermated B73 × Mo17 (IBM) population of maize. Theor Appl Genet 119:1451–1459

Ordas B, Malvar RA, Santiago R (2010) QTL mapping for Mediterranean corn borer resistance in European flint germplasm using recombinant inbred lines. BMC Genomics 11:174–183

Ortega MA, John NA, Boerma HR, Parrott WA (2016) Pyramids of QTLs enhance host–plant resistance and Bt-mediated resistance to leaf chewing insects in soybean. Theor Appl Genet 129:703–715

Palevitz BA (2000) Rice genome gets a boost. Private sequencing yields a rough draft for public. http://www.The-Scientist.com/yr2000/may/palevitz-pl-000501

Papst C, Bohn M, Utz HF, Melchinger AE, Klein D, Eder J (2004) QTL mapping for European corn borer resistance (*Ostrinia nubilalis* Hb.), agronomic and forage quality traits of testcross progenies in early-maturing European maize (*Zea mays* L.) germplasm. Theor Appl Genet 108(8):1545–1554

Paterson AH, Tanksley SD, Sorrells ME (1991) DNA markers in plant improvement. Adv Agron 46:39–90

Phi CN, Fujita D, Yamagata Y, Yoshimura A, Yasui H (2019) High-resolution mapping of GRH6, a gene from Oryza nivara (Sharma et Shastry) conferring resistance to green rice leafhopper (Nephotettix cincticeps Uhler). Breed Sci 69:439–446

Piskur J, Langkjaer RB (2004) Yeast genome sequencing: the power of comparative genomics. Mol Microbiol 53:381–389

Pitrat M, Lecoq H (1982) Relations génétiques entre les resistances par non-acceptation et par antibiose de melon *Aphis gossypii*. Recherche de liaisons avec d'autres gènes. Agronomie 2:503–508

Punnuri S, Huang Y (2017) Identification and confirmation of greenbug resistance loci in an advanced mapping population of sorghum. J Agric Sci 155:1610–1622

Punnuri S, Huang Y, Steets J, Wu Y (2012) Developing new markers and QTL mapping for greenbug resistance in sorghum [*Sorghum bicolor* (L.) Moench]. Euphytica 191:191–203

Rector BG, All JN, Parrott WA, Boerma HR (1998) Identification of molecular markers linked to quantitative trait loci for soybean resistance to corn earworm. Theor Appl Genet 96(6):786–790

Rector BG, All JN, Parrott WA, Boerma HR (1999) Quantitative trait loci for antixenosis resistance to corn earworm in soybean. Crop Sci 39:531–538

Rector BG, All JN, Parrott WA, Boerma HR (2000) Quantitative trait loci for antibiosis resistance to corn earworm in soybean. Crop Sci 40:233–238

Roberts PA, Thomason IJ (1986) Variability in reproduction of isolates of *Meloidogyne incognita* and *M. javanica* on resistant tomato genotypes. Plant Dis 70:547–551

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proc Natl Acad Sci U S A 95:9750–9754

Saka N, Tsuji T, Toyama T, Yano M, Izawa T, Sasaki T (2006) Development of cleaved amplified polymorphic sequence (CAPS) markers linked to a green rice leafhopper resistance gene, *Grh3*(t). Plant Breed 125:140–143

Sama VS, Rawat N, Sundaram RM, Himabindu K, Naik BS, Viraktamath BC, Bentur JS (2014) A putative candidate for the recessive gall midge resistance gene *gm3* in rice identified and validated. Theor Appl Genet 127:113–124

Samayoa LF, Butron A, Malvar RA (2014) QTL mapping for maize resistance and yield under infestation with *Sesamia nonagrioides*. Mol Breed 34(3):1331–1344

Samayoa LF, Malvar RA, McMullen MD (2015) Identification of QTL for resistance to Mediterranean corn borer in a maize tropical line to improve temperate germplasm. BMC Plant Biol 15:265–272

Santiago R, Malvar RA, Barros-Rios J, Samayoa LF, Butrón A (2016) Hydroxycinnamate synthesis and association with Mediterranean corn borer resistance. J Agric Food Chem 64:539–551

Sardesai N, Rajyashri K, Behura SK, Nair S, Mohan M (2001) Genetic, physiological and molecular interactions of rice and its major dipteran pest, gall midge. Plant Cell Tissue Organ Cult 64:115–131

Sardesai N, Nemacheck JA, Subramanyam ES, Williams CE (2005) Identification and mapping of H32, a new wheat gene conferring resistance to Hessian fly. Theor Appl Genet 111:1167–1173

Satish K, Srinivas G, Madhusudhana R, Padmaja PG, Reddy RN, Murali Mohan S, Seetharama N (2009) Identification of quantitative trait loci for resistance to shoot fly in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 8:1425–1439

Schafleitner R, Huang SM, Chu SH, Yen JY, Lin CY, Yan MR, Krishnan B, Liu MS, Lo HF, Chen CY, Chen LFO, Wu DC, Bui TGT, Ramasamy S, Tung CW, Nair R (2016) Identification of single nucleotide polymorphism markers associated with resistance to bruchids (*Callosobruchus* spp.) in wild mungbean (*Vigna radiata* var. *sublobata*) and cultivated *V. radiata* through genotyping by sequencing and quantitative trait locus analysis. BMC Plant Biol 16:159

Schon CC, Michael L, Melchinger AE, Guthrieff WD, Woodman WL (1993) Mapping and characterization of quantitative trait loci affecting resistance against second generation European corn borer in maize with the aid of RFLPs. Heredity 70:648–659

Selvi A, Shanmugasundaram P, Mohan Kumar S, Raja JAJ (2002) Molecular markers for yellow stem borer, *Scirpophaga incertulas* (Walker) resistance in rice. Euphytica 124:371–377

Sharma HC (2009) Biotechnological approaches for pest management and ecological sustainability. CRC Press, Taylor and Francis Group LLC, p 546

Sharma HC, Crouch JH, Sharma KK, Seetharama N, Hash CT (2002) Applications of biotechnology for crop improvement: prospects and constraints. Plant Sci 163:381–395

Sharma HC, Sharma KK, Crouch JH (2004) Genetic transformation of crops for insect resistance: potential and limitations. Crit Rev Plant Sci 23:47–72

Sharopova N, McMullen MD, Schultz L, Schroeder S, Sanchez H, Gardiner J, Bergstrom D, Houchins K, Polacco M, Edwards KJ, Ruf T, Register JC, Brower C, Thompson R, Chin E, Lee M, Liscum III, Cone E, Davis G, Coe EH Jr (2002) Development and mapping of SSR markers for maize. Plant Mol Biol 48:463–481

Sherman F (1998) An introduction to the genetics and molecular biology of the yeast *Saccharomyces cerevisiae*. http://dbb.urmc.rochester.edu/labs/sherman_f/yeast/

Shi A, Chen P, Li DX, Zheng C, Zhang B, Hou A (2009) Pyramiding multiple genes for resistance to soybean mosaic virus in soybean using molecular markers. Mol Breed 23:113–124

Shoemaker DD, Schadt EE, Armour YD, He P, Garrett-Engel PD, McDonayl PM (2001) Experimental annotation of the human genome using microarray technology. Nature 409:922–927

Smith CM (2005) Plant resistance to arthropods—molecular and conventional approaches. Springer, Dordrecht, p 423

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Marek IF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

Souleymane A, Aken'Ova ME, Fatokun CA, Alabi OY (2013) Screening for resistance to cowpea aphid (*Aphis craccivora* Koch) in wild and cultivated cowpea (*Vigna unguiculata* L. Walp.) accessions. Int J Sci Environ Technol 2:611–621

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Staub JE, Serquen FC, Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. Hortic Sci 31(5):729–741

Stoeckli S, Mody K, Patocchi A, Kellerhals M, Dorn S (2009) Rust mite resistance in apple assessed by quantitative trait loci analysis. Tree Genet Genomes 5:257–267

Stromberg LD, Dudley JW, Rufener GK (1994) Comparing conventional early generation selection with molecular marker-assisted selection in maize. Crop Sci 34:1221–1225

Tao YZ, Hardy A, Drenth J, Henzell RG, Franzmann BA, Jordan DR, Butler DG, McIntyre CL (2003) Identifications of two different mechanisms for sorghum midge resistance through QTL mapping. Theor Appl Genet 107(1):116–122

Tar'an B, Thomas E, Michaels TE, Pauls KP (2003) Marker assisted selection for complex trait in common bean (*Phaseolus vulgaris* L.) using QTL-based index. Euphytica 130:423–433

Thein HW, Yamagata Y, Mai TV, Yasui H (2019) Four resistance alleles derived from *Oryza longistaminata* (A. Chev. & Roehrich) against green rice leafhopper, *Nephotettix cincticeps* (Uhler) identified using novel introgression lines. Breed Sci 69:573–584

Valdez VA, Byrne PF, Lapitan NV, Peairs FBP, Bernardo A, Bai G, Haley SD (2012) Inheritance and genetic mapping of Russian wheat aphid resistance in Iranian wheat landrace accession PI 626580. Crop Sci 52:676–682

Vikal Y, Kaur A, Jindal J, Kaur K, Pathak D, Garg T (2020) Identification of genomic regions associated with shoot fly resistance in maize and their syntenic relationships in the sorghum genome. PLoS One 15(6):e0234335

Walker D, Boerma HR, All J, Parrott WL (2002) Combining cry1Ac with QTL alleles from PI 229358 to improve soybean resistance to lepidopteran pests. Mol Breed 9:43–51

Wang C, Yasui H, Yoshimura A, Su C, Zhai H, Wan J (2003) Green rice leafhopper resistance gene transferred through backcrossing and CAPs marker assisted selection. Agric Sci China 2:13–18

Wang C, Yasui H, Yoshimura A, Zhai H, Wan J (2004) Inheritance and QTL mapping of antibiosis to green leafhopper in rice. Crop Sci 44:389–393

Weng Y, Lazar MD (2002) Amplified fragment length polymorphism- and simple sequence repeat-based molecular tagging and mapping of greenbug resistance gene *Gb3* in wheat. Plant Breed 121:218–223

Weng Y, Li W, Devkota RN, Rudd JC (2005) Microsatellite markers associated with two *Aegilops tauschii*-derived greenbug resistance loci in wheat. Theor Appl Genet 110:462–469

Willcox MC, Khairallah MM, Bergvinson D, Crossa J, Deutsch JA, Edmeades GO, Gonzalez-de-Leon D, Jiang C, Jewell DC, Mihm JA, Williams WP, Hoisington D (2002) Selection for resistance to southwestern corn borer using marker-assisted and conventional backcrossing. Crop Sci 42:1516–1528

Wu Y, Huang Y (2008) Molecular mapping of QTLs for resistance to the greenbug *Schizaphis graminum* (Rondani) in *Sorghum bicolor* (Moench). Theor Appl Genet 117:117–124

Wu YQ, Huang Y, Tauer CG, Porter DR (2006) Genetic diversity of sorghum accessions resistant to greenbugs as assessed with AFLP markers. Genome 49:143–149

Xiao J, Grandillo S, Ahn SNK, McCouch SR, Tanksley SD, Li J, Yuan L (1996) Genes from wild rice improve yield. Nature 384:223–224

Xu J (2013) Pyramiding of two BPH resistance genes and *Stv-bi* gene using marker-assisted selection in japonica rice. Crop Breed Appl Biotechnol 13:99–106

Yang M, Cheng L, Yan L, Shu W, Wang X, Qiu Y (2019) Mapping and characterization of a quantitative trait locus resistance to the brown planthopper in the rice variety IR64. Hereditas 156:1–9

Yencho GC, Cohen MB, Byrne PF (2000) Applications of tagging and mapping insect resistance loci in plants. Annu Rev Entomol 45:393–422

Young ND, Kumar L, Menancio-Hautea D, Danesh D, Talekar NS, Shanmugasundaram S, Kim DH (1992) RFLP mapping of a major bruchid resistance gene in mungbean (*Vigna radiata* L. Wilczek). Theor Appl Genet 84:839–844

Yuexiong Z, Gang Q, Qianqian M, Minyi W, Xinghai Y, Zengfeng M, Haifu L, Chi L, Zhenjing L, Fang L, Dahui H, Rongbai L (2020) Identification of major locus *Bph35* resistance to Brown Planthopper in Rice. Rice Sci 27(3):237–245

Zhang G, Gu C, Wang D (2009) Molecular mapping of soybean aphid resistance genes in PI567541B. Theor Appl Genet 118:473–482

Zhao JZ, Fan XL, Shi XP, Zhao RM, Fan YL (1997) Gene pyramiding: an effective strategy of resistance management for *Helicoverpa armigera* and *Bacillus thuringiensis*. Resist Pest Manag 9(2):19–21

Zhao G, Jiang Z, Li D, Han Y, Hu Y, Wu L, Wang Y, Gao Y, Teng W, Li Y, Zeng G, Meng F, Li W (2015) Molecular loci associated with seed isoflavone content may underlie resistance to soybean pod borer (*Leguminivora glycinivorella*). Plant Breed 134:78–84

Zhao L, Abdelsalam NR, Xu Y, Chen MS, Feng Y, Kong L, Bai G (2020) Identification of two novel Hessian fly resistance genes *H35* and *H36* in a hard winter wheat line SD06165. Theor Appl Genet 133:2343–2353

Zhou H, Wang X, Mo Y, Li Y, Yan L, Li Z, Shu W, Cheng L, Huang F, Qiu Y (2019) Genetic analysis and fine mapping of the gall midge resistance gene *Gm5* in rice (*Oryza sativa* L.). Theor Appl Genet 133:2021

Zhu LC, Smith CM, Fritz A, Boyko E, Voothluru P, Gill BS (2005) Inheritance and molecular mapping of new greenbug resistance genes in wheat germplasms derived from *Aegilops tauschii*. Theor Appl Genet 111:831–837

Chapter 4
Glucosinolate-Myrosinase System and Its Role in Specialist and Generalist Insect Herbivores

T. Sathya and Sarwan Kumar

4.1 Introduction

In nature, all plants are armed with some type of protection mechanisms against pest attacks. Those can be biophysical or biochemical adaptations. Biophysical defense includes cuticular waxes, prickles, and thorns, while the latter mechanisms typically contain the synthesis of low molecular weight natural compounds, referred to as secondary metabolites, which might be unfavorable to the organisms attacking plants. Chemical defense compounds may be constitutively present in the plant, i.e., they preexist in anticipation of an insect attack (phytoanticipins), or their biosynthesis may be inducible (phytoalexins) (VanEtten et al. 1994; Mithöfer and Boland 2012). These compounds are stored in inactive form in plants and get activated upon herbivore damage. A number of constitutive glucosinolates are stored as non-active and relatively nontoxic compounds within the plant and are spatially separated from myrosinase. Tissue damage brings them together leading to production of more toxic compounds. This system of glucosinolates and hydrolytic myrosinases is referred to as glucosinolate-myrosinase system. The glucosinolate-myrosinase system is well studied because of the agriculturally important glucosinolate-containing crucifers (Brassicaceae), in addition to the long history of *Arabidopsis thaliana* (thale cress) as a model research organism. It is a major angiosperm family that consists of almost 375 genera and 3200 species (LeCoz and Ducombs 2006). Members of this family offer predominant sources of oilseeds, vegetables, and condiments. The damaged tissue of the *Brassica* plant releases glucosinolates (GLS), which might be then hydrolyzed by myrosinase to toxic isothiocyanates (Halkier

T. Sathya
Department of Entomology, Punjab Agricultural University, Ludhiana, India

S. Kumar (✉)
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India
e-mail: sarwanent@pau.edu

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_4

and Gershenzon 2006). A sudden release of these insecticidal compounds is termed as "mustard oil bomb" (Hopkins et al. 2009). Those compounds affect insect pests of Brassicaceae both by way of antibiosis (direct toxicity) and antixenosis (insects show non-preference to the vegetation) (Hopkins et al. 2009).

4.2 Glucosinolate-Myrosinase System

Glucosinolates are a group of sulfur- and nitrogen-containing glycosides observed exclusively in the order Capparales (Fahey et al. 2001; Halkier and Gershenzon 2006). Till the mid-2018, the number of glucosinolates known from plants, satisfactorily characterized by modern spectroscopic methods (nuclear magnetic resonance spectroscopy and *mass spectrometry*), is 88. In addition, a group of partially characterized structures with highly variable evidence counts for approximately a further 49. Thus, it means the total number of characterized glucosinolates from plants is somewhere between 88 and 137 (Blažević et al. 2020) with many of them being species-specific (Agerbirk and Olsen 2012). Glucosinolate polymorphism is a totally common phenomenon among the various plant species containing such compounds (Kim et al. 2017; Mithen et al. 2010). Glucosinolates (β-thioglucoside-*N*-hydroxysulfates) consist of a β-thioglucose moiety, a sulfonated oxime moiety, and a variable side chain (Fenwick et al. 1983) which makes them nonvolatile and hydrophilic. Depending on their precursor amino acids and the types of modification to the side chain (R group), glucosinolates are divided into three main groups: aliphatic, aromatic or benzenic, and indole. Compounds derived from alanine, leucine, isoleucine, methionine, or valine are referred to as aliphatic glucosinolates, the ones derived from phenylalanine or tyrosine are known as aromatic glucosinolates, and those derived from tryptophan are referred to as indole glucosinolates. The R groups of maximum glucosinolates are modified from those precursor amino acids (Fahey et al. 2001). Methionine-derived glucosinolates have been stated as the most significant class of glucosinolates in *Brassica* vegetables, even though other glucosinolates from three special classes have also been detected in the edible parts of *Brassica* types (Mithen et al. 2003; Cartea and Velasco 2008).

Glucosinolate synthesis is a three-step process involving amino acid chain elongation followed by synthesis of glucon from the amino acid and chain amendment (glucon addition). Many glucosinolates are biosynthesized through sizable adjustments in the aglycone side chains involving a range of chemical modifications which include elongation, hydroxylation, o-methylation, and desaturation, in addition to glycosylation, oxidation, and acylation (Sønderby et al. 2010). Synthesis of glucosinolates happens in cytoplasm of plants followed by storage in vacuoles of various kinds of cells (Mithen 2001). The concentration of glucosinolates varies extensively depending upon species, plant parts, and agronomic and climatic situations (Font et al. 2005; Tripathi and Mishra 2007). A drastic decline in the glucosinolate concentration (specifically aliphatic ones) occurs in *B. napus* seeds during the primary 7 days of imbibition, while *de novo* synthesis of indole glucosinolates

and an aromatic glucosinolate (gluconasturtin) takes place concomitantly. Gluconasturtin is not initially present in the seed. During the following growth period, a few more glucosinolates are additionally synthesized (Clossais-Besnard and Larher 1991). On the other hand, glucosinolates occur in low concentrations in the completely expanded leaves (Porter et al. 1991). With the start of the reproductive phase of plant, there is a reduction in the concentration of glucosinolates in vegetative plant parts as well as in inflorescence, which otherwise has fairly large amounts of these compounds. In contrast to this, at some stage during seed maturation, glucosinolate synthesis takes place in siliques which are then transported to the seeds via pod shells (Rask et al. 2000). The levels of glucosinolates can also be influenced by environmental situations. An increase in the concentration of glucosinolates takes place in *Brassica* plants under drought conditions (Bouchereau et al. 1996; Jensen et al. 1996). But there is no consistent relationship between glucosinolate concentration and water stress since elevated levels of glucosinolates are also found in plants grown under moist conditions in comparison to the ones grown in dry soil (Louda and Mole 1991).

In plant tissue, myrosinase and glucosinolates are stored in separate cellular compartments wherein these are inactive as a result preventing self-toxicity (Jones and Vogt 2001). Myrosinase is a homodimer consisting of subunits with a $(\beta/\alpha)_8$-barrel structure containing eight α-helixes and β-sheets. The structure is stabilized through Zn^{2+} ion incorporated into the center of the dimer and is heavily glycosylated (Burmeister et al. 1997). The enzyme is present in the myrosin cells, observed for the first time in 1884 by Heinricher, who indicated the presence of cells differing in morphology and size in comparison to neighboring cells and suggested that they comprise myrosinase and accordingly named them as myrosin cells. The distribution of myrosin cells differs in individual plant parts as well as plant development stage. High myrosinase activity in the upper parts of roots was reported in mature rape plants, while it was lowest in stems and inflorescences (Andréasson et al. 2001).

As mentioned earlier, myrosinase and glucosinolates are stored in separate cellular compartments. Tissue damage by external factors, e.g., after pest attack or on cutting or grinding, brings myrosinase into close contact with glucosinolates leading to hydrolysis of thioglucosidi bond in glucosinolate structure. This results in cleavage of D-glucose and release of an unstable aglucon-thiohydroximate-*O*-sulfate. Depending on the parent glucosinolate, hydrolysis conditions (pH, temperature), presence of cofactors (e.g., Fe^{2+}), and additional protein elements (e.g., epithiospecifier protein (ESP) and thiocyanate-forming protein (TFP)), the aglucone undergoes rearrangements to form distinct classes of degradation products: isothiocyanates (ITC), thiocyanates, nitriles, epithionitriles (EPT), and oxazolidine-2-thiones (Fig. 4.1) (Rungapamestry et al. 2006).

The formation of unstable intermediate aglucon-thiohydroximate-*O*-sulfate results in the first step of glucosinolate degradation catalyzed by myrosinase. Isothiocyanates are formed by spontaneous rearrangement from the unstable aglucone at neutral pH (6–7). Isothiocyanate production is higher in neutral pH condition than in acidic and alkaline. For example, hydrolysis of gluconapin and sinigrin produces isothiocyanate, namely, 3-butenyl isothiocyanate and 2-propenyl

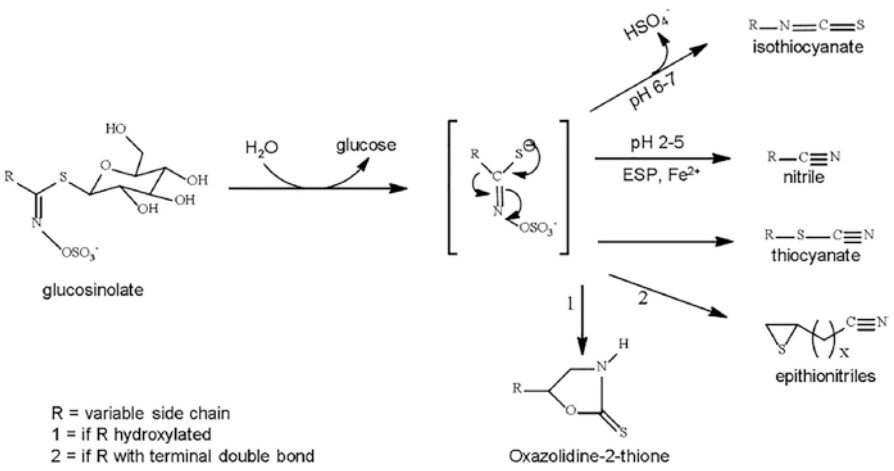

Fig. 4.1 Hydrolysis product of glucosinolates (adapted from Hennig 2013)

isothiocyanate, respectively. Isothiocyanates from indolic group are not much stable than aliphatic and benzenic group, which undergo further modifications. Interestingly, glucosinolates from the indolic group can be broken down independent of myrosinase activation in the physiological condition of the aphid gut to produce nitriles, alcohols, and unstable isothiocyanates that are further metabolized (Agerbirk et al. 2009). Generally, isothiocyanates are lipophilic, volatile, and more toxic than other hydrolysis products of glucosinolates.

At lower pH (<6), epithionitriles and nitriles are formed from thiohydroximate-*O*-sulfonate with the help of epithiospecifier protein. In the presence of Fe^{2+} ions and myrosinase, the recombinant protein catalyzed the formation of epithionitriles from thiohydroximates derived from alkenyl glucosinolates containing double bond between carbon atoms in the side chain (de Torres et al. 2005). Most likely, Fe^{2+} ions enabled the formation of transient bond between thiohydroximate and epithiospecifier protein because this reaction takes place only for thiohydroximate-*O*-sulfonate intermediate and not native glucosinolates (Foo et al. 2000; de Torres et al. 2005). The activity of epithiospecifier protein can be variable in individual plant parts and the development stage. A study on cuckooflower (*Crambe abyssinica*) sprouts suggests that the highest activity of epithiospecifier protein takes place during the second day of sprouting with gradual drop over 3 consecutive days and till the fifth day, when the activity becomes stable at the low level. The changes in the myrosinase activity are similar in trend; but the fluctuations are not as huge as in the case of epithiospecifier protein (Williams et al. 2009). The measurements of epithionitriles liberated in *Brassica* vegetables also revealed the great variability among different plant parts. Interestingly, crushed seeds and sprouts contained higher quantities of these compounds than aerial parts, including edible parts of the same plant (Kołodziejski et al. 2019).

When the glucosinolates are nonalkenyl, nitriles are formed by epithiospecifier protein from the intermediate hydrolysis product of glucosinolates. Apart from epithiospecifier protein, the presence of nitrile-specifier protein in plants also promotes the nitrile production which has different amino acid sequence than that of epithiospecifier protein (Wittstock and Halkier 2002). Nitrile-specifier proteins have been identified in rutabaga (*Brassica napus* L. var. *napobrassica*) (Wittstock and Halkier 2002) and thale cress (*Arabidopsis thaliana*) (Wittstock et al. 2016). Similar to epithiospecifier protein, nitrile-specifier protein also varies with stage and parts of the plants (Wittstock et al. 2016). Interestingly, nitrile-specifier protein has also been reported in large white butterfly, *Pieris brassicae*, that feeds exclusively on *Brassica* plants. *P. brassicae*, being *Brassica* specialist, has evolved mechanism to redirect the degradation of glucosinolates to produce less toxic nitriles with the help of nitrile-specifier protein from otherwise more toxic isothiocyanates produced during myrosinase-catalyzed hydrolysis.

Recently, epithiospecifier modifier protein was recognized in thale cress (*Arabidopsis thaliana*), a myrosinase-associated protein that modifies the activity of other specifier proteins operating in the same plant to promote the formation of isothiocyanates. It was shown to negatively affect the nitrile-to-isothiocyanate ratio in glucosinolate degradation. Even though the mechanism is not much clear, one of the feasible mechanisms is direct interplay of epithiospecifier modifier protein with myrosinase to stimulate the greater production of isothiocyanates and/or with epithiospecifier protein to suppress their activity responsible for epithionitriles and nitrile formation. It has been suspected as an evolutionary mechanism to counter the detoxification mechanism of isothiocyanates by insect herbivores such as the presence of nitrile-specifier protein in *P. brassicae* that redirects the degradation of glucosinolates from production of isothiocyanates to nitriles. However, the activity of epithiospecifier modifier protein on nitrile-specifier protein remains to be examined (Zhang et al. 2006).

Thiocyanates, the hydrolysis products of glucosinolates, are formed after enzymatic degradation of glucosinolates catalyzed by myrosinase with the help of thiocyanate-forming protein. Thiocyanate-forming proteins have been reported in garden cress (*Lepidium sativum*) (Burow et al. 2007), thale cress (*Arabidopsis thaliana*), and broccoli (*Brassica oleracea* L. var. *italica* Plenck) (Burow et al. 2006, 2007; Morant et al. 2008; Williams et al. 2008). The synthesis of thiocyanates occurs very occasionally in plant tissues as compared to isothiocyanates, nitriles, and epithionitriles. Further, only a handful of glucosinolates (such as glucotropaeolin, sinigrin, and glucoerucin) can function as precursors for the formation of suitable thiohydroximes because their chemical structures permit the formation of a strong carbocation form necessary for the reactions leading to thiocyanates (Kuchernig et al. 2011). Depending on the side-chain structure, thiocyanate-forming protein catalyzes the aglucone conversion into either thiocyanates or epithionitriles or nitriles (Kuchernig et al. 2011). Further, environmental conditions such as pH or availability of ions also determine the final product to be formed.

4.3 Impact of Glucosinolate Hydrolysis Products on Specialist and Generalist Insect Pests of Brassicaceae

Insect pests may be either generalist or specialist herbivores. Those which feed on a range of hosts belonging to different families are considered as generalist herbivores. They have greater resource availability and higher capability to exploit new hosts. The possibility of host-plant switching may additionally enhance insect development by minimizing the exposure to any single toxic phytochemical as well as by optimizing the nutrient intake (Bernays and Minkenberg 1997; Behmer 2009). On the other hand, specialist insect pests have a restrained host range which reduces the interspecific competition. They have the ability to counter the single toxic compound in the host plant more efficiently than generalist pest. *Brassica* plants are infested by many generalist or specialist insect herbivores from diverse insect orders. These include *Lipaphis erysimi*, *Brevicoryne brassicae*, *Pieris brassicae*, *P. rapae* and *P. napi*, *Plutella xylostella*, *Athalia lugens proxima*, *Trichoplusia ni*, *Mamestra brassicae*, *Chromatomyia horticola*, *Delia radicum*, *Phyllotreta cruciferae*, *Dasineura brassicae*, *Spodoptera exigua*, *S. littoralis*, *S. gregaria*, and *Helicoverpa armigera* (Agrawal 2000; Traw and Dawson 2002; van Poecke et al. 2003; Reymond et al. 2004; Mewis et al. 2006; Kuśnierczyk et al. 2007; Vogel et al. 2007; Travers-Martin and Müller 2008; Poelman et al. 2008; Bidart-Bouzat and Kliebenstein 2011).

Brassicaceae have evolved glucosinolate-myrosinase system to fend off any insect attack. Among the hydrolysis products of glucosinolates, isothiocyanates are the most reactive and functional metabolites toxic to insects than other hydrolysis products (El Sayed et al. 1996; Borek et al. 1998). Mechanism of their toxicity involves disruption of amino groups of proteins. The lipophilic properties of isothiocyanates facilitate passive diffusion into the insect cell through cell membrane, where they react with proteins to cleave the disulfide bond resulting in impaired catalytic activity (Kawakishi and Kaneko 1985, 1987; Halkier and Gershenzon 2006).

The myrosinase glucosinolate system is a double-edged sword. On one side, it protects the plants from generalist feeders (Rask et al. 2000), while on the other side glucosinolates make plants more attractive to specialist feeders (Renwick 2002; Bjorkman et al. 2011). Further, toxicity of glucosinolate varies depending upon the class (aliphatic, indolic, and benzenic glucosinolates) and the insect involved. Generalist insect pests tobacco hornworm, *Manduca sexta*, and the cabbage looper, *Trichoplusia ni*, are negatively affected only by the presence of aliphatic glucosinolates (Müller et al. 2010), while green peach aphid, *Myzus persicae*, is affected by indolic glucosinolates (de Vos and Jander 2009; Pfalz et al. 2009). In contrast, beet armyworm, *Spodoptera exigua*, is adversely affected by the presence of both aliphatic and indolic glucosinolates (Müller et al. 2010). Further, glucosinolates exhibit exclusive effects depending on the herbivore. For instance, the presence of higher concentrations of sinalbin in cotyledons of *Sinapis alba* exhibits antibiotic resistance to bertha armyworm, *Mamestra configurata*, in terms of low survival and low body weights and repellent effect to the flea beetle, *Phyllotreta cruciferae*. The

lower concentrations found in older leaves did no longer seem to provide any protection against either species. Alternatively, specialist insect herbivores have adapted to use glucosinolates to their advantage. Glucosinolates are known to act as oviposition and feeding stimulants for more than 25 insect species of the orders Coleoptera, Lepidoptera, and Diptera (Hopkins et al. 2009). Many insects such as cabbage aphid, *Brevicoryne brassicae* (Nottingham et al. 1991), and diamondback moth, *Plutella xylostella* (Renwick et al. 2006), carry receptor neurons that could detect isothiocyanates. They are known to stimulate oviposition in large white butterfly, *Pieris brassicae*; small white butterfly, *Pieris rapae* (Renwick et al. 1992; Smallegange et al. 2007); diamondback moth, *Plutella xylostella* (Renwick et al. 2006); and cabbage fly, *Delia radicum* (Roessingh et al. 1992). Higher allyl glucosinolates (sinigrin) induce feeding in cabbage aphid, *Brevicoryne brassicae* (Lankau 2007). Some other plant constituents also act as feeding stimulants collectively along with glucosinolates. Flavonoids and glucosinolates increase the feeding of flea beetle, *Phyllotreta armoraciae* (Nielsen et al. 1979), and diamondback moth, *Plutella xylostella* (van Loon et al. 2002). A number of other hydrolysis products are also known to affect insect feeding/behavior either directly or indirectly. Phenylacetonitrile from benzenic glucosinolate breakdown acts as an indirect plant defense in two different ways, one by repressing the mating of female pierid butterflies (anti-aphrodisiac effect) and another by attracting natural enemies such as the generalist egg parasitoid, *Trichogramma brassicae* (Hymenoptera) (Andersson et al. 2003; Fatouros et al. 2008).

Since glucosinolates play a defensive role in plants, it raises the question that double zero ("00") canola plants which are low in these compounds might be vulnerable to many insects. Such questions may be misplaced because low glucosinolate levels in "00" canola plants were confined primarily to seeds (Milford et al. 1989) and high and low glucosinolate cultivars did not differ in their susceptibility to pod midge (*Dasineura brassicae*), though the level of glucosinolates in green tissue was not determined (Åhman 1982). Extensive studies in India with both *B. juncea* and *B. napus* have shown no reasons to believe that canola-quality cultivars were more susceptible than their non-canola counterparts (Kumar 2019). Theoretically (though there are no supporting references), low glucosinolate plants may be less attractive to specialist insects for which these compounds serve as feeding and oviposition stimulants (Gabrys and Tjallingii 2002; Mewis et al. 2002). This is again supported by the work of Giamoustaris and Mithen (1995) who reported that increase in content of glucosinolates in *B. napus* resulted in increased feeding damage by specialist insects, flea beetles (*Psylliodes chrysocephala*), and greater incidence of small white butterfly (*P. rapae*), while the damage by generalist pests, i.e., pigeons and slugs, was reduced. Further, glucosinolate-rich flower tissues are preferred more by *Pieris brassicae* and sustained higher growth compared to leaf tissues (Smallegange et al. 2007) indicating the selective role of glucosinolate to elicit feeding in this specialist insect and the adaptation of the insect to use these compounds to its advantage.

4.4 Insect Herbivore Adaptation of Glucosinolate-Myrosinase System of Brassicaceae

As discussed above, insects are strongly affected by glucosinolate hydrolysis products. However, a few insect herbivores have adapted to neutralize/detoxify these toxic compounds which permit them to feed on glucosinolate-containing plants. These adaptive processes play their role before and after consumption of food. Insects may either keep away from toxic compounds or feed on glucosinolate-containing plant tissues. Exposure to toxins could be often related to accelerated activity of phase I and phase II detoxification mechanisms. By oxidation, hydrolysis, or reduction, phase I enzymes introduce reactive and polar groups into their substrates. The P450 monooxygenases (P450s), which are commonly known for their role in the metabolism of natural and synthetic insect pesticides, are prominent among phase I enzymes. Following phase I, the activated metabolites of xenobiotics are conjugated with compounds which include glutathione (GSH), sulfate, or glucuronate in phase II reactions. The glutathione-*S*-transferases (GST) are among the best recognized of the phase II enzymes, and increases in their levels are related to resistance to toxins. Some insects can also sequester the toxins in their body to protect them from natural enemies. In insects, behavioral, physiological, and metabolic adaptations may be mixed to conquer the toxic compound.

4.4.1 Before Consumption of Food: Host Plant Selection and Feeding Guilds

Insects require resources for growth and reproduction. Besides the quantity of dietary consumption, the quality of the food consumed can be critical for development time, fecundity, and fitness (Awmack and Leather 2002). The usage of an extensive variety of hosts increases food availability and allows mixtures of different kinds of food, which might also improve nutrient stability (Simpson and Raubenheimer 2001; Berner et al. 2005). Dietary mixing also helps to dilute probably toxic allelochemicals which might be unevenly distributed over different plants (Freeland and Janzen 1974; Bernays and Minkenberg 1997) or even in the same plant, i.e., varying concentrations in different organs and developmental stages (Hoy et al. 1998; Gebrehiwot and Beuselinck 2001), or are induced by the feeding herbivore itself (van Dam et al. 2000). Generalists are known to feed on a wide range of plant species, often from more than one plant family. They are adapted to low to medium levels of various plant defense compounds to avoid ingestion of any single lethal doses of phytotoxin. For example, generalist grasshopper species record higher growth, survival, and fecundity through host plant switching and dietary mixing, while feeding is restricted to a single plant species (Bernays and Minkenberg 1997).

Both generalist and specialist insects do host plant switching and dietary mixing either within the same plant or among the populations of plants or between two plants from different families. Whereas generalists benefit from suppressing any degree of toxicity from plant defense compounds, permitting at least short-term feeding, specialists often suppress only high levels of toxicity and benefit from the presence of low to intermediate levels of plant defense compounds. Sequestering specialists selectively take up and store chemical plant compounds in their own body, benefitting from sequestered compounds because sequestered compounds shield the insect from their enemies (Sect. 4.4.2.5) (Nishida 2002; Ali and Agrawal 2012). Behavioral adaptation via host-plant switching in generalists and selection of toxic plants in specialists also involve trade-offs and fitness costs. Insect herbivores need to invest time and energy to search for an appropriate host (Schultz 1983; Dethier 1988; Despres et al. 2007). Investment costs differ in generalists and specialists and especially seem to depend not only on the level of plant defense compound but also on an excessive degree of the level of activating enzyme. Frequently the more generalists want to interchange host plants, the longer they need to look for suitable host plants, which increases costs. In contrast, specialists need to invest much less time, energy, and thus costs in this case.

Insect herbivores can acquire suitable food through specialist and generalist feeding habits (Schoonhoven et al. 1998). Depending upon the mouthparts, agricultural insect pests are typically divided into two groups: chewing insects and piecing and sucking insects. Those with chewing mouthparts crush the leaf/plant tissue with the help of mandibles, maxillae, labrum, and labium. Examples of chewing insects include grasshoppers (order Orthoptera), beetles (order Coleoptera), and larval Lepidoptera. On the other hand, in sucking insects (order Hemiptera), mandibles and maxillae are modified into a long proboscis protected by a modified labium which penetrates the plant tissue to feed on the plant sap.

Tissue damage during feeding brings together glucosinolates and myrosinases which otherwise are spatially separated in vacuoles of cells and myrosin cells, respectively. Tissue damage depends upon the feeding guild and herbivore species (Textor and Gershenzon 2009).

A guild is defined as a group of species similarly exploiting the same class of environmental resources. In general, there are distinctive guilds of feeding including leaf-chewing, leaf-mining and piercing-sucking (Bernays and Janzen 1988; Sinclair and Hughes 2010). Chewing insects are exposed to more toxic compounds due to higher tissue damage than other feeding guilds, but physiological conditions may favor stabilization or detoxification of plant defense compounds as discussed later. Compared to chewing insects, feeding by leaf miners is limited to parenchymal or epidermal tissues leading to production of canals, mines, or blotches (Sinclair and Hughes 2010). Thus, the overall tissue damage by leaf miners is less than tissue feeders but more than piercing and sucking insects (Schappert and Shore 1999). The limited tissue damage leads to limited production of hydrolysis products compared to chewing insects.

In contrast, sucking insects such as aphids are exposed to little or no plant defense due to their specialized feeding habit. Aphids are specialized phloem sap feeders

which insert their needle-like stylets in the plant tissue keeping off/counteracting the different plant defenses. They withdraw large quantities of phloem sap while maintaining the phloem cells alive. In comparison to the insects with biting and chewing mouthparts which tear the host tissues, aphids penetrate their stylets between epidermal and parenchymal cells to finally reach sieve tubes with slight physical harm to the plants, which is rarely perceived by the host plant (Bhatia et al. 2011). The long and flexible styles travel within the apoplasm across intercellular areas (Giordanengo et al. 2010), while styles also perform intracellular punctures to investigate a cell's internal chemistry (Züst and Agrawal 2016). The excessive stress inside sieve tubes enables in passive feeding (Bhatia et al. 2011). During the stylet penetration and feeding from phloem, aphids produce two types of saliva which are used to form sheath around the stylet (gelling saliva) and to prevent coagulation of proteins which is helpful to defend the feeding site from plant's immune response and efficient feeding (watery saliva), respectively. Aphids feed only on single phloem cells, so the myrosinase-catalyzed breakdown of glucosinolates into active hydrolysis products may not be triggered because glucosinolates and myrosinases are assumed to be placed in separate cells (Barth and Jander 2006). Some specialist insects have adapted to use glucosinolates for their own benefit. For example, cabbage aphid, *Brevicoryne brassicae* (Aphididae), sequesters and stores glucosinolates in its body which are later used in defense against predators (Bridges et al. 2002). Likewise, the generalist green peach aphid, *Myzus persicae* (Aphididae), is capable of ingesting intact glucosinolates which are later excreted out in nontoxic form in the honeydew (Barth and Jander 2006).

Induction of plant defenses also depends on the insect feeding guilds. Induced resistance is a physiological state of enhanced defensive capacity of the plant induced through biological or chemical inducers, which protects plant tissues not exposed to the initial attack against future attack by herbivores that may show greater resistance both locally and systemically (van Loon et al. 1998). While feeding, insects deposit small amounts of saliva/oral secretions at the disrupted tissue. Active components in these fluids (the so-called herbivore-associated elicitors (HAEs) or herbivore-associated molecular patterns (HAMPs)) can be perceived by a large number of plant species as chemical cues (Alborn et al. 1997, 2007; Musser et al. 2002; Schmelz et al. 2009). The perception of herbivore-associated elicitors induces precise defense responses which differ from simple mechanical damage in most of the cases (Mithöfer and Boland 2008; Bonaventure et al. 2011). The perception of herbivore-associated elicitors and herbivore-associated molecular patterns by plants usually affects the activation of particular plant responses in order to protect or tolerate an insect attack. Such reactions include, among others, unique changes in metabolism, gene expression, and plant growth and development patterns (Turlings et al. 1990; Kessler and Baldwin 2002; Bede et al. 2006). During herbivore attack, insect-associated elicitors bind to putative receptors at the plasma membrane and prompt downstream responses such as depolarization of cellular membranes and the activation of Ca^{2+} inflow. Feeding by a few insects induces very strong modifications in cellular membranes and Ca^{2+} influx (Maffei et al. 2004; Arimura et al. 2008). This Ca^{2+} inflow depends on the activity of Ca^{2+} channels; the

reaction can be reduced through either specific Ca^{2+} channel inhibitors or calcium chelators (Maffei et al. 2004, 2006). Modifications in cellular membrane potential and Ca^{2+} influx activate NADPH oxidases in cells that catalyze the production of superoxide such as reactive oxygen species (ROS) (Sagi and Fluhr 2001, 2006). These reactive oxygen species modify amino acids in regulatory proteins as a redox-based mechanism to translate secondary signals into the transcriptional activation of defense-associated genes, in particular lipoxygenases, to initiate the biosynthesis of jasmonic acid (Porta and Rocha-Sosa 2002).

Chewing insects lead to higher degradation of glucosinolates due to greater tissue damage compared to piercing-sucking insects which inflict limited tissue damage (Barth and Jander 2006; Textor and Gershenzon 2009) coupled with active manipulation of plant defenses (Miles 1999; Will et al. 2007, 2009, 2013).

4.4.2 After Consumption of Food

4.4.2.1 pH of Insect Gut

The pH of the insect midgut lumen ranges from a highly acidic pH 3.1 to an extremely alkaline pH 12–14 among different insect orders (Berenbaum 1980; Schultz and Lechowicz 1986; Appel and Joern 1998; Harrison 2001; Cristofoletti et al. 2003). It is promoted by the active secretion of K^+ into the midgut in alternation for H^+ through the proton ATPase pump and by transport of ammonia from the gut lumen into the hemolymph in some insects which are carried out in goblet and collumner cells of the midgut (Weihrauch 2006). Vacuolar proton pumps are reported to occur in many secretory tissues (Huss et al. 2011). A proton pump occurs within the apical membrane of insect salivary glands (Baumann and Walz 2012) and in Malpighian tubule cells and drives the formation of fluid in Malpighian tubules. The pH of insect gut influences the movement of any enzymes secreted into or carried with food. In addition, gut pH might also influence the solubility of ingested components, the toxicity of some potential toxins, and the population of gut microorganisms. The classical example of detoxification of plant toxin by gut pH is tannin detoxification by herbivores. The higher midgut pH in the insects feeding on tannin-rich food may have evolved as a defense mechanism to reduce the toxicity of tannins, which have a tendency to form complex with proteins. The presence of acidic and alkaline pH in the midgut of insect pest of *Brassica*s reduces glucosinolate hydrolysis (El-Shora et al. 2016) which helps the insects to sequester the glucosinolates in their body that are used to protect them from natural enemies (Sect. 4.4.2.5). A numerous insect herbivores with an alkaline/acidic midgut are known to feed on plants not protected by toxins (Berenbaum 1980). Therefore, pH of the midgut probably did no longer rise up as an evolutionary reaction to glucosinolates of plants. Insect herbivores with an alkaline midgut absolutely may have been pre-adapted to feed on plants protected by glucosinolates.

4.4.2.2 Glutathione *S*-Transferases (GST): After Formation of Hydrolysis Product Isothiocyanates

Isothiocyanates are the glucosinolate breakdown products most often encountered by herbivores feeding on glucosinolate-containing plants. Because of their lipophilic nature, isothiocyanates are absorbed passively into epithelial cells. If they are not detoxified at this stage, they would possibly be dangerous to the cells as they easily react with amino groups of proteins and are reported to cleave disulfide bonds though in vitro (Kawakishi and Kaneko 1985, 1987). Isothiocyanates are conjugated with glutathione as quickly as they enter the cells by conjugating enzymes (Kassahun et al. 1997; Traka and Mithen 2009). Conjugating enzyme increases the conjugate water solubility and excretion efficiency. Glutathione (GSH) is an essential biological nucleophile and a reducing agent and is normally found in cells. It is a Glu-Cys-Gly tripeptide which binds with electrophilic centers of isothiocyanates through glutathione *S*-transferases. Isothiocyanate-glutathione conjugates are actively transported out of the cells where they either enter the mercapturic acid pathway for renal excretion or dissociate to release the free isothiocyanates (Al Janobi et al. 2006; Traka and Mithen 2009).

As conjugation with glutathione is a classical and ubiquitous phase II metabolism reaction, it has been investigated whether or not isothiocyanates are detoxified by conjugation with glutathione in insect herbivores. Conjugation with glutathione has been shown to involve in detoxification of isothiocyanates in many generalist herbivores with varying glucosinolate preferences (Schramm et al. 2012). Generalist insect pests such as cotton bollworm *Helicoverpa armigera*, fall armyworm *Spodoptera frugiperda*, cabbage moth *Mamestra brassicae*, and cabbage looper *Trichoplusia ni,* which feed on glucosinolate-containing plants, conjugate poisonous isothiocyanates with glutathione by glutathione-*S*-transferase activity leading to the formation of nonpoisonous products which are excreted in the frass (Schramm et al. 2012). Despite the fact that phase II metabolism response is classical and ubiquitous, glutathione-*S*-transferase activity on isothiocyanates is insect-specific. Extracts acquired from midgut tissue of velvet bean caterpillar, *Anticarsia gemmatalis*—an insect herbivore that does not feed on glucosinolate-containing plants—lacked glutathione-*S*-transferase activity toward isothiocyanates (even after induction), but not on artificial substrates frequently used for glutathione-*S*-transferase activity measurements (Wadleigh and Simon 1988; Yu 1987). This seems to signify that an alternatively specific glutathione-*S*-transferase activity in gut tissue, at least, contributes to isothiocyanate detoxification in the two glucosinolate-feeding species.

Piercing-sucking insects such as aphids, unlike chewing herbivores, cause only minor tissue damage during feeding and are thought to prevent the detonation of the so-called mustard oil bomb to a large extent. These herbivores guide their stylets among individual plant cells to the phloem sieve elements (Tjallingii and Esch 1993). Consequently, glucosinolates present in the phloem and apoplast aren't brought into contact with myrosinases which are localized in separate myrosin cells (Thangstad et al. 2004; Barth and Jander 2006). Thus, green peach aphid, *Myzus*

persicae, is not too much by the presence of glucosinolates in plant tissues. However, indolic glucosinolates breakdown under the conditions present in the insect gut independent of plant myrosinases and have a strong antifeedant effect on *M. persicae* (Kim and Jander 2007; Kim et al. 2008). *M. persicae* is known to harbor a gut-expressed gene with similarity to plant myrosinases (Ramsey et al. 2007). The breakdown products detected in the aphid honeydew contain amino acids and glutathione conjugates that represent lively detoxification products (Ramsey et al. 2010). Induction of glutathione-*S*-transferase activity in response to increasing glucosinolate concentrations has been shown in *M. persicae* (Francis et al. 2005). Interestingly, leaf miner, *Scaptomyza* species (specialist), has also been discovered to excrete glutathione conjugation products with isothiocyanates (Gloss et al. 2014). Glutathione-*S*-transferase-dependent detoxification has an excessive metabolic cost. Glutathione levels in *Spodoptera littoralis* and *Myzus persicae* midgut tissues and hemolymph have been found to drop significantly upon ingestion of isothiocyanates in a dose-dependent manner, suggesting that the available pool of glutathione-*S*-transferase is confined (Kim et al. 2008; Jeschke et al. 2016). These metabolic changes resulted in other metabolic outcomes such as decreased weight and body protein levels and reduction in fecundity, with some of these outcomes ameliorated by supplementation of cysteine (precursor of glutathione) (Jeschke et al. 2016). However, generalist herbivores can modify their feeding behavior upon encountering isothiocyanates to keep away from leaf regions of high constitutive (Shroff et al. 2008) or induced glucosinolates (Perkins et al. 2013).

4.4.2.3 Nitrile-Specifier Protein: After the Formation of Intermediate Hydrolysis Product

Cabbage butterflies, *Pieris* spp., are known to secrete a protein into the gut lumen, specified as nitrile-specifier protein (NSP), which interferes with myrosinase-catalyzed glucosinolate hydrolysis in the ingested plant tissue (Wittstock et al. 2004). This nitrile-specifier protein redirects the hydrolysis from toxic isothiocyanates to simple nitriles which are excreted with the feces, either unchanged or after further metabolism. This mechanism has been identified in numerous different pierid species specialized to feed on glucosinolate-containing plants (Wheat et al. 2007; Wittstock et al. 2004). Nitrile-specifier protein does not have hydrolytic activity on glucosinolates; rather, it acts on the glucosinolate aglucone, the product of plant myrosinase-catalyzed hydrolysis of the thioglucosidi bond. In spite of this purposeful similarity, larval nitrile-specifier protein does not have any structural similarities with plant-specifier proteins. *Arabidopsis thaliana* has AtNSP1 (At3g16400) and AtNSP2 (At2g33070), but in comparison to *Arabidopsis* epithiospecifier protein, *Pieris rapae* nitrile-specifier protein has a low substrate specificity and isn't always dependent on Fe^{2+} (Burow et al. 2006, 2009).

So far, nitrile-specifier protein has most effective been located in glucosinolate-feeding pierid species. Approximately ten million years after the evolution of the glucosinolate-myrosinase system, ancestral pierid insects evolved a key

biochemical adaptation that allowed them to make use of Brassicales plants as their food source (Wheat et al. 2007; Beilstein et al. 2010). This host shift to Brassicales plants becomes facilitated by the evolution of a nitrile-specifier protein, which directs the myrosinase-catalyzed breakdown of glucosinolates in the larval gut to form nitriles, which can be less toxic and reactive than isothiocyanates (Wittstock et al. 2004). These nitriles may be further metabolized prior to excretion. Simple nitriles derived from aliphatic glucosinolates are excreted unchanged, while the nitriles derived from benzenic glucosinolates undergo further metabolism to its sulfate ester (Müller et al. 2003; Wittstock et al. 2004). Phenylacetonitrile shaped from benzylglucosinolate could yield either *N*-phenylaceryglycine or hippuric acid/*N*-benzolylisoserine from the intermediate phenylacetic acid by nitrilase and nitrile hydratase activity, respectively. Although *Pieris* larvae evidently have an efficient method to avoid toxic isothiocyanates, the formation of cyanide ("cyanide bomb") during metabolism of benzenic glucosinolates may also result in toxicity (Stauber et al. 2012). The presence of constitutive β-cyanoalanine synthase and rhodanese in the gut detoxifies the cyanide to nonpoisonous form (van Ohlen et al. 2016). Based on metabolite analyses and the experimentally demonstrated ability of *P. rapae* to survive in cyanide fumigation experiments as well as the facts that benzylglucosinolate was one of the predominant glucosinolates in ancient Brassicales and that ancient Brassicales lack nitrilases involved in alternative pathways, Stauber et al. (2012) proposed that the ability of pierid species to safely handle cyanide contributed to the primary host shift from Fabales to Brassicales that occurred about 75 million years ago and was followed by pierid species diversification.

4.4.2.4 Glucosinolate Sulfatase: Before the Hydrolysis by Myrosinase

Another way to conquer the glucosinolate-myrosinase system could be to rapidly metabolize the intact glucosinolates earlier than they are hydrolyzed by plant myrosinases in the ingested tissue. Given the high levels of myrosinase activity in plant tissues, this will require highly efficient metabolizing enzymes or a myrosinase inhibitor to be present in the mouthparts and/or gut of the herbivore. The enzyme glucosinolate sulfatase (GSS) converts intact glucosinolates to desulfoglucosinolates which are not recognized by myrosinase (Matile 1980). Glucosinolate sulfatase activity has been reported in diamondback moth, *Plutella xylostella*; desert locust, *Schistocerca gregaria*; and turnip sawfly, *Athalia rosae* (Ratzka et al. 2002; Falk and Gershenzon 2007; Opitz et al. 2011). *P. xylostella* larvae possess a glucosinolate sulfatase in their gut that converts all primary classes of glucosinolates to desulfoglucosinolates, which are not amenable to myrosinases (Ratzka et al. 2002). The expression of glucosinolate sulfatase is under tight developmental and tissue-specific regulation: transcripts are constitutively present in the larval gut—the only stage and organ exposed to glucosinolates in the diamondback moth life cycle—and the sulfatase transcripts are absent in other tissues and developmental stages (Ratzka et al. 2002).

Desert locust, *Schistocerca gregaria* (generalist), is also known to possess a glucosinolate sulfatase in the gut with high substrate specificity. Glucosinolate sulfatase activity enables the insect to feed even on *Schouwia purpurea*, a plant with very high concentrations of glucosinolates (Falk and Gershenzon 2007). The glucosinolate sulfatase activity increased tenfold when locusts were fed on *S. purpurea*, while it was reduced when glucosinolates were eliminated from diet. This indicates that glucosinolate sulfatase activity is highly inducible in generalist desert locust, while it is constitutive in the specialist diamondback moth larvae. The interplay between specialized insect enzymes that are active before plant myrosinase and sequestration, as a further adaptation, was proven in the sawfly *Athalia rosae*. Sequestered glucosinolates are hastily turned over and leave the insect body within a day upon diet change (Müller and Wittstock 2005). Therefore, *A. rosae* larvae are required to continuously feed on glucosinolate-containing plants to hold their hemolymph glucosinolate levels. Sawfly larvae take up glucosinolates into their hemolymph from the gut where they are degraded to desulfoglucosinolates by glucosinolate sulfatase and subsequently sulfated at the glucose moiety by means of sulfotransferases (Opitz et al. 2011). Since excess glucosinolates are brought back into the gut and excreted via the frass, it is highly adaptive to earlier conversion within the hemolymph, since these modified glucosinolates in the gut can no longer be hydrolyzed by the remaining plant β-thioglucosidases (Müller 2009; Opitz et al. 2011).

4.4.2.5 Sequestration

Sequestration of plant chemical defenses is another method of insect adaptation to host plant chemistry (Opitz and Müller 2009). In many cases, it has been proven that insects exhibit better defense against their natural enemies after sequestration of defense compounds (Opitz and Müller 2009). For successful sequestration of glucosinolates, insect herbivores should not only possess some form of active uptake mechanism but also need to be capable to avoid glucosinolate hydrolysis. In theory, this could be accomplished by two principal ways: first, the compartmentalization of the glucosinolate-myrosinase system is not disturbed (as in the case of phloem-feeding aphids), and second, the uptake of intact glucosinolates is faster than their hydrolysis by myrosinases. This would require a relatively efficient transport mechanism and/or inhibition of myrosinase. In none of the herbivores that sequester glucosinolates has the precise mechanism of glucosinolate uptake from the gut lumen been reported; however, successful sequestration of intact glucosinolates has been established in both sucking and chewing herbivores. This suggests that both principle methods of retaining intact glucosinolates are operative in insects. To keep away from autotoxicity, a storage site is needed wherein breakdown of glucosinolates is averted till it is needed for defense (Müller 2009). Some insect species synthesize their own myrosinases, saved in a separate compartment away from the sequestered glucosinolates, which can be utilized for activating defense against predators (Beran et al. 2014; Francis et al. 2002).

B. brassicae (*Brassica* specialist) acquires glucosinolates in the hemolymph after phloem feeding with little or no cell disruption. The concentration of glucosinolates in hemolymph is higher in apterous individuals than alates which excrete large amounts of glucosinolates in the honeydew (Kazana et al. 2007). This insect is also known to synthesize its own myrosinase (distinct from plant myrosinase) which is stored in microbodies in flight (thorax) and head muscles (Beran et al. 2014). Endogenous myrosinase has also been reported in turnip aphid, *Lipaphis erysimi* (Bridges et al. 2002). The sequestered glucosinolates not only protect the aphids from attack of predators but also enhance growth and development of aphids. Generation time and fecundity of *Brevicoryne brassicae* correlate positively with concentration of total host glucosinolates (Chaplin et al. 2011; Kos et al. 2012), while composition of glucosinolates in aphid host plants and quantity sequestered can negatively affect the survival of both hoverflies and ladybugs (Francis et al. 2000, 2001; Kazana et al. 2007; Chaplin et al. 2011). Further, glucosinolate sequestration in *L. erysimi* releases isothiocyanates which synergize the action of aphid alarm pheromone *E-β-farnesene* required to initiate aphid dispersion after enemy attack (Dawson et al. 1987). Similar to aphids, flea beetle, *Phyllotreta striolata*, is also known to sequester plant glucosinolates and produce their own myrosinase (the so-called walking mustard oil bomb) (Kazana et al. 2007). The isothiocyanates produced after glucosinolate hydrolysis was found to have pheromone-like activities and set off aggregation behavior in adult beetles at high concentrations (Beran et al. 2011).

Sawfly larvae of the genus *Athalia* are also known to sequester glucosinolates from their host plants (Müller 2009; Opitz et al. 2010). Larval feeding is assumed to destroy the compartmentalization of the glucosinolate-myrosinase system, but larvae acquire intact glucosinolates in their hemolymph in concentrations higher than those in their food (Müller et al. 2001). When attacked by predators, these larvae release droplets of hemolymph (easy bleeding disruption of integument upon touch by predator), and the glucosinolates in the hemolymph probably act as deterrents to predators (Müller et al. 2002). However, so far it is unclear how the larvae manage to take up glucosinolates from their food without glucosinolate breakdown. When the larvae were transferred between plants with different glucosinolate profiles, glucosinolates from the new food were present in the hemolymph after 30 min, while the glucosinolates from the previous food plant were infrequently detectable after 24 h (Müller and Wittstock 2005). However, the feces contained trace quantities of intact glucosinolates arguing for metabolism of glucosinolates before excretion (Müller et al. 2001). Sawfly larvae are known to detoxify glucosinolates to desulfoglucosinolates before excretion (Sect. 4.4.2.4).

4.4.2.6 Symbionts

Symbiosis is extremely important for ecosystems' structure and function. Among the different types of symbioses (mutualistic, commensal, and antagonistic), mutualism is ubiquitous in all types of ecosystems and plays crucial roles in performance

of groups. Among the various symbiotic associations, the most cohesive form is endosymbiosis, wherein one partner is a symbiont (typically microorganisms, together with archaea, bacteria, and fungi) that lives inside the body of and intimately interacts with the other partner called host (normally animals and plants). Some microbes are dangerous or even lethal to the host and are referred to as parasites/pathogens, while others support the host species and appear to be mutualists because of their adaptive metabolic capabilities. Usually, mutualists are known as symbionts, as an antonym for parasites/pathogens (McFall-Ngai et al. 2013). Mutualistic associations with bacteria occur in animals, plants, fungi, or even protists, among which insects are wonderful for the prevalence and excessive variety of the associations they form with symbiotic microorganisms. Insects that feed completely on restricted diets, consisting of plant sap, vertebrate blood, and woody materials, typically own symbiotic microorganisms (mostly bacteria) of their frame, in which the symbionts play pivotal roles in host metabolism by presenting vital nutrients (e.g., critical amino acids and B vitamins) that lack in their ingredients and/or via digesting food substances (Baumann 2005; Brune 2014). Symbiotic bacteria commonly show strict host tissue tropism and are localized in symbiotic organs, even though the localization sample varies among insect species and stages from extracellular in the body cavity to intracellular in specialized cells referred to as mycetocytes/bacteriocytes (Moran and Telang 1998). Those symbionts are normally exceeded from the mother to offspring via sophisticated mechanisms for vertical transmission, which include transovarial transmission, egg-surface contamination, and coprophagy (Salem et al. 2015). For many insect groups, the symbiont phylogeny perfectly mirrors the host phylogeny, indicating that symbiotic associations have been maintained through strict vertical transmission for a protracted length of evolution, resulting in strong host-symbiont interdependency: host insects are afflicted by critical health defects without symbionts, while symbiotic microorganisms are generally unculturable (Kikuchi et al. 2011).

In addition to these dietary metabolic roles, current studies discovered more diverse functions of symbiotic bacteria in insects. Symbiotic bacteria are involved in tolerance to excessive temperature, parasitoid resistance, pathogenic virus protection, toxin synthesis, hardening of cuticle, integument coloration, and even sex determination (Su et al. 2013; Pietri and Liang 2018). In addition to toxin-degrading symbionts, these symbiotic bacteria, unlike the above mentioned nutrient individuals, are not necessary for the growth and reproduction of the host insects. However, such microbial partners play pivotal roles inside the evolution of insects by assisting in tolerance to variation in heterogeneous environments, underpinning the enormous variety of insects in the terrestrial environment. The presence of gut symbionts, *Serratia*, *Providencia*, *Pectobacterium*, and *Acinetobacter*, of the Gammaproteobacteria in cabbage root fly *Delia radicum* is known to degrade the toxic isothiocyanates. Symbionts encode SaxA gene which detoxifies the isothiocyanates by isothiocyanate hydrolase (Welte et al. 2016).

4.5 Conclusion

Glucosinolates have gained special significance in the study of insect-plant interactions as they are found in a wide variety of plants. Moreover, the presence of glucosinolates in *Arabidopsis*, a model organism in genetic studies, has played an important role in developing plant defense models for insect attacks, as well as for various pests. The primary role of myrosinase glucosinolate system in Brassicales is to ensure the protection against pests. Further research is needed to discover the range of stimulatory glucosinolates that possess exclusive side chains. Although a few tests have been carried out on some insect species with moderate range of glucosinolates, they were inadequate to derive generalized structure- activity relationship. In the past decade, research efforts have largely focused on the effect of glucosinolates on insect-plant interactions rather than with other bioactive compounds of plants. For example, glucosinolates are known to interact with plant waxes which in turn have effect on insect-plant interaction.

In addition, the specifier proteins are produced via plant redirecting the spontaneous approaches to biologically less active derivatives. Of what benefit is it to a plant to lower the discharge of poisonous plant secondary metabolites? Similarly, insect adaptation of toxic glucosinolate breakdown products has been reported. Comparative studies on the feature and dynamics of the recently determined enzymatic mechanisms and the variations in their distribution in phylogenetic family as well as far-off insect species can shed light on these fundamental questions. While sequestration has been confirmed for a small number of specialist insects, the dynamics and the mechanism (transporter enzymes) of the sequestration of various glucosinolates and their effects at higher trophic levels warrant further research efforts. At the end, even though glucosinolates are one of the most studied classes of plant compounds in insect-plant interactions, we are far from understanding their precise role in such interactions. Understanding such interactions is a major challenge that requires the use of molecular, biochemical, and ecological techniques.

References

Agerbirk N, Olsen CE (2012) Glucosinolate structures in evolution. Phytochemistry 77:16–45

Agerbirk N, De Vos M, Kim JH, Jander G (2009) Indole glucosinolate breakdown and its biological effects. Phytochem Rev 8(1):101

Agrawal AA (2000) Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos 89:493–500

Åhman I (1982) A comparison between high and low glucosinolate cultivars of summer oilseed rape (*Brassica napus* L.) with regard to their levels of infestation by the brassica pod midge (*Dasineura brassicae* Winn.). Z Angew Entomol (J Appl Entomol) 94:103–109

Al Janobi AA, Mithen RF, Gasper AV, Shaw PN, Middleton RJ, Ortori CA, Barrett DA (2006) Quantitative measurement of sulforaphane, iberin and their mercapturic acid pathway metabolites in human plasma and urine using liquid chromatography–tandem electrospray ionisation mass spectrometry. J Chromatogr B 844:223–234

Alborn T, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH, Tumlinson JH (1997) An elicitor of plant volatiles from beet armyworm oral secretion. Science 276:945–949

Alborn HT, Hansen TV, Jones TH, Bennett DC, Tumlinson JH, Schmelz EA, Teal PE (2007) Disulfooxy fatty acids from the American bird grasshopper *Schistocerca americana*, elicitors of plant volatiles. Proc Natl Acad Sci 104:12976–12981

Ali JG, Agrawal AA (2012) Specialist versus generalist insect herbivores and plant defense. Trends Plant Sci 17:293–302

Andersson J, Borg-Karlson AK, Wiklund C (2003) Antiaphrodisiacs in pierid butterflies: a theme with variation! J Chem Ecol 29:1489–1499

Andréasson E, Jørgensen LB, Höglund AS, Rask L, Meijer J (2001) Different myrosinase and idioblast distribution in Arabidopsis and *Brassica napus*. Plant Physiol 127:1750–1763

Appel HM, Joern A (1998) Gut physicochemistry of grassland grasshoppers. J Insect Physiol 44:693–700

Arimura GI, Garms S, Maffei M, Bossi S, Schulze B, Leitner M, Mithöfer A, Boland W (2008) Herbivore-induced terpenoid emission in *Medicago truncatula*: concerted action of jasmonate, ethylene and calcium signaling. Planta 227:453–464

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Annu Rev Entomol 47:817–844

Barth C, Jander G (2006) Arabidopsis myrosinases TGG1 and TGG2 have redundant function in glucosinolate breakdown and insect defense. Plant J 46:549–462

Baumann P (2005) Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annu Rev Microbiol 59:155–189

Baumann O, Walz B (2012) The blowfly salivary gland–a model system for analyzing the regulation of plasma membrane V-ATPase. J Insect Physiol 58:450–458

Bede JC, Musser RO, Felton GW, Korth KL (2006) Caterpillar herbivory and salivary enzymes decrease transcript levels of *Medicago truncatula* genes encoding early enzymes in terpenoid biosynthesis. Plant Mol Biol 60:519–531

Behmer ST (2009) Insect herbivore nutrient regulation. Annu Rev Entomol 54:165–187

Beilstein MA, Nagalingum NS, Clements MD, Manchester SR, Mathews S (2010) Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. Proc Natl Acad Sci 107:18724–18728

Beran F, Mewis I, Srinivasan R, Svoboda J, Vial C, Mosimann H, Boland W, Büttner C, Ulrichs C, Hansson BS, Reinecke A (2011) Male *Phyllotreta striolata* (F.) produce an aggregation pheromone: identification of male-specific compounds and interaction with host plant volatiles. J Chem Ecol 37:85–97

Beran F, Pauchet Y, Kunert G, Reichelt M, Wielsch N, Vogel H, Reinecke A, Svatoš A, Mewis I, Schmid D, Ramasamy S (2014) *Phyllotreta striolata* flea beetles use host plant defense compounds to create their own glucosinolate-myrosinase system. Proc Natl Acad Sci 111:7349–7354

Berenbaum M (1980) Adaptive significance of midgut pH in larval Lepidoptera. Am Nat 115:138–146

Bernays EA, Janzen DH (1988) Saturniid and sphingid caterpillars: two ways to eat leaves. Ecology 69:1153–1160

Bernays EA, Minkenberg OP (1997) Insect herbivores: different reasons for being a generalist. Ecology 78:1157–1169

Berner D, Blanckenhorn WU, Körner C (2005) Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. Oikos 111:525–533

Bhatia V, Uniyal PL, Bhattacharya R (2011) Aphid resistance in Brassica crops: challenges, biotechnological progress and emerging possibilities. Biotechnol Adv 29:879–888

Bidart-Bouzat MG, Kliebenstein D (2011) An ecological genomic approach challenging the paradigm of differential plant responses to specialist versus generalist insect herbivores. Oecologia 167:677

Bjorkman M, Klingen I, Birch ANE, Bones AM, Bruce TJA, Johansen TJ, Meadow R, Molmann J, Seljasen R, Smart LE, Stewart D (2011) Phytochemicals of Brassicaceae in plant protection and human health—influences of climate, environment and agronomic practice. Phytochemistry 72:538–556

Blažević I, Montout S, Burčul F, Olsen CE, Burow M, Rollin P, Agerbirk N (2020) Glucosinolate structural diversity, identification, chemical synthesis and metabolism in plants. Phytochemistry 169:112100. https://doi.org/10.1016/j.phytochem.2019.112100

Bonaventure G, Schuck S, Baldwin IT (2011) Revealing complexity and specificity in the activation of lipase-mediated oxylipin biosynthesis: a specific role of the *Nicotiana attenuata* GLA1 lipase in the activation of jasmonic acid biosynthesis in leaves and roots. Plant Cell Environ 34:1507–1520

Borek V, Elberson LR, McCaffrey JP, Morra MJ (1998) Toxicity of isothiocyanates produced by glucosinolates in Brassicaceae species to black vine weevil eggs. J Agric Food Chem 46:5318–5323

Bouchereau A, Clossais-Besnard N, Bensaoud A, Leport L, Renard M (1996) Water stress effects on rapeseed quality. Eur J Agron 5:19–30

Bridges M, Jones AM, Bones AM, Hodgson C, Cole R, Bartlet E, Wallsgrove R, Karapapa VK, Watts N, Rossiter JT (2002) Spatial organization of the glucosinolate–myrosinase system in brassica specialist aphids is similar to that of the host plant. Proc R Soc B 269:187–191

Brune A (2014) Symbiotic digestion of lignocellulose in termite guts. Nat Rev Microbiol 12:168–180

Burmeister WP, Cottaz S, Driguez H, Iori R, Palmieri S, Henrissat B (1997) The crystal structures of *Sinapis alba* myrosinase and a covalent glycosyl–enzyme intermediate provide insights into the substrate recognition and active-site machinery of an S-glycosidase. Structure 5:663–676. https://doi.org/10.1016/s0969-2126(97)00221-9

Burow M, Markert J, Gershenzon J, Wittstock U (2006) Comparative biochemical characterization of nitrile-forming proteins from plants and insects that alter myrosinase-catalysed hydrolysis of glucosinolates. FEBS J 273:2432–2446

Burow M, Bergner A, Gershenzon J, Wittstock U (2007) Glucosinolate hydrolysis in *Lepidium sativum* - identification of the thiocyanate-forming protein. Plant Mol Biol 63:49–61

Burow M, Losansky A, Müller R, Plock A, Kliebenstein DJ, Wittstock U (2009) The genetic basis of constitutive and herbivore-induced ESP-independent nitrile formation in Arabidopsis. Plant Physiol 149:561–574

Cartea ME, Velasco P (2008) Glucosinolates in Brassica foods: bioavailability in food and significance for human health. Phytochem Rev 7:213–229

Chaplin KR, Kliebenstein DJ, Chiem A, Morrill E, Mills NJ, Kremen C (2011) Chemically mediated tritrophic interactions: opposing effects of glucosinolates on a specialist herbivore and its predators. J Appl Ecol 48:880–887

Clossais-Besnard N, Larher F (1991) Physiological role of glucosinolates in *Brassica napus*—concentration and distribution pattern of glucosinolates among plant organs during a complete life cycle. J Sci Food Agr 56:25–38

Cristofoletti PT, Ribeiro AF, Deraison C, Rahbé Y, Terra WR (2003) Midgut adaptation and digestive enzyme distribution in a phloem feeding insect, the pea aphid *Acyrthosiphon pisum*. J Insect Physiol 49:11–24

Dawson GW, Griffiths DC, Pickett JA, Wadhams L, Woodcock CM (1987) Plant-derived synergists of alarm pheromone from turnip aphid, *Lipaphis (Hyadaphis) erysimi* (Homoptera, Aphididae). J Chem Ecol 13:1663–1671

de Torres Zabala M, Grant M, Bones AM, Bennett R, Lim YS, Kissen R, Rossiter JT (2005) Characterisation of recombinant epithiospecifier protein and its over-expression in *Arabidopsis thaliana*. Phytochemistry 66:859–867

de Vos M, Jander G (2009) *Myzus persicae* (green peach aphid) salivary components induce defence responses in *Arabidopsis thaliana*. Plant Cell Environ 32:1548–1560

Despres L, David JP, Gallet C (2007) The evolutionary ecology of insect resistance to plant chemicals. Trends Ecol Evol 22:298–307

Dethier VG (1988) The feeding behavior of a polyphagous caterpillar (*Diacrisia virginica*) in its natural habitat. Can J Zool 66:1280–1288

El Sayed G, Louveaux A, Mavratzotis M, Rollin P, Quinsac A (1996) Effects of glucobrassicin, epiprogoitrin and related breakdown products on locusts feeding: *Schouwia purpurea* and desert locust relationships. Entomol Exp Appl 78:231–236

El-Shora HM, El-Shobaky AM, El-Atrozy MM (2016) Activity of purified bacterial myrosinase and its essential residues. Int J Curr Microbiol Appl Sci 5:567–578

Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5–1

Falk KL, Gershenzon J (2007) The desert locust, *Schistocerca gregaria* detoxifies the glucosinolates of *Schouwia purpurea* by desulfation. J Chem Ecol 33:1542–1555

Fatouros NE, Broekgaarden C, Bukovinszkine'Kiss G, van Loon JJ, Mumm R, Huigens ME, Dicke M, Hilker M (2008) Male-derived butterfly anti-aphrodisiac mediates induced indirect plant defense. Proc Natl Acad Sci 105:10033–10038

Fenwick GR, Heaney RK, Mullin WJ, VanEtten CH (1983) Glucosinolates and their breakdown products in food and food plants. Crit Rev Food Sci Nutr 18:123–201

Font R, Del Rio-Celestion M, Rosa E, Aires A, De Hardo-Bailon A (2005) Glucosinolate assessment in *Brassica oleracea* leaves by near-infrared spectroscopy. J Agric Sci 143:65–73

Foo HL, Grønning LM, Goodenough L, Bones AM, Danielsen BE, Whiting DA, Rossiter JT (2000) Purification and characterisation of epithiospecifier protein from *Brassica napus*: enzymic intramolecular sulphur addition within alkenyl thiohydroximates derived from alkenyl glucosinolate hydrolysis. FEBS Lett 468:243–246

Francis F, Haubruge E, Hastir P, Gaspar C (2001) Effect of aphid host plant on development and reproduction of the third trophic level, the predator Adalia bipunctata (Coleoptera: Coccinellidae). Environ Entomol 30:947–952

Francis F, Lognay G, Wathelet JP, Haubruge E (2002) Characterisation of aphid myrosinase and degradation studies of glucosinolates. Arch Insect Biochem Physiol 50:173–182

Francis F, Vanhaelen N, Haubruge E (2005) Glutathione-s-transferases in the adaptation to plant secondary metabolites in the *Myzus persicae* aphid. Arch Insect Biochem Physiol 58:166–174. Published in Collaboration with the Entomological Society of America

Frédéric, FRANCIS Eric, HAUBRUGE Charles, GASPAR (2000) Influence of host plants on specialist / generalist aphids and on the development of Adalia bipunctata (Coleoptera: Coccinellidae). European Journal of Entomology 97(4) 481–485 https://doi.org/10.14411/eje.2000.074

Freeland WJ, Janzen DH (1974) Strategies in herbivory by mammals: the role of plant secondary compounds. Am Nat 108:269–289

Gabrys B, Tjallingii WF (2002) The role of sinigrin in host plant recognition by aphids during initial plant penetration. Entomol Exp Appl 104:89–93

Gebrehiwot L, Beuselinck PR (2001) Seasonal variations in hydrogen cyanide concentration of three Lotus species. J Agron 93:603–608

Giamoustaris A, Mithen R (1995) The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. *oleifera*) on its interaction with specialist and generalist pests. Ann Appl Biol 126:347–363

Giordanengo P, Brunissen L, Rusterucci C, Vincent C, van Bel A, Dinant S, Girousse C, Faucher M, Bonnemain JL (2010) Compatible plant-aphid interactions: how aphids manipulate plant responses. C R Biol 333:516–523

Gloss AD, Vassao DG, Hailey AL, Nelson Dittrich AC, Schramm K, Reichelt M, Rast TJ, Weichsel A, Cravens MG, Gershenzon J, Montfort WR (2014) Evolution in an ancient detoxification pathway is coupled with a transition to herbivory in the Drosophilidae. Mol Biol Evol 31:2441–2456

Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 57:303–333

Harrison JF (2001) Insect acid-base physiology. Annu Rev Entomol 46:221–250

Hennig K (2013) Plant science meets food science: genetic effects of glucosinolate degradation during food processing in Brassica. PhD dissertation, Wageningen University, The Netherlands

Hopkins RJ, van Dam NM, van Loon JJ (2009) Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annu Rev Entomol 54:57–83

Hoy CW, Head GP, Hall FR (1998) Spatial heterogeneity and insect adaptation to toxins. Annu Rev Entomol 43:571–594

Huss M, Vitavska O, Albertmelcher A, Bockelmann S, Nardmann C, Tabke K, Tiburcy F, Wieczorek H (2011) Vacuolar H+-ATPases: intra-and intermolecular interactions. Eur J Cell Biol 90:688–695

Jensen CR, Mogensen VO, Mortensen G, Fieldsend JK, Milford GFJ, Andersen MN, Thage JH (1996) Seed glucosinolate, oil and protein contents of field-grown rape (*Brassica napus* L.) affected by soil drying and evaporative demand. Field Crop Res 47:93–105

Jeschke V, Gershenzon J, Vassão DG (2016) A mode of action of glucosinolate-derived isothiocyanates: detoxification depletes glutathione and cysteine levels with ramifications on protein metabolism in *Spodoptera littoralis*. Insect Biochem Mol Biol 71:37–48

Jones P, Vogt T (2001) Glycosyltransferases in secondary plant metabolism: tranquilizers and stimulant controllers. Planta 213:164–174

Kassahun K, Davis M, Hu P, Martin B, Baillie T (1997) Biotransformation of the naturally occurring isothiocyanate sulforaphane in the rat: identification of phase I metabolites and glutathione conjugates. Chem Res Toxicol 10:1228–1233

Kawakishi S, Kaneko T (1985) Interaction of oxidized glutathione with allyl isothiocyanate. Phytochemistry 24:715–718

Kawakishi S, Kaneko T (1987) Interaction of proteins with allyl isothiocyanate. J Agric Food Chem 35:85–88

Kazana E, Pope TW, Tibbles L, Bridges M, Pickett JA, Bones AM, Powell G, Rossiter JT (2007) The cabbage aphid: a walking mustard oil bomb. Proc R Soc B 274:2271–2277. https://doi.org/10.1098/rspb.2007.0237

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol 53:299–328

Kikuchi Y, Hosokawa T, Fukatsu T (2011) Specific developmental window for establishment of an insect-microbe gut symbiosis. Appl Environ Microbiol 77:4075–4081

Kim JH, Jander G (2007) *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. Plant J 49:1008–1019

Kim JH, Lee BW, Schroeder FC, Jander G (2008) Identification of indole glucosinolate breakdown products with antifeedant effects on *Myzus persicae* (green peach aphid). Plant J 54:1015–1026

Kim MJ, Chiu YC, Kim NK, Park HM, Lee CH, Juvik JA, Ku KM (2017) Cultivar-specific changes in primary and secondary metabolites in pak choi (*Brassica rapa*, Chinensis group) by methyl jasmonate. Int J Mol Sci 18(5):1004. https://doi.org/10.3390/ijms18051004

Kołodziejski D, Piekarska A, Hanschen FS, Pilipczuk T, Tietz F, Kusznierewicz B, Bartoszek A (2019) Relationship between conversion rate of glucosinolates to isothiocyanates/indoles and genotoxicity of individual parts of Brassica vegetables. Eur Food Res Technol 245(2):383–400. https://doi.org/10.1007/s00217-018-3170-9

Kos M, Houshyani B, Achhami BB, Wietsma R, Gols R, Weldegergis BT, Kabouw P, Bouwmeester HJ, Vet LE, Dicke M, van Loon JJ (2012) Herbivore-mediated effects of glucosinolates on different natural enemies of a specialist aphid. J Chem Ecol 38(1):100–115

Kuchernig JC, Backenköhler A, Lübbecke M, Burow M, Wittstock U (2011) A thiocyanate-forming protein generates multiple products upon allylglucosinolate breakdown in *Thlaspi arvense*. Phytochemistry 72(14–5):1699–1709

Kumar S (2019) Susceptibility of canola and non-canola cultivars of rapeseed-mustard to mustard aphid, *Lipaphis erysimi* (Kaltenbach). In: Souvenir and Abstracts, '4th National Brassica

conference-innovative approaches in oilseed Brassica towards self-sufficiency', 01–03 Feb 2019, CSAUAT, Kanpur, p 70

Kuśnierczyk A, Winge P, Midelfart H, Armbruster WS, Rossiter JT, Bones AM (2007) Transcriptional responses of *Arabidopsis thaliana* ecotypes with different glucosinolate profiles after attack by polyphagous *Myzus persicae* and oligophagous *Brevicoryne brassicae*. J Exp Bot 58(10):2537–2552

Lankau RA (2007) Specialist and generalist herbivores exert opposing selection on a chemical defense. New Phytol 175(1):176–184

LeCoz C, Ducombs G (2006) Plants and plant products. In: Frosch PJ, Menne T, Lepottevin JP (eds) Contact dermatitis, 4th edn. Springer, Berlin/Heidelberg, pp 751–800

Louda S, Mole S (1991) Glucosinolates, chemistry and ecology. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary plant metabolites, vol 1, 2nd edn. Academic, San Diego, pp 123–164

Maffei M, Bossi S, Spiteller D, Mithöfer A, Boland W (2004) Effects of feeding *Spodoptera littoralis* on lima bean leaves. I. Membrane potentials, intracellular calcium variations, oral secretions, and regurgitate components. Plant Physiol 134(4):1752–1762

Maffei ME, Mithöfer A, Arimura GI, Uchtenhagen H, Bossi S, Bertea CM, Cucuzza LS, Novero M, Volpe V, Quadro S, Boland W (2006) Effects of feeding *Spodoptera littoralis* on lima bean leaves. III. Membrane depolarization and involvement of hydrogen peroxide. Plant Physiol 140(3):1022–1035

Matile PH (1980) The mustard oil bomb compartmentation of the myrosinase system. Biochem Physiol Pflanz 175(8–9):722–731

McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U (2013) Animals in a bacterial world, a new imperative for the life sciences. Proc Natl Acad Sci 110(9):3229–3236

Mewis I, Ulrich C, Schnitzler WH (2002) The role of glucosinolates and their hydrolysis products in oviposition and host-plant finding by cabbage webworm, *Hellula undalis*. Entomol Exp Appl 105(2):129–139

Mewis I, Tokuhisa JG, Schultz JC, Appel HM, Ulrichs C, Gershenzon J (2006) Gene expression and glucosinolate accumulation in *Arabidopsis thaliana* in response to generalist and specialist herbivores of different feeding guilds and the role of defense signaling pathways. Phytochemistry 67(22):2450–2462

Miles PW (1999) Aphid saliva. Biol Rev 74:41–85

Milford GFJ, Fieldsend JK, Porter AJR, Rawlinson CJ, Evans EJ, Bilsborrow PE (1989) Changes in glucosinolate concentrations during the vegetative growth of single- and double-low cultivars of winter oilseed rape. Asp Appl Biol 23:83–90

Mithen R (2001) Glucosinolates–biochemistry, genetics and biological activity. J Plant Growth Regul 34(1):91–103

Mithen R, Faulkner K, Magrath R, Rose P, Williamson G, Marquez J (2003) Development of isothiocyanate-enriched broccoli, and its enhanced ability to induce phase 2 detoxification enzymes in mammalian cells. Theor Appl Genet 106(4):727–734

Mithen R, Bennett R, Marquez J (2010) Glucosinolate biochemical diversity and innovation in the Brassicales. Phytochemistry 71(17–18):2074–2086

Mithöfer A, Boland W (2008) Recognition of herbivory-associated molecular patterns. Plant Physiol 146(3):825–831

Mithöfer A, Boland W (2012) Plant defense against herbivores: chemical aspects. Annu Rev Plant Biol 63:431–450

Moran NA, Telang A (1998) Bacteriocyte-associated symbionts of insects. Bioscience 48(4):295–304

Morant AV, Bjarnholt N, Kragh ME, Kjærgaard CH, Jørgensen K, Paquette SM, Piotrowski M, Imberty A, Olsen CE, Møller BL, Bak S (2008) The β-glucosidases responsible for bioactivation of hydroxynitrile glucosides in *Lotus japonicus*. Plant Physiol 147(3):1072–1091

Müller C (2009) Interactions between glucosinolate-and myrosinase-containing plants and the sawfly *Athalia rosae*. Phytochem Rev 8(1):121–134
Müller C, Wittstock U (2005) Uptake and turn-over of glucosinolates sequestered in the sawfly *Athalia rosae*. Insect Biochem Mol Biol 35(10):1189–1198
Müller C, Agerbirk N, Olsen CE, Boevé JL, Schaffner U, Brakefield PM (2001) Sequestration of host plant glucosinolates in the defensive hemolymph of the sawfly *Athalia rosae*. J Chem Ecol 27(12):2505–2516
Müller C, Boevé JL, Brakefield PM (2002) Host plant derived feeding deterrence towards ants in the turnip sawfly *Athalia rosae*. In: Proc 11th international symposium on insect-plant relationships. Springer, Dordrecht, pp 153–157
Müller C, Agerbirk N, Olsen CE (2003) Lack of sequestration of host plant glucosinolates in *Pieris rapae* and *P. grarricae*. Chemoecology 13(1):47–54
Müller R, de Vos M, Sun JY, Sønderby IE, Halkier BA, Wittstock U, Jander G (2010) Differential effects of indole and aliphatic glucosinolates on lepidopteran herbivores. J Chem Ecol 36(8):905–913
Musser RO, Hum-Musser SM, Eichenseer H, Peiffer M, Ervin G, Murphy JB, Felton GW (2002) Caterpillar saliva beats plant defences. Nature 416(6881):599–600
Nielsen JK, Larsen LM, Sørensen H (1979) Host plant selection of the horseradish flea beetle *Phyllotreta armoraciae* (Coleoptera: Chrysomelidae): identification of two flavonol glycosides stimulating feeding in combination with glucosinolates. Entomol Exp Appl 26(1):40–48
Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. Annu Rev Entomol 47(1):57–92
Nottingham SF, Hardie J, Dawson GW, Hick AJ, Pickett JA, Wadhams LJ, Woodcock CM (1991) Behavioral and electrophysiological responses of aphids to host and nonhost plant volatiles. J Chem Ecol 17:1231–1242
Opitz SE, Müller C (2009) Plant chemistry and insect sequestration. Chemoecology 19(3):117
Opitz SE, Jensen SR, Müller C (2010) Sequestration of glucosinolates and iridoid glucosides in sawfly species of the genus *Athalia* and their role in defense against ants. J Chem Ecol 36(2):148–157
Opitz SE, Mix A, Winde IB, Müller C (2011) Desulfation followed by sulfation: metabolism of benzylglucosinolate in *Athalia rosae* (Hymenoptera: Tenthredinidae). Chem BioChem 12(8):1252–1257
Perkins LE, Cribb BW, Brewer PB, Hanan J, Grant M, de Torres M, Zalucki MP (2013) Generalist insects behave in a jasmonate-dependent manner on their host plants, leaving induced areas quickly and staying longer on distant parts. Proc R Soc B 280(1756):20122646. https://doi.org/10.1098/rspb.2012.2646
Pfalz M, Vogel H, Kroymann J (2009) The gene controlling the indole glucosinolate modifier1 quantitative trait locus alters indole glucosinolate structures and aphid resistance in Arabidopsis. Plant Cell 21(3):985–999
Pietri JE, Liang D (2018) The links between insect symbionts and insecticide resistance: causal relationships and physiological tradeoffs. Ann Entomol Soc 111(3):92–97
Poelman EH, Broekgaarden C, Van Loon JJ, Dicke M (2008) Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. Mol Ecol 17(14):3352–3365
Porta H, Rocha-Sosa M (2002) Plant lipoxygenases: physiological and molecular features. Plant Physiol 130(1):15–21
Porter AJR, Morton AM, Kiddle G, Doughty KJ, Wallsgrove RM (1991) Variation in the glucosinolate content of oilseed rape (*Brassica napus* L.). I. Effects of leaf age and position. Ann Appl Biol 118:461–467
Ramsey JS, Wilson AC, de Vos M, Sun Q, Tamborindeguy C, Winfield A, Malloch G, Smith DM, Fenton B, Gray SM, Jander G (2007) Genomic resources for *Myzus persicae*: EST sequencing, SNP identification, and microarray design. BMC Genomics 8(1):423

Ramsey JS, Rider DS, Walsh TK, De Vos M, Gordon KH, Ponnala L, Macmil SL, Roe BA, Jander G (2010) Comparative analysis of detoxification enzymes in *Acyrthosiphon pisum* and *Myzus persicae*. Insect Mol Biol 19:155–164

Rask L, Andreasson E, Ekbom B, Eriksson S, Pontoppidan B, Meijer J (2000) Myrosinase: gene family evolution and herbivore defence in Brassicaceae. Plant Mol Biol 42:93–113

Ratzka A, Vogel H, Kliebenstein DJ, Mitchell-Olds T, Kroymann J (2002) Disarming the mustard oil bomb. Proc Natl Acad Sci 99(17):11223–11228

Renwick JAA (2002) The chemical world of crucivores: lures, treats and traps. Entomol Exp Appl 104:35–42

Renwick JAA, Radke CD, Sachdev-Gupta K, Städler E (1992) Leaf surface chemicals stimulating oviposition by *Pieris rapae* (Lepidoptera: Pieridae) on cabbage. Chemoecology 3:33–38

Renwick JAA, Haribal M, Gouinguenè S, Städler E (2006) Isothiocyanates stimulating oviposition by the diamondback moth, *Plutella xylostella*. J Chem Ecol 32:755–766

Reymond P, Bodenhausen N, Van Poecke RM, Krishnamurthy V, Dicke M, Farmer EE (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell 16(11):3132–3147

Roessingh P, Städler E, Fenwick GR, Lewis JA, Nielsen JK, Hurter J, Ramp T (1992) Oviposition and tarsal chemoreceptors of the cabbage root fly are stimulated by glucosinolates and host plant extracts. Entomol Exp Appl 65(3):267–282

Rungapamestry V, Duncan AJ, Fuller Z, Ratcliffe B (2006) Changes in glucosinolate concentrations, myrosinase activity, and production of metabolites of glucosinolates in cabbage (*Brassica oleracea* var *capitata*) cooked for different durations. J Agric Food Chem 54(20):7628–7634

Sagi M, Fluhr R (2001) Superoxide production by plant homologues of the gp91phox NADPH oxidase. Modulation of activity by calcium and by tobacco mosaic virus infection. Plant Physiol 126(3):1281–1290

Sagi M, Fluhr R (2006) Production of reactive oxygen species by plant NADPH oxidases. Plant Physiol 141(2):336–340

Salem H, Florez L, Gerardo N, Kaltenpoth M (2015) An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. Proc R Soc B 282(1804):20142957. https://doi.org/10.1098/rspb.2014.2957

Schappert PJ, Shore JS (1999) Effects of cyanogenesis polymorphism in *Turnera ulmifolia* on *Euptoieta hegesia* and potential Anolis predators. J Chem Ecol 25(6):1455–1479

Schmelz EA, Engelberth J, Alborn HT, Tumlinson JH, Teal PE (2009) Phytohormone-based activity mapping of insect herbivore-produced elicitors. Proc Natl Acad Sci 106(2):653–657

Schoonhoven LM, Jermy T, Van Loon JJ (1998) Insect-plant biology: from physiology to evolution. Chapman & Hall

Schramm K, Vassão DG, Reichelt M, Gershenzon J, Wittstock U (2012) Metabolism of glucosinolate-derived isothiocyanates to glutathione conjugates in generalist lepidopteran herbivores. Insect Biochem Mol Biol 42(3):174–182

Schultz JC (1983) Habitat selection and foraging tactics of caterpillars in heterogeneous trees. In: Variable plants and herbivores in natural and managed systems. pp 61–90

Schultz JC, Lechowicz MJ (1986) Hostplant, larval age, and feeding behavior influence midgut pH in the gypsy moth (*Lymantria dispar*). Oecologia 71(1):133–137

Shroff R, Vergara F, Muck A, Svatoš A, Gershenzon J (2008) Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. Proc Natl Acad Sci 105(16):6196–6201

Simpson SJ, Raubenheimer D (2001) The geometric analysis of nutrient–allelochemical interactions: a case study using locusts. Ecology 82(2):422–439

Sinclair RJ, Hughes L (2010) Leaf miners: the hidden herbivores. Austral Ecol 35(3):300–313

Smallegange R, van Loon J, Blatt S, Harvey J, Agerbirk N, Dicke M (2007) Flower vs. leaf feeding by *Pieris brassicae*: glucosinolate rich flower tissues are preferred and sustain higher growth rate. J Chem Ecol 33:1831–1844

Sønderby IE, Geu-Flores F, Halkier BA (2010) Biosynthesis of glucosinolates–gene discovery and beyond. Trends Plant Sci 15(5):283–290

Stauber EJ, Kuczka P, Van Ohlen M, Vogt B, Janowitz T, Piotrowski M, Beuerle T, Wittstock U (2012) Turning the 'mustard oil bomb' into a 'cyanide bomb': aromatic glucosinolate metabolism in a specialist insect herbivore. PLoS One 7(4):e35545

Su Q, Zhou X, Zhang Y (2013) Symbiont-mediated functions in insect hosts. Commun Integr Biol 6(3):e23804

Textor S, Gershenzon J (2009) Herbivore induction of the glucosinolate–myrosinase defense system: major trends, biochemical bases and ecological significance. Phytochem Rev 8(1):149–170

Thangstad OP, Gilde B, Chadchawan S, Seem M, Husebye H, Bradley D, Bones AM (2004) Cell specific, cross-species expression of myrosinases in *Brassica napus*, *Arabidopsis thaliana* and *Nicotiana tabacum*. Plant Mol Biol 54(4):597–611

Tjallingii WF, Esch TH (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiol Entomol 18(3):317–328

Traka M, Mithen R (2009) Glucosinolates, isothiocyanates and human health. Phytochem Rev 8(1):269–282

Travers-Martin N, Müller C (2008) Matching plant defence syndromes with performance and preference of a specialist herbivore. Funct Ecol 22(6):1033–1043

Traw BM, Dawson TE (2002) Differential induction of trichomes by three herbivores of black mustard. Oecologia 131(4):526–532

Tripathi MK, Mishra AS (2007) Glucosinolates in animal nutrition: a review. Anim Feed Sci Technol 132:1–27

Turlings TC, Tumlinson JH, Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250(4985):1251–1253

van Dam NM, Hadwich K, Baldwin IT (2000) Induced responses in *Nicotiana attenuata* affect behavior and growth of the specialist herbivore *Manduca sexta*. Oecologia 122(3):371–379

van Loon LC, Bakker PA, Pieterse CM (1998) Systemic induced resistance by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

van Loon JJ, Wang CZ, Nielsen JK, Gols R, Qiu YT (2002) Flavonoids from cabbage are feeding stimulants for diamondback moth larvae additional to glucosinolates: chemoreception and behaviour. In: Proc 11th international symposium on insect-plant relationships. Springer, Dordrecht, pp 27–34

van Ohlen M, Herfurth AM, Kerbstadt H, Wittstock U (2016) Cyanide detoxification in an insect herbivore: molecular identification of β-cyanoalanine synthases from *Pieris rapae*. Insect Biochem Mol Biol 70:99–110

van Poecke RM, Roosjen M, Pumarino L, Dicke M (2003) Attraction of the specialist parasitoid *Cotesia rubecula* to *Arabidopsis thaliana* infested by host or non-host herbivore species. Entomol Exp Appl 107(3):229–236

vanEtten HD, Mansfield JW, Bailey JA, Farmer EE (1994) Two classes of plant antibiotics: phytoalexins versus phytoanticipins. Plant Cell 6(9):1191

Vogel H, Kroymann J, Mitchell-Olds T (2007) Different transcript patterns in response to specialist and generalist herbivores in the wild Arabidopsis relative *Boechera divaricarpa*. PLoS One 2(10):e1081

Wadleigh RW, Simon JY (1988) Detoxification of isothiocyanate allelochemicals by glutathione transferase in three lepidopterous species. J Chem Ecol 14(4):1279–1288

Weihrauch D (2006) Active ammonia absorption in the midgut of the tobacco hornworm *Manduca sexta* L.: transport studies and mRNA expression analysis of a Rhesus-like ammonia transporter. Insect Biochem Mol Biol 36(10):808–821

Welte CU, de Graaf RM, van den Bosch TJ, Op den Camp HJ, van Dam NM, Jetten MS (2016) Plasmids from the gut microbiome of cabbage root fly larvae encode SaxA that catalyses the conversion of the plant toxin 2-phenylethyl isothiocyanate. Environ Microbiol 18(5):1379–1390

Wheat CW, Vogel H, Wittstock U, Braby MF, Underwood D, Mitchell-Olds T (2007) The genetic basis of a plant–insect coevolutionary key innovation. Proc Natl Acad Sci 104(51):20427–20431

Will T, Tjallingii WF, Thönnessen A, van Bel AJE (2007) Molecular sabotage of plant defense by aphid saliva. Proc Natl Acad Sci U S A 104:10536–10541

Will T, Kornemann SR, Furch ACU, Tjallingii WF, van Bel AJE (2009) Aphid watery saliva counteracts sieve-tube occlusion: a universal phenomenon? J Exp Biol 212:3305–3312

Will T, Furch AC, Zimmermann MR (2013) How phloem-feeding insects face the challenge of phloem-located defenses. Front Plant Sci 29(4):336

Williams DJ, Critchley C, Pun S, Nottingham S, O'Hare TJ (2008) Epithiospecifier protein activity in broccoli: the link between terminal alkenyl glucosinolates and sulphoraphane nitrile. Phytochemistry 69(16):2765–2773

Williams DJ, Critchley C, Pun S, Chaliha M, O'Hare TJ (2009) Differing mechanisms of simple nitrile formation on glucosinolate degradation in *Lepidium sativum* and *Nasturtium officinale* seeds. Phytochemistry 70(11–12):1401–1409

Wittstock U, Halkier BA (2002) Glucosinolate research in the Arabidopsis era. Trends Plant Sci 7(6):263–270

Wittstock U, Agerbirk N, Stauber EJ, Olsen CE, Hippler M, Mitchell-Olds T, Gershenzon J, Vogel H (2004) Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proc Natl Acad Sci 101(14):4859–4864

Wittstock U, Meier K, Dörr F, Ravindran BM (2016) NSP-dependent simple nitrile formation dominates upon breakdown of major aliphatic glucosinolates in roots, seeds, and seedlings of *Arabidopsis thaliana* Columbia-0. Front Plant Sci 7:1821

Yu SJ (1987) Biochemical defense capacity in the spined soldier bug (*Podisus maculiventris*) and its lepidopterous prey. Pestic Biochem Physiol 28(2):216–223

Zhang Z, Ober JA, Kliebenstein DJ (2006) The gene controlling the quantitative trait locus EPITHIOSPECIFIER MODIFIER1 alters glucosinolate hydrolysis and insect resistance in Arabidopsis. Plant Cell 18(6):1524–1536

Züst T, Agrawal AA (2016) Mechanisms and evolution of plant resistance to aphids. Nat Plants 2(1):1–9

Chapter 5
Advances in Molecular Techniques of Insect Resistance in Cereal Improvement

S. Aarthi, V. Selvanarayanan, and Amarjit S. Tanda

5.1 Introduction

Given the importance of cereal crops in human diets, food security cannot be achieved without a significant rise in production. In the last few years, molecular genetic manipulation of plants, in addition to breeding programmes, has given numerous beneficial technologies in the genetic enhancement of critical crop species. Globally, biotech crop output grew from 1.7 million hectares to 170 million hectares between 1996 and 2012 (Kilian et al. 2003; Andersen and Lubberstedt 2003). The desired crop yield can be accomplished in a variety of ways, not just through conventional breeding (Vasil 1998). It is predicted that the production of cereal grains must be doubled by the year 2025, and almost tripled by 2050, in order to satisfy the food needs of the twenty-first century. Like other crops, cereal crops also suffer due to ravages of insect pests, which are considered as an important limiting factor in their production. According to the Food and Agriculture Organization of the United Nations (FAO), losses inflicted by insect pests globally in cereal crops are estimated at 19–30%. In view of the burgeoning population in the world, increasing crop productivity became a dire necessity. Since prehistoric times, cereal grains have been considered the resource of human nutrition. Innovative breeding and selection methods, such as those used in the production of hybrid maize and green revolution wheat and rice varieties, have greatly improved quality and productivity, with an average grain yield increase of 2.1% per year over the past three decades. In certain nations, however, there is a higher need for cereal grains due to population

S. Aarthi · V. Selvanarayanan (✉)
Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

A. S. Tanda
School of Horticulture, University of Western Sydney, Richmond, NSW, Australia

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_5

growth, economic development and changing dietary patterns. According to estimates, food consumption will double by 2025 and treble by 2050 (Vasil 1998).

Plant breeding has resulted in massive increases in cereal crop production over the last several decades, leading to the development of hybrid maize, wheat and rice cultivars (Borlaug and Doswell 2001). But increased pestilence was witnessed in the high-yielding crop varieties. In India, pest-related losses in cereal crops have increased by 15.9% during the green revolution compared to losses prior to the revolution. Over 200 pests attack various grains, and insects that were previously unknown or of little consequence have suddenly become important pests, as well as numerous new pests. According to Oerke et al. (1994), the overall crop losses caused by all pest groups amount to 51.4% in rice owing to the biotype development of several important insects such as BPH and gall midge.

As a result of recent advancements in tissue culture and molecular biology (biotechnology), modified crops that are resistant to a range of biotic and abiotic stressors are now possible. In breeding of cereal crops, molecular technology and genetic engineering paved the way for adopting an extensive range of new approaches for improving the efficiency of selection strategies. By means of molecular marker-based genetic analysis of BPH resistance, resistant gene from the wild rice *Oryza officinalis* was found promising. To date, 29 major BPH resistance genes were recognised from indica cultivars and wild rice species. In maize, nine quantitative trait loci (QTL) were identified for leaf-feeding insects and seven for stalk tunneling insects such as the European corn borer and south-western corn borer. In wheat, seven QTL were detected against the orange blossom midge. The single-nucleotide polymorphism (SNP) is closely linked with QTL, which is used for marker-assisted selection. Two major QTLs on chromosomes 1H and 3H and existence of an additional QTL on chromosome 2H in barley germplasm revealed resistance to the Russian wheat aphid. So, effectiveness of marker-assisted selection for key insects can be increased significantly when progressing from a QTL-based approach towards a genome-wide approach. Genome editing using the CRISPR/Cas9 system has been effectively demonstrated in several crops, including rice, wheat, maize and barley. The two eye pigmentation genes in *Nilaparvata lugens* Stal are knocked out by CRISPR/Cas9, a valuable tool for the study of functional genomics and pest management in this planthopper species. For developing new sources of resistance to rice tungro disease, mutations in eIF4G were generated using the CRISPR/Cas9 system. Cereals, like other plant species, benefit from tissue culture and molecular methods that allow for the introduction, integration and expression of genes of interest in cells that may be regenerated into normal and fruitful plants. This chapter provides detailed information about the molecular tools and techniques of insect resistance in cereal crops**.**

5.2 Molecular Techniques in Exploring Insect Resistance in Cereals

In traditional breeding, genotype selection is based on visual and phenotypic selection, which is often effective for qualitative qualities but ineffective for quantitative traits. It is owing to the fact that many genes and abiotic variables regulate quantitative characteristics with continuous changes (Makkar and Bentur 2017). Modern resistant breeding programmes attempt to create new rice varieties by combining traditional breeding methods with molecular approaches, thanks to significant advances in biotechnology. Conventional breeding takes five to six generations to transfer a characteristic within a species into high-yielding, regionally suited cultivars, and this approach necessitates evaluating a more number of progenies to choose the plants with the best combination of features. The most challenging characteristics to deal with in the field using direct phenotypic selection are quantitative traits with low heredity; nevertheless, the use of DNA markers for indirect selection has a lot of promise (Huang et al. 2014a, b, c). Rice is the oldest agricultural species whose genome has been completely sequenced (International Rice Genome Sequencing Project 2005).

The area of plant resistance to arthropods has been transformed into a new age in the last 30 years, with the introduction and application of molecular tools, providing enormous potential for ongoing creation of the newest crop cultivars with genes for robust arthropod resistance (Smith 2005; Ni et al. 2010). The structural and functional genomics analysis of cereal genomes, which has spanned both basic and applied aspects over the last two decades, has deepened our understanding of gene networks for cereal development, from existing genomic, molecular maps, and expressed sequence tag (EST) sequences to gene product interactions and QTL information.

5.2.1 QTL (Quantitative Trait Loci)

Quantitative trait loci (QTL) genetic mapping is an exceptionally capable molecular technique for working with quantitative characteristics (Smith 2005; Yencho et al. 2000). QTL mapping aids in the improvement of molecular markers and the marker-assisted introgression of resistance characteristics into commercially important crop cultivars (Varshney et al. 2005; Bergelson and Roux 2010). The most effective approach for identifying and categorising new insect-resistant genes is QTL mapping. The development of powerful molecular genetic techniques has allowed genome-wide association studies to examine the genomic variation that underpins insect resistance variation (Madhusudhana 2015; Chan et al. 2010; Kump et al. 2011). Since Yencho et al. (2000) included QTL in six crop genera for resistance to ten arthropod species from the orders Coleoptera, Hemiptera and Lepidoptera, numerous arthropod crop resistances QTL have been identified. Entomologists,

breeders and molecular biologists utilise DNA markers to build genetic linkage maps of resistance genes in the wheat crop (to *Mayetiola destructor, Diuraphis noxia and Schizaphis graminum*).

Marker-assisted selection (MAS) and genetic engineering breeding (GEB) are the two main methods used in molecular resistance breeding (Rao et al. 2014). QTLs are found in a range of rice chromosomes through crossings between susceptible and resistant types using a variety of mapping populations. qBPH6 (t) was found between two markers RM568 and RM469 on chromosome 6 of rice line IR71033–121-15 (Jairin et al. 2007). qBPH3 was identified and mapped between markers t6 and f3 on chromosome 3 in rice line IR02W101 (Hu et al. 2015). Several QTLs, including qBPH4, qBPH4.2, qBPH4.3 and qBPH4.4 from IR02W101, IR65482-17-511 and Salkathi, have been discovered on chromosome 4 (Hu et al. 2015). Distinct germplasm sources contribute different gene imparting resistance, as shown in the study by Krakowsky et al. (2002), where De811 and B52 include completely different genomic areas for European corn borer resistance (ECB). Maize is the first crop for which a complete molecular map has been created (Helentjaris et al. 1986).

Bohn et al. (2000) identified QTLs in early developing European dent germplasm produced from a hybrid of D06 (resistant) and D408 (sensitive) ECB resistance in F3 families. Five QTLs were discovered for stalk damage resistance, accounting for 50% of the genotypic difference, and six for tunnel length. The relentless research led to the discovery of nine QTLs for leaf-feeding resistance in the first-generation ECB on chromosomes 1, 2, 4, 5, 6 and 8, as well as seven QTLs for stalk tunnelling resistance in the second-generation ECB on chromosomes 2, 5, 6, 8 and 9 (Jampatong et al. 2002; Sharopova et al. 2001). Krakowsky et al. (2004) used 191 RILs (recombinant inbred lines) of maize population generated from B73 (sensitive) and De8 (resistant) to find 10 QTLs imparting resistance to stalk tunnelling by the ECB, which explained 42% of the phenotypic variance. QTLs for leaf-feeding resistance to the south-western corn borer (SWCB) and fall armyworm (FAW) were mapped by Brooks et al. (2007). They discovered that the QTL on chromosomes 6, 7 and 9 was dependable for insect resistance.

QTLs determining resistance to the Mediterranean corn borer are mapped using IBM (maize inbred lines) population generated from a cross B73 Mo17 (Ordas et al. 2009) and RIL (recombinant inbred lines) population (Ordas et al. 2010). Two recombinant inbred line (RIL) populations were developed and utilised for molecular mapping in detection of blossom midge resistance genes and QTL in wheat breeding. Apart from the QTL on 7D, seven were found on chromosomes 2D, 4A, 4D and 7D, all of which had positive alleles from resistant parents (Zhang et al. 2020). The single-nucleotide polymorphism (SNP) markers AX-109543456, AX-108942696 and AX-110928325 were found to be strongly related to the QTL for orange wheat blossom midge resistance and will be utilised in MAS (Joukhadar et al. 2013). Qss.msub-3BL, QTL on chromosome 3B, has been found to control the majority of stem solidness variance in crossings between solid- and hollow-stem genotypes (Cook et al. 2004).

Cook et al. (2017) discovered that the rescue derived solid-stem haplotype at the Qss.msub-3BL gene was prevalent in contemporary wheat stem sawfly-resistant cultivars using single-nucleotide polymorphisms (SNPs). SNP markers linked to sunn pest resistance have been linked to the Rht-B1 gene, according to Zanke et al. (2014). The Hessian fly (HF), Russian wheat aphid (RWA), *Eurygaster integriceps* sunn pest (SP), wheat stem sawfly (WSSF) and cereal Chrysomelidae (CLB) have 26 loci across the wheat genome coupled to genes conferring resistance to those pests, 20 of which are potential QTL with significance values ranging from 5×10^{-3} and 10^{-11} (Joukhadar et al. 2013). Simplified composite interval mapping identified a QTL on chromosome 5 for cereal aphids in barley and two QTLs on chromosomes 2 and 5 for the Russian wheat aphid (*Diuraphis noxia*) (Nieto-Lopez and Blake 1994). Multiple genes are involved in the control of Russian wheat aphid resistance in barley (Mornhinweg et al. 2017). In wheat, five QTL and eleven Dn (*Diuraphis noxia*) resistance genes imparting antixenosis, antibiosis and tolerance have been identified (Burd et al. 2006, Ricciardi et al. 2010).With the currently existing QDn. unlp-2H and QDn.unlp-1H genes in barley that impact the aphid cycle, these two novel genes imparting aphid tolerance will result in gene pyramiding to increase the genetic foundation of protection against this aphid pest (Tocho et al. 2013). The major QTL on barley chromosome 3H generated from F1 of the cross Lina × 5172-28:4, which exhibited segregation for resistance to avian cherry-oat aphid, was mapped using a double haploid population (Cheung et al. 2010). Combining this QTL on chromosome 3H with another significant QTL on chromosome 2H resulted in a more long-lasting and high degree of resistance. Moharramipour et al. (1997) discovered an important QTL on chromosome 1 that conferred field resistance to a varied population of maize leaf aphids (*Rhopalosiphum maidis* Fitch) and avian cherry-oat aphids as dominating species with minimal presence of *Sitobian graminum* and *Sitobion akebiae* (7H).

5.2.2 Molecular Markers and Its Applications

Molecular markers and its applications play a major role in the development of genetics research and technology. Now, comprehensive molecular genetics maps are also available for the most important cereal species (Varshney et al. 2004). There is, however, a desire to include more markers in the genetic maps of rye, oats and millet species. Furthermore, among the different types of molecular markers, simple sequence repeat (SSR or microsatellite) markers have proven to be the best choice for a variety of applications, particularly breeding (Gupta and Varshney 2000).

SNPs, or single-nucleotide polymorphisms, are a type of molecular marker that is more abundant in the genome and may be automated for high-throughput genotyping. Furthermore, diversity array technology (DArT) markers are a high-throughput marker system that may be utilised to generate a full genome map without the need for sequence information (as is the case with SNP markers) for the crop (Kilian et al. 2003). Such genome-wide maps are going to be helpful for

establishing marker-trait associations, not solely through linkage analysis however conjointly through association mapping. The accessibility of sequence information for genes has enabled the development of molecular markers from the transcribed region of the genome, which are generally referred to as 'genic' or 'functional' markers (FMs), because a putative function can usually be deduced for such markers, thanks to genome- and EST-sequencing projects (Andersen and Lubberstedt 2003). FMs are a chief resource for measuring practical variation in wild or breeding populations and for discovering genome evolution through comparative mapping, in addition to being beneficial for identifying the 'perfect' or 'ideal' markers in marker-assisted selection.

5.2.3 Marker-Assisted Selection (MAS)

Marker-assisted selection (MAS) might be a powerful tool for the indirect selection of complex characteristics at an early stage before the generation's production, speeding up traditional plant breeding and enabling the creation of features that can't be enhanced simply by conventional techniques (Ribaut and Hoisington 1998). Because of the effectiveness of MAS, a large number of genes and QTLs that confer tolerance to each abiotic and biotic stress in wheat have been identified and tagged (Jahoor et al. 2004). Though the potential benefits of MAS are significant, the implementation of this approach in crop breeding programmes (Tuberosa and Salvi 2004) has been slow.

In addition, numerous programmes and initiatives are underway to perform MAS in breeding, such as molecular breeding programmes in wheat and barley in Australia (Langridge 2005) and 'MASWheat' (http://maswheat.ucdavis.edu/index.htm). Characterising markers associated with various pest and disease resistance genes in rice is done using a combination of molecular marker methods and bulk segregant analysis (Blair and McCouch 1997). Selvi et al. (2002) looked at the feasibility of using microsatellite markers to tag genes for yellow stem borer resistance. A cross between the moderately resistant variety W1263 and the extremely sensitive variety Co43 yielded an F2 mapping population. A higher number of genes/QTLs controlling pest resistance have been identified using molecular markers (Brar and Khush 2013). Rice cultivars that are resistant to BPH are developed by combining (or 'pyramided') the resistance genes using both MAS and traditional breeding methods. Since the advancement of molecular markers (such as SSR, InDel and SNPs) and functional genomics, the number of resistance genes has increased, and a few have been cloned. The better cultivars, on the other hand, have single resistance genes that are losing effectiveness due to the development of new biotypes (Jena and Kim 2010).

In rice, pyramiding of resistance genes or QTLs has proven to be an efficient strategy for generating disease and pest-resistant lines (Singh et al. 2015). Furthermore, molecular markers were used to map four WBPH resistance genes in Sinna Sivappu, which were designated as wbph 9(t), wbph 10(t), wbph 11(t) and

WBPH12(t) (Ramesh et al. 2014). Many sources of gall midge resistance have been identified, as well as the 11 genes linked to Asian rice gall midge biotype resistance; all except GM9 and GM10 have been mapped (Yasala et al. 2012). Gall midge resistance is managed by a minimum of ten resistance genes, of which eight have been tagged and mapped (Kumar et al. 2005). In a variety of rice varieties, flanking markers are used to identify the resistance genes Gm1 and Gm 2. (Himabindu et al. 2010). Six of the genes (Bph1, bph2, Bph14, Bph15, Bph18 and bph19) have been mapped in advanced genetic research (Zhang 2007), and the genes Bph1, bph2 and Bph18 are utilised for MAS of BPH resistance in temperate japonica and tropical indica rice cultivars (Jena et al. 2006). Grh1, Grh2, Grh3, Grh4, Grh5 and Grh6 are rice green leafhopper resistance genes that are found on chromosomes 5, 11, 6, 3, 8 and 4 (Fujita et al. 2006). SSR markers are connected to these resistance genes. Willcox et al. (2002) combined a marker-assisted backcross breeding with QTL mapping for leaf-feeding resistance to the first-generation SWCB. On chromosomes 7, 9 and 10, three putative QTLs associated with leaf-feeding resistance to the first-generation SWCB were discovered, accounting for 28% of the overall phenotypic variance.

Flint-Garcia et al. (2005) and Samayoa et al. (2015) similarly came to the conclusion that MAS may be used to introduce resistance attributes without sacrificing yield. Using SSRs and restriction fragment length polymorphism molecular markers, QTLs for maize weevil resistance were discovered in an F2 population (García-Lara et al. 2009). As a first step towards marker validation, certain markers are evaluated across a range of cultivars (e.g. Dweikat et al. 1997 for Hessian fly resistance genes; Ogbonnaya et al. 2001 for Cereal cyst nematode Cre1 and Cre3). Using 75 RAPD primers, Malik et al. (2013) investigated four barley genotypes during bulk segregant analysis for corn leaf aphid resistance research. OPAC-01, a RAPD primer, was discovered to be a closely related marker for corn leaf aphid resistance in barley.

5.2.4 Transgenics

Toxins from the soil bacterium *Bacillus thuringiensis* are now the most frequently used insecticidal toxins produced in commercial transgenic plants (Gatehouse 2008). Over the last 30 years, transgenes from *B. thuringiensis* (Bt) that determine insecticidal crystalline proteins and alternative genes for proteins that cause toxicity or inhibit arthropod growth (proteinase inhibitors, amylase inhibitors, lectins, chitinases) have been successfully expressed within the genomes of many crop plants. Cry1Ab and Cry1F are the two distinct Bt delta-endotoxins expressed in registered Bt PIPs for lepidopteran management in corn.

In contrast to the bacterial isolates from which they were generated, each of those corn hybrids produces somewhat shortened forms of either the Cry1Ab delta-endotoxin or the Cry1F delta-endotoxin (USEPA 2001). Non-plant transgenes expressing various Bt toxins are introduced into plants in a minimum of ten genes

(Schuler et al. 1998). Bt cotton, maize and rice cultivars that are categorically resistant to a wide range of lepidopteran pests are now commonplace in agriculture. Each Bt and non-Bt transgenic plant is commonly referred to as an insecticidal plant and has a high level of antibiosis in terms of plant resistance.

Insect virulence (the ability to overcome Bt) to Bt toxins is well-documented within the laboratory (Tabashnik et al. 2003). In the field, the longevity of Bt transgenes with success extended through the utilisation of insect resistance management programmes focused on nontransgenic refuge areas that allow survival of individual arthropods homozygous for susceptibility to the Bt toxin (Gassmann et al. 2009). Bt cotton and maize production have substantial economic profit to producers of non-Bt crops (Hutchison et al. 2010; Wu et al. 2008).

Field assessments of transgenic rice expressing Bt genes, primarily cry1A, have been published since 2000 (Tu et al. 2000; Ye et al. 2009). Chewing insects, such as Lepidoptera, can be controlled by transgenic rice expressing Bt toxins. Bt transgenic rice, on the other hand, showed no enhanced resistance to sap-sucking insects (Gatehouse 2008). Cry1Ab-based maize hybrids have been widely used to combat the ECB (Koziel et al. 1993) and Cry3Bb against root cutworms. The stem borer larvae and Egyptian cotton leafworm (*Spodoptera littoralis*) larvae were resistant to transgenic maize and rice plants expressing the fusion protein, but plants expressing the unaltered Cry1Ac were vulnerable to both insects (Mehlo et al. 2005; Gatehouse 2008). A transgenic maize hybrid containing six insect resistance genes active against corn rootworm and lepidopterous pests (for rootworm, cry34Ab1 + cry35Ab1, modified cry3Bb1; for lepidoptera, cry1F, cry1A.105, cry2Ab2), provide a long-lasting solution to both (Gatehouse 2008).

Transgenic rice and maize plants containing the coding sequences for the endotoxin Cry1Ac and therefore the galactose-binding domain of the nontoxic ricin B chains were resistant to stem borer (*Chilo suppressalis*) and leaf armyworm (*S. littoralis*) larvae (Mehlo et al. 2005). Several Bt insecticidal proteins have been identified, demonstrating their efficacy against a variety of pests. Cry34/Cry35, a single-chain vegetative insecticidal protein, and Vip3, a single-chain vegetative insecticidal protein, are known to be active against lepidopterous insect larvae and rootworms (coleopteran), respectively, having a wider range of toxicity than previous Cry proteins (Moellenbeck et al. 2001; Fang et al. 2007). Transgenic wheat containing two insecticidal genes, cryIA(c) and pta, in a single transformation event offers a promising insect control potential (Yu and Wei 2008). From the outcome of biotin sequestration, avidin's insecticidal action arise (Morgan et al. 1993). The avidin-engineered maize plants produced more than 2.0% more avidin of total protein in seed and shown great resistance to the red flour beetle, *Tribolium castaneum*, and other coleopteran pests (Kramer et al. 2000). Transgenics that produce dsRNA against the V-type ATPase of the corn rootworm suppressed mRNA within the insect and reduced damage when compared to controls in maize (Baum et al. 2007).

5.2.5 Host-Induced Gene Silencing (HIGS)

Small interfering RNA (siRNA) protects insect pests in transgenic plants including wheat, barley and other minor grains. For wheat grain aphid RNAi, four targets were selected from a total of 66 unigenes (Wang et al. 2015). Heat-shock protein 90 (HSP90) has been proven to be an excellent target in several investigations (Will and Vilcinskas 2013). Insect hatching was stopped by silencing the chitin synthase gene, as well as knocking out the segmentation gene, which prohibited insects from eating (Aranda et al. 2000).

Silencing of key genes, including ecdysone receptor (EcR) and ultraspiracle protein (USP), in grain aphids (*Sitobion avenae* F.) via the trans-generational approach decreases the existence and egg-laying capacity; also, silencing of the matrix metalloproteinase MMP-2 gene in gut development has positive outcomes (Yan et al. 2016). Injection in grain aphids causes catalase, acetylcholinesterase1, cytochrome c oxidase, salivary protein DSR33, serine protease 1 DSR48 and olfactory co-receptor genes to be knocked down, confirming a variety of RNAi targets (Qi et al. 2019). Individual genes can not only be targeted by RNAi, but their promoters are ideal components for regulating gene expression and may thus be employed in the RNAi approach. The feeding and dissemination of aphids were greatly decreased by transgenic production of siRNA of the structural sheath protein (SHP), which is an important component of the leaf sheath in barley (Carolan et al. 2010). HIGS is becoming an environmentally benign and suitable commercial solution for managing insect pests of crop plants.

5.2.6 Genes and Protein's Role in Insect Resistance

The most important development in plant biotechnology is the advancement of genetic transformation techniques based on recombinant DNA technology to convert genes from unrelated sources into economically significant crop plants to enhance insect pest resistance (Dhaliwal and Uchimiya 1999). Various proteins with insecticidal activity have been used to generate intrinsic pest resistance in genetically modified plants. Protease inhibitors, plant lectins, ribosome-inactivating proteins, secondary plant metabolites, Bt and related species' vegetative insecticidal proteins (Vip) and small RNA viruses can all be combined with Bt genes to create transgenic plants for pest management (Hilder and Boulter 1999).

For transgenic rice with enhanced stem borer resistance, the cowpea trypsin inhibitor (CpTi) transgene is used (Brar and Khush 2018). In transgenic rice, the expression of the mannose-specific lectin gene has been used to defend against a variety of homopterans, coleopterans and lepidopterous insects (Wu et al. 2002; Nagadhara et al. 2003). Chaitanya, a well-known rice variety that has been modified with the snowdrop (*Galanthus nivalis*) lectin gene, GNA (*Galanthus nivalis* agglutinin), has given resistance to hopper pests such as the brown plant hopper (BPH),

white-backed plant hopper (WBPH) and green leaf hopper (GLH) (Nagadhara et al. 2003).

For sorghum plants with the cry1Ac gene for resistance to *C. partellus*, the spotted stem borer A wound-inducible promoter from the maize protease inhibitor gene (mpi) is in charge (Seetharama et al. 2003; Harshavardhan et al. 2002; Girijashankar 2005). Transgenic technology can pyramid resistance genes to multiply the effectiveness of helpful genes in sorghum crops with multiple insect pest resistances, and biotechnology can modify metabolic pathways to increase the amounts of different flavonoids that play a significant role in insect pest resistance (Zhuang et al. 2011). In response to mechanical wounding and insect damage, several enzyme-like proteinase inhibitor genes are expressed. Cordero et al. (1994) described both the local and systemic activation of MPI, a maize protease inhibitor gene. Zhu-Salzman et al. (2004) compared expression patterns of 672 cDNAs within seedling tissues before and after green bug infestation to assess transcriptional alterations in a sorghum variety.

Proteinase inhibitor (Pi) proteins, which are small proteins with a molecular weight of 4–25 kDa, interfere with insect digestion. Jongsma et al. (1994) demonstrated that a low amount of trypsin inhibitor expression (0.16% of total protein) in transgenic plants alters the makeup of digestive enzymes in insect guts, resulting in inhibitor resistance in some insects. In comparison to untransformed management, the survival rate of Angoumois grain moths (*Sitotroga cerealella*) grown on transgenic wheat seeds expressing the trypsin inhibitor BTI-CMe decreased. According to Hoffmann et al. (1992), a lepidopteran pest's early embryonic stage was the most vulnerable to death after feeding on transgenic tobacco expressing a trypsin inhibitor. Because 1RS carries resistance genes to disease-causing organisms, translocations involving the short arm of chromosome 1 of rye, 1RS, have a significant impact on wheat variety performance (Marais et al. 1994). The genomes of wheat and rye are believed to contain 75% repetitive DNA (Rimpau et al. 1978). Repeated DNA peculiar to rye appears to exist as a result of sequences inside repetitive areas that have rapidly diverged from those of wheat during evolution. The only sources of resistance found so far for generating resistant barley cultivars are two single dominant green bug resistance genes, Rsg1a (in 'Post 90') and Rsg2b (in PI 426756), which show comparable phenotypes when challenged by a variety of green insect biotypes (Porter et al. 2007).

5.2.7 Genome Editing: CRISPR/CAS9

CRISPR/Cas9, which consists of a guide RNA (small RNA fragment) linked to a DNA endonuclease known as Cas9, is regarded as the most valuable and easiest genome editing technology (Weeks et al. 2016). The structure of gRNA (guide RNA) is made up of two parts: CRISPR-derived RNA (crRNA) and trans-activating RNA (tracrRNA). Because of its acceptance, economic effectiveness, shorter time

required and enhanced with targeted targeting, the CRISPR/Cas9 technique has been successful in cereal crops (Xu et al. 2016).

By targeting the viral genome or the host genome, the CRISPR/Cas9 technology has been effectively used to develop virus resistance in plants. CRISPR/Cas9 was used to create mutations in eIF4G in the *Rice tungro spherical virus*-susceptible variety IR64, which is commonly grown in tropical Asia, in order to generate a novel source of resistance to rice tungro disease. The mutation rates varied from 36.0 to 86.6%, depending on the target location, and the alterations were effectively passed down to the following generations (Zaidi et al. 2016). Site-specific nucleases (SSNs) are commonly employed in genome editing to change the target sites of genes. It was also utilised to make germ line mutations in two eye pigmentation genes in *N. lugens*, resulting in mosaic eyes in all individuals with white and faintly coloured ommatidia. PCR and RNA interference-based knock-down studies backed it up even further (Xue et al. 2018). CRISPR/Cas9-mediated gene editing is most feasible in hemipteran insects, making it a useful tool for functional genomics and pest control research.

5.2.8 Importance of Biolistic Transformation

Microprojectile bombardment, also known as biolistics, is a more contemporary and flexible technique for grain transformation. Even if the transformed cells are regenerated, competent cells that show genetic variants are still required for this technique. It entails firing microparticles with the necessary DNA into cells. The DNA is extracted from the particles using an unknown process, and it finally inserts itself into the cell's (typically nuclear) genome. *Agrobacterium tumefaciens*, a naturally occurring bacterium, is the most common contemporary cereal transformation method.

Cereals were long thought to be non-hosts of the *Agrobacterium* genus because they were not infected in vivo or in vitro. The genus *Agrobacterium* attaches to cereal cells and produces substances that trigger *Agrobacterium* virulence genes, causing the bacteria to move their T-DNA within the cell (Tinland et al. 1995). Microprojectile bombardment of the cultivar Tarom molaii resulted in the expression of an engineered cry1Ab gene for stem borer resistance in rice (Ghareyazie et al. 1997). Using biolistics, Alam et al. (1998) inserted an artificial cry1Ab gene into embryogenic calli of Vaidehi, a deepwater Indica rice variety. Because of the first report of lowland rice bred with the Bt gene, this is frequently asserted. Microprojectile bombardment and protoplast systems have also been used to introduce the shortened cry1Ab gene into various rice cultivars (Indica and Japonica) (Datta et al. 1998). Ho et al. (2006) demonstrated resistance to yellow stem borer in rice based on the efficacies of a hybrid Bt gene (cry1A/cry1Ac) regulated by the rice actin-1 promoter and obtained by biolistic transformation. Using the particle bombardment technique, Rahman et al. (2007) assessed s16 totally different transgenic lines expressing 1 (cry1Ac or cry2A), 1 (cry1Ac + cry2A) and 3 genes

(cry1Ac + cry2A+ gna) that exhibited the greatest degree of resistance against lepidopteran insects.

5.3 Integration of Insect Resistance Genes into IPMT Programs

Hundreds of insect resistance genes are deployed in improved cultivars across the world (Smith 2005), but cultivars are coupled with various ways to successfully implement IPMT programmes at the farm level (Stout 2007). Plant resistance, along with biological management and pesticides, is one of the many management strategies used in Asia's very successful rice IPM programmes (Franzmann et al. 2008). In Asian rice varieties, single *N. lugens* resistance genes, gene pyramids and seasonal rotations have all been successful (Alam and Cohen 1998).

Commercial hybrids with antibiosis resistance to *Stenodiplosis sorghicola* have been successfully coupled with diverse management approaches, such as variable planting dates, synthetic pesticides and biological management in Australian sorghum production (Franzmann et al. 2008).

5.4 Conclusion

In agricultural research, the joint efforts of entomologists, plant breeders and molecular biologists to develop insect-resistant cultivars during the last several years have been critical. They use the genetic diversity of wild and closely related species of world crop germplasm to identify genes that exhibit resistance to the major herbivory. Insect resistance has drastically reduced insecticides use, resulting in a better environment and the ability to keep global food supply afloat. Insect resistance can be developed by means of any molecular tools, and it must be monitored for presence of capable genes in newly developing resistant breaking biotypes. It is better to use the best molecular tools such as markers to monitor the biotypes. For diverse insect pests, germplasm collections from all over the world must be examined on a regular basis. The molecular genetic knowledge acquired from these initiatives, as well as the application of new DNA technologies (Kopp et al. 1998), may hasten substantial advances in plant resistance—molecular genetics research. Due to the rising global population and need for food, it is imperative to expand the productivity and production of cereal crops in order to meet future demands. Because of their proven environmental benefits, insect resistant crops must continue to be important components of food supply. The combination of improved germplasm collection, curation and maintenance and rapidly emerging new molecular genetic technologies will open up numerous opportunities for interdisciplinary research efforts to

identify and develop new sources of insect resistance, encouraging us to develop enough to meet our future needs globally.

References

Alam SN, Cohen MB (1998) Detection and analysis of QTLs for resistance to the brown planthopper, Nilaparvata lugens, in a double-haploid rice population. Theor Appl Genet 97:1370–1379

Alam MF, Datta KE, Abrigo A, Vasquez D, Senadhira, Datta SK (1998) Production of transgenic deepwater indica rice plants expressing a synthetic *Bacillus thuringiensis* cryIA (b) gene with enhanced resistance to yellow stem borer. Plant Sci 135(1):25–30

Andersen JR, Lubberstedt T (2003) Functional markers in plants. Trends Plant Sci 8:554–560

Aranda M, Marquessouza H, Bayer T, Tautz D (2000) The role of the segmentation gene hairy in Tribolium. Dev Genes Evol 218:465–477

Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T (2007) Control of coleopteran insect pests through RNA interference. Nat Biotechnol 25(11):1322–1326

Bergelson J, Roux F (2010) Towards identifying genes underlying ecologically relevant traits in Arabidopsis Thaliana. Nat Rev Genet 11:867–879

Blair MW, McCouch SR (1997) Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial leaf blight resistance gene xa-5. Theor Appl Genet 85:174–184

Bohn M, Schulz B, Kreps R (2000) QTL mapping for resistance against the European corn borer (*Ostrinia nubilalis* H.) in early maturing European dent germplasm. Theor Appl Genet 101:907–917

Borlaug NE, Doswell C (2001) The unfinished green revolution—the future role of science and technology in feeding the developing world. Paper presented at seeds of opportunity conference at School of Oriental and African Studies, London, UK, May 31–June 1 2001

Brar DS, Khush GS (2013) Biotechnological approaches for increasing productivity and sustainability of rice production. Academic, San Diego, pp 151–175

Brar DS, Khush GS (2018) Wild relatives of rice: a valuable genetic resource for genomics and breeding research. In: The wild Oryza genomes. pp 1–25

Brooks TD, Shaun Bushman B, Paul Williams W, McMullen MD, Buckley PM (2007) Genetic basis of resistance to fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) leaf-feeding damage in maize. J Econ Entomol 100(4):1470–1475

Burd JD, Porter DR, Puterka GJ, Haleyand SD, Peairs FB (2006) Biotypic variation among north American Russian wheat aphid (Homoptera: Aphididae) populations. J Econ Entomol 99(5):1862–1866

Carolan JC, Fitzroy CIJ, Ashton PD, Douglas AE, Wilkinson TL (2010) The secreted salivary proteome of the pea aphid *Acyrthosiphon pisum* characterised by mass spectrometry. Proteomics 9:2457–2467

Chan EKF, Rowe HC, Kliebenstein DJ (2010) Understanding the evolution of defense metabolites in Arabidopsis thaliana using genome-wide association mapping. Genetics 185:991–1007

Cheung WY, Di Giorgio L, Åhman I (2010) Mapping resistance to the bird cherry-oat aphid (*Rhopalosiphum padi*) in barley. Plant Breed 129(6):637–646

Cook JP, Wichman DM, Martin JM, Bruckner PL, Talbert LE (2004) Identification of microsatellite markers associated with a stem solidness locus in wheat. Crop Sci 44(4):1397–1402

Cook JP, Blake NK, Heo HY, Martin JM, Weaver DK, Talbert LE (2017) Phenotypic and haplotype diversity among tetraploid and hexaploid wheat accessions with potentially novel insect resistance genes for wheat stem sawfly. Plant Genome 10(1):2016–2003

Cordero MJ, Raventós D, San Segundo B (1994) Expression of a maize proteinase inhibitor gene is induced in response to wounding and fungal infection: systemic wound-response of a monocot gene. Plant J 6(2):141–150

Datta K, Vasquez A, Tu J, Torrizo L, Alam MF, Oliva N, Abrigo E, Khush GS, Datta SK (1998) Constitutive and tissue-specific differential expression of the cryIA (b) gene in transgenic rice plants conferring resistance to rice insect pest. Theor Appl Genet 97(1–2):20–30

Dhaliwal HS, Uchimiya H (1999) Genetic engineering for disease and pest resistance in plants. Plant Biotechnol 16(4):255–261

Dweikat I, Ohm H, Patterson F, Cambron S (1997) Identification of RAPD markers for 11 Hessian fly resistance genes in wheat. Theor Appl Genet 94(3–4):419–423

Fang M, Motavalli PP, Kremer RJ, Nelson KA (2007) Assessing changes in soil microbial communities and carbon mineralization in Bt and non-Bt corn residue-amended soils. Appl Soil Ecol 37(1–2):150–160

Flint-Garcia SA, Thuillet AC, Yu J, Pressoir G, Romero SM (2005) Maize association population: a high-resolution platform for quantitative trait locus dissection. Plant J 44:1054–1064

Franzmann BA, Hardy AT, Murray DAT, Henzell RG (2008) Host-plant resistance and biopesticides: ingredients for successful integrated pest management (IPM) in Australian sorghum production. Aust J Exp Agric 48:1594–1600

Fujita D, Doi K, Yoshimura AH, Yasui H (2006) Molecular mapping of a novel gene, Grh5, conferring resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice, *Oryza sativa* L. Theor Appl Genet 113(4):567–573

García-Lara S, Khairallah MM, Vargas M, Bergvinson DJ (2009) Mapping of QTL associated with maize weevil resistance in tropical maize. Crop Sci 49(1):139–149

Gassmann AJ, Carriere Y, Tabashnik BE (2009) Fitness costs of insect resistance to *Bacillus thuringiensis*. Annu Rev Entomol 54:147–163

Gatehouse JA (2008) Biotechnological prospects for engineering insect-resistant plants. Plant Physiol 146:881–887

Ghareyazie B, Alinia F, Menguito CA, Rubia LG, de Palma JM, Liwanag EA, Cohen MB, Khush GS, Bennett J (1997) Enhanced resistance to two stem borers in an aromatic rice containing a synthetic cryIA (b) gene. Mol Breed 3(5):401–414

Girijashankar V (2005) Effect of promoters on Bt transgene expression in sorghum (*Sorghum bicolor* Moench). Ph.D Biotechnology thesis, Dept of Biotechnology, Jawaharlal Nehru Technological University, Hyderabad, AP, India

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163–185

Harshavardhan D, Rani TS, Ugalanathan K, Seetharama N (2002) An improved protocol for regeneration of *Sorghum bicolor* from isolated shoot apices. Plant Biotechnol 19(3):163–171

Helentjaris T, Weber T, Wright S (1986) Use of monosomics to map cloned DNA fragments in maize. Proc Natl Acad Sci U S A 83:6035–6039

Hilder VA, Boulter D (1999) Genetic engineering of crop plants for insect resistance—a critical review. Crop Prot 18(3):177–191

Himabindu K, Suneetha K, Sama VSAK, Bentur JS (2010) A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. Euphytica 174(2):179–187

Ho NH, Baisakh N, Oliva N, Datta K, Frutos R, Datta SK (2006) Translational fusion hybrid Bt genes confer resistance against yellow stem borer in transgenic elite Vietnamese rice *(Oryza sativa* L.) cultivars. Crop Sci 46(2):781–789

Hoffmann MP, Zalom FG, Wilson LT, Smilanick JM, Malyj LD, Kiser J, Hilder VA, Barnes WM (1992) Field evaluation of transgenic tobacco containing genes encoding *Bacillus thuringiensis*∂-endotoxin or cowpea trypsin inhibitor: efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae). J Econ Entomol 85(6):2516–2522

Hu J, Xiao C, Cheng M, Ga G, Zhang Q, He Y (2015) Fine mapping and pyramiding of brown planthopper resistance genes QBph3 and QBph4 in an introgression line from wild rice *O. officinalis*. Mol Breed 35(1):1–10

Huang Q, Duan Z, Yang J, Ma X, Zhan R, Xu H, Chen W (2014a) SNP typing for germplasm identification of Amomum villosum Lour. Based on DNA barcoding markers. PLoS One 9(12):e114940

Huang Y, Sharma HC, Dhillon MK (2014b) Bridging conventional and molecular genetics of sorghum insect resistance. In: Genomics of the Saccharinae. Springer, New York, pp 367–389

Huang S, Wang L, Liu L, Fu Q, Zhu D (2014c) Nonchemical pest control in China rice: a review. Agron Sustain Dev 34(2):275–291

Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW (2010) Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330:222–225

International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. Nature 436:793–800

Jahoor A, Eriksen L, Backes G (2004) QTLs and genes for disease resistance in barley and wheat. In: Cereal genomics. Springer, Dordrecht, pp 199–251

Jairin J, Teangdeerit SN, Leelagud P, Phengrat K, Vanavichi A, Toojindra T (2007) Detection of brown planthopper resistance genes from different rice mapping populations in the same genomic location. Sci Asia 33:347–352

Jampatong C, McMullen MD, Barry BD (2002) Quantitative trait loci for first- and second-generation European corn borer resistance from the maize inbred line Mo47. Crop Sci 42:584–593

Jena KK, Kim SM (2010) Current status of brown planthopper (BPH) resistance and genetics. Rice 3(2):161–171

Jena KK, Jeung JU, Lee JH (2006) High-resolution mapping of a new brown planthopper (BPH) resistance gene, Bph18(t), and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theor Appl Genet 112:288–297

Jongsma MA, Bakker PL, Visser B, Stiekema WJ (1994) Trypsin inhibitor activity in mature tobacco and tomato plants is mainly induced locally in response to insect attack, wounding and virus infection. Planta 195(1):29–35

Joukhadar R, El-Bouhssini M, Jighly A, Ogbonnaya FC (2013) Genome-wide association mapping for five major pest resistances in wheat. Mol Breed 32(4):943–960

Kilian A, Huttner E, Wenzl P, Jaccoud D, Carling J, Caig V, Evers M, Heller-Uszynska K, Cayla C, Patarapuwadol S, Xia L (2003) The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. In: Proceedings of the international congress in the wake of the double helix: from the green revolution to the gene revolution. pp 443–461

Kopp MU, de Mello AJ, Manz A (1998) Chemical amplification: continuous-flow PCR on a chip. Science 280:1046–1048

Koziel MG, Beland GL, Bowman C, Carozzi NB, Crenshaw R, Crossland L, Dawson J, Desai N, Hill M, Kadwell S, Launis K (1993) Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Biotechnology 11(2):194–200

Krakowsky MD, Brinkman MJ, Woodman-Clikeman WL, Lee M (2002) Genetic components of resistance to stalk tunneling by the European corn borer in maize. Crop Sci 42(4):1309–1315

Krakowsky MD, Lee M, Woodman-Clikeman WL, Long MJ, Sharopova N (2004) QTL mapping of resistance to stalk tunneling by the European corn borer in RILs of maize population B73× De81. Crop Sci 44(1):274–282

Kramer KJ, Morgan TD, Throne JE, Dowell FE, Bailey M, Howard JA (2000) Transgenic avidin maize is resistant to storage insect pests. Nat Biotechnol 18(6):670–674

Kumar A, Jain A, Sahu RK, Shrivastava MN, Nair S, Mohan M (2005) Genetic analysis of resistance genes for the rice gall midge in two rice genotypes. Crop Sci 45(4):1631–1635

Kump KL, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR, Oropeza-Roas MA, Zwonitzer JC, Kresovich S, McMullen MD, Ware D, Balint-Kurti PJ (2011) Genome-wide association study

of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43(2):63–168
Langridge P (2005) Molecular breeding of wheat and barley. In: Tuberosa R et al (eds) In the wake of double helix: from the green revolution to the gene revolution. Avenue Media, pp 279–286
Madhusudhana R (2015) Application of DNA markers for genetic improvement. In: Madhusudhana R, Rajendra Kumar P, Patil JV (eds) Molecular breeding. Springer, New Delhi, pp 71–100
Makkar GS, Bentur JS (2017) Breeding for stem borer and gall midge resistance in rice. In: Arora A, Sandhu S (eds) Breeding insect resistant crops for sustainable agriculture. Springer Nature, Singapore, pp 323–352
Malik R, Kumar R, Verma RPS (2013) Molecular markers based bulk segregant analysis for corn leaf aphid resistance in barley (*Hordeum vulgare*). Progress Agric 13(2):172–177
Marais GF, Horn M, Du Torr F (1994) Intergeneric transfer (rye to wheat) of a gene (s) for Russian wheat aphid resistance. Plant Breed 113(4):265–271
Mehlo L, Gahakwa D, Nghia PT, Loc NT, Capell T, Gatehouse J, Gatehouse A, Christou P (2005) An alternative strategy for sustainable pest resistance in genetically enhanced crops. Proc Natl Acad Sci U S A 102:7812–7816
Moellenbeck DJ, Peters ML, Bing JW, Rouse JR, Higgins LS, Sims L, Nevshemal T, Marshall L, Ellis RT, Bystrak PG, Lang BA (2001) Insecticidal proteins from Bacillus thuringiensis protect corn from corn rootworms. Nat Biotechnol 19(7):668–672
Moharramipour S, Tsumuki H, Sato K, Yoshida H (1997) Mapping resistance to cereal aphids in barley. Theor Appl Genet 94:592–596
Morgan TD, Oppert B, Czapla TH, Kramer KJ (1993) Avidin and streptavidin as insecticidal and growth inhibiting dietary proteins. Entomol Exp Appl 69(2):97–108
Mornhinweg DW, Hammon RW, Obert DE (2017) Registration of 'Mesa' Russian wheat aphid–resistant winter feed barley. J Plant Regist 11(2):85–88
Nagadhara D, Ramesh S, Pasalu IC, Rao YK, Krishnaiah NV, Sarma NP, Bown DP, Gatehouse JA, Reddy VD, Rao KV (2003) Transgenic indica rice resistant to sap-sucking insects. Plant Biotechnol J 1(3):231–240
Ni X, Li X, Chen Y, Guo F, Feng J, Zhao H (2010) Metamorphosis of cisgenic insect resistance research in the transgenic crop era. In: Liu T-X, Kang L (eds) Recent advances in entomological research: from molecular biology to pest management. High. Educ. Press, Beijing, pp 157–169. 500 p
Nieto-Lopez RM, Blake TK (1994) Russian wheat aphid resistance in barley: inheritance and linked molecular markers. Crop Sci 34(3):655–659
Oerke EC, Dehne HW, Schonbeck F, Weber A (1994) Crop production and crop protection estimated losses in major food and cash crops. Elsevier, Amsterdam
Ogbonnaya FC, Subrahmanyam NC, Moullet O, De Majnik J, Eagles HA, Brown JS, Eastwood RF, Kollmorgen J, Appels R, Lagudah ES (2001) Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. Aust J Agric Res 52(12):1367–1374
Ordas B, Malvar RA, Santiago R, Sandoy G, Romay MC, Butron A (2009) Mapping of QTL for resistance to the Mediterranean corn borer attack using the intermated B73× Mo17 (IBM) population of maize. Theor Appl Genet 119(8):1451–1459
Ordas B, Malvar RA, Santiago R, Butron A (2010) QTL mapping for Mediterranean corn borer resistance in European flint germplasm using recombinant inbred lines. BMC Genomics 11(1):1–10
Porter DR, Burd JD, Mornhinweg DW (2007) Differentiating greenbug resistance genes in barley. Euphytica 153(1):11–14
Qi T, Guo J, Peng H, Liu P, Kang Z, Guo J (2019) Host-induced gene silencing: a powerful strategy to control diseases of wheat and barley. Int J Mol Sci 20(1):206
Rahman MU, Rashid H, Shahid AA, Bashir K, Husnain T, Riazuddin S (2007) Insect resistance and risk assessment studies of advanced generations of basmati rice expressing two genes of Bacillus thuringiensis. Electron J Biotechnol 10(2):241–251

Ramesh K, Padmavathi G, Deen R, Pandey MK, Lakshmi VJ, Bentur JS (2014) Whitebacked planthopper *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae) resistance in rice variety Sinna Sivappu. Euphytica 200(1):139–148

Rao Y, Li Y, Qian Q (2014) Recent progress on molecular breeding of rice in China. Plant Cell Rep 33(4):551–564

Ribaut JM, Hoisington D (1998) Marker-assisted selection: new tools and strategies. Trends Plant Sci 3:236–239

Ricciardi M, Tocho E, Tacaliti MS, Vasicek A, Giménez DO, Paglione A, Simmonds J, Snape JW, Cakir M, Castro AM (2010) Mapping quantitative trait loci for resistance against Russian wheat aphid (*Diuraphis noxia*) in wheat (*Triticum aestivum* L.). Crop Pasture Sci 61(12):970–977

Rimpau J, Smith D, Flavell R (1978) Sequence organisation analysis of the wheat and rye genomes by interspecies DNA/DNA hybridisation. J Mol Biol 123(3):327–359

Samayoa LF, Malvar RA, McMullen MD, Butrón A (2015) Identification of QTL for resistance to Mediterranean corn borer in a maize tropical line to improve temperate germplasm. BMC Plant Biol 15:265

Schuler TH, Poppy GM, Kerry BR, Denholm I (1998) Insect-resistant transgenic plants. Trends Biotechnol 16(4):168–175

Seetharama N, Mythili PK, Rani TS, Harshavardhan D, Ranjani A, Sharma HC (2003) Tissue culture and alien gene transfer in sorghum. In: Jaiwal PK, Singh R (eds) Plant genetic engineering: improvement of food crops, vol 2. Sci Tech Publishing LLC, pp 235–265

Selvi A, Shanmugasundaram P, Mohankumar S, Raja JAJ, Sadasivam S (2002) Microsatellite markers for yellow stem borer, *Scirpophaga incertulas* (Walker) resistance in rice. Plant Cell Biotechnol Mol Biol 3(3&4):117–124

Sharopova N, Woodman WL, Long MJ (2001) Genetic mapping and analysis of quantitative trait loci in maize for resistance to stalk tunnelling by the European corn borer. Crop Sci 41:835–845

Singh AK, Singh VK, Singh A, Ellur RK, Pandian RTP, Gopala Krishnan S (2015) Introgression of multiple disease resistance into a maintainer of Basmati rice CMS line by marker-assisted backcross breeding. Euphytica 203:97–107

Smith CM (2005) Plant resistance to arthropods: molecular and conventional approaches. Springer, Dordrecht. 423 p

Stout MJ (2007) Types and mechanisms of rapidly induced plant resistance to herbivorous arthropods. In: Walters D, Newton A, Lyon G (eds) Induced resistance for plant defence. Blackwell, Oxford, pp 89–107

Tabashnik BE, Carrière Y, Dennehy TJ, Morin S, Sisterson MS, Roush RT, Shelton AM, Zhao JZ (2003) Insect resistance to transgenic Bt crops: lessons from the laboratory and field. J Econ Entomol 96(4):1031–1038

Tinland B, Schoumacher F, Gloeckler V, Bravo-Angel AM, Hohn B (1995) The *Agrobacterium tumefaciens* virulence D2 protein is responsible for precise integration of T-DNA into the plant genome. EMBO J 14(14):3585–3595

Tocho E, Börner A, Lohwasser U, Castro AM (2013) Mapping and candidate gene identification of loci determining tolerance to greenbug (*Schizaphis graminum*, Rondani) in barley. Euphytica 191:173–182

Tu J, Zhang G, Datta K, Xu C, He Y, Zhang Q, Khush GS, Datta SK (2000) Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* δ-endotoxin. Nat Biotechnol 18(10):1101–1104

Tuberosa R, Salvi S (2004) QTLs and genes for tolerance to abiotic stress in cereals. In: Gupta PK, Varshney RK (eds) Cereal genomics. Kluwer Academic Publishers, pp 253–315

USEPA (2001) Bt plant-incorporated protectants. October 15, 2001 Biopesticides Registration Action Document. http://www.epa.gov/pesticides/biopesticides

Varshney RK, Korzun V, Börner A (2004) Molecular maps in cereals: methodology and progress. In: Cereal genomics. Springer, Dordrecht, pp 35–82

Varshney RK, Graner A, Sorrells ME (2005) Genomics assisted breeding for crop improvement. Trends Plant Sci 10:621–630

Vasil IK (1998) Biotechnology and food security for the 21st century: a real world perspective. Nat Biotechnol 16:99–400

Wang D, Liu Q, Li X, Sun Y, Wang H, Xia L (2015) Double-stranded RNA in the biological control of grain aphid (*Sitobion avenae* F.). Funct Integr Genomics 15:211–223

Weeks DP, Spalding MH, Yang B (2016) Use of designer nucleases for targeted gene and genome editing in plants. Plant Biotechnol J 14:483–495

Will T, Vilcinskas A (2013) Aphid-Proof Plants: biotechnology-based approaches for aphid control. Adv Biochem Eng Biotechnol 136:179–203

Willcox MC, Khairallah MM, Bergvinso D, Crossa J, Deutsch JA, Edmeades GO, González-de-León D, Jiang C, Jewell DC, Mihm JA, Williams WP, Hoisington D (2002) Selection for resistance to southwestern corn borer using marker-assisted and conventional backcrossing. Crop Sci 42:1516–1528

Wu G, Cui H, Ye G, Xia Y, Sardana R, Cheng X, Li Y, Altosaar I, Shu Q (2002) Inheritance and expression of the cry1Ab gene in Bt (*Bacillus thuringiensis*) transgenic rice. Theor Appl Genet 104(4):727–734

Wu KM, Lu YH, Feng HQ, Jiang YY, Zhao JZ (2008) Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. Science 321:1676–1678

Xu R, Yang Y, Qin R, Li H, Qiu C, Li L, Wei P, Yang J (2016) Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. J Genet Genome Res 43:529

Xue WH, Xu N, Yuan XB, Chen HH, Zhang JL, Fu SJ, Zhang CX, Xu HJ (2018) CRISPR/Cas9-mediated knockout of two eye pigmentation genes in the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). Insect Biochem Mol Biol 93:19–26

Yan T, Chen H, Sun Y, Yu X, Xia L (2016) RNA interference of the ecdysone receptor genes EcR and USP in grain aphid (*Sitobion avenae* F.) affects its survival and fecundity upon feeding on wheat plants. Int J Mol Sci 17:2098

Yasala AK, Rawat N, Sama VK, Himabindu K, Sundaram RM, Bentur JS (2012) In silico analysis for gene content in rice genomic regions mapped for the gall midge resistance genes. Plant Omics 5(4):405–413

Ye R, Huang H, Yang Z, Chen T, Liu L, Li X, Chen H, Lin Y (2009) Development of insect-resistant transgenic rice with Cry1C*-free endosperm. Pest Manag Sci 65(9):1015–1020

Yencho GC, Cohen MB, Byrne PF (2000) Applications of tagging and mapping insect resistance loci in plants. Annu Rev Entomol 45:393–422

Yu Y, Wei Z (2008) Increased oriental armyworm and aphid resistance in transgenic wheat stably expressing *Bacillus thuringiensis* (Bt) endotoxin and Pinellia ternata agglutinin (PTA). Plant Cell Tissue Organ Cult 94(1):33–44

Zaidi SS, Tashkandi M, Mansoor S, Mahfouz MM (2016) Engineering plant immunity: using CRISPR/Cas9 to generate virus resistance Frontiers in Plant. Science 7:1673

Zanke CD, Ling J, Plieske J, Kollers S, Ebmeyer E, Korzun V, Argillier O, Stiewe G, Hinze M, Neumann K, Ganal MW, Röder MS (2014) Whole genome association mapping of plant height in winter wheat (*Triticum aestivum* L.). PLoS One 9(11):e113287

Zhang Q (2007) Strategies for developing green super rice. Proc Natl Acad Sci 104(42):16402–16409

Zhang L, Geng M, Zhang Z, Zhang Y, Yan G, Wen S, Liu G, Wang R (2020) Molecular mapping of major QTL conferring resistance to orange wheat blossom midge (*Sitodiplosis mosellana*) in Chinese wheat varieties with selective populations. Theor App Genet 133(2):491–502

Zhuang X, Köllner TG, Zhao N, Li G, Jiang Y, Zhu L, Ma J, Degenhardt J, Chen F (2011) Dynamic evolution of herbivore-induced sesquiterpene biosynthesis in sorghum and related grass crops. Plant J 69:70–80

Zhu-Salzman K, Salzman RA, Ahn JE, Koiwa H (2004) Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. Plant Physiol 134(1):420–431

Chapter 6
Evolution of Constitutive and Induced Resistance in Plants Against Insect Herbivory

Abdul Rasheed War, Mohd Yousuf War, Barkat Hussain, and Hari C. Sharma

6.1 Introduction

Insect herbivores pose a great threat to crop plants, and the plants have responded to insect damage by developing a number of traits to counterattack the herbivores. The plant defence against insect herbivory could be either constitutive or induced in response to insect damage. Host plant resistance to insects is expressed as antixenosis, antibiosis, or tolerance. Antixenosis to insects is expressed in terms of oviposition/feeding non preference due to morphological traits or the chemical stimuli emitted by the host plant; antibiosis or adverse effects on insects are due to the poor nutritional quality of the host plant and/or adverse effects of the secondary metabolites on development and survival of the insects, whilst tolerance is expressed in terms of the ability of the host plant to withstand a certain degree of insect damage without any adverse effects on yield by either putting up more plant growth or producing axillary tillers in response to insect damage. The secondary metabolites or morphological changes induced in response to insect damage constitutes the induced resistance to insect herbivores (Fig. 6.1). These secondary metabolites though occur

A. R. War (✉)
Crop Health Sciences, Jubilee Hills, Hyderabad, Telangana, India

M. Y. War
Department of Botany, Degree College, Sopore, India

B. Hussain
Division of Entomology, SKUAST-K, Srinagar, Jammu and Kashmir, India

H. C. Sharma (✉)
International Crops Research Institute for the Semi-Arid (ICRISAT), Tropics, Patancheru, Telangana, India

Dr YS Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_6

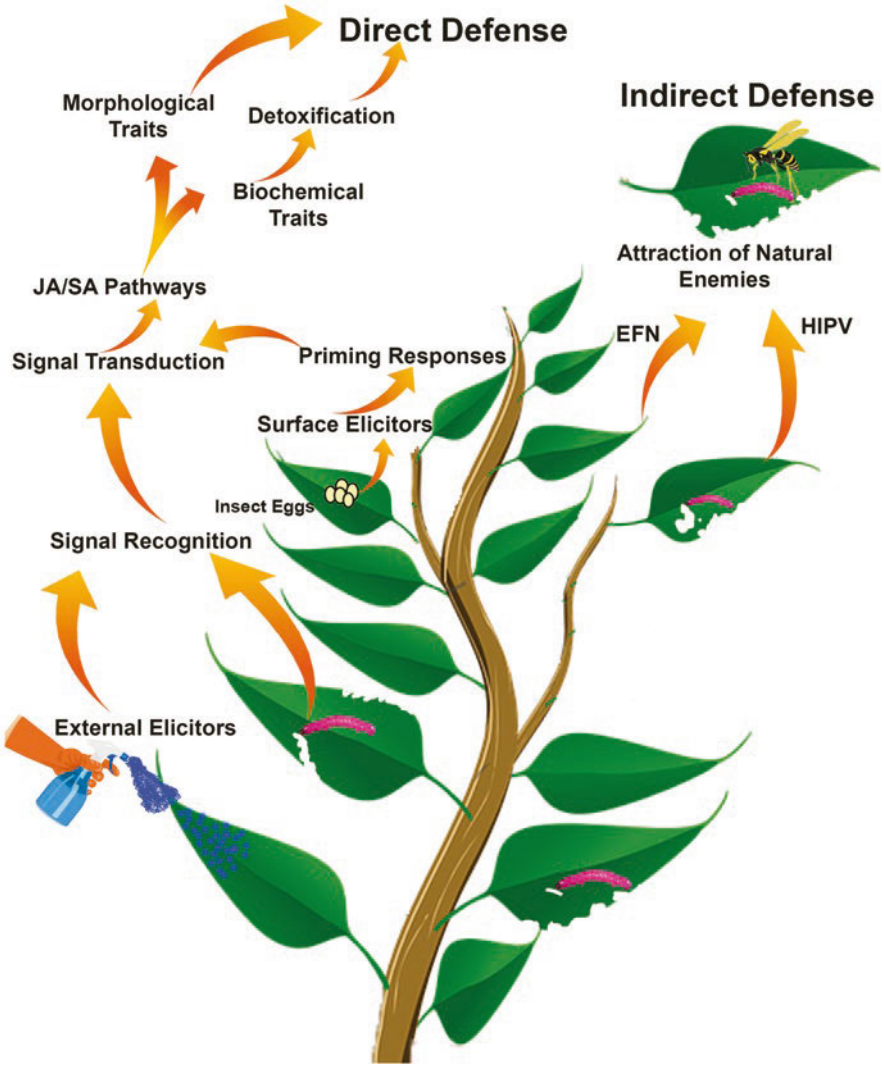

Fig. 6.1 Plant defence against insect pests (*EPF* extrafloral nectar, *HIPV* herbivore-induced plant volatiles, *JA* jasmonic acid, *SA* salicylic acid) (Source, War et al. 2018)

constitutively in host plant, but their production and accumulation in the plant host occur in response to insect damage.

Coevolution is a reciprocal evolutionary change. The allelic frequency changes within a population of interacting species are driven by natural selection (Janzen 1980). Natural selection depends on time, space, and organizational scales (Blomberg and Garland 2002; Jablonka and Lamb 2014). Coevolution occurs between the two species when they impact each other's life cycle or when a species

evolves in reciprocity with another species (Thompson 1999). Plant-herbivore coevolution could be macro- or microevolution, depending on how prolific they were during independent development.

Plant defences against insect pests can be classified as resistance, tolerance, phenological escape, or overcompensation (Agrawal 2000). Tolerance does not affect the insect herbivores but reduces the negative effects of herbivory on plant fitness, and this is largely a plant response to natural selection. Genotypic variability and environmental interactions affect the plant tolerance to herbivore attack. Compared to healthy plants, genotypes with tolerance to herbivore attack show faster growth and higher photosynthetic capacity (Agrawal 2000; Stowe et al. 2000). The insect-resistant plants deter the insect pests or have adverse effects on their development and survival, and thus, limit the population increase of the herbivore (Fineblum and Rausher 1995; Stowe et al. 2000).

6.2 Components of Resistance to Insects

6.2.1 Plant Secondary Metabolites

The genetic basis of plant defensive traits and insect adaptations are being studied by eco-genomic tools in many insect–plant systems (Schranz et al. 2009). These tools help better understand the constitutive and inducible defences in plant systems by studying the polymorphic traits or utilizing transgenic approaches to understand ecological consequences and functional genomics (Schranz et al. 2009). During the coevolution, the generalist and specialist insect herbivores have adapted to plant defences by the evolution of some candidate genes which enable them to adapt to the toxic plant secondary metabolites and/or overcome other defensive traits of the host plant. For example, a specialist insect herbivore *Drosophila sechellia* Tsacas and Baechli has adapted to recognize the odours from fruits of *Morinda citrifolia* L. by expressing higher levels of neurons *ab3*-sensitive to hexanoate esters and *ab3B*-sensitive to 2-heptanone, respectively (Ibba et al. 2010). The bruchid beetle *Caryedes brasiliensis* (Thun.) grubs feed on *Dioclea megacarpa* Rolfe seeds containing L-canavanine, a toxic non-protein amino acid. L-canavanine and arginine are distinguished in these insects by a modified tRNA synthetase (Rosenthal et al. 1976).

Both generalist and specialist insect herbivores withstand the plant defensive traits, be it morphological and/or toxic secondary metabolites by utilizing a number of adaptive strategies. Insect counteradaptations to plant defensive traits could be morphological, behavioural, or biochemical (Howe and Jander 2008; War et al. 2013a, b, 2018). Insects possess a strong olfactory system to recognize the host plant for oviposition and feeding (Hansson and Stensmyr 2011). Furthermore, antennae, proboscis, and maxillary palpi of insects' olfactory system are important chemosensory organs involved in perceiving the host plant cues (by olfaction and taste) (Bruce and Pickett 2011). The recognition of host plant cues is mediated by

specific proteins, which are activated during herbivore damage. The proteins include odourant-binding proteins (OBPs), olfactory receptors (ORs), and gustatory receptors (GRs). The sensory cues activate chemosensory neurons after the chemical cues are solubilized and transported by the OBPs (Leal 2013). The insects respond rapidly to chemical cues by ORs, which detect cues and recognize the airborne odours as well (Getahun et al. 2012; Missbach et al. 2014).

Various genes are up-and/or downregulated in insects in response to biotic and abiotic stresses that contribute to the evolution of OBPs and ORs/GRs (Guo and Kim 2007; Vieira et al. 2007). For example, the evolution of OBP in *Drosophila sechellia* Tsacas and Baechli results in a loss of repellence towards acids in *Morinda citrifolia* L., and the perception of key volatiles from the host plant. This occurs due to gene regulation for OBPs and chemosensory stimuli (Matsuo et al. 2007; Kopp et al. 2008). Similarly, in *Bombyx mori* (L.), specific receptors of GR gene family perceive plant secondary metabolites and allow the insects to adapt to them (Wanner and Robertson 2008). In *Heliconius melpomene* (L.), GRs are highly expressed in gustatory sensilla and regulate plant-specific oviposition (Briscoe et al. 2013). During coevolution, some insects not only avoid the plant defensive traits, but they also utilize them for their benefit as well. The sequestration of plant secondary metabolites is one of the most important adaptation of insects to plant toxic secondary metabolites. For example, turnip sawfly *Athalia rosae* (L.) utilizes the plant tissues that contain defensive traits as a photosynthesis reservoir of nutrients for its own growth and development (Opitz et al. 2010). Additionally, the insects also modify the host plants' toxic phenols in the galls for growth and development. During the course of sequestration of glucosinolates, insects convert them into desulfoGS sulfates instead of highly toxic isothiocyanates (Opitz et al. 2010). Toxic nicotine from tobacco plants is used by *Manduca sexta* (L.) as a defence against its parasitoids (Harvey et al. 2007).

The gene and genome duplication in plants has led to the development of functional traits, resulting in enzyme complexes involved in secondary metabolism (Benderoth et al. 2006). Most of the interactions between insect herbivores and plants arise from coevolution between plant and insect assemblages in which the host develops new or modified traits in a highly specialized system (Janz 2011; Wilson et al. 2012). There are several well-studied examples that show a tight coevolution and co-cladogenesis between insect herbivory and the plants (Farrell and Mitter 1990; Cruaud et al. 2012). Though the plant traits determine the suitability of food for the insects, the plant defensive traits make them good indicators of insect diversity, distribution, and host phylogeny (Becerra 1997; Koricheva et al. 2004).

One of the most diverse groups of organisms on earth comprises of insects. Currently, it has been estimated that there are 1,053,578 named insect species (Roskov et al. 2018), a large fraction of which are plant feeders (Jaenike 1990). Insect pests have developed a number of strategies/adaptations to withstand the hostility of plants' defensive traits. Some of these adaptations include but are not limited to interspecific factors such as resistance to predators (e.g., by sequestration of plant defences) (Petschenka and Agrawal 2016; War et al. 2020), mate-finding (Colwell 1986), and competition for resources (e.g., reproductive interference

(Nishida et al. 2015). The specialist insect herbivores are more likely to evolve due to limited food availability, resulting in greater encounters with specialized plant defensive traits. On the other hand, the generalist insect herbivores do not evolve as rapidly as the specialists due to habitat heterogeneity (Jones and Agrawal 2017), greater resource availability (Bernays and Minkenberg 1997), and physiological constraints for nutritional requirements using a single source of food (Bernays 1998). Specialization to a smaller number of plant hosts enables the insect herbivores to become well adapted to the host plant defences, whilst the generalist herbivores that feed on multiple host species with a choice of food are less likely to adapt to host defensive traits (Becerra 1997). Therefore, the insect herbivores that are unspecialized are often excluded from host plants that possess strong defensive traits (Bernays 1998; Benderoth et al. 2006). The fitness impact of herbivores in insect–plant interaction indicates how successful the coevolutionary process is likely to take place between the two.

Glucosinolates are highly important plant secondary metabolites involved in plant defence against insect herbivores. When glucosinolates encounter the enzyme myrosinase, hydrolyzation occurs, which leads to the formation of isothiocyanates produced by the breakdown of glucosinolates. When insects feed on cruciferous plants, glucosinolates and myrosinase come together due to tissue damage and form isothiocyanates. Diamondback moth *Plutella xylostella* (L.) gut releases sulfatase enzymes that convert isothiocyanates and nitriles into desulfoglucosinolates, due to which glucosinolates and myrosinase enzymes in plant tissues do not come together (Ratzka et al. 2002). *Brassica juncea* (L.) Czern. plants having different glucosinolate profiles and myrosinase activity are resistant to both generalist and specialist insect herbivores. The generalists including *Spodoptera eridania* Stoll feeds on low glucosinolate plants, whilst the specialists such as *P. xylostella* feed on low myrosinase plants (Li et al. 2000). The cabbage white butterfly *Pieris rapae* (L.) contains a specific gut protein that redirects the toxic isothiocyanates into a safe and nontoxic nitrile (Wittstock et al. 2004). In *Passiflora auriculata* Kunth (passion vine), toxic cyanogenic glucosides in leaves are converted to less toxic thiols by the specialist butterfly *Heliconius sara* (Fab.) (Engler et al. 2000). Additionally, the butterfly also releases nitrogen whilst converting toxic glucosinolates into thiols, which are then used in the insect's primary metabolic processes.

6.2.2 Coevolution of Phenols in Plant–Insect Systems

Phenols are considered as potent plant defensive traits against herbivores (Howe and Jander 2008; Scott et al. 2010; War et al. 2012). Based on their structure, plant phenols are classified as simple phenols (i.e., catechols and hydroxybenzoic acid derivatives), condensed tannins, and lignins. The intermediate molecular weight phenols are called flavonoids and stilbenes. Though phenols play diverse roles in plant systems, most of them are employed in defence against biotic stresses. Some of these phenols confer resistance against insect herbivory, whilst others are involved

in attracting pollinators or some provide mechanical support to plants. Phenols that are commonly involved in plant defence against insect herbivores include caffeic and ferulic acid (simple phenols), coumarins, psoralen, umbelliferone (phenylpropanoid lactones), vanillin, and salicylic acid (benzoic acid derivatives). Phenols occur constitutively in plant tissues; however, their accumulation is induced in response to insect damage (Rani and Ravibabu 2011; Bhonwong et al. 2009; Taggar et al. 2014). Phenols are either directly toxic to insect pests or act as feeding deterrents, and thus, providing direct defence to plants (Atteyat et al. 2012; Dixit et al. 2017; War et al. 2013a, b, 2020). They affect the insect growth and development by their own toxicity and/or by oxidizing peroxidases or polyphenol oxidases to toxic compounds including quinones (Bhonwong et al. 2009). Phenolic compounds or their oxidative products cross-link with side chains of proteins and amino acids, thereby, reducing the nutritional quality of the host plant (Constabel et al. 1995; Thipyapong and Steffens 1997). Some phenols also act as attractants to the natural enemies of insect pests, thereby mediating indirect plant defence (Heil 2008). The cereal aphid *Rhopalosiphum padi* does not prefer feeding on wheat cultivars with high phenol content (Leszczynski 1995). Phenols are induced in plants by herbivory and/or application of phytohormones. For example, in groundnut plants, *H. armigera* infestation and jasmonic acid application increase the amounts of phenols in host tissue (War et al. 2015). The growth of oak moth *Operophtera brumata* L. larvae is reduced by benzoic acid-derived salicylates (Ruuhola et al. 2001). In cotton, gossypol phenolic pigment is toxic to *Heliothis virescens* (Fab.) (Maxwell et al. 1965) and deters other insects as well (Abou-Donia 1989). Tannins constitute an important group of secondary metabolites utilized by plants against insect herbivory. Amongst tannins, condensed tannins are mostly involved in herbivore defence. Condensed tannins are either directly toxic to insects or indirectly deter feeding by them. Tannins bind to midgut proteins and gut enzymes of insects, by covalent or hydrogen bonds causing metal ion chelation and protein precipitation, leading to lesions in insect gut (Howe and Jander 2008; Barbehenn and Constabel 2011; War et al. 2013a, b, 2018). They occur constitutively in plant tissues and are induced by herbivory and application of elicitors (Barbehenn and Constabel 2011; War et al. 2015, 2018). Some of plant system in which tannin defence has been studied include *Quercus* spp., *Pinus sylvestris* L. (Roitto et al. 2009), *Populus* spp. (Stevens and Lindroth 2005). The deterrent effects of tannins have been reported against *Euproctis chrysorrhoea* (L.), *Aphis craccivora* Koch, *Schistocerca gregaria* (Forsk.) Kuhn, *H. armigera*, *Lymantria dispar* (L.), and *Operophtera brumata* (L.) (Feeny 1968; Bernays 1981; Grayer et al. 1992; War et al. 2015). The bitter taste of cucurbitacin makes the host plant tissue hostile for the lepidopterans, beetles, and mites by either directly affecting insect growth and development or indirectly as oviposition deterrents (Agrawal et al. 1999; Balkema-Boomstra et al. 2003). Some cucurbitacins are phagostimulants to some insect pests, including *S. exigua*, which attained a luxurious growth on *Cucumis sativus* L. genotypes with elevated levels of cucurbitacins (Barrett and Agrawal 2004). Similarly, cucurbitacins B and D attract leaf beetles, *Cerotoma arcuate* (Oliv.), and *Diabrotica speciose* Germar (Nishida et al. 1986; Nishida and Fukami 1990). Some insects of genus *Aulacophora* sequester cucurbitacins (Nishida et al. 1992).

During coevolution, insect herbivores have adapted to the toxic tannins in plants, and even implicate them for their growth and development (Zhu-Salzman and Zeng 2015; War et al. 2018). The growth of *Anacridium melanorhodon* (Walk., F) increased by 15% after feeding on a diet incorporated with tannins (Eswaran and Jindal 2013). It has been suggested that lower oxygen and higher pH in insect gut inhibits oxidation of tannins to toxic compounds (Johnson and Barbehenn R 2000). Some insects have adapted to tannins by absorbing them through the peritrophic membrane and polymerizing them to be finally removed as polyphenols (Kopper et al. 2002). In grasshoppers, antioxidative α-tocopherol, glutathione, and ascorbate have been found to reduce tannin toxicity (Krishnan and Sehnal 2006); however, in the desert locust, *S. gregaria,* ultrafiltration of tannins in theca protects them from the toxicity of tannins (Bernays and Chamberlain 1980).

6.2.3 Detoxifying Enzymes

During the process of coevolution, plant toxic secondary metabolites that are considered highly important in plant defence against herbivory have suffered counter-adaptation by a number of insect pests. During this interaction, insects produce detoxifying enzymes either constitutively or are induced after ingesting these compounds, to detoxify them into non-toxic or less toxic compounds (Francis et al. 2005; Scott et al. 2010; Saha et al. 2012; War et al. 2013a). The insect gut detoxifying enzymes that are mostly involved in counteradaptation to plant defence traits are cytochrome P450 monooxygenases (P450s), esterases (EST), and glutathione *S*-transferases (GSTs). They have been reported in a number of insects including *Sitobion avenae* Fab.*, Acyrthosiphon pisum* Harris and *Myzus persicae* (Sul.)*, H. armigera, P. xylostella*, hoverfly, leaf beetles, leafhoppers, aphids, *Trichoplusia ni* (Hüb.), and bruchids (Zhu-Salzman et al. 2003; Francis et al. 2005; Ramsey et al. 2010; Scott et al. 2010; War et al. 2013a). Increased activities of esterases and cytochrome P450 have been observed in *M. persicae* after feeding in tobacco plants (Cabrera-Brandt et al. 2010; Puinean et al. 2010). The GSTs are involved in metabolizing isothiocyanates in *M. persicae* (Francis et al. 2005). The phenolic glycosides from different host plants induce GST activity in *L. dispar* larvae, tea mosquito bug, *Helopeltis theivora* (Sign.), and *Malacosoma disstria* (Hub.) (Hemming and Lindroth 2000; Saha et al. 2012).

6.2.4 Plant Cues and Insect–Plant Interaction

Insects use cues to identify suitable plant hosts, and this occurs very quickly as insects possess a sophisticated system for processing the sensory input for host plant recognition (Martin et al. 2011). Insect response to host plant cues to identify suitable host plant is very quick since the insects encounter host order plumes for a fraction of a second due to their patchy structure (Bruce and Pickett 2011). The

response to individual compounds is different than that to a blend of host cues, and the insects are more sensitive to the latter (Riffell et al. 2009; Webster et al. 2010). The response of insects to combinations of cues is highest, and more information is gathered from the moisture as compared to the individual compounds (War et al. 2011). Plants respond to the herbivore attack by producing volatile compounds known as phytoalexins. When an insect attacks a plant, a downstream cascade in plant cells is induced resulting in the release of specific volatiles. Plant volatiles occur constitutively in plants but are induced in response to herbivory (herbivore-induced plant volatiles, HIPVs). The released HIPVs from plants depend on the attaching insect species and the insect–plant interaction. The important volatiles released include terpenes, terpenoids, methyl salicylate, green leaf volatiles, etc. Terpenes are modified unsaturated compounds comprising of isoprene units and are considered as the most important group of volatiles involved in insect–plant interaction. To date, more than 40,000 terpenes are known (Howe and Herde 2015). Isoprene and monoprenes are the most abundant terpenoids in plant volatiles. The induction of plant volatiles in response to herbivory occurs by insect elicitors in oral secretions/saliva and in the ovipositional fluid (Wu et al. 2007; Howe and Jander 2008). HIPVs are involved in both direct and indirect plant defence, by deterring the insect pests and by attracting pests' natural enemies. Though most of the volatiles are involved in indirect plant defence, some plant volatiles such as indoles (E)-β-caryophyllene) are toxic to the herbivores suggesting that the primary role of volatiles might have been toxicity against the attackers and might have evolved for attracting natural enemies of the herbivores (Turlings and Erb 2018).

6.2.5 Protease Inhibitors in Plants and Insect Adaptation

In insects, dietary proteins are broken down to amino acids required for growth and development, by enzymes known as proteases by cleaving the peptide bonds. Plant PIs target digestive proteases and interfere with the insect digestion and nutritional balance, thus reducing their growth and development (Koiwa et al. 1997; Gatehouse 2011). Protease inhibitors (PIs) are an important component of defensive traits employed by plants against field and storage insect pests (Zhu-Salzman et al. 2003; Parde et al. 2012; Zhu-Salzman and Zeng 2015; Jadhav et al. 2016). Protease inhibitors are considered as crucial plant defensive traits against insect pests (Kessler and Baldwin 2002). The concept of PIs was proposed by Green and Ryan after reporting that potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) leaves rapidly accumulated the PIs locally and systemically when infested by Colorado potato beetles (Green and Ryan 1972). The PI-expressing transgenic tobacco lines containing a PI-encoding gene construct linked to CaMV 35S promoter from cowpea (cowpea trypsin inhibitor, CpTI) expressed constitutively high levels of CpTI (approximately 1% of total soluble protein) and were resistant to tobacco budworm *H. virescens* apart from other lepidopteran insects (Hilder et al. 1987). This induces PIs in plants for resistance against insect herbivores (Duan et al. 1996; Altpeter et al.

1999). In transgenic tobacco, constitutive overexpression of tomato inhibitor-II and potato inhibitor-II by genetically transformed genes significantly reduced insect growth and development (Johnson et al. 1989; McManus et al. 1994). Later PI-inducing genes were transferred into many crops including rice and wheat for resistance against insect herbivores (Duan et al. 1996; Altpeter et al. 1999).

Protease inhibitors act by inhibiting the activity of digestive enzymes in insect gut, thereby impairing digestion which results in reduced growth and development. The enzymes targeted by protease inhibitors include cysteine, serine, metallocarboxypeptidases, and aspartate proteinases (War et al. 2012, 2013a; Parde et al. 2012; Jadhav et al. 2016). The inhibition of proteolytic enzymes in insect guts by PIs renders the insects deficient in essential amino acids, resulting in the extension of their developmental period, fecundity reduction, and mortality. When insects feed on PI-based diet, a feedback mechanism is initiated and hyperproduction of digestive proteases occurs, resulting in a shortage of essential amino acids. The effect of PIs on insect growth due to shortage of essential amino acids is not restored even after supplying amino acids with the diet, suggesting that other than amino acid scarcity, some other mechanisms of toxicity by PIs may be present (Zhu-Salzman and Zeng 2015). Though several transgenics were developed successfully with PIs to increase resistance against insect herbivory, this strategy for crop protection could not be commercialized fully except for cotton SGK-321 developed in China combined with CpTI (cowpea trypsin inhibitor) transgene and Bt toxin b (Jia et al. 2004). Under high selection pressure, resistance development in insect pests has been reported to these PIs, and to compensate for low nutritional quality, they overconsume transgenic plant parts. Furthermore, insects that feed on transgenic plants showed more weight than those that feed on non-transgenics.

To withstand the toxic effect of protease inhibitors, insects have developed counteradaptations to them (Zhu-Salzman and Zeng 2015). Insects utilize a number of ways to counter the PIs, which include the production of PI-insensitive proteases (Bayes et al. 2006; Zhu-Salzman and Zeng 2015), utilization of alternative proteases to hydroxyl, and detoxify the PIs and de novo synthesis of existing proteases and their regulation (Zhu-Salzman et al. 2003). Some of the insects that produce PI-insensitive proteases include *Agrotis ipsilon* (Huf.), *S. exigua*, *H. zea*, *Leptinotarsa decemlineata* Say, *C. maculatus,* and *T. ni* (Volpicella et al. 2003; Zhu-Salzman et al. 2003; Gruden et al. 2004; Brioschi et al. 2007). New protease synthesis, regulation of existing proteases and PI degradation have been reported on *H. armigera, Spodoptera frugiperda* (JE Smith), and *P. xylostella* (Gatehouse et al. 1997; Brioschi et al. 2007; Yang et al. 2009). In cabbage flea beetle, *Psylliodes chrysocephala* L., the cysteine proteinase inhibitor, oryzacystatin I in oilseed rape is countered by increased protease activity when fed on the plants expressing high levels of PI (Girard et al. 1998). Similarly, in *H. armigera* and *T. ni* feeding on soybean Kunitz inhibitor-containing diet and in *S. exigua* feeding on transgenic tobacco plants, potato PI2 is overexpressed, thus converting sensitive proteases into insensitive ones (Jongsma et al. 1995; Bown et al. 1997; Broadway 1997). The red flour beetle shifts mainly when fed on diets containing cysteine PI. The *Callosobruchus maculatus* (Fab.) showed the presence of cathepsin L-like cysteine against scN

(Zhu-Salzman et al. 2003). Besides this, *C. maculatus* and *Tribolium castaneum* (Herb.) showed a slight shift from cysteine proteases to minor serine proteases and from cysteine proteases to aspartic proteases, respectively (Zhu-Salzman et al. 2003; Ahn et al. 2007; Oppert et al. 2010). In *C. maculatus*, a number of genes encoding proteins and carbohydrates are up-and/or downregulated in response to soybean cystatin (a cysteine protease inhibitor, scN) (Zhu-Salzman et al. 2003; Ahn et al. 2007). The concentration of cysteine and aspartic PIs in potato leaves is increased after the application of jasmonic acid.

The gut proteolytic activity of Colorado potato beetles is reduced by these PIs; however, the Colorado potato beetles produce increased amounts of uninhibited proteases to withstand the PI pressure (Bolter and Jongsma 1995). A wide spectrum of gut proteases in insect pests enables them to counter the dietary PIs. The co-existence of PIs and PI-insensitive proteases in insects with their upregulation in response to ingested PIs is highly beneficial to insects and is supposed to be driven by some feedback mechanism. However, the downregulation of some of the sensitive proteases in insect gut could benefit both plants and insects. The resource allocation by both plants and insects in producing PIs and insensitive proteases, respectively, is highly monitored. Plants spend less energy on PI production and are synthesized only when needed. On the other hand, insects do not waste any resources on inhibited proteases. Plants always look for a short and perfect strategy to lower gut proteases in insects that would result in reduced development and high mortality in the target pest. The hijacking of protease regulation in insect gut could be the next step in coevolution but not without insects ready for the next strategy to combat the same.

6.3 Ecological Costs

Plant secondary metabolites involved in plant defence against insect herbivory occur either constitutively in plants and/or induced by insect attack. These metabolites are employed by plants against various stresses depending on their genetic variability. Though most of the plant secondary metabolites are synthesized de novo after plants encounter the stresses, especially insect herbivory, resource allocation is incurred for the same which may have some effect on the primary growth and development of the host plant (Stamp 2003; Siemens et al. 2010). However, since the secondary metabolites are induced in response to the stresses, thus, are produced only when in demand, which means the resource allocation should not have any major effect on the plant's primary growth and development (Karban et al. 1999). The cost involved in the production of secondary metabolites in plants against herbivory has been studied in a few plant systems (Karban et al. 1999). For instance, glucosinolate production in knockout Arabidopsis mutants of *Arabidopsis thaliana* (L.) Heynh. has affected the plant growth by allocating about 15% of photosynthetic energy (Siemens et al. 2010). Though in some cases as for example in *A. thaliana*, a substantial cost is incurred in induced plant defence against herbivory, which seems a concern for plant systems, some authors believe that the cost is compensated by the defence against various stresses (Agrawal et al. 1999; Bekaert et al.

2012). One more concern regarding the cost of induced plant defence is the reduction in pollinator attraction in some plant systems as some insect repellents may also repel pollinators (Agrawal et al. 1999).

Apart from the ecological cost incurred by plant systems in mounting defence against insect herbivory, insect pests, in turn, have been coevolving by adapting to these defences. The insect adaptations manifested through sequestration and detoxification also incur costs which affect insect growth and development, behaviour, reproduction, survival, or immunity (Després et al. 2007; Schwenke et al. 2016). The ecological cost incurred depends on the insect–plant interaction and the adaptive strategy of the insect pest (Després et al. 2007). It has been reported that higher cost is incurred in specialist insect herbivores than the generalists that incur higher costs for adapting to plant toxins, but they are well protected from natural enemies during the interaction (Forister et al. 2012). The cardenolide-containing milkweed plants harbour milkweed bugs *Oncopeltus fasciatus* (Dallas), monarch butterflies *D. plexippus,* and milkweed aphids *Aphis nerii* Boyer de Fon., which are not preferred by predatory birds (Brower and Moffitt 1974), mantids (Paradise and Stamp 1991), and spiders (Petschenka et al. 2011), respectively. The cardenolides and their concentrations differentially impact the cardenolide–aphid interaction (Desneux et al. 2009; Colvin and Yeargan 2013).

6.4 Coevolution and Genetic Variability Amongst Plants and Insects

Plants face biotic and abiotic stresses, and the plant response depends on the genetic variability amongst the genotypes, and the environment (Zhou et al. 2010; War et al. 2012; Gloss et al. 2013). Plants have developed several physical and chemical defences against insect herbivory. In *A. pisum* genome, the loci have diverged based on the preference and/or nonpreference to the host, suggesting that insect adaptation to plant defensive traits maintain some genetic variability amongst the host races (Jaquiery et al. 2012; Via et al. 2012). Similarly, in insect pests such as the large pine weevil, *Hylobius abietis*—a specialist, the allele frequencies differ at a few loci (Manel et al. 2009). Defensive strategies in plants against insect herbivory and counteradaptation by insects are essential to maintain genetic variability within and amongst different populations of plants and the herbivores.

6.5 Conclusions

During coevolution, plants and insects have been defending each other from the defence traits. This coevolution has led to the production of morphological traits and toxic compounds in plants and counter adaptation by the insect pests. Counter adaptation by the insect pests to host plant defences has posed a major challenge for developing pest-resistant cultivars for pest management. It is highly important to

understand resource allocation and the costs incurred by each partner in insect–plant systems to develop rational strategies for pest management. The genes coding defensive traits in both plants and insects need to be identified to understand different transduction pathways involved in the development of these traits to devise appropriate strategies for developing cultivars with resistance to insects for sustainable crop production.

References

Abou-Donia MB (1989) Gossypol. In: Cheeke PR (ed) Toxicants of plant origin, phenolics, vol 5. CRC Press, Boca Raton, pp 2–22

Agrawal AA (2000) Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. Trends Plant Sci 5:309–313

Agrawal AA, Gorski PM, Tallamy DW (1999) Polymorphism in plant defense against herbivory: constitutive and induced resistance in Cucumis sativus. J Chem Ecol 25:2285–2304

Ahn JE, Guarino LA, Zhu-Salzman K (2007) Seven-up facilitates insect counter-defense by suppressing cathepsin B expression. FEBS J 274:2800–2814

Altpeter F, Diaz I, McAuslane H, Gaddour K, Carbonero P, Vasil IK (1999) Increased insect resistance in transgenic wheat stably expressing trypsin inhibitor CMe. Mol Breed 5:53–63

Atteyat M, Abu-Romann S, Abu-Darwish M, Ghabeish I (2012) Impact of flavonoids against woolly apple aphid, Eriosoma lanigerum (Hausmann) and its sole parasitoid, Aphelinus mali (Hald.). JouJ Agri Sci 4:227–236

Balkema-Boomstra AG, Zijlstra S, Verstappen FW, Inggamer H, Mercke PE, Jongsma MA, Bouwmeester HJ (2003) Role of cucurbitacin C in resistance to spider mite (Tetranychus urticae) in cucumber (*Cucumis sativus* L.). J Chem Ecol 29:225–235

Barbehenn RV, Constabel PC (2011) Tannins in plant-herbivore interactions. Phytochemistry 72:1551–1565

Barrett RD, Agrawal AA (2004) Interactive effects of genotype, environment, and ontogeny on resistance of cucumber (*Cucumis sativus*) to the generalist herbivore, *Spodoptera exigua*. J Chem Ecol 30:37–51

Bayes A, de la Vega MR, Vendrell J, Aviles FX, Jongsma MA, Beekwilder J (2006) Response of the digestive system of Helicoverpa zea to ingestion of potato carboxypeptidase inhibitor and characterization of an uninhibited carboxypeptidase B. Insect Biochem Mol Biol 36:654–664

Becerra JX (1997) Insects on plants: chemical trends in host use. Science 276:253–256

Bekaert M, Edger PP, Hudson CM, Pires JC, Conant GC (2012) Metabolic and evolutionary costs of herbivory defense: systems biology of glucosinolate synthesis. New Phytol 196:596–605

Benderoth M, Textor S, Windsor AJ, Mitchell-Olds T, Gershenzon J, Kroymann J (2006) Positive selection driving diversification in plant secondary metabolism. Proc Natl Acad Sci U S A 103:9118–9123

Bernays EA (1981) Plant tannins and insect herbivores: an appraisal. Ecol Entomol 6:353–360

Bernays EA (1998) Evolution of feeding behavior in insect herbivores. Bioscience 48:35–44

Bernays EA, Chamberlain DJ (1980) A study of tolerance of ingested tannin in *Schistocerca gregaria*. J Insect Physiol 26:415–420

Bernays EA, Minkenberg OPJM (1997) Insect herbivores: different reason for being a generalist. Ecology 78:1157–1169

Bhonwong A, Stout MJ, Attajarusit J, Tantasawat P (2009) Defensive role of tomato polyphenol oxidases against cotton bollworm (Helicoverpa armigera) and beet armyworm (Spodoptera exigua). J Chem Ecol 35:28–38

Blomberg SP, Garland T (2002) Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. J Evol Biol 15:899–910

Bolter CJ, Jongsma MA (1995) Colorado potato beetles (*Leptinotarsa decemlineata*) adapt to proteinase inhibitors induced in potato leaves by methyl jasmonate. J Insect Physiol 41:1071–1078
Bown DP, Wilkinson HS, Gatehouse JA (1997) Differentially regulated inhibitor-sensitive and insensitive protease genes from the phytophagous insect pest, Helicoverpa armigera, are members of complex multigene families. Insect Biochem Mol Biol 27:625–638
Brioschi D, Nadalini LD, Bengtson MH, Sogayar MC, Moura DS, Silva-Filho MC (2007) General up regulation of Spodoptera frugiperda trypsins and chymotrypsins allows its adaptation to soybean proteinase inhibitor. Insect Biochem Mol Biol 37:1283–1290
Briscoe AD, Macias-Muñoz A, Kozak KM, Walters JR et al (2013) Female behaviour drives expression and evolution of gustatory receptors in butterflies. PLoS Genet 9:e1003620
Broadway RM (1997) Dietary regulation of serine proteinases that are resistant to serine proteinase inhibitors. J Insect Physiol 43:855–874
Brower LP, Moffitt CM (1974) Palatability dynamics of cardenolides in the monarch butterfly. Nature 249:280–283
Bruce TJ, Pickett JA (2011) Perception of plant volatile blends by herbivorous insects–finding the right mix. Phytochemistry 72:1605–1611
Cabrera-Brandt MA, Fuentes-Contreras E, Figueroa CC (2010) Differences in the detoxification metabolism between two clonal lineages of the aphid *Myzus persicae* (Sulze) (Hemiptera: Aphididae) reared on tobacco (*Nicotiana tabacum* L.). Chilean J Agric Res 70:567–575
Colvin SM, Yeargan KV (2013) Effects of milkweed host species on interactions between Aphis nerii (Hemiptera: Aphididae) and its parasitoids. J Kansas Entomol Soc 86:193–205
Colwell RK (1986) Population structure and sexual selection for host fidelity in the speciation of hummingbird flower mites. In: Samuel K, Eviatar N (eds) Evolutionary processes and theory. Academic, Orlando, pp 475–495
Constabel CP, Bergey DR, Ryan CA (1995) Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway. Proc Natl Acad Sci U S A 92:407–411
Cruaud A, Ronsted N, Chantarasuwan B, Chou LS, Clement WL et al (2012) An extreme case of plant-insect codiversification: figs and fig-pollinating wasps. Syst Biol 61:1029–1047
Desneux N, Barta RJ, Hoelmer KA, Hopper KR, Heimpel GE (2009) Multifaceted determinants of host specificity in an aphid parasitoid. Oecologia 160:387–398
Després L, David JP, Gallet C (2007) The evolutionary ecology of insect resistance to plant chemicals. Trends Ecol Evol 22:298–307
Dixit G, Praveen A, Tripathi T, Yadav VK, Verma PC (2017) Herbivore responsive cotton phenolics and their impact on insect performance and biochemistry. J Asia Pac Entomol 20:341–351
Duan X, Li X, Xue Q, Abo-el-Saad M, Xu D, Wu R (1996) Transgenic rice plants harboring an introduced potato proteinase inhibitor II gene are insect resistant. Nat Biotechnol 14:494–498
Engler HS, Spencer KC, Gilbert LE (2000) Preventing cyanide release from leaves. Nature 406:144–145
Eswaran SV, Jindal A (2013) Grasshoppers—generalists to specialists? Resonance 18:810–816
Farrell B, Mitter C (1990) Phylogenesis of insect- plant interactions: have Phyllobrotica (Coleoptera: Chrysomelidae) and the Lamiales diversified in parallel? Evolution 44:1389–1403
Feeny PP (1968) Effect of oak leaf tannins on larval growth of the winter moth Operophtera brumata. J Insect Physiol 14:805–817
Fineblum WL, Rausher MD (1995) Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature 377:517–520
Forister ML, Dyer LA, Singer MS, Stireman JO, Lill JT (2012) Revisiting the evolution of ecological specialization, with emphasis on insect-plant interactions. Ecology 93:981–991
Francis F, Vanhaelen N, Haubruge E (2005) Glutathione S-transferases in the adaptation to plant secondary metabolites in the Myzus persicae aphid. Arch Insect Biochem Physiol 58:166–174
Gatehouse JA (2011) Prospects for using proteinase inhibitors to protect transgenic plants against attack by herbivorous insects. Curr Protein Pept Sci 12:409–416
Gatehouse AMR, Davison GM, Newell CA, Merryweather A et al (1997) Transgenic potato plants with enhanced resistance to the tomato moth, Lacanobia oleracea: growth room trials. Mol Breed 3:49–63

Getahun MN, Wicher D, Hansson BS, Olsson SB (2012) Temporal response dynamics of Drosophila olfactory sensory neurons depends on receptor type and response polarity. Front Cell Neurosci 6:54

Girard C, Le Metayer M, Bonade-Bottino M, Pham-Delegue MH, Jouanin L (1998) High level of resistance to proteinase inhibitors may be conferred by proteolytic cleavage in beetle larvae. Insect Biochem Mol Biol 28:229–237

Gloss AD, Nelson Dittrich AC, Goldman-Huertas B, Whiteman NK (2013) Maintenance of genetic diversity through plant-herbivore interactions. Curr Opin Plant Biol 16:443–450

Grayer RJ, Kimmins FM, Padgham DE, Harborne JB, Ranga Rao DV (1992) Condensed tannin levels and resistance in groundnuts (*Arachis hypogaea* (L.)) against *Aphis craccivora* (Koch). Phytochemistry 31:3795–3800

Green TR, Ryan CA (1972) Wound-induced proteinase inhibitor in plant leaves—possible defense mechanism against insects. Science 175:776–777

Gruden K, Kuipers AGJ, Guncar G, Slapar N, Strukelj B, Jongsma MA (2004) Molecular basis of Colorado potato beetle adaptation to potato plant defence at the level of digestive cysteine proteinases. Insect Biochem Mol Biol 34:365–375

Guo S, Kim J (2007) Molecular evolution of Drosophila odorant receptor genes. Mol Biol Evol 24:1198–1207

Hansson BS, Stensmyr MC (2011) Evolution of insect olfaction. Neuron 72:698–711

Harvey JA, Van Dam NM, Witjes LMA, Solerro R, Gols R (2007) Effects of dietary nicotine on the development of an insect herbivore, its parasitoid and secondary hyperparasitoid over four trophic levels. Ecol Entomol 32:15–23

Heil M (2008) Indirect defence via tritrophic interactions. New Phytol 178:41–61

Hemming JDC, Lindroth RL (2000) Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxification activities. Environ Entomol 29:108–1115

Hilder VA, Gatehouse AMR, Sheerman SE, Barker RF, Boulter D (1987) A novel mechanism of insect resistance engineered into tobacco. Nature 330:160–163

Howe GA, Herde M (2015) Interaction of plant defense compounds with the insect gut: new insights from genomic and molecular analyses. Curr Opin Insect Sci 9:62–68

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59:41–66

Ibba I, Angioy AM, Hansson BS, Dekker T (2010) Macroglomeruli for fruit odors change blend preference in *Drosophila*. Naturwissenschaften 97:1059–1066

Jablonka E, Lamb MJ (2014) Evolution in four dimensions: genetic, epigenetic, behavioral, and symbolic variation in the history of life, 2nd edn. MIT Press, Cambridge

Jadhav AR, War AR, Nikam AN, Adhav AS et al (2016) Capsicum annuum proteinase inhibitor ingestion negatively impacts the growth of sorghum pest *Chilo partellus* and promotes differential protease expression. Biochem Biophys Rep 8:302–309

Jaenike J (1990) Host specialization in phytophagous insects. Annu Rev Ecol Syst 21:243–273

Janz N (2011) Ehrlich and raven revisited: mechanisms underlying codiversification of plants and enemies. Annu Rev Ecol Evol Syst 42:71–89

Janzen DH (1980) When is it coevolution. Evolution 34:611–612

Jaquiery J, Stoeckel S, Nouhaud P, Mieuzet L et al (2012) Genome scans reveal candidate regions involved in the adaptation to host plant in the pea aphid complex. Mol Ecol 21:5251–5264

Jia SR, Guo SD, An DC, Xia GX (2004) Transgenic cotton. Science Press, Beijing, pp 172–183

Johnson KS, Barbehenn R V (2000) Oxygen levels in the gut lumens of herbivorous insects. J Insect Physiol 46:897–903

Johnson R, Narvaez J, An G, Ryan C (1989) Expression of proteinase inhibitors I and II in transgenic tobacco plants: effects on natural defense against *Manduca sexta* larvae. Proc Natl Acad Sci U S A 86:9871–9875

Jones PL, Agrawal AA (2017) Learning in insect pollinators and herbivores. Annu Rev Ecol Evol Syst 62:53–71

Jongsma MA, Bakker PL, Peters J, Bosch D, Stiekema WJ (1995) Adaptation of Spodoptera exigua larvae to plant proteinase inhibitors by induction of gut proteinase activity insensitive to inhibition. Proc Natl Acad Sci U S A 92:8041–8045

Karban R, Agrawal AA, Thaler JS, Adler LS (1999) Induced plant responses and information content about risk of herbivory. Trends Ecol Evol 14:443–447
Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol 53:299–328
Koiwa H, Bressan RA, Hasegawa PM (1997) Regulation of protease inhibitors and plant defense. Trends Plant Sci 2:379–384
Kopp A, Barmina O, Hamilton AM, Higgins L, McIntyre LM, Jones CD (2008) Evolution of gene expression in the Drosophila olfactory system. Mol Biol Evol 25:1081–1092
Kopper BJ, Jakobi VN, Osier TL, Lindroth RL (2002) Effects of paper birch condensed tannin on white marked tussock moth (Lepidoptera: Lymantriidae) performance. Environ Entomol 31:10–14
Koricheva J, Nykänen H, Gianoli E (2004) Meta-analysis of tradeoffs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? Am Nat 163:64–75
Krishnan N, Sehnal F (2006) Compartmentalization of oxidative stress and antioxidant defense in the larval gut of *Spodoptera littoralis*. Arch Insect Biochem Physiol 63:1–10
Leal WS (2013) Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Annu Rev Entomol 58:373–391
Leszczynski B (1995) The influence of phenolic compounds on the preference of winter wheat cultivars by cereal aphids. Insect Sci Appl 6:157–158
Li Q, Eigenbrode SD, Stringham GR, Thingarajah MR (2000) Feeding and growth of *Plutella xylostella* and *Spodoptera eridania* on *Brassica juncea* with varying glucosinolate concentrations and myrosinase activities. J Chem Ecol 26:2401–2419
Manel S, Conord C, Després L (2009) Genome scan to assess the respective role of host-plant and environmental constraints on the adaptation of a widespread insect. BMC Evol Biol 9:288
Martin JP, Beyerlein A, Dacks AM, Reisenman CE, Riffell JA, Lei Hand Hildebrand JG (2011) The neurobiology of insect olfaction: sensory processing in a comparative context. Prog Neurobiol 95:427–447
Matsuo T, Sugaya S, Yasukawa J, Aigaki T, Fuyama Y (2007) Odorantbinding proteins OBP57d and OBP57e affect taste perception and host-plant preference in *Drosophila sechellia*. PLoS Biol 5:985–996
Maxwell FG, Lafever HN, Jenkins JN (1965) Blister beetles on glandless cotton. J Econ Entomol 58:792–798
McManus MT, White DW, McGregor PG (1994) Accumulation of a chymotrypsin inhibitor in transgenic tobacco can affect the growth of insect pests. Transgenic Res 3:50–58
Missbach C, Dweck HK, Vogel H, Vilcinskas A, Stensmyr MC, Hansson BS, Grosse-Wilde E (2014) Evolution of insect olfactory receptors. Elife 3:e02115
Nishida R, Fukami H (1990) Sequestration of distasteful compounds by some pharmacophagous insects. J Chem Ecol 16:151–164
Nishida R, Fukami H, Tanaka Y, Magalhães BP, Yokoyama M, Blumenschein A (1986) Isolation of feeding stimulants of Brazilian leaf beetles (*Diabrotica speciosa* and *Cerotoma arcuata*) from the root of *Ceratosanthes hilariana*. Agric Biol Chem 50:2831–2836
Nishida R, Yokoyama M, Fukami H (1992) Sequestration of cucurbitacin analogs by New and Old World chrysomelid leaf beetles in the tribe Luperini. Chemoecology 3:19–24
Nishida T, Takakura K, Iwao K (2015) Host specialization by reproductive interference between closely related herbivorous insects. Popul Ecol 57:273–281
Opitz SE, Jensen SR, Müller C (2010) Sequestration of glucosinolates and iridoid glucosides in sawfly species of the genus *Athalia* and their role in defense against ants. J Chem Ecol 36:148–157
Oppert B, Elpidina EN, Toutges M, Mazumdar-Leighton S (2010) Microarray analysis reveals strategies of *Tribolium castaneum* larvae to compensate for cysteine and serine protease inhibitors. Comp Biochem Phys D 5:280–287
Paradise CJ, Stamp NE (1991) Prey recognition time of praying mantids (Dictyoptera: Mantidae) and consequent survivorship of unpalatable prey (Hemiptera: Lygaeidae). J Insect Behav 4:265–273

Parde VD, Sharma HC, Kachole MS (2012) Potential of protease inhibitors in wild relatives of pigeonpea against the cotton bollworm/legume pod borer, *Helicoverpa armigera*. Am J Plant Sci 3:627–635

Petschenka G, Agrawal AA (2016) How herbivores coopt plant defenses: natural selection, specialization, and sequestration. Curr Opin Insect Sci 14:17–24

Petschenka G, Bramer C, Pankoke H, Dobler S (2011) Evidence for a deterrent effect of cardenolides on Nephila spiders. Basic Appl Ecol 12:260–267

Puinean AM, Foster SP, Oliphant L, Denholm I, Field LM, Millar NS, Williamson MS, Bass C (2010) Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid Myzus persicae. PLoS Genetics 6:e1000999

Ramsey JS, Rider DS, Walsh TK, De Vos M, Gordon KH, Ponnala L, Macmil SL, Roe BA, Jander G (2010) Comparative analysis of detoxification enzymes in Acyrthosiphon pisum and Myzus persicae. Insect Mol Biol 19:155–164

Rani PU, Ravibabu MV (2011) Allelochemicals in castor (*Ricinus communis*) plants and their impact on pest larval feeding as antiherbivore defensive. Allelopathy J 27:263–276

Ratzka A, Vogel H, Kliebenstein DJ, Mitchell-Olds T, Kroymann J (2002) Disarming the mustard oil bomb. Proc Natl Acad Sci U S A 99:11223–11228

Riffell JA, Lei H, Christensen TA, Hildebrand JG (2009) Characterization and coding of behaviorally significant odor mixtures. Curr Biol 19:335–340

Roitto M, Rautio P, Markkola A, Julkunen-Tiitto R, Varama M, Saravesi K, Tuomi J (2009) Induced accumulation of phenolics and sawfly performance in scots pine in response to previous defoliation. Tree Physiol 29:207–216

Rosenthal GA, Dahlman DL, Janzen DH (1976) A novel means for dealing with L-canavanine, a toxic metabolite. Science 192:256–258

Roskov Y, Abucay L, Orrell T, Nicolson D et al (2018) Species 2000 & ITIS catalogue of life, 2018 annual checklist. Digital resource at www.catalogueoflife.org/annual-checklist/2018. Species 2000: Naturalis, Leiden, the Netherlands

Ruuhola T, Tikkanen O, Tahvanainen O (2001) Differences in host use efficiency of larvae of a generalist moth, Operophtera brumata on three chemically divergent Salix species. J Chem Ecol 27:1595–1615

Saha D, Mukhopadhyay A, Bahadur M (2012) Effect of host plants on fitness traits and detoxifying enzymes activity of Helopeltis theivora, a major sucking insect pest of tea. Phytoparasitica 40:433–444

Schranz ME, Manzaneda AJ, Windsor AJ, Clauss MJ, Mitchell-Olds T (2009) Ecological genomics of Boechera stricta: identification of a QTL controlling the allocation of methionine- vs branched chain amino acid-derived glucosinolates and levels of insect herbivory. Heredity 102:465–474

Schwenke RA, Lazzaro BP, Wolfner MF (2016) Reproduction–immunity trade-offs in insects. Annu Rev Entomol 61:239–256

Scott MI, Thaler SJ, Scott GF (2010) Response of a generalist herbivore Trichoplusia ni to jasmonate-mediated induced defense in tomato. J Chem Ecol 36:490–499

Siemens DH, Keck AG, Ziegenbein S (2010) Optimal defense in plants: assessment of resource allocation costs. Evol Ecol 24:1291–1305

Stamp N (2003) Out of the quagmire of plant defense hypotheses. Q Rev Biol 78:23–55

Stevens MT, Lindroth RL (2005) Induced resistance in the indeterminate growth of aspen (*Populus tremuloides*). Oecologia 145:298–306

Stowe KA, Marquis RJ, Hochwender CG, Simms EL (2000) The evolutionary ecology of tolerance to consumer damage. Annu Rev Ecol Syst 31:565–595

Taggar GK, Gill RS, Gupta AK, Singh S (2014) Induced changes in the antioxidative compounds of black gram (*Vigna mungo* (L.) Hepper) genotypes due to infestation by *Bemisia tabaci* (Gennadius). J Environ Biol 35:1037–1045

Thipyapong P, Steffens JC (1997) Tomato polyphenol oxidase: differential response of the polyphenol oxidase F promoter to injuries and wound signals. Plant Physiol 115:409–418

Thompson JN (1999) What we know and do not know about coevolution: insect herbivores and plants as a test case. In: Olff H, Brown VK, Drent RH (eds) Herbivores: between plants and predators. Blackwell Science, Oxford, pp 7–30

Turlings TCJ, Erb M (2018) Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. Annu Entomol 63:433–452

Via S, Conte G, Mason-Foley C, Mills K (2012) Localizing F(ST) outliers on a QTL map reveals evidence for large genomic regions of reduced gene exchange during speciation-with-gene-flow. Mol Ecol 21:5546–5560

Vieira FG, Sánchez-Gracia A, Rozas J (2007) Comparative genomic analysis of the odorant-binding protein family in 12 Drosophila genomes: purifying selection and birth-and-death evolution. Genome Biol 8:R235

Volpicella M, Ceci LR, Cordewener J, America T, Gallerani R et al (2003) Properties of purified gut trypsin from Helicoverpa zea, adapted to proteinase inhibitors. Eur J Biochem 270:10–19

Wanner KW, Robertson HM (2008) The gustatory receptor family in the silkworm moth *Bombyx mori* is characterized by a large expansion of a single lineage of putative bitter receptors. Insect Mol Biol 17:621–629

War AR, Sharma HC, Paulraj MG, War MY, Ignacimuthu S (2011) Herbivore induced plant volatiles: their role in plant defense for pest management. Plant Signal Behav 6:1973–1978

War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC (2012) Mechanisms of plant defense against insect herbivores. Plant Signal Behav 7:1306–1320

War AR, Sharma HC, Paulraj MG, Hussain B, Buhroo AA, War MY, Ignacimuthu S, Sharma HC (2013a) Effect of plant secondary metabolites on *Helicoverpa armigera*. J Pest Sci 86:399–408

War AR, Hussain B, Sharma HC (2013b) Induced resistance in groundnut by jasmonic acid and salicylic acid through alteration of trichome density and oviposition by *Helicoverpa armigera* (Lepidoptera: Noctuidae). AoB Plants 5:plt053. https://doi.org/10.1093/aobpla/plt053

War AR, Paulraj MG, Ignacimuthu S, Sharma HC (2015) Induced resistance to *Helicoverpa armigera* through exogenous application of jasmonic acid and salicylic acid in groundnut, *Arachis hypogaea*. Pest Manag Sci 71:72–82

War AR, Buhroo AA, Hussain B, Ahmad T, Nair, RM, Sharma HC (2020) Plant defense and insect adaptation with reference to secondary metabolites. In: Co-Evolution of Secondary Metabolites (Eds. Mérillon J-M, Ramawat KG). Springer Publishers. pp 795–822 https://doi.org/10.1007/978-3-319-76887-8_60–1

War AR, Taggar GK, Hussain B, Taggar MS, Nair RM, Sharma HC (2018) Plant defence against herbivory and insect adaptations. AoB Plants 10:ply037. https://doi.org/10.1093/aobpla/ply037

Webster B, Bruce T, Pickett J, Hardie J (2010) Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. Anim Behav 79:451–457

Wilson JS, Forister ML, Dyer LA, O'Connor JM et al (2012) Host conservatism, host shifts and diversification across three trophic levels in two Neotropical forests. J Evol Biol 25:532–546

Wittstock U, Agerbirk N, Stauber EJ, Olsen CE, Hippler M, Mitchell-Olds T, Gershenzon J, Vogel H (2004) Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proc Natl Acad Sci U S A 101:4859–4864

Wu J, Hettenhausen C, Meldau S, Baldwin IT (2007) Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of Nicotiana attenuata. The Plant Cell 19:1096–1122

Yang L, Fang Z, Dicke M, van Loon JJ, Jongsma MA (2009) The diamondback moth, Plutella xylostella, specifically inactivates Mustard Trypsin Inhibitor 2 (MTI2) to overcome host plant defence. Insect Biochem Mol Biol 39:55–61

Zhou X, Ma C, Li M, Sheng C, Liu H, Qiu X (2010) CYP9A12 and CYP9A17 in the cotton bollworm, Helicoverpa armigera: sequence similarity, expression profile and xenobiotic response. Pest Manag Sci 66:65–73

Zhu-Salzman K, Zeng R (2015) Insect response to plant defensive protease inhibitors. Annu Rev Entomol 60:233–252

Zhu-Salzman K, Koiwa H, Salzman RA, Shade RE, Ahn JE (2003) Cowpea bruchid Callosobruchus maculatus uses a three-component strategy to overcome a plant defensive cysteine protease inhibitor. Insect Mol Biol 12:135–145

Chapter 7
Biotechnological Interventions for Creating Novel Resistance Against Major Insect Pests of Rice

Pavneet Kaur, Kumari Neelam, Ankita Babbar, and Yogesh Vikal

7.1 Introduction

Rice is considered one of the most important cereal crops in the Asia-Pacific region. It has been estimated that half the world's population subsists wholly or partially on rice. Rice is mainly grown in tropical and subtropical areas worldwide spanning north at 53° N latitude and toward south at 39° S latitude and from sea level to altitudes of 3000 m. The warm and humid environment in which rice is grown is conducive to the proliferation of insects and pests. Globally, there are around 100 insect species to which rice plant remains vulnerable from sowing till harvest. The attack of insect pests is one of the major yield-limiting factors in rice causing up to 20–30% yield losses annually (Salim et al. 2001). Insects are the most abundant life form on earth, and their continuous evolution has become a major constraint to the global production of food and fiber. Insect pests, as a part of the natural ecosystem, pose serious constraints to the world's agricultural produce and thereby hamper the food security levels. Currently, many of the crops are suffering a yearly loss of about 36 billion USD in India due to insect pests (Dhaliwal et al. 2015; Rathee and Dalal 2018). In addition to direct impacts on yield, insects also reduce yields by making crops more susceptible to disease-causing pathogens (Haq et al. 2004). The insects/pests hamper the crop by negatively targeting the physiological and metabolic pathways at the different growth phases of rice. Several insects attack during the nursery stage leading to thrips (*Stenchaetothrips uniformis*), green leafhopper (*Nephotettix malayanus* and *N. virescens*), rice caseworm (*Nymphula depunctalis*), paddy stem borer (*Scirpophaga incertulas*), and swarming caterpillar (*Spodoptera mauritia*). In rice, a different range of biotic stress develops as a result of the infestation of insects

P. Kaur · K. Neelam (✉) · A. Babbar · Y. Vikal
School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India
e-mail: kneelam@pau.edu; yvikal-soab@pau.edu

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_7

in major field conditions, including stem borer (*Sesamia inferens*, *Scirpophaga incertulas*, *S. innotata*, *Chilo suppressalis, C. polychrysus*, *C. auricilius*), gall midge (*Orseolia oryzae*), swarming caterpillar (*Spodoptera mauritia*), leaf folder (*Cnaphalocrocis medinalis*), rice horned caterpillar (*Melanitis leda ismene* Cramer and *Mycalesis* sp.), yellow hairy caterpillar (*Psalis pennatula*), grasshopper (*Hieroglyphus banian*), rice hispa (*Dicladispa armigera*), whorl maggot (*Hydrellia philippina* Ferino), green leafhopper (*Nephotettix nicropictus*, *N. malayanus,* and *N. virescens*), brown planthopper (*Nilaparvata lugens*), white-backed planthopper (*Sogatella furcifera*), mealy bug (*Brevennia rehi*), rice earhead bug (*Leptocorisa acuta*), and thrips (*Stenchaetothrips biformis*) (Plate 7.1).

The infestation of various insects follows different modes of action in order to infect the host plant. Majority of the insects are classified as chewing insects, piercing insects, and sucking insects. Chewing damage is caused by insects with mouthparts that lead to mechanical damage of tissues, thereby promoting ingestion. The latter type includes hoppers, responsible for invading plant cells and sucking nutrients from vascular tissues. However, the extend of disease occurrence is highly dependent on the severity and exposure frequency of insects.

Over the years, the widespread use of insecticides/pesticides has led to the evolution of pesticide-resistant insects and reduction in beneficial insect population, along with the harmful impact on food safety, humans, and the environment (Fitt 1994; Gatehouse et al. 1994; Gunning et al. 1991; Haq et al. 2004). These problems have led researchers to develop different insect control approaches using various tools and techniques of genetic engineering, molecular biology, and plant biotechnology that are more environmentally friendly. The various techniques used in terms of biotechnological aspects have been successfully devised in various crops for crop improvement, viz., attaining herbicide tolerance in soybean, cotton, corn, and canola crops (Gianessi 2005). Herbicide tolerance has been proven to be beneficial for farmers by increasing crop productivity and environmental benefits for soil and water quality and eliminating the need for manual removal of weeds. The current biotechnological approaches significantly aim for improving abiotic and biotic stress tolerance in various crops worldwide. Similarly, plant biotechnology targets a varied number of regulatory components associated with the growth and development of crops aiding in their evolution and domestication, by improving their respective quality and yield attributes. Another aspect of biotechnology involves genomic hybrid breeding, providing a promising approach for attaining true superior hybrids with the minimum cost expense (Plate 7.2).

Considerable progress has been made in the past to incorporate resistance against insects/pests of rice. All these methodologies exploit the prevailing phenomenon of host plant resistance in an environmentally favorable manner. The significant insect-pest damage in the case of economically valuable crops, like cotton, tobacco, tomato, corn, sorghum, sunflower, pulses, rice, maize, and wheat, can be reduced by employing the modern biotechnological tools through critical analysis and engineering of biological processes. In the insect research field, biotechnological tools have been applied to study various issues, such as insect identification, insect

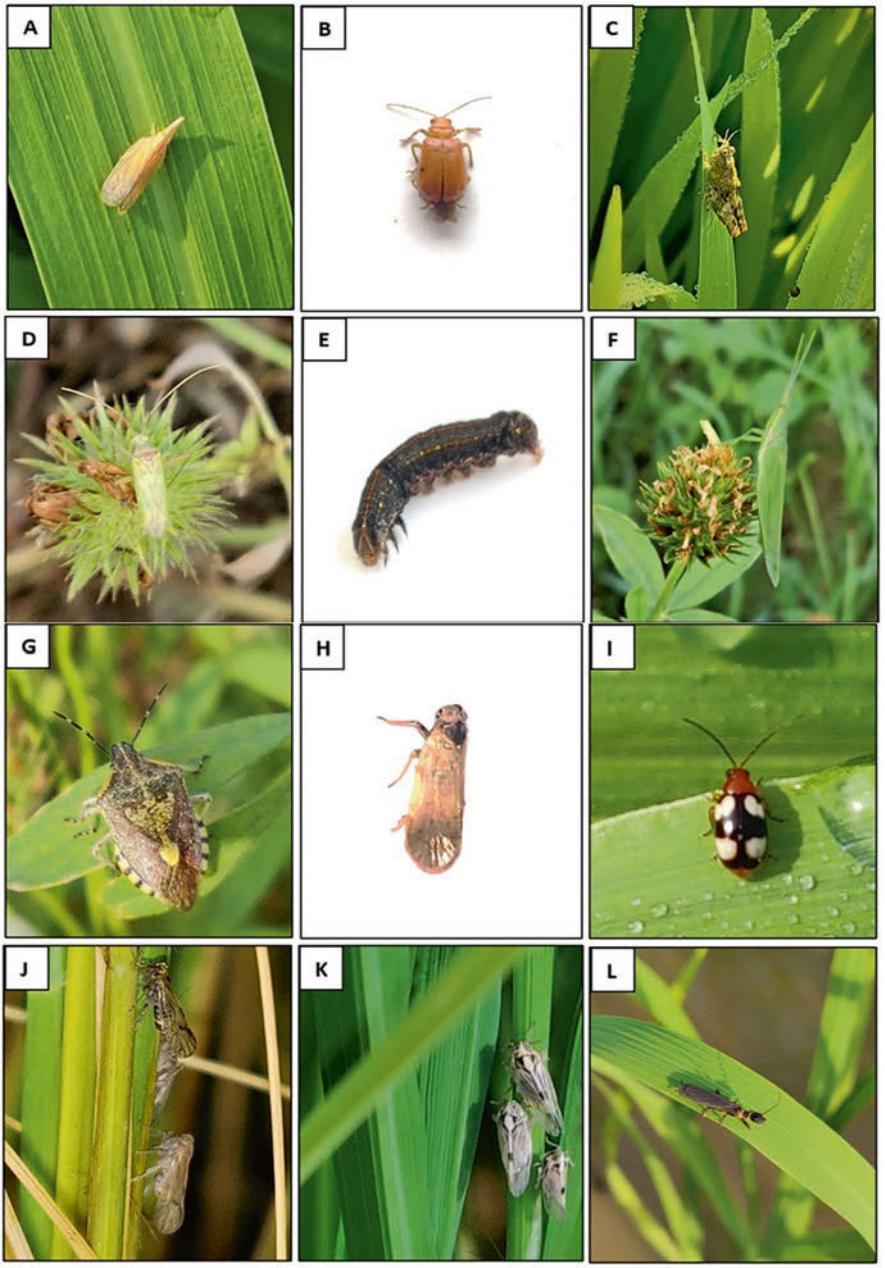

Plate 7.1 (**a**) Leaf hopper. (**b**) Stem borer. (**c**) Pygmy grasshopper. (**d**) Chinch bug. (**e**) Armyworm. (**f**) Chinese grasshopper. (**g**) Stink bug. (**h**) Rice delphacid. (**i**) Rice hispa. (**j**) Brown planthopper. (**k**) White-backed planthopper. (**l**) Rice thrip

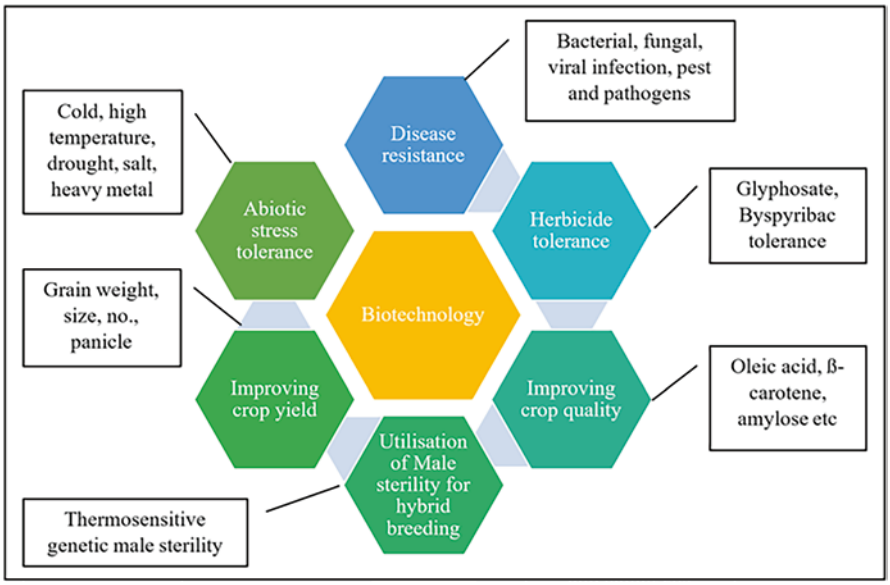

Plate 7.2 Applications of biotechnology in different aspects of crop improvement

control, and insect genetic relationships. It has a significant role in improving the potency and cost-effectiveness and in expanding the markets for bioinsecticides (Talukdar 2013). Genetic modification of the crops through biotechnology can potentially provide a much larger array of novel insecticidal genes along with conventional breeding. Since the commercialization of genetically modified crops in 1996, farmers have adopted the technology at such a dramatic rate, that in 2011, 16.7 million farmers in 29 counties planted 160 million hectares of biotech crops. In India alone, Bt cotton has increased cotton yields by up to 60% and has reduced insecticide sprays by around half. This in turn has led to an income increase of up to the US $11.9 billion per annum (James 2011). Thus, the insect control strategies that integrate advanced knowledge in biotechnology will contribute to the sustainability of agriculture. Extensive knowledge regarding the genotype of insect-resistant rice using biotechnological approaches unveils a wide range of molecular mechanisms that can open new avenues in the field of improvement.

Crop protection through effective management of insect pests and pathogens has remained the primary target for various advances in biotechnology. These advances could take place by progressing in genetic engineering and molecular biology, which have resulted in identification, isolation, characterization, and modification of resistance genes from diverse biological sources. Employment of DNA-based markers provides additional efficiency and precision via marker-assisted selection for the introgression of various resistant genes in rice cultivars. Recombinant DNA

(rDNA) technology has significantly expanded conventional crop protection by providing dramatic improvement in manipulating genes from diverse and exotic sources and inserting them into microorganisms and crop plants to confer resistance to insect pests and increased effectiveness of biocontrol agents. The availability of fully characterized genes, in turn, led to the development of plant biotechnology, making the transgenic expression of such genes possible in crop plants. Several such genes have already been exploited in different crop plants irrespective of any genetic barrier. However, only a limited number of such genes have afforded desired field resistance to transgenic plants against limited insect pest species. Currently, biotechnology is being applied for the precise characterization of insect pest species as well as the identification and characterization of novel genes for meaningful insect resistance. RNA interference (RNAi), on the other, hand has emerged as a powerful technique for downregulating gene expression in insects, whereas CRISPR Cas involves genome editing techniques for understanding the functions of target genes in diverse organisms. Additionally, a systematic study of the complete repertoire of metabolites/chemicals of any organism has given birth to a new area of research called "metabolomics." Integration of genomics and proteomics with metabolomics will enrich our understanding of the gene-function relationship that can be utilized in achieving crop improvement with a view to insect resistance. In this chapter, we will discuss various insect pests of rice, along with the biotechnological interventions, viz., genetic engineering, genomics, and the functional genomics approaches for managing the yield losses of rice.

7.2 Insects of Rice

The suitable environment favoring rice production promotes the proliferation of insects hampering its growth. These insects are enemies of rice production responsible for the reduction in total rice produce. The crop is attacked by more than 100 insect species, infesting varied plant parts by its specialized infesting organs and toxins (Table 7.1). Diverse insects attack the rice crop at a different stage of the life cycle. Majority of insects infesting rice plants attack during the vegetative stage belonging to the order Hemiptera, Homoptera, Orthoptera, Thysanoptera, Coleoptera, Lepidoptera, and Diptera. Among the insects attacking during the reproductive stage, green-horned caterpillar belongs to the minor pests of rice as its severity is too low. Among all insects, planthoppers, leafhopper, and leaf folders account for the cause of major alarming threats to rice production. Timely identification of insects is a key for accurate disease management strategy. The morphological identification of all these insects is aided by DNA barcoding differentiating insects in distinct species.

Table 7.1 Various insect-pests attacking rice crop at different developmental stages

Stage	Order	Family	Insect	Damage	Symptoms
Vegetative stage	Hemiptera	Pentatomidae	Black bugs *(Macropes excavatus)*	Phloem sap feeder	Stunting, wilting, chlorotic lesions, fewer tillers, panicles affected, and unfilled spikelet
	Homoptera	Pseudococcidae	Mealybugs *(Brevennia rehi)*	Infest leaf sheath and act by sucking sap	Yellowing, stunting, small leaves, irregular tillering, and underdeveloped panicles
	Orthoptera	Acrididae	Grasshoppers *(Hieroglyphus banian)*	Cutoff leaf areas and panicles	Excessive foliage damage
		Tettigoniidae	Field crickets *(Gryllus pennsylvanicus)*	Feeds on seeds, tillers and roots	Patches appear, cutting off of tillers at ground level
	Thysanoptera	Thripidae	Rice thrips *(Stenchaetothrips biformis)*	Tearing of the leaf tissues	Silvery streaks, wilting, stunting, curling, and discoloration of leaves and seedling death
	Coleoptera	Chrysomelidae	Rice hispa *(Dicladispa armigera)*	Adults target the upper surface of the leaf blade	White streaks and patches, withering of infected parts, reduced leaf area, less vigorous, stunted
	Lepidoptera	Pyralidae	Rice leaf folder *(Cnaphalocrocis medinalis)*	Larvae fold the leaves in the longitudinal direction with threadlike silk stitches	White stripe damage of leaf and vigor and photosynthetic ability hampered
			Rice caseworms *(Parapoynx stagnalis)*	Larvae responsible for scrapping chlorophyll from leaves	Cutting of leaf tips and visible as cylindrical tubes
		Noctuidae	Rice green semiloopers *(Naranga diffusa)*	Young and mature larvae scrape tissue from leaf blades and feed on leaf edges	Scraped leaves exposing lower epidermis and damaged leaf edges
			Armyworms *(Spodoptera frugiperda)* and cutworms *(Agrotis ipsilon)*	Larval feeding results in skeletonizing leaf blades	Leaves and panicle detachment
		Pyralidae and Noctuidae	Rice stem borers *(Scirpophaga innotata)*	Larval boring and feeding in the leaf sheath	Discolored areas, apical central leaf whorl browning and drying leading to affected tillers (dead heart), whitish empty panicles (whiteheads)

	Diptera	Diopsidae	Stalked-eyed flies *(Teleopsis dalmanni)*	Maggots feed on the growing zone of plant	Central whorl does not open, dries, and dies (dead heart)
		Cecidomyiidae	Rice gall midge *(Orseolia oryzae)*	Infestation of plants in seedbed only	Tillers appear in the form of tubular galls
		Ephydridae	Rice whorl maggots *(Hydrellia sasakii)*	Feeds on inner margins of unfurled leaves	Discolored areas, dried, drooped, wilted and deformed leaves, stunting, less tillering, delayed panicle initiation and maturity
Reproductive stage	Lepidoptera	Satyridae	Green horned caterpillars *(Melanitis leda ismene)*	Larvae feed on leaf margins and blades	Removal of leaf tissue and veins
		Hesperiidae	Rice skippers *(Parnara mathias)*	Leaf tissue removal followed by leaf rolling	Removal of leaf tissue and veins and rolling of two edges of leaf tied with silken threads
	Homoptera	Delphacidae	Planthoppers: Brown planthopper *(Nilaparvata lugens)* White-backed planthopper *(Sogatella furcifera)* Small brown planthopper *(Laodelphax striatellus)*	Sap-sucking insects targeting xylem and phloem; transmits various viruses	Complete drying of plant, heavy infestation responsible for hopperburn (complete drying out)
		Cicadellidae	Leafhoppers *(Nephotettix nigropictus)*		
Ripening stage	Hemiptera	Pentatomidae	Stink bugs *(Halyomorpha halys)*	Feeds on endosperm of developing grains	Brown spots, shriveled and empty grains, discoloration of grains

7.3 Biotechnological Approaches

With the advent of genetic engineering and several tools of biotechnology, viz., genetic engineering tissue culture (anther culture, embryo culture), genetic transformation for insect resistance, inhibitors of several digestive enzymes, marker-assisted selection (MAS) for plant resistance to insect, pyramiding of resistant genes into a single cultivar, and development of insect-resistant plants using RNAi and CRISPR Cas have been accelerated. The acceptability of biotechnology products may be greater along with the increase in better understanding of biotechnological processes.

7.3.1 Genetic Engineering

The expanding knowledge regarding the genome and harboring genes has prompted advancement in the development of transgenics for the incorporation of resistance-conferring genes in commercially important rice varieties. Tissue culture offers the potential to contribute to the improvement of crop plants through the manipulation of plants at the cellular level. With the commencement of genetic transformation, it has become possible to replicate and introduce genes into the crop plants to produce resistance to insect pests. Insect-resistant genetically modified crops are offering great benefits for farmers. Gene resistance against various insects has been introduced into crop plants, such as maize, cotton, potato, tobacco, potatoes, rice, broccoli, lettuce, walnuts, apples, alfalfa, and soybean (Griffiths 1998). As the products of most transgenes are ingested by the insect pest and therefore act through the gut, most of the focus has been on transgene-encoded proteins that target the insect midgut and/or the peritrophic membrane to disrupt digestion or nutrition (Czapla and Lang 1990; Hopkins and Harper 2001; Murdock et al. 1990; Eisemann et al. 1994; Harper et al. 1998). Generally, the detrimental effects on larval and insect growth result from limited assimilation of nutrients (Williams 1999; Lopes et al. 2004; Zavala and Baldwin 2004; Silva et al. 2006). The use of transgenic plants that express insecticidal agents thus reduces the population of insect pests, usage of chemical insecticide, and the ecological damage they may cause (Schuler et al. 1998). To date, the most successful transgenes for insect control have been the genes encoding insecticidal toxins from the soil bacterium *Bacillus thuringiensis* (Table 7.2).

Bt cotton has been genetically adapted by the accumulation of one or more genes from general soil bacteria, *Bacillus thuringiensis*. These genes produce insecticidal proteins, and therefore, genetically transformed plants generate one or more toxins. Bollworms are responsible for 60–70% of damage to cotton plants. Boll guard I and Boll guard II exhibited a reduction in the number of damaged bolls of 61 and 95%, respectively, compared with the conventional variety (Estruch et al. 1996). *VIP 3A + Cry 1Ab* expressing line gives the maximum mortality of susceptible and resistant strain of *Heliothis virescens* as compared to individual toxin expressing line and

Table 7.2 Bt transgenic plants expressing genes for insect resistance

Crop	Gene(s) for insect resistance	Target insect	References
Tobacco	*Magi6 peptide*	*Spodoptera frugiperda*	Hernandez-Campuzano et al. (2009)
	cry1Ac and *cry3A*	*Helicoverpa armigera* Hubner	Yuan et al. (2017a, b)
	cry1Ac and *cry2A*	*Phthorimaea operculella* Zeller	Bakhsh et al. (2018)
	SmchiC	*Botrytis cinerea* and *S. frugiperda*	Navarro-González et al. (2019)
	Arginine kinase	*Helicoverpa armigera* Hubner	Ai et al. (2019)
	Vigna mungo protease inhibitor (*VmPI*)	*Spodoptera litura*	Mudiyappanayar and Koundal (2020)
Tomato	*Proteinase inhibitor 2* (*Pin2*)	*Tuta absoluta* (Meyrick)	Hamza et al. (2018)
	cry2AX1	*H. armigera* and *S. litura*	Sushmitha et al. (2018)
Potato	*cry1Ab*	*P. operculella* Zeller	Salehian et al. (2021a)
	cry3A	Colorado potato beetle	Salehian et al. (2021b)
Sugarcane	*Vip3A*	*Chilo infuscatellus*	Riaz et al. (2020)
Maize	*Cry1Ab/Cry2Aj*	*Ostrinia furnacalis*, *H. armigera,* and *Mythimna separata*	Liu et al. (2018)
	Cry1Ab, Vip3Aa20	*S. frugiperda*	Eghrari et al. (2021)
Rice	*cry1Ac and CpTI*	*Chilo suppressalis, Cnaphalocrocis medinalis,* and *Scirpophaga incertulas*	Han et al. (2008)
	Maize proteinase inhibitor and *potato carboxypeptidase inhibitor* fusion gene	*C. suppressalis*	Quilis et al. (2014)
Rice	*miR-14*	*C. suppressalis*	He et al. (2019)
Rice	*Asal*	*Sogatella furcifera* (WBPH), *Nephotettix* sp. (GLH), and *Nilaparvata lugens* (BPH)	Yarasi et al. (2008)
	Asal and *Galanthus nivalis* (*gna*) lectin genes	*S. furcifera*, *Nephotettix* sp., and *Nilaparvata lugens* (BPH)	Bharathi et al. (2011)
	Dioscorea batatas tuber lectin 1 (DB1)	*N. lugens*	Yoshimura et al. (2012)
	Asal	*N. lugens*	Chandrasekhar et al. (2014)
	Cry1Ac::Asal	*S. incertulas*, *C. medinalis,* and *N. lugens*	Boddupally et al. (2018)
	Cry1Ab and *Vip3A* fusion protein	*C. suppressalis* and *C. medinalis*	Xu et al. (2018a, b)

non-*Bt* line. *Bt* is very specific to particular insect pests and does not have any direct effect on any of the nontargeted beneficial insects. *Bt* rice provides resistance against various stem borers such as the following: striped stem borer (*Chilo suppressalis*), yellow stem borer (*Scirpophaga incertulas*), and pink stem borer (*Sesamia inferens*). More than 70 transgenic *Bt* rice lines of three selected cultivars, IR64, Pusa Basmati-1, and Karnal local, have been produced using the artificial shortened *Bt* gene, *cry1Ac*. The *Bt* brinjal provides resistance against brinjal shoot and fruit borer. The first transgenic brinjal carried a synthetic *Bt-cry1Ab* gene. At all locations, the *Bt* variety (MHB *Bt*) had significantly less brinjal fruit and shoot borer larvae and percent fruit damage. The transgenic *Bt* tomato expressing *Cry1Ab* protein, *CpTi* gene, etc. is effective against *Helicoverpa armigera*. Leaf-specific overexpression of the potato PI–II and carboxypeptidase inhibitors (PCI) results in resistance to *Heliothis obsoleta* and *Liriomyza trifolii* larvae in homozygote tomato lines expressing high levels of the transgenes. The transgenic sugarcane lines were generated expressing *Vip3A* toxin driven by polyubiquitin promoter for resistance against sugarcane stem borer. A direct correlation was observed between the *Vip3A* protein and *Vip3A* transgene expression in the transgenic sugarcane lines. In in vitro insect bioassay on V1, *Vip3A* transgenic sugarcane lines exhibited high resistance to *C. infuscatellus* with up to 100% mortality compared to the control sugarcane line. Thus, a single copy insertion of the *Vip3A* gene in transgenic sugarcane lines renders them resistant to borer, and these lines can be potentially used for the generation of insect-resistant transgenic sugarcane and could also be employed in gene pyramiding with *Bt* toxin to prolong resistance (Riaz et al. 2020).

Han et al. (2008) reported genetically modified rice lines containing *cry1Ac* and *CptI* (cowpea trypsin inhibitor) to provide resistance against *Chilo suppressalis*, *Cnaphalocrocis medinalis*, and *Scirpophaga incertulas* pests for rice. The transgenics so developed reveals fluctuation in disease reaction toward the survival of *Sesamia inferens* (Pink Stem borer) larvae. Thus, further investigations were devised to delay its population density. Quilis et al. (2014) explained the role of proteinase inhibitors including maize proteinase inhibitor (MPI) and potato carboxypeptidase inhibitor (PCI) in insect resistance. Their fusion, followed by an introduction to rice plants, revealed a reduction in larval weight of *C. suppressalis* (striped stem borer), which is a major pest of rice. Also, the plants expressing *mpi-pci* fusion gene display enhanced resistance against *Magnaporthe oryzae,* the causal organism for rice blast. Thus, the fusion gene was reported to provide resistance for insects and pathogens as well in rice. He et al. (2019) demonstrated the transgenic lines with overexpressing *miR-14*, an insect-specific mRNA leading to the death of striped stem borer individuals. The *miR-14* has been reported to regulate metamorphosis in a variety of insects (Jayachandran et al. 2013; Liu et al. 2013; Varghese and Cohen 2007). Its overexpression resulted in interference with normal metamorphosis development of the insect by eliminating the functions of ecdysone after molting. Developing transgenic insect-resistant rice lines using miRNA significantly broadens the scope of target genes for pest control. Yarasi et al. (2008) reported the introduction of *Allium sativum* leaf lectin gene (*asal*) into indica rice cultivars susceptible to brown planthopper (BPH), green leafhopper (GLH), and white-backed planthopper (WBPH).

The calli were cocultivated with *Agrobacterium* comprising of pSB111 vector harboring *asal*, along with the herbicide resistance gene *bar*, under the control of CaMV35S promoter. The bioassay involving the expression of foreign gene reveals entomotoxic effects on BPH, GLH, and WBPH insects, with their decreasing survival, development, and fecundity of the insects. Also, the *asal* transgenic rice lines are a promising source of resistant cultivars. Among the sap-sucking pests, Bharathi et al. (2011) demonstrated the positive correlation of transgenic rice plants bearing pyramided *asal* and *gna* (*Galanthus nivalis*) lectin genes with the enhanced resistance conferred by the plant. Against BPH, transgenic lines have been developed, harboring *Dioscorea batatas* tuber lectin 1, and *asal* gene shows a high level of resistance against *Nilaparvata lugens* independently reported by Yoshimura et al. (2012) and Chandrasekhar et al. (2014). Boddupally et al. (2018) reported transgenic rice plants with Cry1Ac: ASAL fusion protein to provide resistance against the yellow stem borer (YSB), leaf folder (LF), and brown planthopper (BPH). The bioassays revealed 100%, 80–100%, and 70–80% mortality rate of pests of YSB, LF, and BPH, respectively. The study implied the enhanced efficacy of *Cry1Ac::Asal* fusion protein in minimizing pest population and providing insect resistance. Similarly, Xu et al. (2018a, b) reported the expression of the fusion protein of *Cry1Ab* and *Vip3A* protein in transgenic rice lines displayed efficient resistance against two major pests, viz., *C. suppressalis* and *C. medinalis.* Henceforth, these studies imply the role of transgenic rice plants harbors the significant potential for insect resistance management following various tissue culture and genetic engineering protocols.

7.3.2 Marker-Assisted Selection (MAS)

Locating and identifying genes of interest responsible for resistance is crucial for breeding insect-resistant varieties. The molecular marker-assisted selection of crops is one of the most fundamental applications of biotech tools. This progress has been facilitated by the construction of high-density genetic maps of certain plants and insects. Researchers have utilized molecular markers in crops linked to genes expressing resistance to several major insect pests. Molecular markers have been effectively applied for rice improvement. The main advantages of molecular markers include consistency, biosafety, time-saving, and efficient and accurate selection of complex traits (Jena and Mackill 2008). Application of molecular markers includes selecting the plants harboring specific genomic regions responsible for the expression of traits of interest (Das et al. 2017). The identified molecular markers are either linked to a single major gene for resistance or a group of loci controlling the expression of quantitative resistance known as quantitative trait loci (QTL). The first known case of QTL mapping for plant resistance to insects was in tomato, *Lycopersicon esculentum* Mill. The wild species of tomato, *L. hirsutum f. glabratum*, conferring resistance to arthropod pests had a principal toxic factor, viz., 2-tridecanone (2-TD). A mapping population of 74 F_2 individuals was evaluated for the

amount of 2-TD, and the marker loci on three different linkage groups were found associated with expression levels of 2-TD. In case of yellow stem borer (YSB) resistance, the detection of major quantitative trait loci could be of considerable value for insect resistance breeding programs, since their incorporation in susceptible genotypes permits a direct increase of the resistance level in the improved genotypes. Identification of markers associated with YSB resistance facilitates selection in applied breeding given the inherent difficulties in field-based screening for this pest. Linkage analysis with the F_2 phenotypic scores and RAPD data revealed that the RAPD markers K6695, C1320, and AH5660 were at a distance of 12.8 cM, 15.2 cM, and 14.9 cM, respectively, from the gene (s) of interest (Kammar and Nitin 2019).

At present, considerable attention has been focused on the resourceful wild species of rice for breeding purposes. The genus *Oryza* harbors 22 wild and 2 cultivated species. Among these, wild accessions represent an exclusive collection of rich germplasm bearing huge potential in crop improvement. Khush states that cultivated and wild species belong to different categories of genome, viz., AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, and HHKK. Wide hybridization has been successfully applied since many years for providing resistance against various biotic and abiotic stresses in rice. It has been used to delimit the genotypes possessing exclusive properties for providing resistance, and thus selection of such genomes allows precise introgression for disease resistance. We will discuss some of the examples in the next paragraph.

The wild relative of rice, *O. australiensis* (accession 100,882), belonging to the EE genome displayed strong resistance and thus serves as a potential source of BPH resistance development. The *BPH10* and *BPH18* identified from *O. australiensis* harbor resistance to four biotypes of BPH, both belonging to the long arm of chromosome 12. Also, another QTL named *qBPH4.2* was found on the short arm of chromosome 4 and narrowed down to a 300 kb genomic region of the Nipponbare genome bracketed by RM261 and S1 markers (Hu et al. 2015a). *O. officinalis* has been found a significantly important source for BPH resistance comprising of *bph11*, *BPH12*, *BPH13*, *BPH14*, *BPH15*, *qBPH3*, and *qBPH4*. This wild species has been reported for the successful identification and introgression of various resistance gene(s)/QTLs WBPH7, WBPH8, *qSBPH3d, qSBPH7a*, and *qSBPH12b* against other planthoppers, viz., WBPH and SBPH. *O. rufipogon* stands as a progenitor of present-day cultivated rice possessing enriched genetic diversity and, thus, a significant reservoir for crop improvement programs in rice. This wild relative harbors diverse QTLs contributing tolerance toward various biotic and abiotic stresses (Ma et al. 2015; Vaughan et al. 2003; Xiao et al. 1998). BPH resistance from *O. minuta* belonging to BBCC genome has been successfully transferred to cultivated rice, henceforth responsible for providing a wide spectrum BPH resistance. Three dominant genes *BPH20*, *BPH21*, and *BPH23* have been reported for successful introgression from *O. minuta*. Also, *O. glaberrima* belonging to the cultivated rice category has been reported as a resistance source for BPH, GRH, and GLH. Apart from the usefulness of *O. nivara* genome against various abiotic

stresses, it has been successfully used to derive BPH resistance in the form of *BPH34* gene.

Collard and Mackill (2007) have reviewed the application of molecular markers in various rice improvement programs with superior advantages of molecular markers in terms of time, consistency, biosafety efficiency and accuracy. A diverse set of DNA markers have been effectively employed to identify resistance gene(s)/QTLs following MAS for integrating different resistance gene(s)/QTLs into the rice cultivars lacking the desired disease tolerance traits. Various genes and QTLs were identified from a wide rice germplasm worldwide against BPH, WBPH, SBPH, gall midge, green rice leafhopper, green leafhopper, and rice leaf folder for developing resistant varieties (Table 7.3).

Table 7.3 Details of the donor resources along with linked markers used in MAS

Source	Gene (s)/QTLs name	Chr	Linked markers	References
Cheongcheongbyeo	*BPH1*	12L	pBPH4-14	Cha et al. (2008)
ASD7	*bph2*	12L	RM1246-463	Sun et al. (2006)
Rathu Heenati	*BPH3*, *BPH17*	6S, 4S	RM1929-8072, RM8213-5953	Jairin et al. (2007b) Sun et al. (2005)
Babawee	*bph4*	6S	RM589-586	Jairin et al. (2010)
ARC10550	*bph5*	–	–	Khush et al. (1985)
Swarnalata	*BPH6*	4L	RM16994-119	Kabir and Khush (1988), Qiu et al. (2010)
T12	*bph7*	12L	RM3448-313	Kabir and Khush (1988), Qiu et al. (2014)
Chin Saba	*bph8*	–	–	Nemoto et al. (1989)
Pokkali	*BPH9*	12L	InD2-RsaI	Nemoto et al. (1989), Zhao et al. (2016)
O. australiensis	*BPH10*, *BPH18*, *qBPH4.2*	12L, 12L, 4S	RG457-CDO459, BIM3-BN162, RM261-XC4-27	Ishii et al. (1994) Ji et al. (2016) Hu et al. (2015a)
O. officinalis	*bph11, BPH12, BPH13, BPH14, BPH15*	3L, 4S, 3S, 3L, 4S	G1318, RM16459-1305, RZ892-RG191, S M1-G1318, RG1-RG2	Hirabayashi et al. (1998) Qiu et al. (2012) Renganayaki et al. (2002) Du et al. (2009) Yang et al. (2004)
	qBPH3, qBPH4	3, 4	t6-f3, P17-xc4-27	Hu et al. (2015b)
	WBPH7, WBPH8	3, 4	R1925-G1318, R288-S11182	Tan et al. (2004)
	qSBPH3d, qSBPH7a, qSBPH12b	3, 7, 12	RM218-745, RM7012-6338, RM463-6256	Zhang et al. (2014)

(continued)

Table 7.3 (continued)

Source	Gene (s)/QTLs name	Chr	Linked markers	References
M1635–7	*BPH16*	12	RM6732-R10289	Hirabayashi et al. (2004)
O. rufipogon	*bph18(t), bph19(t), bph22(t), bph23(t), bph24(t), BPH27, bph29, bph30, BPH36*	4L, 12, 4, 8, –, 4L, 6S, 10S, 4S	RM273-6506, RM17, RM8212-261, RM2655-3572, –, RM16846-16853, BYL8-BID2, RM222-244, RM16465-16502	Li et al. (2006) Li et al. (2006) Hou et al. (2011) Hou et al. (2011) Deen et al. (2010) Huang et al. (2013) Wang et al. (2015) Yang et al. (2012) Li et al. (2019)
	qWPH2, qWBPH5, qWBPH9	2, 5, 9	RM1285-555, RM3870-RZ70, RG451-RM245	Chen et al. (2010a, b)
	GRH5	8	RM3754-3761	Fujita et al. (2006)
AS20–1	*bph19(t)*	3S	RM6308, RM3134	Chen et al. (2006)
O. minuta	*BPH20(t), BPH21(t), BPH23(t)*	4S, 12L,	B42:B4, M510, RM5953, S12094A-B122	Rahman et al. (2009) Rahman et al. (2009) Ram et al. (2010)
O. glaberrima	*BPH22(t)*	–	–	Ram et al. (2010)
	qGRH9	9	RM215-RM2482	Fujita et al. (2010)
ADR52	*bph25, BPH26*	6S, 12L	S00310-RM8101, DS72B4-DS173B	Myint et al. (2012) Tamura et al. (2014)
	WBPH3	–	–	Hernandez and Khush (1981)
Balamawee	*BPH27(t)*	4L	Q52, Q20	He et al. (2013)
DV85	*BPH28(t)/QBPH11*	1L	InDel55, InDel66	Wu et al. (2014)
AC-1613	*BPH30*	4S	SSR28, SSR69	Wang et al. (2018)
CR2711–76	*BPH31*	3L	PA26, RM2334	Prahalada et al. (2017)
PTB33	*BPH32*	6S	RM19291, RM8072	Ren et al. (2016)
KOLAYAL	*BPH33*	4S	H99, H101	Hu et al. (2018)
O. nivara	*BPH34*	4L	RM16994, RM17007	Kumar et al. (2018)
IR64	*BPH37*	1	RM302, YM35	Yang et al. (2019)
Khazar	*BPH38(t)*	1L	SNP-693369, id10112165	Balachiranjeevi et al. (2019)
Salkathi	*qBPH4.3*	4	RM551, RM335	Mohanty et al. (2017)
	qBPH4.4	4	RM335, RM5633	
IR71033–121-15	*qBPH6(t)*	6	RM469, RM568	Jairin et al. (2007a)
Nagina 22	*WBPH1*	7	–	Sidhu et al. (1979)
ARC10239	*WBPH2*	6	RZ667	Angeles et al. (1981), Liu et al. (2002)

(continued)

Table 7.3 (continued)

Source	Gene (s)/QTLs name	Chr	Linked markers	References
Podiwi A8	*wbph4*	–	–	Hernandez and Khush (1981)
N'Diang Marie	*WBPH5*	–	–	Wu and Khush (1985)
Guiyigu	*WBPH6*	11	RM167	Li et al. (2004)
Sinna Sivappu	*wbph9(t), wbph10(t), wbph11(t), WBPH12(t)*	6, 12, 4, 4	RM589-539, SSR12-17.2-RM28487, RM3643-1223, RM16592-16649	Ramesh et al. (2014)
Asominori	*Ovc, qOVA-1-3, qOVA-4, qOVA-5-1, qOVA-5-2*	6, 1, 4, 5, 5	R2373-C946, XNpb346-C112, R1854, XNpb251-R3313, C1268	Yamasaki et al. (2003)
Chuanjiang 06	*qWL6*	6	M3, M5	Yang et al. (2014)
	qRLF-3, qRLF-4, qRLF-8	3, 4, 8	RM1022-7, RM3276-255, RM72-331	Rao et al. (2010)
IR54751	*qWBPH3.2, qWBPH11*	3, 11	InDel3-23-InDel3-26, DJ53973-SNP56	Fan et al. (2018)
Mudgo	*qSBPH2b, qSBPH3d, qSBPH12a*	2, 3, 12	RM29-5791, RM5442-3199, I12-17, RM3331	Duan et al. (2009)
Kasalath	*qSBPH2, qSBPH3, qSBPH8, qSBPH11*	2, 3, 8, 11	R712-R1843, C1135-C80, R1943-C390, G257-S2260	Duan et al. (2010)
N22	*qSBPH2, qSBPH3, qSBPH5, qSBPH7, qSBPH11*	2, 3, 5, 7, 11	RM263-1385, RM22-545, RM153-413, RM234-429, RM209-RM21	Wang et al. (2013)
9194	*qSBPH1, qSBPH5, qSBPH8, qSBPH9*	1, 5, 8, 9	RM3738-8236, RM18452-163, RM210-3845, RM257-160	Sun et al. (2017)
WR24	*qSBPH5, qSBPH7, qSBPH10*	5, 7, 10	Indel 5–11, RM3664, RM6403-234, RM25664-228	Xu et al. (2018b)
W1263	*GM1*	9S	RM444-219	Biradar et al. (2004)
Phalguna	*GM2*	4	RM241-317	Himabindu et al. (2007)
RP2068-18-3-5	*gm3*	4	RM17480-gm3SSR4	Sama et al. (2014)
Abhaya	*GM4*	8L	RM22551-22562	Divya et al. (2015)

(continued)

Table 7.3 (continued)

Source	Gene (s)/QTLs name	Chr	Linked markers	References
ARC5984	*GM5*	12	RM101-309	Dubey and Chandel (2010)
Duokang #1	*GM6*	4L	RG214-RG476	Katiyar et al. (2001)
RP2333–156-8	*GM7*	4	F8LB-SA598	Sardesai et al. (2002)
Aganni	*GM8*	8S	RM22685-22709	Divya et al. (2018)
Line9	*GM9*		–	Shrivastava et al. (2003)
BG 380–2	*GM10*	–	–	Kumar et al. (2005)
CR57-MR1523	*GM11*	12	RM28574-28706	Himabindu et al. (2010)
IR24	*GRH1*	5	R569-C309	Kadowaki et al. (2003)
DV85	*GRH2*	11	R2458-C50	Kadowaki et al. (2003)
Rantaj emas 2	*GRH3*	6	C288B-C133A	Saka et al. (2006)
DV85	*GRH4*	3	C1186-R2982	Kadowaki et al. (2003)
SML17, IRGC105715	*GRH6*	4	RM8213-C708	Fujita et al. (2004), Tamura et al. (2004)
Maddani Karuppan	*GLH 7*	–	–	Rezaul Karim and Pathak (1982)
DV85	*glh8*	–	–	Ghani and Khush (1998)
IR28	*GLH 9*	–	–	Angeles and Khush (1999)
IR36	*glh10*	–	–	Angeles and Khush (2000a)
IR20965-11-3-3	*GLH 11*	–	–	
ARC10313	*GLH 12*	–	–	Angeles and Khush (2000b)
Asmaita	*GLH 13*	–	–	
ARC11554	*GLH 14*	4	Y3635-RZ262	Sebastian et al. (1996)
Taichung Native 1	*qRLF-1*	1	RM3412-6716	Rao et al. (2010)
	qRLF-2	2	RM207-48	

7.3.3 Gene Pyramiding

Improved insect resistance has also been achieved through the employment of multiple resistance genes in a single plant, also known as gene stacking or gene pyramiding. Multiple insect-resistant genes stacking in the transgenic *Bt* crops have been employed to confer resistance to the insects and herbicides. The first transgenic *Bt* crop (cotton) with stacked genes, *Cry1Ac* and *Cry2Ab2*, registered for use in the USA in 2002, was Bollgard II. These stacked genes in the transgenic cotton have been very effective against the pink bollworms (*Pectinophora gossypiella*)

(Stefey et al. 2009). These genes (*Cry1Ac* and *Cry1C*), also stacked in transgenic Bt broccoli, had the potential to delay resistance to the diamondback moth (*Plutella xylostella*) more effectively than the transgenic plants with single Bt gene (Zhao et al. 2003). Wang et al. (2017) developed LuoYang69 restorer line of 93–11, harboring two pyramided BPH resistance genes, *BPH6* and *BPH9*, using marker-assisted selection. The resultant line displays an enhanced resistance reaction toward BPH. He et al. (2020) reported pyramiding of *BPH3*, *BPH14*, *BPH18*, and *BPH32* resistance genes in Guang 8B rice variety. The study suggested additional increase in resistance level by the introduction of four genes. Venkanna et al. (2018) provided evidence for stacking three gall midge resistance genes—*Gm1*, *gm3*, and *Gm8*—in an improved line WGL-1068, developed as the F5 generation of the cross between Kavya (susceptible cultivar) and gall midge-resistant introgression line Samba Mahsuri. Apart from gall midge resistance, the improved line possesses high-yielding and fine-grain characters better than elite variety Kavya. Wang et al. (2017) developed LuoYang69 restorer line of 93-11 harboring two pyramided BPH resistance genes *BPH6* and *BPH9* using marker-assisted selection. The resultant line displays an enhanced resistance reaction towards BPH. He et al. (2020) reported pyramiding of *BPH3*, *BPH14*, *BPH18* and *BPH32* resistance genes in Guang 8B rice variety. The study suggested an additional increase in resistance level by the introduction of 4 genes. Venkana et al. (2018) provided evidence for stacking 3 gall midge resistance genes; *Gm1*, *gm3* and *Gm8* in an improved line WGL-1068 developed as F5 generation of the cross between Kavya (susceptible cultivar) with gall midge resistant introgression line Samba Mahsuri. Apart from gall midge resistance, the improved line possesses high yielding and fine-grain characters better than elite variety Kavya. Jena et al. (2017) developed 25 NILs, among which 16 lines belonged to multiple resistance gene combinations. Apart from these, multiple disease resistance programs have revolutionized breeding programs recently. Reinke et al. (2018) developed various moderately resistant lines, harboring brown planthopper, rice stripe virus, rice blast, and bacterial blight-resistant genes in different combinations. Following the marker-assisted selection, the MR lines selected were encompassing *BPH18*, *qSTV11*^{SG}, *Pib* and *Pik*, and *Xa40* or *Xa3* to provide stable resistance with effect on major agronomic traits. The pyramiding of genes harbors profound antibiosis reactions during BPH infestation as compared to single resistance gene bearing lines. This way, critically developed pyramided lines can act as a rich genetic source for breeding purposes in light of insect resistance (Plate 7.3).

7.3.4 Functional Genomics

Functional genomics emerges as an advanced field of biotechnology that has presented diverse platforms in agricultural research programs. Rice is considered as a model plant for functional genomics studies owing to its smaller genome, sequenced genome, vast transformation methodologies, and abundant germplasm availability (Jiang et al. 2012). Among the rice germplasm, the availability of wild relatives, rice

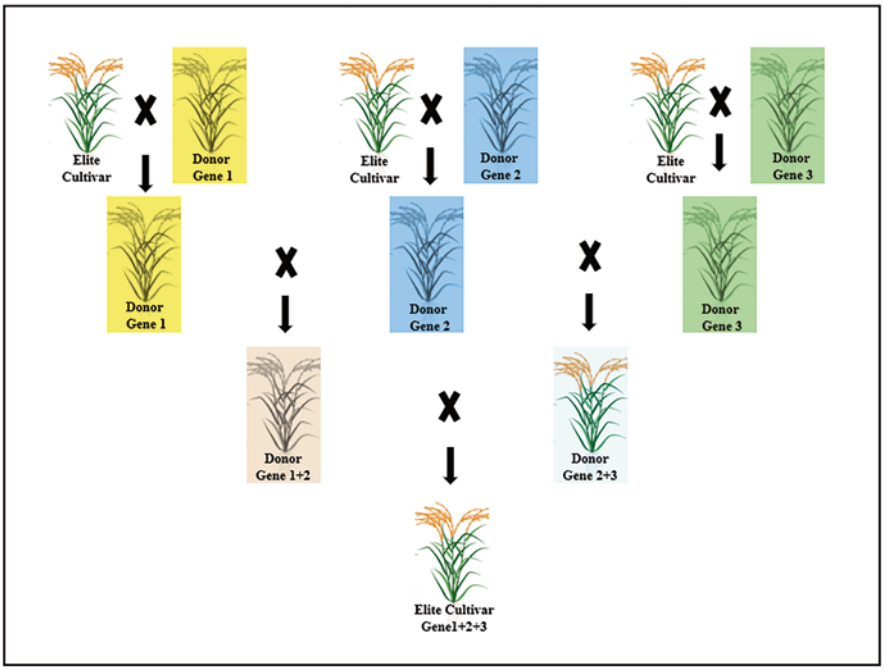

Plate 7.3 Gene pyramiding in elite rice cultivar with multiple insect resistance genes

mutant libraries and rice genome-based databases, opens new avenues for functional genomics studies relating to other crop plants as well. This field deals with the functional characterization of various genes in the genome, which is obtained through gain or loss of functions in plants. Wei and Chen (2018) presented a report focusing on the comparison of the basic helix-loop-helix transcription factors (bHLH) in *Arabidopsis*, rice, maize, and wheat. The comparative functional genomics studies were carried using available genome assembly databases. Among the family, different subfamilies confirmed their role in iron uptake, anther development, disease tolerance via different defense pathways, and secondary metabolite production. The resultant information regarding constitutive and differentially expressing bHLH can serve as a hub of its functional characterization in *Gramineae* species, thus contributing toward molecular breeding approaches. The fungus *Magnaporthe oryzae* is considered to secrete proteins that are responsible for disease reactions in rice plants. However, the functions of effector proteins are not explored in a way to enhance disease resistance. Guo et al. (2019) deduced the functional aspect of various proteins of the fungus, following transient expression assays of 98 in planta-expressed *M. oryzae*. The researcher devised eight novel proteins, MoCDIP6 to MoCDIP13, responsible for rice blast owing to death. Thus, similar studies can help to accelerate the understanding of mechanisms underlying the pathogenic infection, which in turn can be utilized as a key source for

developing resistant cultivars. Among the genes pertaining to host, Li et al. (2020) utilized the transgenic plants with insect-inducible promoters as an important strategy for resistance against the striped rice stem borer (*Chilo suppressalis*). This first reporter of SSB-inducible promoter states the upregulation of *hydroperoxide lyase* gene (*OsHPL2*) post insect feeding. Thus, cloning strategy was directed toward the promoter of this gene, devising the promoter and positive regulatory regions exhibiting SSB larval mortality. Thus, functional information related to the host as well as pathogen genes and promoters can serve as a potential source for accelerating the insect-resistant rice cultivars.

7.3.5 RNA Interference (RNAi)

Since the discovery that dsRNA can silence genes, RNAi has been developed as an effective tool for regulating gene expression (Vogel et al. 2019). This approach bears significant potential in the field of crop improvement due to its preferential target specificity and low negative environmental effect (Chung et al. 2021). RNAi or gene silencing has been used to inhibit virus replication in transgenic plants and has the potential to be developed commercially for insect management also. RNAi constructs directed toward targeting insect-derived genes are considered as a promising approach for agricultural pest control (Chung et al. 2021). Insect genes can be downregulated by injection of dsRNA or by oral administration of high concentrations of exogenously supplied dsRNA as part of an artificial diet, but a much more efficient method of delivering dsRNA is needed before RNAi technology can be used to control pests in the field (Mao et al. 2007; Bettencourt et al. 2002). Before now, a very sensitive RNAi response has been observed in the Western corn rootworm (WCR) *D. virgifera virgifera*, to oral administration of dsRNA and the first RNAi-based insecticides for the control of this insect have already been approved by the US Environmental Protection Agency (EPA). This plant-incorporated protectant (PIP) employs pyramid strategy where several different *Bt* proteins (crystalline toxins) and dsRNA targeting the WCR Snf7 gene, will be expressed in the plant (Head et al. 2017). Contrarily, downregulation of *Snf7*, a gene that plays an essential role in protein trafficking, will also result in mortality (Bolognesi et al. 2012). So this integrated strategy is intended to target the insect while also reducing the chances for insects to develop resistance against the PIP (Head et al. 2017). As RNAi is a growing tool within the field of biotechnology, it will definitely show up as a strong insecticidal strategy for crop improvement (Kunte et al. 2019).

Insect genes that serve as a target for successful RNAi constructs include the following: gene encoding enzymes of basal insect metabolism, effectors responsible for plant defense suppression, detoxifying and digestive enzymes, genes involved in detoxification of defensive secondary metabolites of the hosts, etc. (Chung et al. 2021). He et al. (2019) reported the expression of artificial miRNAs in transgenic rice, providing profound resistance to *Chilo suppressalis* (rice stem borer). The course of action involved in the process includes high mortality and developmental

defects, owing to targeting the ecdysone receptor of insects. In addition, Kola et al. (2019) determined that the knockdown of acetylcholinesterase gene of *Scirpophaga incertulas* (rice yellow stem borer) using dsRNA construct in transgenic rice leads to reduced larval weight. Thus, the genome of insects and pests carrying specific genes facilitating the disease occurrence can be targeted by different constructs following specific delivery methodologies to cure the potential spread of disease. Recently, nanoparticles, such as chitosan, liposomes, and cationic dendrimers, offer advantages in delivering dsRNA/small interfering (si)RNA (siRNA) to improve RNAi efficiency, thus promoting the development and practice of RNAi-based insect management strategies (Yan et al. 2021) (Table 7.4).

Table 7.4 RNAi for insect resistance

Target pest	Target gene	Function	Effect	References
BPH	*Entomomyces delphacidicola arginine-succinate lyase* (*EdArg4*)	Arginine biosynthesis	Delayed nymphal development, thickened wings, enlarged antennae, legs, and anal tubes in adults	Yuan et al. (2017a, b)
	Trehalase (TRE)	Wing bud formation and molting	Deformed wings	Zhang et al. (2017)
	20-Hydroxyecdysone	Molting and metamorphosis	Decrease in transcript level, reduction in fecundity	Yu et al. (2014)
	Vacuolar ATP synthase subunit E (V-ATPase-E, 21E01)	Membrane transporter binding protein	Decreased expression of target gene	Li et al. (2011)
	Hexose transporter, carboxypeptidase, trypsin like serine protease	Transport of glucose, hydrolysis of protein	Depletion in transcript level and no effect on larval survival	Zha et al. (2011)
	Trehalose phosphate synthase (TPS)	Production of trehalose-6-phosphate	Decreased survival rate	Chen et al. (2010a, b)
Yellow stem borer	*CytochromeP450 derivative (CYP6)* and *aminopeptidase N (APN)*	Metabolism of insecticides and protein digestion	Detrimental effect on larval growth and development	Kola et al. (2016)
WBPH	*Halloween gene disembodied (dib)*	Encodes cytochrome P450 monooxygenase CYP302AI (22-hydroxylase) which plays a role in ecdysteroidogenesis	Reduction in *dib* and *EcR* (ecdysone receptor) transcript, development and survival of nymphs was impaired	Wan et al. (2014)

7.3.6 CRISPR Cas

Clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated gene *Cas9* represent a valuable system for specific editing of genes in diverse species. So far, genome editing has been demonstrated in model species, like *Arabidopsis*, as well as important crops, like rice, wheat, maize, etc. Genome editing system has unfolded several possibilities that enable precise and efficient targeted modifications in diverse agronomic traits, including durable resistance against insect pests and pathogens. CRISPR/Cas9 mediated editing has been used to generate insect- and pathogen-resistant crops by knocking out of host susceptibility genes, exploiting the effector-target interaction, engineering synthetic immune receptor eliciting broad-spectrum resistance, etc. Modification of insect genomes through CRISPR/Cas9 has been used either to create gene drive or to counteract resistance to various insecticides. Lu et al. (2018) reported the knockdown of *CYP71A1* (encoding tryptamine 5-hydroxylase) following CRISPR/Cas9 methodology, leading to an increased level of salicylic acid and decreased serotonin levels, thereby providing resistance against BPH in rice (Du et al. 2020). Further, expressing insecticidal bacterial genes, anti-nutritional proteins like protease inhibitors, lectins, host-delivered RNAi and the modification of defense-signaling pathways can be utilized for insect resistance (Bisht et al. 2019). The experiment conducted by Li et al. (2020) demonstrates five genes, *OsWRKY2*, *OsWRKY14*, *OsWRKY26*, *OsWRKY69*, and *OsWRKY93*, induced in response to *Magnaporthe oryzae* infection. The increased transcript level of *OsWRKY93* pertains to resistance conferred against *M. oryzae* in rice. The results were validated with the development of *oswrky93-1* CRISPR knockout mutant's susceptibility toward *M. oryzae* infection. These results clearly indicate that the senescence-inducible gene *OsWRKY93* is also a positive regulator of the defense response and can be utilized for attaining resistance against *M. oryzae*.

7.3.7 Proteomics and Metabolomics

Proteomics and metabolomics are the two new emerging omics technologies that have the potential to provide complete information on the biological and metabolic processes of an organism. These technologies have been successfully exploring the differences in gene expression, protein and metabolite abundance, and modification of the posttranslational protein and providing a different level of views for the cellular processes that occur in cells. A proteomics and metabolomics study was executed on four wheat cultivars against wheat stem sawfly (WSS) infestation. Using liquid chromatography-mass spectrometry, the reported cultivars were infested with WSS, and variations in stem proteins and metabolites were detected. The proteome

included 1830 proteins, contributing in five major biological processes, i.e., metabolic processes and stimuli response, metabolome spanning eight chemical super classes of alkaloids, benzenoids, and lipids. Following infestation, the varieties under study showed molecular response to WSS. The data validated variation in the wheat stem molecular response against WSS infestation that supports different breeding approaches for insect resistance in wheat (Lavergne et al. 2020).

Henceforth, studying the proteome and metabolome level of the plant is critical to understand the host response under biotic stress. Erb and Kliebenstein (2020) proposed that metabolites involved in defense reactions in rice include volatile indole, glucosinolates, benzoxazinoids, phenylpropanoid phytoalexins, diterpenoid phytoalexins, and phenylamine. Kang et al. (2019) conducted a comparative metabolomics analysis to reveal the differences in metabolite profiles of susceptible rice cultivar (TN1) and two resistant cultivars (IR36 and IR56) in response to BPH infestations. The gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) investigations reveal the differentially expressed metabolites that included the defense-related metabolites, viz., induction of cyanoamino acids and lipid metabolism in IR36 and changes in thiamine, taurine, and hypotaurine metabolism in IR56 during BPH infection. Apart from these, quercetin and spermidine content were elevated in TN1 and IR36 owing harm to BPH insects. Thus, differences in metabolite profile upon BPH infestations reveal the metabolic mechanism and pathways that can be exploited as a resource for effective pest control. Furthermore, Uawisetwathana et al. (2019) reported the increment in flavonoid glycosides level subjected to resistant reaction in rice against BPH. Apart from BPH, Cheah et al. (2020) reported the proteomic analysis aided by SWATH-MS to identify the proteome profile of Qingliu and TN1 under the attack of *Cnaphalocrocis medinalis*. The results described the overrepresentation of proteins involved in photosynthesis, amino acid metabolic processes, and processes involving secondary metabolites. Also, Dong et al. (2017) reported comparative analysis of protein profiles in the leaf sheath of Pf9279-4 and 02428 representing small brown planthopper (*Laodelphax striatellus* Fallén, Homoptera, Delphacidae)-resistant and susceptible genotypes. The protein expression profile of both genotypes reveals that proteins induced by SBPH feeding were majorly employed in photosynthesis, cell wall-related proteins, amino acid metabolism, stress response, energy metabolism, carbohydrate metabolic process, and transcriptional regulation. The resistant genotype revealed a higher level of superoxide dismutase and glutathione and a defense pathway governed by salicylic acid. Liu et al. (2016) revealed that resistant rice plants infected with *Cnaphalocrocis medinalis* and *Chilo suppressalis*, respectively, displayed induction of photosynthesis that activated the biosynthesis of certain amino acids and metabolites. The differential proteome and metabolome levels among the host-adapted and non-adapted pathogens infer the knowledge regarding the adaptability of pathogens in terms of rice resistance at the proteomics and metabolomics level.

7.4 Integrated Pest Management

Biotechnology in the context of insect pest management can provide controlled, specific, and early by-products for insect pest control, which will have more substantial implications for agriculture than simply improved IPM. Currently, biotechnology is being applied for the precise characterization of insect pest species as well as identification and characterization of novel genes from the host for significant insect resistance. The development of insect-resistant crop varieties suppressing insect pest abundance with minimal environmental loss is the main aim of insect pest management. Till now, many resistant genes have been identified from host plants and diverse exotic sources and inserted into microorganisms and crop plants to confer resistance to insect pests and have improved understanding of gene action and metabolic pathways. For example, the insecticidal *Cry* family genes from *Bacillus thuringiensis* expressing insecticidal Cry proteins (*Bt* toxins) are deployed against an equally vast range of insect pest species. A parallel search on other possible non-*Bt* insect-resistant proteins has identified a large number of genes, holding great potential to interfere with the development and nutrition of different insect pests. Important gene(s), which have attracted scientific attention for rendering similar insect resistance potential in different crop plants, are *vegetative insecticidal proteins (VIPs)* (produced by different bacterial species including *B. cereus* and *B. thuringiensis*, toxic to coleopteran and lepidopteran insects), biotin-binding proteins (avidin and streptavidin are insect growth-inhibiting proteins whose genes could potentially be expressed in plants to provide inbuilt resistance to insect pests.), chitinases (target chitin in the peritrophic membrane of the midgut, causing a reduction in survival and growth), proteinase inhibitors (interfere with the activity of midgut proteinases, causing nutritional limitations), bean α-amylase inhibitors (α-amylase inhibitor peptides from some legume seeds impart resistance to coleopteran seed weevils), plant lectins (constitute direct defense responses in plants against attack by phytophagous insects), and scorpion and spider Venoms (exert a neurotoxic effect in other insect species) (Gupta and Jindal 2014). Biotechnology, as applied to insects now, provides ample opportunities for the identification and utilization of new genes to open a new field for their exploitation in effective insect pest control. The future prospects for biotechnological applications to mediate crop protection against insects using novel approaches along with wide-scale adoption of genetically modified biotech crops worldwide have formed high potential of biotechnology for the improvement of crops.

7.5 Conclusion

Biotechnology has been central to the acceleration of crop improvement over the last two decades. Among the most impactful biotechnology-derived traits, insect-pest resistance has greatly contributed to the worldwide increase in agricultural

productivity and stabilization of food security. The existence of multiple insect pests simultaneously in the field becomes inopportune for the plant survival; thus, incorporation of broad-spectrum resistance genes is required to minimize the loss and investment of rice farmers in the future. The methodologies in biotechnology and molecular biology serve as tools in developing resistant varieties to hasten crop improvement. For the past decades, rapid technological advances have made the discovery and analysis of plant and insect genomes accessible for research and improvement. Diverse techniques, like genetic engineering, wide hybridization, MAS, RNAi, and CRISPR, have provided a boost in identifying putative insect effectors, cloning insect resistance genes, selecting traits that are difficult to measure and observe, and revealing the key components of plant-insect resistance signals. Advances in biotechnology techniques like MAS have already been used to pyramid multiple insect resistance genes to cultivate durable, broad-spectrum insect resistance rice. At the same time, the new emerging technologies such as CRISPR/Cas9 gene editing to convert insect-susceptible alleles to insect resistance alleles, in vivo, provide the potential to design crops that can be patched in real time to combat evolving pests. Recent development in RNAi has provided an efficient means for identification and functional analysis of new plant genes, which are specifically expressed in response to the insect-pest attacks. Furthermore, the emerging biotechnological technologies will enhance the insect resistance and regulate plant immunity in rice varieties. However, in order to fully exploit the enormous potential of biotechnology, appropriate biosafety regulatory frameworks need to be effectively implemented. These integrated approaches can commute the dynamic threat of insects and ably contribute to sustainable development.

References

Ai XY, Ren S, Liu N, Huang L, Liu XN (2019) Transgenic tobacco expressing dsRNA of the arginine kinase gene exhibits enhanced resistance against *Helicoverpa armigera*. Bull Insectol 72(1):115–124

Angeles ER, Khush GS (1999) A new gene for resistance to green leafhopper, *Nephotettix virescens* (distant) in rice. Rice Genet News 16:93–94

Angeles ER, Khush GS (2000a) Genetic analysis of resistance to green leafhopper, *Nephotettix virescens* (distant), in three varieties. Plant Breed 119:446–448

Angeles ER, Khush GS (2000b) Genetics of resistance to green leafhopper in five cultivars of rice, *Oryza sativa* L. SABRAO J Breed Genet 32:1–4

Angeles ER, Khush GS, Heinrichs EA (1981) New genes for resistance to white-backed planthopper in rice. Crop Sci 21:47–50

Bakhsh A, Dinç T, Hussaın T, Demirel U, Aasım M, Çalışkan M (2018) Development of transgenic tobacco lines with pyramided insect resistant genes. Turk J Biol 42(2):174–186

Balachiranjeevi CH, Prahalada GD, Mahender A, Jamaloddin M, Sevilla MA, Marfori-Nazarea C, Vinarao R, Sushanto U, Baehaki SE, Li ZK, Ali J (2019) Identification of a novel locus, *BPH38*(t), conferring resistance to brown planthopper (*Nilaparvata lugens* Stal.) using early backcross population in rice (*Oryza sativa* L.). Euphytica. https://doi.org/10.1007/s10681-019-2506-2

Bettencourt R, Terenius O, Faye I (2002) *Hemolin* gene silencing by ds-RNA injected into Cecropia pupae is lethal to next generation embryos. Insect Mol Biol 11:267–271

Bharathi Y, Kumar V, Pasalub IC, Balachandranb SM, Reddya VD, Rao KV (2011) Pyramided rice lines harbouring *Allium sativum* (*asal*) and *Galanthus nivalis* (*gna*) lectin genes impart enhanced resistance against major sap-sucking pests. J Biotechnol 152:63–71

Biradar SK, Sundaram RM, Thirumurugan T, Bentur JS, Amudhan S, Shenoy VV, Mishra B, Bennett J, Sarma NP (2004) Identification of flanking SSR markers for a major rice gall midge resistance gene *Gm1* and their validation. Theor Appl Genet 109:1468–1473

Bisht DS, Bhatia V, Bhattacharya R (2019) Improving plant-resistance to insect-pests and pathogens: the new opportunities through targeted genome editing. Semin Cell Dev Biol 96:65–76

Boddupally D, Tamirisa S, Gundra SR, Vudem DR, Khareedu VR (2018) Expression of hybrid fusion protein (Cry1Ac::ASAL) in transgenic rice plants imparts resistance against multiple insect pests. Sci Rep 8:8458

Bolognesi R, Ramaseshadri P, Anderson J, Bachman P, Clinton W, Flannagan R, Ilagan O, Lawrence C, Levine S, Moar W, Mueller G, Tan J, Uffman J, Wiggins E, Heck G, Segers G (2012) Characterizing the mechanism of action of double-stranded RNA activity against Western corn rootworm (*Diabrotica virgifera virgifera* LeConte). PLoS One 7:1–6

Cha YS, Ji H, Yun DW, Ahn BO, Lee MC, Suh SC, Lee CS, Ahn EK, Jeon YH, Jin ID, Sohn JK, Koh HJ, Eun MY (2008) Fine mapping of the rice *Bph1* gene, which confers resistance to the brown planthopper (*Nilaparvata lugens* Stål), and development of STS markers for marker-assisted selection. Mol Cells 26:146–115

Chandrasekhar K, Vijayalakshmi M, Vani K, Kaul T, Reddy MK (2014) Phloem-specific expression of the lectin gene from *Allium sativum* confers resistance to the sap-sucker *Nilaparvata lugens*. Biotechnol Lett 36:1059–1067

Cheah BH, Lin HH, Chien HJ, Liao CT, Liu LY, Lai CC, Lin YF, Chuang WP (2020) SWAtH-MS-based quantitative proteomics reveals a uniquely intricate defense response in *Cnaphalocrocis medinalis* resistant rice. Sci Rep 10:1–11

Chen J, Wang L, Pang X, Pan Q (2006) Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph19(t)*. Mol Gen Genomics 275:321–329

Chen J, Huang DR, Wang L, Liu GJ (2010a) Identification of quantitative trait loci for resistance to whitebacked planthopper, *Sogatella furcifera*, from an interspecific cross *Oryza sativa* × *O. rufipogon*. Breed Sci 60:153–159

Chen J, Zhang D, Yao Q, Zhang J, Dong X, Tian H, Chen J, Zhang W (2010b) Feeding-based RNA interference of a trehalose phosphate synthase gene in the brown planthopper, *Nilaparvata lugens*. Insect Mol Biol 19(6):777–786

Chung SH, Feng H, Jander G (2021) Engineering pest tolerance through plant-mediated RNA interference. Curr Opin Plant Biol. https://doi.org/10.1016/j.pbi.2021.102029

Collard BCY, Mackill DJ (2007) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc Lond Ser B Biol Sci 17:1–16

Czapla TH, Lang BA (1990) Effects of plant lectins on the larval development of European corn borer (Lepidoptera: Pyralidae) and Southern corn rootworm (Coleoptera: Chrysomelidae). J Econ Entomol 83:2480–2485

Das G, Patra JK, Baek K-H (2017) Insight into MAS: a molecular tool for development of stress resistant and quality of rice through gene stacking. Front Plant Sci. https://doi.org/10.3389/fpls.2017.00985

Deen R, Ramesh K, Gautam SK, Rao YK, Lakshmi VJ, Viraktamath BC, Brar DS, Ram T (2010) Identification of new gene for BPH resistance introgressed from *O. rufipogon*. Rice Genet Newsl 25:70–71

Dhaliwal GS, Vikas J, Bharathi M (2015) Crop losses due to insect pests: global and Indian scenario. Indian J Entomol 77:165–168

Divya D, Himabindu K, Nair S, Bentur JS (2015) Cloning of a gene encoding LRR protein and its validation as candidate gall midge resistance gene *Gm4*, in rice. Euphytica 203:185–195

Divya D, Sahu N, Nair S, Bentur JS (2018) Map-based cloning and validation of a gall midge resistance gene, *Gm8*, encoding a proline-rich protein in the rice variety Aganni. Mol Biol Rep 45:2075–2086

Dong Y, Fang X, Yang Y, Xue G-P, Chen X, Zhang W, Wang X, Yu C, Zhou J, Mei Q, Fang W, Yan C, Chen J (2017) Comparative proteomic analysis of susceptible and resistant rice plants during early infestation by small brown planthopper. Front Plant Sci. https://doi.org/10.3389/fpls.2017.01744

Du B, Zhang WL, Liu B, Hu J, Wei Z, Shi ZY, He RF, Zhu LL, Chen RZ, Han B, He GC (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. Proc Natl Acad Sci U S A 106:22163–22168

Du B, Chen R, Guo J, He G (2020) Current understanding of the genomic, genetic, and molecular control of insect resistance in rice. Mol Breed 40:24–48

Duan CX, Cheng ZJ, Lei CL, Zhai HQ, Wan J (2009) Analysis of QTLs for resistance to small brown planthopper (*Laodelphax striatellus* Fallén) in rice (*Oryza sativa* L.) using an F_2 population from a cross between Mudgo and Wuyujing 3. Acta Agron Sin 35:388–394

Duan CX, Su N, Cheng ZJ, Lei CL, Wang JL, Zhai HQ, Wan J (2010) QTL analysis for the resistance to small brown planthopper (*Laodelphax striatellus* fallen) in rice using backcross inbred lines. Plant Breed 129:63–67

Dubey M, Chandel G (2010) In silico survey and characterization of resistance gene analogues (RGAs) in the genomic regions encompassing gall midge resistance genes *Gm4* and *Gm5* in rice (*Oryza sativa* L.). Plant Omics J 3:140–148

Eghrari K, Oliveira SC, Nascimento AM, Queiroz B, Fatoretto J, Souza BH, Fernandes OA, Môro GV (2021) The implications of homozygous *vip3Aa20*- and *cry1Ab*-maize on Spodoptera frugiperda control. J Pest Sci. https://doi.org/10.1007/s10340-021-01362-7

Eisemann CH, Donaldson RA, Pearson RD, Cadogan LC, Vuocolo T, Tellam RL (1994) Larvicidal activity of Lectins on *Lucilia cuprina*—mechanism of action. Entomol Exp Appl 72:1–10

Erb M, Kliebenstein D (2020) Plant secondary metabolites as defenses, regulators and primary metabolites—the blurred functional trichotomy. Plant Physiol 184:39–52

Estruch JJ, Warren GW, Mullins MA, Nye GJ, Craig JA, Koziel MG (1996) A novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. Proc Natl Acad Sci U S A 93:5389–5394

Fan D, Liu Y, Zhang H, He J, Huang F, Huang S, Wu B, Liu D, Wen P, Liu L, Jiang L, Cheng X, Wan J (2018) Identification and fine mapping of *qaWBPH11* conferring resistance to white-backed planthopper (*Sogatella furcifera* Horvath) in rice (*Oryza sativa* L.). Mol Breed. https://doi.org/10.1007/s11032-018-0846-6

Fitt GP (1994) Cotton pest management: part 3. An Australian perspective. Annu Rev Entomol 39:532–562

Fujita D, Doi K, Yoshimura A, Yasui H (2004) Introgression of a resistance gene for green rice leafhopper from *Oryza nivara* into cultivated rice, *Oryza sativa* L. Rice Genet Newsl 21:64–66

Fujita D, Doi K, Yoshimura A, Yasui H (2006) Molecular mapping of a novel gene, *Grh5*, conferring resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice *Oryza sativa* L. Theor Appl Genet 113:567–573

Fujita D, Doi K, Yoshimura A, Yasui H (2010) A major QTL for resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) derived from African rice (*Oryza glaberrima* Steud.). Breed Sci 60:336–341

Gatehouse AM, Hilder VA, Powell KS, Wang M, Davison GM, Gatehouse LN, Down RE, Edmonds HS, Boulter D, Newell CA, Merryweather A, Hamilton WD, Gatehouse JA (1994) Insect-resistant transgenic plants: choosing the gene to do the 'job'. Biochem Soc Trans 22:944–949

Ghani MU, Khush GS (1998) A new gene for resistance to green leafhopper *Nephotettix virescens* (distant) in rice. J Genet 67:151–159

Gianessi LP (2005) Economic and herbicide use impacts of glyphosate-resistant crops. Pest Manag Sci 61:241–245

Griffiths W (1998) Will genetically modified crops replace agrochemicals in modern agriculture? Pestic Outlook 9:6–8

Gunning RV, Easton CS, Balfe ME, Ferris IG (1991) Pyrethroid resistance mechanisms in Australian *Helicoverpa armigera*. Pestic Sci 33:473–490

Guo X, Zhong D, Xie W, He Y, Zheng Y, Lin Y, Chen Z, Han Y, Tian D, Liu W, Wang F, Wang Z, Chen S (2019) Functional identification of novel cell death-inducing effector proteins from *Magnaporthe oryzae*. Rice. https://doi.org/10.1186/s12284-019-0312-z

Gupta VK, Jindal V (2014) Biotechnological approaches for insect pest management. In: Abrol DP (ed) Integrated pest management. https://doi.org/10.1016/B978-0-12-398529-3.00018-X

Hamza R, Pérez-Hedo M, Urbaneja A, Rambla JL, Granell A, Gaddour K, Beltrán JP, Cañas LA (2018) Expression of two barley proteinase inhibitors in tomato promotes endogenous defensive response and enhances resistance to *Tuta absoluta*. BMC Plant Biol. https://doi.org/10.1186/s12870-018-1240-6

Han L, Liu P, Wu K, Peng Y, Wang F (2008) Population dynamics of *Sesamia inferens* on transgenic rice expressing *Cry1Ac* and *CpTI* in Southern China. Environ Entomol 37(5):1361–1370

Haq SK, Atif SM, Khan RH (2004) Protein proteinase inhibitor genes in combat against insects, pests, and pathogens: natural and engineered phytoprotection. Arch Biochem Biophys 431:145–159

Harper MS, Hopkins TL, Czapla TH (1998) Effect of wheat germ agglutinin on formation and structure of the peritrophic membrane in European corn borer (*Ostrinia nubilalis*) larvae. Tissue Cell 30:166–176

He J, Liu YQ, Liu YL, Jiang L, Wu H, Kang H, Liu S, Chen L, Liu X, Cheng X, Wan J (2013) High-resolution mapping of brown planthopper (BPH) resistance gene *Bph27(t)* in rice (*Oryza sativa* L.). Mol Breed 31:549–557

He K, Xiao H, Sun Y, Ding S, Situ G, Li F (2019) Transgenic microRNA-14 rice shows high resistance to rice stem borer. Plant Biotechnol J 17:461–471

He L, Zou L, Huang Q, Sheng X, Wu W, Hu J (2020) Development of InDel markers of *Bph3* and pyramiding of four brown planthopper resistance genes into an elite rice variety. Mol Breed 40:95–105

Head GP, Carroll MW, Evans SP, Rule DM, Willse AR, Clark TL, Storer NP, Flannagan RD, Samuel LW, Meinke LJ (2017) Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. Pest Manag Sci 73:1883–1899

Hernandez JE, Khush GS (1981) Genetics of resistance to white-backed planthopper in some rice (*Oryza sativa* L.) varieties. Oryza 18:44–50

Hernandez-Campuzano B, Suarez R, Lina L, Hernandez V, Villegas E, Corzo G, Iturriaga G (2009) Expression of a spider venom peptide in transgenic tobacco confers insect resistance. Toxicon 53:122–128

Himabindu K, Sundaram RM, Neeraia CN, Mishra B, Bentur JS (2007) Flanking SSR markers for allelism test for the Asian rice gall midge (*Orseolia oryzae*) resistance genes. Euphytica 157:267–279

Himabindu K, Suneetha K, Sama VSAK, Bentur JS (2010) A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. Euphytica 174:179–187

Hirabayashi H, Angeles ER, Kaji R, Ogawa T, Brar DS, Khush GS (1998) Identification of the brown planthopper resistance gene derived from *O. officinalis* using molecular markers in rice. Breed Sci 1:48–51

Hirabayashi H, Ideta O, Sato H, Takeuchi Y, Ando I, Nemoto H, Imbe T, Brar DS, Ogawa T (2004) Identification of a resistance gene to brown planthopper derived from *Oryza minuta* in rice. Breed Res 6:285–288

Hopkins TL, Harper MS (2001) Lepidopteran peritrophic membranes and effects of dietary wheat germ agglutinin on their formation and structure. Arch Insect Biochem Physiol 47:100–109

Hou LY, Yu P, Xu Q, Yuan XP, Yu HY, Wang YP, Wang CH, Wan G, Tang SX, Peng ST, Wei XH (2011) Genetic analysis and preliminary mapping of two recessive resistance genes to brown planthopper, *Nilaparvata lugens* Stål in rice. Rice Sci 18:238–242

Hu J, Xiao C, Cheng M, Gao G, Zhang Q, He Y (2015a) A new finely mapped *Oryza australiensis*-derived QTL in rice confers resistance to brown planthopper. Gene 561:132–137

Hu J, Xiao C, Cheng M, Gao G, Zhang Q, He Y (2015b) Fine mapping and pyramiding of brown planthopper resistance genes *QBph3* and *QBph4* in an introgression line from wild rice *O. officinalis*. Mol Breed. https://doi.org/10.1007/s11032-015-0228-2

Hu J, Chang X, Zou L, Tang W, Wu W (2018) Identification and fine mapping of *Bph33*, a new brown planthopper resistance gene in rice (*Oryza sativa* L.). Rice. https://doi.org/10.1186/s12284-018-0249-7

Huang D, Qiu Y, Zhang Y, Huang F, Meng J, Wei S, Li R, Chen B (2013) Fine mapping and characterization of *BPH27*, a brown planthopper resistance gene from wild rice (*Oryza rufipogon* Griff.). Theor Appl Genet 126:219–229

Ishii T, Brar DS, Multani DS, Khush GS (1994) Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O sativa*. Genome 37:217–221

Jairin J, Teangdeerith SN, Leelagud P, Phengrat K, Vanavichi A, Toojindra T (2007a) Detection of brown planthopper resistance genes from different rice mapping populations in the same genomic location. Sci Asia 33:347–352

Jairin J, Teangdeerith SN, Leelagud P, Phengrat K, Vanavichit A, Toojinda T (2007b) Physical mapping of *Bph3*, a brown planthopper resistance locus in rice. Maejo Int J Sci Technol 1:166–177

Jairin J, Sansen K, Wonboon W, Kothcharerk J (2010) Detection of a brown planthopper resistance gene *bph4* at the same chromosomal position of *Bph3* using two different genetic backgrounds of rice. Breed Sci 60:71–75

James C (2011) Global status of commercialized biotech/GM crops. ISAAA Brief No. 43 ISAAA: Ithaca, NY https://www.isaaa.org/resources/publications/briefs/43/

Jayachandran B, Hussain M, Asgari S (2013) Regulation of *Helicoverpa armigera* ecdysone receptor by miR-14 and its potential link to baculovirus infection. J Invertebr Pathol 114:151–157

Jena KK, Mackill DJ (2008) Molecular markers and their use in marker-assisted selection in Rice. Crop Sci 48:1266–1276

Jena KK, Jeung JU, Lee JH, Choi HC, Brar DS (2006) High-resolution mapping of a new brown planthopper (BPH) resistance gene, *Bph18*(t), and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theor Appl Genet 112:288–297

Jena KK, Hechanova SL, Verdeprado H, Prahalada GD, Kim S-R (2017) Development of 25 near-isogenic lines (NILs) with ten BPH resistance genes in rice (*Oryza sativa* L.): production, resistance spectrum, and molecular analysis. Theor Appl Genet 130:2345–2360

Ji H, Kim SR, Kim YH, Suh JP, Park HM, Sreenivasulu N, Misra G, Kim SM, Hechanova SL, Kim H, Lee GS, Yoon UH, Kim TH, Lim H, Suh SC, Yang J, An G, Jena KK (2016) Map based cloning and characterization of the *BPH18* gene from wild rice conferring resistance to brown planthopper (BPH) insect pest. Sci Rep. https://doi.org/10.1038/srep34376

Jiang Y, Cai Z, Xie W, Long T, Yu T, Zhang Q (2012) Rice functional genomics research: progress and implications for crop genetic improvement. Biotechnol Adv 30:1059–1070

Kabir MA, Khush GS (1988) Genetic analysis of resistance to brown planthopper resistance gene in rice (*Oryza sativa* L.). Plant Breed 100:54–58

Kadowaki M, Yoshimura A, Yasui H (2003) RFLP mapping of antibiosis to rice green leafhopper. In: Khush GS, Brar DS, Hardy B (eds) Advances in rice genetics. International Rice Research Institute, Los Baños, pp 270–272

Kammar V, Nitin KS (2019) Molecular marker-assisted selection of plant genes for insect resistance. In: Experimental techniques in host-plant resistance. pp 267–273

Kang, K, Yue L, Xia X (2019) Comparative metabolomics analysis of different resistant rice varieties in response to the brown planthopper *Nilaparvata lugens* Hemiptera: Delphacidae. Metabolomics. https://doi.org/10.1007/s11306-019-1523-4

Katiyar SK, Tan Y, Huang B, Chandel G, Xu Y, Zhang Y, Xie Z, Bennett J (2001) Molecular mapping of gene *Gm6*-(t) which confers resistance against four biotypes of Asian rice gall midge in China. Theor Appl Genet 103:953–961

Khush GS, Karim ANMR, Angeles ER (1985) Genetics of resistance of rice cultivar ARC10550 to Bangladesh brown planthopper biotype. J Genet 64:121–125

Kola VSR, Renuka P, Padmakumari AP, Mangrauthia SK, Balachandran SM, Babu VR, Madhav MS (2016) Silencing of CYP6 and APN genes affects the growth and development of rice yellow stem borer, *Scirpophaga incertulas*. Front Physiol. https://doi.org/10.3389/fphys.2016.00020

Kola VSR, Pichili R, Padmakumari AP, Mangrauthia SK, Balachandran SM, Madhav MS (2019) Knockdown of *acetylcholinesterase (AChE)* gene in rice yellow stem borer, *Scirpophaga incertulas* (Walker) through RNA interference. Agric Gene. https://doi.org/10.1016/j.aggene.2019.100081

Kumar A, Jain A, Sahu RK, Shrivastava MN, Nair S, Mohan M (2005) Genetic analysis of resistance genes for the rice gall midge in two rice genotypes. Crop Sci 45:1631–1635

Kumar K, Sarao PS, Bhatia D, Neelam K, Kaur A, Mangat GS, Brar DS, Singh K (2018) High-resolution genetic mapping of a novel brown planthopper resistance locus, *Bph34* in *Oryza sativa* L. × *Oryza nivara* (Sharma & Shastry) derived interspecific F_2 population. Theor Appl Gene 131:1163–1171

Kunte N, McGraw E, Bell S, Held D, Avila L (2019) Prospects, challenges and current status of RNAi through insect feeding. Pest Manag Sci. https://doi.org/10.1002/ps.5588

Lavergne FD, Broeckling CD, Brown KJ, Cockrell DM, Haley SD, Peairs FB, Pearce S, Lisa M, Wolfe LM, Jahn CE, Heuberger AL (2020) Differential stem proteomics and metabolomics profiles for four wheat cultivars in response to the insect pest Wheat Stem Sawfly. J Proteome Res 19:1037–1051

Li X, Zhai H, Wan J, Ma L, Zhuang J, Liu G, Yang C (2004) Mapping of a new gene *Wbph6(t)* resistant to the white-backed planthopper, *Sogatella furcifera*, in rice. Rice Sci 11:86–90

Li R, Li L, Wei S, Wei Y, Chen Y, Bai D, Yang L, Huang F, Lu W, Zhang X, Li X, Yang X, Wei Y (2006) The evaluation and utilization of new genes for brown planthopper resistance in common wild rice (*Oryza rufipogon* Griff.). Mol Plant Breed 4:365–371

Li J, Chen Q, Lin Y, Jiang T, Wua G, Huaa H (2011) RNA interference in *Nilaparvata lugens* (Homoptera: Delphacidae) based on dsRNA ingestion. Pest Manag Sci 67:852–859

Li Z, Xue Y, Zhou H, Li Y, Usman B, Jiao X, Wang X, Liu F, Qin B, Li R, Qiu Y (2019) High resolution mapping and breeding application of a novel brown planthopper resistance gene derived from wild rice (*Oryza. rufipogon* Griff). Rice 12:41–54

Li H, Wang Z, Han K, Guo M, Zou Y, Zhang W, Ma W, Hua H (2020) Cloning and functional identification of a *Chilo suppressalis*-inducible promoter of rice gene, *OsHPL2*. Pest Manag Sci. https://doi.org/10.1002/ps.5872

Liu Z, Liu G, Sogawa K, Zhuang J, Chen S, Zheng K (2002) Mapping the gene *Wbph2* in ARC10239 resistant to the white-backed planthopper *Sogatella furcifera* in rice. Chin J Rice Sci 16:311–314

Liu Y, Yang L, Nie Z, Lu X, Lu Z, Chen J, Yu W, Xiang WU, Zhou ZY (2013) Upregulation and expression of *Bombyx mori* bmo-miR-14 and prediction of its target genes. China Agric Sci 46:1263–1271

Liu Q, Wang X, Tzin V, Romeis J, Peng Y, Li Y (2016) Combined transcriptome and metabolome analyses to understand the dynamic responses of rice plants to attack by the rice stem borer *Chilo suppressalis* (Lepidoptera: Crambidae). BMC Plant Biol 16:1–17

Liu MM, Zhang XJ, Gao Y, Shen ZC, Lin CY (2018) Molecular characterization and efficacy evaluation of a transgenic corn event for insect resistance and glyphosate tolerance. J Zhejiang Univ Sci B 19:610–619

Lopes AR, Juliano MA, Juliano L, Terra WR (2004) Coevolution of insect trypsins and inhibitors. Arch Insect Biochem Physiol 55:140–152

Lu H, Luo T, Fu H, Wang L, Tan Y, Huang J, Wang Q, Ye G, Gatehouse A, Lou Y, Shu Q (2018) Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. Nat Plants 4:338–344

Ma Y, Dai X, Xu Y, Luo W, Zheng X, Zeng D, Pan Y, Lin X, Liu H, Zhang D, Xiao J, Guo X, Xu S, Niu Y, Jin J, Zhang H, Xu X, Li L, Wang W, Qian Q, Ge S, Chong K (2015) *COLD1* confers chilling tolerance in rice. Cell 160:1209–1221

Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, Wang LJ, Huang YP, Chen XY (2007) Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nat Biotechnol 25:1307–1313

Mohanty SK, Panda RS, Mohapatra SL, Nanda A, Behera L, Jena M, Sahu RK, Sahu SC, Mohapatra T (2017) Identification of novel quantitative trait loci associated with brown planthopper resistance in the rice landrace Salkathi. Euphytica. https://doi.org/10.1007/s10681-017-1835-2

Mudiyappanayar J, Koundal KR (2020) Constitutive expression of protease inhibitor gene isolated from black gram (*Vigna mungo* L.) confers resistance to *Spodoptera litura* in transgenic tobacco plants. Indian J Biotechnol. http://nopr.niscair.res.in/handle/123456789/55655

Murdock LL, Huesing JE, Nielsen SS, Pratt RC, Shade RE (1990) Biological effects of plant lectins on the cowpea weevil. Phytochemistry 29:85–89

Myint KK, Fujita D, Matsumura M, Sonoda T, Yoshimura A, Yasui H (2012) Mapping and pyramiding of two major genes for resistance to the brown planthopper (*Nilaparvata lugens* Stål) in the rice cultivar ADR52. Theor Appl Genet 124:495–504

Navarro-González SS, Ramírez-Trujillo JA, Peña-Chora G, Gaytán P, Roldán-Salgado A, Corzo G, Lina-García LP, Hernández-Velázquez VM, Suárez-Rodríguez R (2019) Enhanced tolerance against a fungal pathogen and insect resistance in transgenic tobacco plants overexpressing an *endochitinase* gene from Serratia marcescens. Int J Mol Sci. https://doi.org/10.3390/ijms20143482

Nemoto H, Ikeda R, Kaneda C (1989) New gene for resistance to brown planthopper, *Nilaparvata lugens* Stål, in rice. Jpn J Breed 39:23–28

Prahalada GD, Shivakumar N, Lohithaswa HC, Sidde Gowda DK, Ramkumar G, Kim SR, Ramachandra C, Hittalmani S, Mohapatra T, Jena KK (2017) Identification and fine mapping of a new gene, *BPH31* conferring resistance to brown planthopper biotype 4 of India to improve rice, *Oryza sativa* L. Rice. https://doi.org/10.1186/s12284-017-0178-x

Qiu Y, Guo J, Jing S, Zhu L, He G (2010) High-resolution mapping of the brown planthopper resistance gene *Bph6* in rice and characterizing its resistance in the 9311 and Nipponbare near isogenic backgrounds. Theor Appl Genet 121:1601–1611

Qiu Y, Guo J, Jing S, Zhu L, He G (2012) Development and characterization of japonica rice lines carrying the brown planthopper-resistance gene *BPH12* and *BPH6*. Theor Appl Genet 124:485–494

Qiu Y, Guo J, Jing S, Zhu L, He G (2014) Fine mapping of the rice brown planthopper resistance gene *BPH7* and characterization of its resistance in the 93-11 background. Euphytica 198:369–379

Quilis J, López-García B, Meynard D, Guiderdoni E, Segundo S (2014) Inducible expression of a fusion gene encoding two proteinase inhibitors leads to insect and pathogen resistance in transgenic rice. Plant Biotechnol J 12(3):367–377

Rahman ML, Jiang W, Chu SH, Qiao Y, Ham TH, Woo MO, Lee J, Khanam MS, Chin JH, Jeung JU, Brar DS, Jena KK, Koh HJ (2009) High-resolution mapping of two brown planthopper resistance genes, *Bph20(t)* and *Bph21(t)*, originating from *Oryza minuta*. Theor Appl Genet 119:1237–1246

Ram T, Deen R, Gautam SK, Ramesh K, Rao YK, Brar DS (2010) Identification of new genes for brown planthopper resistance in rice introgressed from *O. glaberrima* and *O. minuta*. Rice Genet Newsl 25:67–69

Ramesh K, Padmavathi G, Deen R, Pandey MK, Jhansi Lakshmi V, Bentur JS (2014) Whitebacked planthopper *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae) resistance in rice variety Sinna Sivappu. Euphytica 200:139–148

Rao YC, Dong GJ, Zeng DL, Hua J, Zeng LJ, Gao ZY, Zhang GH, Guo LB, Qian Q (2010) Genetic analysis of leaf folder resistance in rice. J Genet Genomics 37:325–331

Rathee M, Dalal P (2018) Emerging insect pests in Indian agriculture. Indian J Entomol 80(2):267–228

Reinke R, Kim SM, Kim BK (2018) Developing japonica rice introgression lines with multiple resistance genes for brown planthopper, bacterial blight, rice blast, and rice stripe virus using molecular breeding. Mol Gen Genomics 293:1565–1575

Ren J, Gao F, Wu X, Lu X, Zeng L, Lv J, Sun X, Luo H, Ren G (2016) *Bph32*, a novel gene encoding an unknown SCR domain-containing protein, confers resistance against the brown planthopper in rice. Sci Rep. https://doi.org/10.1038/srep37645

Renganayaki K, Fritz AK, Sadasivam S, Pammi S, Harrington SE, McCouch SR, Kumar SM, Reddy AS (2002) Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, *O. sativa*. Crop Sci 42:2112–2117

Rezaul Karim ANM, Pathak MD (1982) New genes for resistance to green leafhopper, *Nephotettix virescens* (distant) in rice *Oryza sativa* L. Crop Prot 1:483–490

Riaz S, Nasir IA, Bhatti MU, Adeyinka OS, Toufiq N, Yousaf I, Tabassum B (2020) Resistance to *Chilo infuscatellus* (Lepidoptera: Pyraloidea) in transgenic lines of sugarcane expressing *Bacillus thuringiensis* derived Vip3A protein. Mol Biol Rep 47:2649–2658

Saka N, Tsuji T, Toyama T, Yano M, Izawa T, Sasaki T (2006) Development of cleaved amplified polymorphic sequence (CAPS) markers linked to a green rice leafhopper resistance gene, *Grh3(t)*. Plant Breed 125:140–143

Salehian H, Rahnama H, Dezhsetan S, Babaei S (2021a) Constitutive expression of a synthetic *cry1Ab* gene confers resistance to potato tube moth (*Phthorimaea operculella* Zellar) larva. Crop Breed Appl Biotechnol. https://doi.org/10.1590/1984-70332021v21n1a9

Salehian H, Rahnama H, Dezhsetan S, Babaei S (2021b) Constitutive expression of *cry3A* gene in transgenic potato plants for resistance to Colorado Potato Beetle (CPB). Potato Res. https://doi.org/10.1007/s11540-021-09500-5

Salim M, Masud SA, Ramzan M (2001) Integrated pest management of basmati rice. In: Rice of the world: Breeding, production and marketing. FAO, Rome

Sama VSAK, Rawat N, Sundaram RM, Himabindu K, Naik BS, Viratamath BC, Bentur JS (2014) A putative candidate for the recessive gall midge resistance gene *gm3* in rice identified and validated. Theor Appl Genet 127:113–124

Sardesai N, Kumar A, Rajyashri KR, Nair S, Mohan M (2002) Identification and mapping of an AFLP marker liked to *Gm7*, a gall midge resistance gene and its conversion to a SCAR marker for its utility in marker aided selection in rice. Theor Appl Genet 105:691–698

Schuler TH, Poppy GM, Kerry BR, Denholm I (1998) Insect-resistant transgenic plants. Trends Biotechnol 16:168–175

Sebastian LS, Ikeda R, Huang N, Imbe T, Coffman WR, McCouch SR (1996) Molecular mapping of resistance to rice spherical virus and green leafhopper. Phytopathology 86:25–30

Shrivastava MN, Kumar A, Bhandarkar S, Shukla BC, Agrawal KC (2003) A new gene for resistance in rice to Asian rice gall midge (*Orseolia oryzae* wood Mason) biotype 1 population at Raipur, India. Euphytica 130:143–145

Sidhu GS, Khush GS, Medrano FG (1979) A dominant gene in rice for resistance to white-backed planthopper and its relationship to other plant characteristics. Euphytica 28:227–232

Silva FC, Alcazar A, Macedo LL, Oliveira AS, Macedo FP, Abreu LR, Santos EA, Sales MP (2006) Digestive enzymes during development of *Ceratitis capitate* (Diptera: Tephritidae) and effects of SBTI on its digestive serine proteinase targets. Insect Biochem Mol Biol 36:561–569

Stefey K, Gray M, Estes R (2009) Traits for insect control with transgenic Bt Corn: what, why, and how now and in the future. In: The proceedings of the 2009 University of Illinois Corn & Soybean Classics

Sun L, Su C, Wang C, Zhao H, Wan J (2005) Mapping of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. Breed Sci 55:39139–39136

Sun L, Wang C, Su C, Liu Y, Zhai H, Wan J (2006) Mapping and marker-assisted selection of a brown planthopper resistance gene *bph2* in rice (*Oryza sativa* L.). Acta Genet Sin 33:717–273

Sun Z, Liu Y, Xiao S, Hu J, Pan G, He J, Xu T, Huang J, Qiu Z, Fan D, Zhang L, Liu L, Jiang L, Cheng X, Zhao H, Wan J (2017) Identification of quantitative trait loci for resistance to rice black-streaked dwarf virus disease and small brown planthopper in rice. Mol Breed. https://doi.org/10.1007/s11032-017-0669-x

Sushmitha J, Bamishaiye EI, Balakrishnan N, Varanavasiappan S, Arul L, Kumar SM, Joel AJ, Sudhakar D (2018) Efficacy of *cry2AX1* gene in transgenic tomato plants against *Helicoverpa armigera* and *Spodoptera litura*. Madras Agric J 105:341–345

Talukdar D (2013) Modern biotechnological approaches in insect research. Int Res J Sci Eng 1(3):71–78

Tamura K, Fukuta Y, Hirae M, Oya S, Ashikawa I, Yagi T (2004) RFLP mapping of a new resistance gene for green rice leafhopper in Kanto PL10. Rice Genet Newsl 21:62–64

Tamura Y, Hattori M, Yoshioka H, Yoshioka M, Takahashi A, Wu J, Sentoku N, Yasui H (2014) Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa* L. ssp. indica cultivar ADR52. Sci Rep. https://doi.org/10.1038/srep05872

Tan G, Weng Q, Ren X, Huang Z, Zhu L, He G (2004) Two white-backed planthopper resistance genes in rice share the same loci with those for brown planthopper resistance. Heredity 92:212–217

Uawisetwathana U, Chevallier OP, Xu Y, Kamolsukyeunyong W, Nookaew I, Somboon T, Toojinda T, Vanavichit A, Goodacre R, Elliott CT, Karoonuthaisiri N (2019) Global metabolite profiles of rice brown planthopper-resistant traits reveal potential secondary metabolites for both constitutive and inducible defenses. Metabolomics. https://doi.org/10.1007/s11306-019-1616-0

Varghese J, Cohen SM (2007) microRNA miR-14 acts to modulate a positive autoregulatory loop controlling steroid hormone signaling in Drosophila. Genes Dev 21:2277–2282

Vaughan DA, Morishima H, Kadowaki K (2003) Diversity in the *Oryza genus*. Curr Opin Plant Biol 6:139–146

Venkanna V, Hari Y, Rukminidevi K, Chandra BS, Raju J, Malathi S, Reddy PRR (2018) Markers assisted selection for pyramiding of gall midge resistance genes in Kavya, a Popular Rice Variety. Int J Curr Microbiol App Sci 7(4):745–753

Vogel E, Santos D, Mingels L, Verdonckt T-W, Broeck JV (2019) RNA interference in insects: protecting beneficials and controlling pests. Front Physiol. https://doi.org/10.3389/fphys.2018.01912

Wan P, Jia S, Li N, Fan J, Li G (2014) RNA interference depletion of the Halloween gene disembodied implies its potential application for management of planthopper *Sogatella furcifera* and *Laodelphax striatellus*. PLoS One. https://doi.org/10.1371/journal.pone.0086675

Wang Q, Liu Y, Hu J, Zhang Y, Xie K, Wang B, Tuyen le Q, Song Z, Wu H, Liu Y, Jiang L, Liu S, Cheng X, Wang C, Zhai H, Wan J (2013) Detection of quantitative trait loci (QTLs) for resistances to small brown planthopper and rice stripe virus in rice using recombinant inbred lines. Int J Mol Sci 14:8406–8421

Wang Y, Cao L, Zhang Y, Cao C, Liu F, Huang F, Qiu Y, Li R, Lou X (2015) Map-based cloning and characterization of *BPH29*, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice. J Exp Bot 66:6035–6045

Wang Y, Jiang W, Liu H, Zeng Y, Du B, Zhu L, He G, Chen G (2017) Marker assisted pyramiding of *Bph6* and *Bph9* into elite restorer line 93–11 and development of functional marker for *Bph9*. Rice 10:51–63

Wang H, Shi S, Guo Q, Nie L, Du B, Chen R, Zhu L, He G (2018) High-resolution mapping of a gene conferring strong antibiosis to brown planthopper and developing resistant near-isogenic lines in 9311 background. Mol Breed. https://doi.org/10.1007/s11032-018-0859-1

Wei K, Chen H (2018) Comparative functional genomics analysis of bHLH gene family in rice, maize and wheat. BMC Plant Biol. https://doi.org/10.1186/s12870-018-1529-5

Williams IS (1999) Slow-growth, high-mortality—a general hypothesis, or is it? Ecol Entomol 24:490–495

Wu CF, Khush GS (1985) A new dominant gene for resistance to white-backed planthopper in rice. Crop Sci 25:505–509

Wu H, Liu Y, He J, Liu Y, Jiang L, Liu L, Wang C, Cheng X, Wan J (2014) Fine mapping of brown planthopper (*Nilaparvata lugens* Stål) resistance gene *Bph28(t)* in rice (*Oryza sativa* L.). Mol Breed 33:909–918

Xiao J, Li J, Grandillo S, Ahn SN, Yuan L, Tanksley SD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150:899–909

Xu C, Cheng J, Lin H, Lin C, Gao J, Shen Z (2018a) Characterization of transgenic rice expressing fusion protein *Cry1Ab/Vip3A* for insect resistance. Sci Rep. https://doi.org/10.1038/s41598-018-34104-4

Xu T, Liu Y, Zhang L, Liu L, Wang C, Hu J, Sun Z, Pan G, Xiao S, He J, Huang J, Qiu Z, Fan D, Jiang L, Cheng X, Zhai H, Wan J (2018b) Mapping of quantitative trait loci associated with rice black-streaked dwarf virus disease and its insect vector in rice (*Oryza sativa* L.). Plant Breed 137:698–705

Yamasaki M, Yoshimura A, Yasui H (2003) Genetic basis of ovicidal response to white-backed planthopper (*Sogatella furcifera* Horváth) in rice (*Oryza sativa* L.). Mol Breed 12:133–143

Yan S, Ren BY, Shen J (2021) Nanoparticle-mediated double-stranded RNA delivery system: a promising approach for sustainable pest management. Insect Sci 28:21–34

Yang H, You A, Yang Z, Zhang F, He R, Zhu L, He G (2004) High-resolution genetic mapping at the *Bph15* locus for brown planthopper resistance in rice (*Oryza sativa* L.). Theor Appl Genet 110:182–191

Yang L, Li RB, Li YR, Huang FK, Chen YZ, Huang SS, Huang LF, Liu C, Ma ZF, Huang DH, Jiang JJ (2012) Genetic mapping of *bph20(t)* and *bph21(t)* loci conferring brown planthopper resistance to *Nilaparvata lugens* Stål in rice (*Oryza sativa* L.). Euphytica 183:161–171

Yang Y, Xu J, Leng Y, Xiong G, Hu J, Zhang G, Huang L, Wang L, Guo L, Li J, Chen F, Qian Q, Zeng D (2014) Quantitative trait loci identification, fine mapping and gene expression profiling for ovicidal response to white-backed planthopper (*Sogatella furcifera* Horváth) in rice (*Oryza sativa* L.). BMC Plant Biol. https://doi.org/10.1186/1471-2229-14-145

Yang M, Cheng L, Yan L, Shu W, Wang X, Qiu Y (2019) Mapping and characterization of a quantitative trait locus resistance to the brown planthopper in the rice variety IR64. Hereditas. https://doi.org/10.1186/s41065-019-0098-4

Yarasi B, Sadumpati V, Immanni CP, Vudem DR, Khareedu VR (2008) Transgenic rice expressing *Allium sativum leaf agglutinin (Asal)* exhibits high-level resistance against major sap-sucking pests. BMC Plant Biol. https://doi.org/10.1186/1471-2229-8-102

Yoshimura S, Komatsu M, Kaku K, Hori M, Ogawa T, Muramoto K, Kazama T, Ito Y, Toriyama K (2012) Production of transgenic rice plants expressing *Dioscorea batatas tuber lectin 1* to confer resistance against brown planthopper plant. Biotechnology 29:501–504

Yu R, Xu X, Liang Y, Tian H, Pan Z, Jin S, Wang N, Zhang W (2014) The insect ecdysone receptor is a good potential target for RNAi based pest control. Int J Biol Sci 10(10):1171–1180

Yuan S, Dong Y, Zhang N, Ren Y, Yang M, Gao B (2017a) Construction of high-efficiency transformation vector with multiple insect-resistance genes and expression in tobacco. Acta Physiol Plant. https://doi.org/10.1007/s11738-016-2338-9

Yuan SY, Li GQ, Wan PJ, Fu Q, Lai FX, Mu LL (2017b) Knockdown of a putative *argininosuccinate lyase* gene reduces arginine content and impairs nymphal development in *Nilaparvata lugens*. Insect Biochem Physiol. https://doi.org/10.1002/arch.21385

Zavala JA, Baldwin IT (2004) Fitness benefits of trypsin proteinase inhibitor expression in Nicotiana attenuata are greater than their costs when plants are attacked. BMC Ecol. https://doi.org/10.1186/1472-6785-4-11

Zha WJ, Peng XX, Chen RZ, Du B, Zhu LL, He GC (2011) Knockdown of midgut genes by dsRNA-transgenic plant-mediated RNA interference in the hemipteran insect *Nilaparvata lugens*. PLoS One. https://doi.org/10.1371/journal.pone.0020504

Zhang W, Dong Y, Yang L, Ma B, Ma R, Huang F, Wang C, Hu H, Li C, Yan C, Chen J (2014) Small brown planthopper resistance loci in wild rice (*Oryza officinalis*). Mol Gen Genomics 289:373–382

Zhang L, Qiu LY, Yang HL, Wang HJ, Zhou M, Wang SG, Tang B (2017) Study on the effect of wing bud chitin metabolism and its developmental network genes in the brown planthopper, *Nilaparvata lugens*, by knockdown of TRE gene. Front Physiol. https://doi.org/10.3389/fphys.2017.00750

Zhao JZ, Cao J, Li Y, Collins HL, Roush RT, Earle ED, Shelton AM (2003) Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. Nat Biotechnol 21:1493–1497

Zhao Y, Huang J, Wang Z, Jing S, Wang Y, Ouyang Y, Cai B, Xin XF, Liu X, Zhang C, Pan Y, Ma R, Li Q, Jiang W, Zeng Y, Shangguan X, Wang H, Du B, Zhu L, Xu X, Feng YQ, He SY, Chen R, Zhang Q, He G (2016) Allelic diversity in an NLR gene *BPH9* enables rice to combat planthopper variation. Proc Natl Acad Sci U S A 113:12850–12855

Chapter 8
Antixenosis and Antibiosis Mechanisms of Resistance to Turnip Aphid, *Lipaphis erysimi* (Kaltenbach) in Brassica: Conventional and Biotechnological Approaches

Neha Panwar and Sarwan Kumar

8.1 Introduction

The Brassicaceae family, consisting of about 375 genera and 3200 species, is one of the earliest groups of cultivated plants (LeCoz and Ducombs 2006). The members of this family are a source of vegetables, oilseeds and condiments. Various biotic and abiotic stresses limit the production and productivity of these crops. Out of the various insect pests, turnip aphid, *Lipaphis erysimi* (Kaltenbach), is the most damaging, which causes 35.4–91.3% reduction in yield with the average yield losses of around 56.2% (Ram et al. 2017). At present, systemic insecticides are used to manage this pest. Although these insecticides are very effective, they have the associated problems like residue problem in oil and cake, environmental pollution and development of insecticide resistance. The past two decades have witnessed an increased interest in finding alternate solutions for aphid management. One such strategy is host plant resistance. Various efforts have been made to develop aphid-resistant brassica plants. Crop wild relatives of Brassicas, such as *Brassica fruticulosa*, *B. montana* and *Rorippa indica*, have been found to contain certain resistance genes. With the advancement of biotechnology, genes from these non-crossable gene pools can be incorporated into the cultivated ones. Further, the use of novel biotechnological techniques like gene silencing can complement the conventional breeding in efforts to develop aphid-resistant plants. This chapter provides a complete overview of the conventional and biotechnological tools used to develop aphid-resistant plants.

N. Panwar
Department of Entomology, Punjab Agricultural University, Ludhiana, India

S. Kumar (✉)
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India
e-mail: sarwanent@pau.edu

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_8

8.2 Aphid Biology and Behaviour

More than 4000 species of aphids have been reported worldwide, and about 250 species are economically important from agriculture's point of view (Bhatia et al. 2011). In case of brassicas, three aphid species, namely, cabbage aphid (*Brevicoryne brassicae*), green peach aphid (*Myzus persicae*) and turnip aphid (*L. erysimi*), are the important pests. The green peach aphid is polyphagous and can be regarded as a generalist feeder, whereas the other two feed exclusively on brassicaceous crops and are regarded as specialist feeders (Blackman and Eastop 2000). The turnip aphid causes damage not only by sucking plant sap but also by acting as a vector of various viruses. It has been reported as a vector of about 13 viruses, including *Cauliflower mosaic virus, Beet mosaic virus, Radish mosaic virus* and *Cabbage black ring spot virus* (Adhab and Schoelz 2015). Both nymphs and adults damage the crop by sucking the phloem sap. Although damage can occur at any crop growth stage, severe damage generally occurs during flowering and pod setting (Bakhetia 1991). Telescoping of generations, parthenogenetic viviparity, wing dimorphism, minimum tissue damage (during feeding) and shorter life cycle are some of the adaptations that make them so serious pests of crop and vegetable Brassicas.

8.2.1 Feeding Mechanism of Aphids

The feeding mechanism of aphids is highly specialized that can overcome the plant defences easily (Mondal 2020). The needle-like stylets pierce through the host's sieve elements to reach the phloem sap (Bhattacharya 2019). During the penetration process, aphids position their stylets between epidermal and parenchymal cells in such a way that there is the least mechanical damage to the host, so that the damage-associated defence responses are lowered to a great extent (Bhatia et al. 2011).

In addition to this, aphids also produce two types of saliva (Louis et al. 2012). The dense gelling saliva is produced initially during the penetration process, which helps in developing an intercellular stylet path. It not only lowers the wounding damage and the damage-associated host responses (Louis et al. 2012) but also acts as a physical barrier for the stylets by creating a protective sheath around it (Felton and Eichenseer 1999). The other type of saliva produced is watery saliva (Cherqui and Tjallingii 2000; Powell 2005). It is released after phloem puncture and contains various effector proteins, which play an important role in phenol detoxification and the maintenance of redox potential (Campbell and Dreyer 1990; Miles and Oertli 1993; Miles 1999). Protein clogging is also prevented by this saliva, helping the pest to feed continuously for long hours (Will and van Bel 2006; Will et al. 2009). Apart from this, calcium-binding proteins are also present in the aphid saliva, which

destabilize the plant defence responses associated with calcium activation and allow the aphids to feed systemically (Will et al. 2007).

It is believed that various effector proteins are present in the aphid saliva, which downregulate the plant defence responses and play a key role in the successful colonization by aphids (Elzinga and Jander 2013). Several potential effectors have been identified from the aphid saliva with the help of bioinformatics and proteomics (Rodriguez and Bos 2013). Aphid virulence can be affected either positively or negatively by these effector molecules (Jaouannet et al. 2014). Effector molecules like C002 and Armet are essential for survival, feeding and colonization of pea aphid, *Acyrthosiphon pisum* (Mutti et al. 2008; Wang et al. 2015). Effectors Mp1, Mp2, Mp55, Mp56, Mp57 and Mp58 increase fecundity of the green peach aphid (Pitino and Hogenhout 2013; Elzinga et al. 2014). Similarly, salivary effectors, like Me10, Me23 and Me47, are crucial for fecundity of potato aphid (Atamian et al. 2013; Kettles and Kaloshian 2016). On the other hand, certain aphid effector proteins such as Mp10 and Mp42 from *M. persicae* negatively affect the aphid virulence when overexpressed in *Nicotiana benthamiana* (Bos et al. 2010).

8.2.2 Aphid Life Cycle

Aphids have two different types of life cycle: heteroecious and autoecious. Heteroecious life cycle is also referred to as host alternating life cycle, as aphids utilize two different hosts in this type of life cycle. Mated females lay eggs on the primary host in summer, whereas only parthenogenetic reproduction takes place on secondary host in winter. On the other hand, in autoecious life cycle (also referred to as non-host-alternating), both sexual and parthenogenetic reproduction take place on the same host (Bhatia et al. 2011). In case of turnip aphid, overwintering egg stage is generally absent, and they reproduce parthenogenetically throughout the year (Blackman and Eastop 2007).

8.2.3 Crosstalk Between Various Signalling Molecules

Various plant signalling molecules like jasmonic acid, salicylic acid, abscisic acid and gibberellic acid and free radicals, like nitric oxide and hydrogen peroxide, interact with each other, both synergistically and antagonistically, and lead to an optimum plant defence strategy (Morkunas et al. 2011). Specialist aphids are able to manipulate this crosstalk between plant molecules by upregulation of SA-dependent pathway and downregulation of JA-dependent pathway, leading to severe aphid infestation (Giordanengo et al. 2010).

8.3 Host Plant Resistance: Plant Defences Against Turnip Aphid

Brassica plants have a wide array of biophysical and biochemical defence mechanisms against aphids. These defences can be either constitutive or induced in nature. Constitutive defences are already present in the plants before herbivory, whereas induced defences are activated after the perception of herbivory by plants. Defences can also be categorized as direct or indirect. Direct defences are those which affect the pest directly by production of toxins, repellents, etc., whereas indirect defences lead to the attraction of natural enemies which ultimately have a negative effect on pest population. The biophysical and biochemical bases of resistance are discussed in the following subsections.

8.3.1 Biophysical Defences

Various morphological and anatomical features, like epicuticular wax, trichomes, depth of vascular bundles, etc., play a key role in deciding whether a plant is a suitable host or not. The epicuticular wax acts as a first line of defence in plant-insect interactions. Waxiness imparts resistance against turnip aphid. It hinders the aphids from reaching the undersurface of leaf (Ahman 1990). However, Angadi et al. (1987) reported that non-waxy plants had lesser *L. ersimi* infestation as compared to waxy plants, and this non-waxiness is characterized by a single dominant gene, which can be bred into cultivated varieties to develop aphid-tolerant plants. Lal et al. (1999) also found that non-waxy stem of two germplasm lines, viz., B-85 glossy and RW-White glossy, was responsible for imparting resistance against turnip aphid, whereas Lamb et al. (1993) reported that leaf waxiness did not have any effect on aphid feeding. Leaf surface wax also plays a key role in tritrophic interactions and indirect defences. Muratori et al. (2006) found that aphid cuticular wax (related to plant surface waxes) influences the host recognition behaviour of aphid parasitoid, *Aphidius rhopalosiphi*.

Different insect pests show different responses to plant trichomes. Trichomes are short hairlike structures, which play a key role in host plant resistance against insects. Aside from various factors like length, density, shape, softness or hardness, growth direction of trichomes is important in imparting resistance against insects. Insect responses may vary according to species, but the general response is the avoidance or rejection of plants by insects for feeding or oviposition (Dalin et al. 2008). Trichomes can be either simple or glandular. Simple trichomes affect the insects directly by acting as a barrier for insect feeding or oviposition, whereas glandular trichomes, which produce sesquiterpenes, alkanes, acyl sugars or other chemicals, may act as repellent or toxicant for the insects (Handley et al. 2005).

In oilseeds rape, surface characteristics of the leaf such as epidermis thickness, bushiness and length of trichomes negatively affected the aphid feeding behaviour by acting as the first line of defence (Hao et al. 2020). They reported that cultivar 'Dehezayou8' with a thinner upper epidermis and short trichomes suffered from more cabbage aphid infestation. Longer trichomes imparted resistance against the sugarcane aphid, *M. sacchari,* in sorghum cultivars (Chang et al. 2008). Strawberry cultivars having bushy trichomes on the abaxial leaf surface were found resistant to the green peach aphid, *M. persicae* (Jiang et al. 2006). When all glandular parts and secretions of trichomes were mechanically wiped out from *Solanum* species, the green peach aphid recovered the ingestion (Alvarez et al. 2006). In certain cases, trichomes may also have negative effects on the natural enemies of aphids. Wietsma (2010) reported that leaf trichomes reduced the performance of *Arabidopsis thaliana* lines by negatively affecting the movement of an aphid predator, *Episyrphus balteatus* (De Geer).

8.3.2 Biochemical Defences

8.3.2.1 Phytoanticipins and Phytoalexins

Plants produce a large number of secondary metabolites to defend themselves against a variety of biotic and abiotic stresses. These secondary metabolites can be divided into two groups, viz., phytoanticipins and phytoalexins. The former are constitutively present in plants even before herbivory, whereas the latter are synthesized after herbivory (Vanetten et al. 1994; Morant et al. 2008). However, there is no clear distinction between phytoanticipins and phytoalexins. Some compounds may act as phytoanticipins in one species while phytoalexins in others (Dixon 2001).

Terpenoids, flavonoids, alkaloids, lignans and phenolic acids play a key role in plant defences against insects. Phenolics act as feeding deterrents for a wide range of pests in *B. napus* plants (Meisner and Mitchell 1984; Muir et al. 1999). Various workers have reported the negative effect of phenols on aphids in artificial diet assays. Leszczynski et al. (1995) have reported that ingestion of phloem sap was drastically reduced when grain aphids were fed with wheat seedlings treated with methoxyphenols. As a result of reduced feeding, varieties with high terpenoid concentration accounted for lower fecundity and longer pre-reproductive period of aphids.

Flavonoids, like flavonols, flavan 3-ols, flavones, flavanones, isoflavonoids and proanthocyanidins, also affect the behaviour, growth and development of aphids. Lattanzio et al. (2000) reported that cowpea varieties with a higher flavonoid concentration were resistant to cowpea aphid, *A. craccivora*.

8.3.2.2 Glucosinolates

Glucosinolates, also known as mustard oil glucosides, are sulphur containing specialized metabolites present in about 17 plant families with a total of about 4700 species (Edger et al. 2018). They are produced by almost all the members of brassicaceae and play a key role in plant defences (Humphrey et al. 2016). Till date, 88 different glucosinolates have been characterized (Blažević et al. 2020). These brassica glucosinolates can be categorized into three types: aliphatic, indole and benzenic glucosinolates (Chhajed et al. 2020). They exist with myrosinases (β-thioglucosidases), which hydrolyse the glucosinolates to form different bioactive compounds (Angelino et al. 2015). This specialized glucosinolate-myrosinase system, also known as 'mustard oil bomb', is a two-component defence system (Ratzka et al. 2002).

Tissue damage due to insect feeding leads to mixing of these glucosinolates and myrosinases. This rapid mixing initiates the formation of an unstable aglycone (thiohydroximate-*O*-sulphate intermediate), which ultimately leads to the production of various biologically active compounds like thiocyanates, isothiocyanates and nitriles by undergoing elimination of the sulphate group (Chhajed et al. 2019). These biologically active metabolites are pungent and act as a repellents and toxicants for various insect herbivores. For example, gut cathepsin protease activity of corn earworm (*Helicoverpa armigera*) is reduced by isothiocyanate derived from 2-propenylglucosinolate, causing reduced growth and death of pest (Agnihotri et al. 2018).

However, certain specialist pests of brassicas, like *Pieris*, *Plutella* and *Lipaphis*, have adapted to these glucosinolate-producing plants by using these glucosinolates and hydrolysis products as cues for feeding and oviposition. These specialists have developed various strategies to disarm this 'mustard oil bomb'. For example, specialists like *Plutella xylostella* have sulfatases enzymes in their gut, which convert glucosinolates into desulfo form after ingestion, which is non-toxic to the larvae (Jeschke et al. 2017). Yang et al. (2020) reported that horseradish flea beetle (*Phyllotreta armoraciae*) overcomes the negative effect of toxic glucosinolates by rapidly eliminating ingested glucosinolates via sequestration and excretion.

Phloem feeders like aphids and whiteflies, because of their specialized mouthparts, minimize contact with myrosin cells and reduce their vulnerability to the so-called mustard oil bomb (Ratzka et al. 2002; Malka et al. 2016). The turnip aphid can even synthesize its own glucosidase which can be stored in the flight muscles.

8.3.2.3 Volatile Compounds

Plants release a variety of volatile organic compounds upon herbivory which play a key role in plant-insect interactions and plant-plant communication (Baldwin et al. 2002). These volatiles are a blend of derivatives of fatty acids (also known as green

leaf volatiles), terpenoids (monoterpenoids, diterpenoids, sesquiterpenoids), derivatives of amino acids, phenylpropanoids and benzenoids. They play a key role in mediating tritrophic interactions by attracting the natural enemies of the herbivore. This form of indirect defence is also referred to as 'call or cry for help'. For example, volatile z-jasmone released after turnip aphid feeding acts as an attractant for its parasitoid (Birkett et al. 2000). The aphid parasitoid *Diaeretiella rapae* is also attracted to the turnip plants as a result of volatiles released after aphid feeding (Blande et al. 2007).

8.4 Screening for Aphid Resistance

Screening is the most important step in the development of insect-resistant cultivar. Phenotyping of plants during segregating generations is the most difficult and tedious part of screening. All the screening material must be infested with an equal and optimal number of insects, so that the plants categorized as resistant are truly resistant and not a result of insect escape (pseudoresistance). Screening under field conditions is generally based upon the injury caused by the insects to plants. For aphid resistance screening, different injury symptoms, like yellowing, curling, crinkling of leaves, drying of inflorescence, injury of developing pods etc., are used to grade the resistant and susceptible genotypes or cultivars. The most adopted screening index for turnip aphid is the one given by Bakhetia and Sandhu (1973). The major drawback of using this screening technique is that it is not able to differentiate between the different phenologies of different genotypes. For example, the early flowering genotypes, which escape the pest attack due to asynchrony between the peak population of pest and susceptible stage of plant, can be falsely considered as resistant. Despite these limitations, it is the widely used method for field screening for resistance against turnip aphid in India.

Traditional phenotype-based screening is a time-consuming technique. There is a need to develop more reliable, quick and easy methods for screening aphid-resistant genotypes. Automated video tracking can be used to phenotype the resistant and susceptible plants. Although this method can screen a large number of genotypes at a single time, it is not able to detect the actual resistance as it uses leaf discs instead of intact plants (Kloth et al. 2015). Electrical penetration graphs (EPG) technique can also be used to screen plants. This technique eliminates the limitation of automated video tracking by using intact plants, but its high equipment cost limits its use (Tjallingii 1988; Trebicki et al. 2012).

Traditional phenotype-based screening can be augmented with the help of recent molecular techniques to increase the efficacy and speed of screening. Recently, a pathogen-responsive gene panel was developed by Sandeep Raj et al. (2017), which could be used in the future for expression-assisted resistance screening against turnip aphid.

8.5 Breeding for Aphid Resistance

Domesticated plants are different from their wild relatives as a result of selection or breeding for various desirable economic traits, like higher yields, biotic and abiotic stress resistance, sweet fruits, faster growth, etc. The earliest examples of insect-resistant cultivar dates back to the 1780s, viz., underhill cultivar of wheat resistant to *Mayetiola destructor* and Winter Majetin cultivar of apple resistant to *Eriosoma lanigerum* (Panda and Khush 1995). However, the conventional breeding methods for insect resistance were more formalized after the rediscovery of Mendelian law of heredity, and the science of host plant resistance came into the limelight after the pioneer work of Painter (Painter 1951). During the past 70 years, HPR had emerged as an important component of integrated pest management strategy, as it is ecologically as well as economically sound. This insect-resistance breeding had changed the global scenario of agricultural crops by increasing the quality and quantity of crops during the green revolution in the 1960s (Smith and Clement 2010). A classic example is the development of multiple pest-resistant rice cultivar IR36, which had increased annual income of Asian rice farmers by approximately $1 billion (Smith 2005).

With the advances in biotechnology and molecular breeding, the science of plant breeding has blossomed. Transgenic crops, like *Bt* cotton, maize and soybean, have changed the global agricultural scenario. *Bt* toxins are only effective against lepidopteran and coleopteran pests and do not have much effect on aphids. However, various alternative genes, like Mi-1.2, Vat genes, lectin-related genes, etc., are useful in transgenic breeding for aphid resistance. The recent advances in conventional and transgenic breeding for aphid resistance in brassicaceae are discussed in the upcoming subsections.

8.5.1 Conventional Breeding Approaches

Various morphological, physiological and biochemical factors are associated with insect resistance. Different breeding methods, like selection, pedigree method, single seed descent method, inter-varietal hybridization, induced mutagenesis or autotetraploidy, are used to incorporate these useful traits conferring resistance in cultivated lines. Various workers have reported that colchicine-induced tetraploid *B. rapa* and *B. napus* strains were more resistant to turnip aphid in comparison to diploids (Rajan 1961; Singh et al. 1965; Jarvis 1970; Gill and Bakhetia 1985; Kalra et al. 1987). Antibiosis was responsible for the resistance, but these strains were not cytogenetically stable. Lammerink (1968) did recurrent selection of crosses between Purple Top White Globe and Sjodin turnip to breed *L. erysimi*-resistant cultivar.

Another possible strategy to breed aphid-resistant cultivar can be the use of crop wild relatives. In wild germplasm and landraces, the susceptible plants die till

harvest, if there is a severe pest attack, and only resistant plants survive. This unintentional selection process taking place over several hundred years in the nature leads to the development of insect-resistant sources or germplasm. These crop wild relatives can be exploited in the breeding programs for insect resistance breeding. These wild relatives contain resistance genes, but they are low yielders. These resistant genes can be introgressed into the cultivated lines using different modern breeding techniques.

In the case of turnip aphid, no resistance source is available in cultivated brassica germplasm (Yadava and Singh 1999; Dutta et al. 2005; Bandopadhyay et al. 2013). However, certain wild relatives of brassica are known to confer resistance against these crucifer specialists. To the best of our knowledge, only three wild germplasms, viz., *Brassica fruticulosa*, *B. montana* and *Rorippa indica*, have been found resistant to turnip aphid. An attempt was made by Kumar et al. (2011) to introgress resistance from *B. fruticulosa* to *B. juncea*. *R. indica* plants, another wild relative of brassica crops, can also survive heavy aphid colonization. Successful introgression has also been reported from *R. indica* to *B. juncea* by somatic hybrids and their backcross progenies (Mandal 2003; Dutta 2007). A novel *Rorippa indica* defensin (RiD) obtained from this plant is responsible for the aphicidal activity of this wild crucifer (Bandopadhyay et al. 2013; Sarkar et al. 2016).

8.5.2 Biotechnological Tools

8.5.2.1 Lectins

Plant lectins have shown promising results for the development of transgenic lines (Vandenborre et al. 2011). Lectins cause increased mortality of aphids by disrupting the digestive system of insects. The aphicidal property of lectins increases with increasing concentration in transgenic lines (Yu et al. 2014). A high concentration of lectins in *B. fruticulosa* was responsible for imparting resistance against turnip aphid (Kumar et al. 2011).

Transgenic plants with upregulation of genes coding for *N*-acetylglucosamine lectins were found resistant to green peach aphid (Gatehouse et al. 1996; Birch et al. 1999), grain aphid (Stoger et al. 1999), tobacco aphid (Wu et al. 2012) and mustard aphid (Kanrar et al. 2002). Transgenic maize plants expressing snowdrop lectin (*Galanthus nivalis* L. agglutinin; GNA) showed 46.9% reduced nymphal production of corn leaf aphid (*Rhopalosiphum maidis* Fitch) (Wang et al. 2005).

Another group of lectins, which can be used as a potential biotechnological tool against aphids, is *Pinellia ternata* agglutinin (PTA). This lectin is derived from a Chinese medicinal plant. This lectin had shown aphicidal activity against cotton and peach aphid in artificial diet assays (Huang et al. 1997; Pan et al. 1998).

8.5.2.2 Protease Inhibitors

Protease inhibitors constitute a major component of plant defence system against herbivory. Upon ingestion, they inhibit protein digestion in insects leading to amino acid deficiency (Ceci et al. 2003). These PIs can be used in breeding transgenic crops for insect resistance. The effect of cysteine PI and serine PI is well-documented against various chewing insects, but not much development has been made in the case of sucking insects (Yu et al. 2014). Aphids primarily use cysteine proteases for digesting proteins. Hence, transgenic plants with cysteine PIs are used for developing aphid-resistant plants. Various artificial diet assays have shown that cysteine PI oryzacystatin (OC-I) inhibits the growth of cotton aphid, peach aphid and potato aphid (Rahbe et al. 2003; Azzouz et al. 2005). Transgenic eggplants and oilseed rape upregulating OC-I gene showed reduced aphid infestation (Rahbe et al. 2003; Ribeiro et al. 2006). Similarly, transgenic *Arabidopsis* plants with barley cysteine PIs HvCPI-6 gene showed aphicidal activity against the green peach aphid and pea aphid (Carrillo et al. 2011). The major limitations for the use of this technology are the possible negative effects on other beneficial insects and development of resistance in aphids (Carlini and Grossi-de-Sa 2002).

8.5.2.3 *Bt* and Other Toxins

Bt transgenics have been used for managing lepidopteran and coleopteran pests since a long time. *Bt* cotton, maize and soybean have been of great benefit in solving the global agricultural problems (Gatehouse et al. 2011). However, these Bt toxins are not much effective against hemipteran pests. In the case of sap sucking aphids, low level of toxicity is mainly due to toxin instability in the gut and low levels of binding (Chougule and Bonning 2012). This limitation has been overcome by the Chougule et al. (2013) with the use of a gut-binding peptide. The binding and toxicity of *Bt* increased in the artificial assay when they added or replaced a 12-amino acid pea aphid gut-binding peptide with the amino acids in one of the three loops of Cyt 2Aa.

Another possible strategy for using insect-specific toxins can be the use of toxins, which act directly in the haemocoel of insects. Development of aphid-resistant plants can be made with the help of coat protein of an aphid-vectored plant virus. Bonning et al. (2014) found that the transgenic *Arabidopsis* plants with an insect haemocoel acting toxin were resistant to the four aphid species (pea aphid, bird cherry-oat aphid, soybean aphid and green peach aphid). This toxin was made by the fusion of coat protein of a luteovirus (aphid-vectored virus) and peptide ω-hexatoxin-Hv1a (spider-derived insect-specific toxin). Since these luteovirid virions can also enter the haemocoel of non-vector aphids like the bird cherry-oat aphid, they can be used for imparting resistance against a wide range of aphid species (Gray and Banerjee 1999). In addition to this, only aphids can transmit luteovirids, making them specific to aphids and safer to non-target organisms (Bonning et al. 2014).

8.5.2.4 Plant Resistance Genes

Till now, only two aphid resistance (R) genes have been used for the transgenic development. Both these genes belong to the NBS-LRR family. The Mi-1.2 gene from wild tomato (*Lycopersicon peruvianum*) shows resistance against potato aphid (Rossi et al. 1998). One another gene Vat (virus-aphid transmission) isolated from melon had shown negative effects on the fitness and fertility of melon/cotton aphid (Dogimont et al. 2014). Both Mi-1.2 and Vat are dominant R genes, but their modes of action are completely different. Mi-1.2 provides resistance by reducing aphid feeding, whereas Vat gene leads to reduced aphid fitness and survival. Various workers have reported that NBS-LRR family genes can play an important role in aphid resistance, but there is a major problem with the use of these resistance genes. These genes provide only species and biotype-specific resistance. For example, Mi-1.2 gene could not provide resistance against potato aphid when it was transferred to eggplant.

Some other examples of candidate R genes for aphid resistance are Ra gene in lettuce against lettuce grain aphid (Wroblewski et al. 2007), Rag1–Rag3 gene in soybean against soybean aphid (Kim et al. 2010; Zhang et al. 2010; Jun et al. 2012) and AKR, TTR and AIN gene in barrel clover against blue green aphid, spotted alfalfa aphid and pea aphid (Klingler et al. 2005, 2007, 2009). R genes Pto and Pti1 belonging to the cytoplasmic serine/threonine kinase family have also shown resistance against the Russian wheat aphid (Afzal et al. 2008).

8.5.2.5 Other Plant Secondary Metabolites

Plant-aphid interactions are mediated by a number of volatile and non-volatile secondary metabolites, which are released by the plant. Various biochemically derived plant metabolites, like phenolics, alkaloids, cyanogenic glycosides, foliar phenolic esters (rutin, chlorogenic acid, etc.), steroids, flavonoids, saponins, etc., play a key role in protection against these sap suckers (Mello and Silva-Filho 2002; Sharma et al. 2000). The pathway involved in the production of these metabolites is regulated by multiple enzymes, like phenylalanine ammonia lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO), making the single gene manipulations for transgenics quite difficult (Hilder and Boulter 1999). However, few key genes encoding for their biosynthesis pathway have been identified and are successfully used to make aphid-resistant transgenic crops. One such example is transgenic chrysanthemum plant, which overexpresses caffeine production genes. This plant secondary metabolite belonging to purine alkaloid family plays a key role in defences against various biotic stresses. Therefore, transgenic chrysanthemum with three enhanced caffeine production genes like coffee (*Coffea arabica*) *N*-methyl transferases genes [7-methylxanthosine synthase 1 (CaXmt1), monomethylxanthine methyltransferase (CaMxmt1) and 3,7-dimethylxanthine *N* methyltransferase (CaDxmt1)] was found resistant to cotton aphids (Kim et al.

2011). Similarly, transgenic tobacco with more β-glucosidases production genes was found to be more aphid resistant as compared to untransformed control (Jin et al. 2011).

Various volatile secondary metabolites also play an important role in plant-aphid interactions, by either acting as an insect repellent or by providing olfactory cues in compatible host recognition (Baldwin 2010). Apart from providing direct defences, these volatiles also play a key role in activating indirect defences by attracting natural enemies. For example, plants produce cis-jasmone upon detection of a threat. Bruce et al. (2008) reported that *Arabidopsis* plants showed differential responses for specialist and generalist aphids when they were induced with this cis-jasmone. The specialist aphid, *L. erysimi*, was attracted to induced plants, whereas the generalist aphid, *M. persicae*, was repelled. However, the reverse responses were noticed for their respective parasitoids, i.e., the generalist parasitoid, *A. ervi*, was attracted and the specialist parasitoid, *Diaeretiella rapae*, was unaffected. Therefore, plants with a high volatile emission can be engineered to provide direct as well as indirect defences against aphids.

Terpenoids are also a major component of plant volatile blends. They not only repel aphids but also attract biocontrol agents of aphids (Kopke et al. 2008). Certain plant-derived terpenoids, like benzoquinone, citronellol, farnesol, geraniol and linalool, act as feeding deterrents at low level and toxic at high level (Gutierrez et al. 1997; Burgueno-Tapia et al. 2008; Halbert et al. 2009). Thus, they can be used as a potential target for engineering transgenic plants with enhanced aphid resistance. Transgenic tobacco plants with enhanced levels of a diterpene cembratriene-ol effectively provided resistance against aphids by silencing P450 gene (Wang et al. 2001). Similarly, transgenic *Arabidopsis* plants with enhanced expression of recombinant linalool/neradiol synthase gene (FaNES1) significantly repelled the green peach aphids (Aharoni et al. 2003)**.**

(E)-*β*-farnesene is an aphid alarm pheromone made up of sesquiterpene hydrocarbon. This pheromone is released when aphids are attacked by their natural enemies. Various plants, like Douglas fir, yuzu, sweet wormwood and peppermint, also have genes encoding for E*β*F synthesis (Huber et al. 2005; Maruyama et al. 2001; Picaud et al. 2005; Crock et al. 1997; Prosser et al. 2006). These genes can be exploited to produce transgenic plants, which can act as a non-toxic repellent for aphids. Beale et al. (2006) reported that *Arabidopsis* plants overexpressing E*β*F synthase gene not only repelled the green peach aphid but also attracted its parasitoids. Transgenic tobacco with upregulation of E*β*F synthase genes also repelled aphids and attracted green lacewing predator (Yu et al. 2012, 2013). Verma et al. (2015) isolated E*β*F synthase gene from *Mentha arvensis* and transformed into *B. juncea*. They reported that transgenic plants overexpressing this gene had lesser *L. erysimi* infestation as compared to untransformed wild plants. These results show the practical applications of E*β*F producing transgenic plants in agriculture.

8.5.2.6 Ribosome-Inactivating Proteins

Ribosome-inactivating proteins (RIPs) are toxic protein synthesis inhibitors that act upon the ribosome. RIPs are cytotoxic *N*-glycosidases that cleave nucleotide N-C glycosidic bonds. They are found in various plants and play an active role in defences against insect pests. These plant proteins can inactivate foreign ribosome with the help of an enzymatic mechanism (Roberts and Selitrennikoff 1986). The artificial diet assays have been done to prove their insecticidal activity. Shahidi-Noghabi et al. (2008) reported the efficacy of *Sambucus nigra* agglutinin I (SNA-I), a type 2 RIP isolated from elderberry (*S. nigra* L.) bark, against two aphid species, *A. pisum* and *Myzus nicotianae*. They found that the survival and fecundity of pea aphid, *A. pisum*, was significantly reduced when they were fed with different concentrations of this RIP in artificial diets. Also, tobacco plants with upregulation of SNA-I gene reduced the survival and fertility of tobacco aphid, *M. nicotianae*. Hamshou et al. (2016) also reported the aphicidal activity of transgenic tobacco plants overexpressing RIP production genes against the green peach aphid, *M. persicae*. In addition to this, they also found sublethal effects on the surviving aphids like reduced fecundity and net reproductive rate.

8.5.2.7 RNA Interference

RNA interference (RNAi) or RNA silencing is a gene suppression phenomenon, which involves the cleavage of dsRNA into small interfering RNA (siRNA) molecules with the help of the dicer enzyme (Yu et al. 2016). After cleavage, these siRNA molecules join a RNA-induced silencing complex (RISC), where argonaute proteins (catalytic components of RISC) use these siRNAs to silence the complementary target messenger RNA (mRNA) via degradation or/and transcriptional repression. This technique has been successfully used to suppress gene expression through highly specific depletion of target transcripts. This phenomenon was first described in *Caenorhabditis elegans* (Fire et al. 1998), for which they got a Nobel Prize in Physiology or Medicine in 2006. Later, it was found that many eukaryotes have also conserved this phenomenon.

In *C. elegans,* exogenous siRNA molecules can be delivered through four methods, i.e. injection, feeding, soaking and in vivo delivery. Microinjection and oral feeding can be used to downregulate the genes via RNAi in aphids. Several workers have reported different genes, which can be targeted by using this technology. Possamai et al. (2007) reported that there was 40% decrease in the gene expression of two marker genes (calreticulin and cathepsin-L) when the pea aphid was microinjected with dsRNA. Mutti et al. (2008) also found that in the case of pea aphid, microinjection of 21–23 nt siRNA caused transcript knockdown in just 3 days. Such knockdown aphids were found to feed less on faba bean plants as a result of reduced contact time with phloem sap in the sieve elements.

RNAi can also be used to engineer transgenic plants with increased resistance to insect pests. This can be done by transforming the host plant with a transgene that encodes a hairpin RNA targeting essential gene of the insect pest. This technique has been successfully exploited in *Arabidopsis* and tobacco for the development of aphid-resistant plants. Transgenic *Arabidopsis* and tobacco plants with silencing of Rack-1 and COO2 gene showed 60% knockdown of these genes and reduction of peach aphid feeding on these plants (Pitino et al. 2011). This was the first example of host plant-mediated RNAi in aphids. Bhatia et al. (2012) reported that transgenic *Arabidopsis* plants expressing dsRNA of a serine protease gene resulted in the reduced gut protease activity and fecundity of *M. persicae*. Similarly, green peach aphid reproduction was reduced drastically when fed on transgenic tobacco plants with dsRNA of gap gene hunchback (Mao and Zeng 2014). Carboxylesterases (CbES) are enzymes which can hydrolyse the esters of insecticides like carbamates and pyrethroids. CbE E4 gene is primarily involved in imparting resistance to the wheat aphid against a number of pesticides. Silencing of this gene using transgenic wheat-mediated RNAi resulted in the reduced tolerance of wheat aphids against phoxim insecticide (Xu et al. 2014). However, there is a high risk of off-target silencing which raises biosafety concerns with the use of this technology. Only highly specific aphid genes should be targeted, which have no orthologs with non-target natural enemies and human beings.

8.6 Conclusion

Genetic engineering can be used to incorporate different desirable genes in a single event. Different approaches with different modes of action can be integrated to develop an effective insect-resistant cultivar. For example, a transgenic plant can be developed with more secondary metabolite production gene and siRNA of an important aphid survival gene. Such plants will repel most of the aphids, and the few aphids which will otherwise feed on the plants will be killed due to downregulation of an important survival gene.

Till now, various efforts have been made to develop aphid-resistant cultivar. However, major emphasis is on developing resistant cultivar, which completely eliminates the aphid population. Such approach can lead to biotype development due to high selection pressure on the aphids. In the context of integrated pest management also, focus should be on those mechanisms, which keep the pest population below the economic threshold level. Moderately resistant variety can easily fit into the IPM module and provide more sustainable pest control.

References

Adhab MA, Schoelz JE (2015) Report of the turnip aphid, *Lipaphis erysimi* (Kaltenbach, 1843) from Missouri, USA. J Plant Prot Res 55:327–332. https://doi.org/10.1515/jppr-2015-0035

Afzal AJ, Wood AJ, Lightfoot DA (2008) Plant receptor-like serine threonine kinases: roles in signaling and plant defense. Mol Plant-Microbe Interact 21:507–517. https://doi.org/10.1094/MPMI-21-5-0507

Agnihotri AR, Hulagabali CV, Adhav AS, Joshi RS (2018) Mechanistic insight in potential dual role of sinigrin against *Helicoverpa armigera*. Phytochemistry 145:121–127. https://doi.org/10.1016/j.phytochem.2017.10.014

Aharoni A, Giri AP, Deuerlein S, Griepink F, de Kogel WJ, Verstappen FW, Verhoeven HA, Jongsma MA, Schwab W, Bouwmeester HJ (2003) Terpenoid metabolism in wild-type and transgenic Arabidopsis plants. Plant Cell 15:2866–2884. https://doi.org/10.1105/tpc.016253

Ahman I (1990) Plant surface characteristics and movements of two Brassica-feeding aphids, *Lipaphis erysimi* and *Brevicoryne brassicae*. In: Symposia Biologica Hungaria No. 39. Publishing House of Hungarian Academy of Sciences, Budapest, pp 119–125

Alvarez AE, Tjallingii WF, Garzo E, Vleeshouwers V, Dicke M, Vosman B (2006) Location of resistance factors in the leaves of potato and wild tuber-bearing *Solanum* species to the aphid *Myzus persicae*. Entomol Exp Appl 121:145–157. https://doi.org/10.1111/j.1570-8703.2006.00464.x

Angadi SP, Singh JP, Anand IJ (1987) Inheritance on non-waxiness and tolerance to aphids in Indian mustard. Short communication. J Oilseeds Res 4(2):265–267

Angelino D, Dosz EB, Sun J, Hoeflinger JL, Van Tassell ML, Chen P, Harnly JM, Miller MJ, Jeffery EH (2015) Myrosinase-dependent and independent formation and control of isothiocyanate products of glucosinolate hydrolysis. Front Plant Sci 6:831. https://doi.org/10.3389/fpls.2015.00831

Atamian HS, Chaudhary R, Cin VD, Bao E, Girke T, Kaloshian I (2013) *In planta* expression or delivery of potato aphid *Macrosiphum euphorbiae* effectors Me10 and Me23 enhances aphid fecundity. Mol Plant Microbe Interact 26(1):67–74. https://doi.org/10.1094/MPMI-06-12-0144-FI

Azzouz H, Cherqui A, Campan EDM, Rahbe Y, Duport G, Jouanin L, Kaiser L, Giordanengo P (2005) Effects of plant protease inhibitors, oryzacystatin I and soybean Bowman–Birk inhibitor, on the aphid *Macrosiphum euphorbiae* (Homoptera, Aphididae) and its parasitoid *Aphelinus abdominalis* (Hymenoptera, Aphelinidae). J Insect Physiol 51:75–86. https://doi.org/10.1016/j.jinsphys.2004.11.010

Bakhetia DRC (1991) Insect pests. In: Chopra VL, Prakash S (eds) Oilseed Brassicas in Indian agriculture. Vikas Publishing House, New Delhi, pp 211–240

Bakhetia DRC, Sandhu RS (1973) Differential response of Brassica species/varieties to the aphid, *Lipaphis erysimi* (Kalt.) infestation. J Res Punjab Agric Univ 10:272–279

Baldwin IT (2010) Plant volatiles. Curr Biol 20:R392–R397

Baldwin IT, Kessler A, Halitschke R (2002) Volatile signaling in plant-plant-herbivore interactions: what is real? Curr Opin Plant Biol 5:351–354. https://doi.org/10.1016/S1369-5266(02)00263-7

Bandopadhyay L, Basu D, Sikdar SR (2013) Identification of genes involved in wild crucifer *Rorippa indica* resistance response on mustard aphid, *Lipaphis erysimi* challenge. PLoS One 8(9):e73632. https://doi.org/10.1371/journal.pone.0073632

Beale MH, Birkett MA, Bruce TJ, Chamberlain K, Field LM, Huttly AK, Martin JL, Parker R, Phillips AL, Pickett JA (2006) Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. Proc Natl Acad Sci U S A 103:10509–10513. https://doi.org/10.1073/pnas.0603998103

Bhatia V, Uniyal PL, Bhattacharya R (2011) Aphid resistance in Brassica crops: challenges, biotechnological progress and emerging possibilities. Biotechnol Adv 29(6):879–888. https://doi.org/10.1016/j.biotechadv.2011.07.005

Bhatia V, Bhattacharya R, Uniyal PL, Singh R, Niranjan RS (2012) Host generated siRNAs attenuate expression of serine protease gene in *Myzus persicae*. PLoS One 7:e46343. https://doi.org/10.1371/journal.pone.0046343

Bhattacharya S (2019) Brassica-aphid interaction: challenges and prospects of genetic engineering for integrated aphid management. Physiol Mol Plant Pathol. https://doi.org/10.1016/j.pmpp.2019.101442

Birch ANE, Geoghegan IE, Majerus MEN, McNicol JW, Hackett CA, Gatehouse AMR, Gatehouse JA (1999) Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. Mol Breed 5:75–83. https://doi.org/10.1023/A:1009659316170

Birkett MA, Campbell CAM, Chamberlain K, Guerrieri E, Hick AJ, Martin JL, Matthes M, Napier JA, Pettersson J, Pickett JA, Poppy GM, Pow EM, Pye BJ, Smart LE, Wadhams GH, Wadhams LJ, Woodcock CM (2000) New roles for cis-jasmone as an insect semiochemical and in plant defense. Proc Natl Acad Sci U S A 97:9329–9334. https://doi.org/10.1073/pnas.160241697

Blackman RL, Eastop VF (2000) Aphids on the world's crops: an identification and information guide, 2nd edn. Wiley, Chichester

Blackman RL, Eastop VF (2007) Taxonomic issues. In: van Emden HF, Harrington R (eds) Aphids as crop pests. CABI, Wallingford, pp 1–29

Blande J, Pickett J, Poppy G (2007) A comparison of semiochemically mediated interactions involving specialist and generalist brassica feeding aphids and the braconid parasitoid *Diaeretiella rapae*. J Chem Ecol 33:767–779. https://doi.org/10.1007/s10886-007-9264-7

Blažević I, Montaut S, Burcul F, Olsen CE, Burow M, Rollin P, Agerbirk N (2020) Glucosinolate structural diversity, identification, chemical synthesis and metabolism in plants. Phytochemistry 169:112100. https://doi.org/10.1016/j.phytochem.2019.112100

Bonning BC, Pal N, Liu S, Wang Z, Sivakumar S, Dixon PM, King GF, Miller WA (2014) Toxin delivery by the coat protein of an aphid-vectored plant virus provides plant resistance to aphids. Nat Biotechnol 32:102–105. https://doi.org/10.1038/nbt.2753

Bos JI, Prince D, Pitino M, Maffei ME, Win J, Hogenhout SA (2010) A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). PLoS Genet 6(11):e1001216. https://doi.org/10.1371/journal.pgen.1001216

Bruce TJ, Matthes MC, Chamberlain K, Woodcock CM, Mohib A, Webster B, Smart ME, Birkett MA, Pickett JA, Napier JA (2008) cis Arabidopsis genes that affect the chemical ecology of multitrophic interactions with aphids and their parasitoids. Proc Natl Acad Sci U S A 105:4553–4558. https://doi.org/10.1073/pnas.0710305105

Burgueno-Tapia E, Castillo L, Gonzalez-Coloma A, Joseph-Nathan P (2008) Antifeedant and phytotoxic activity of the sesquiterpene p-benzoquinone perezone and some of its derivatives. J Chem Ecol 34:766–771. https://doi.org/10.1007/s10886-008-9495-2

Campbell BC, Dreyer DL (1990) The role of plant matrix polysaccharides in aphid-plant interactions. In: Campbell RK, Eikenbary RD (eds) Aphid-plant genotype interactions. Elsevier, Amsterdam, pp 149–169

Carlini CR, Grossi-de-Sa MF (2002) Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon 40:1515–1539. https://doi.org/10.1016/s0041-0101(02)00240-4

Carrillo L, Martinez M, Alvarez-Alfageme F, Castanera P, Smagghe G, Diaz I, Ortego F (2011) A barley cysteine-proteinase inhibitor reduces the performance of two aphid species in artificial diets and transgenic Arabidopsis plants. Transgenic Res 20:305–319. https://doi.org/10.1007/s11248-010-9417-2

Ceci LR, Volpicella M, Rahbé Y, Gallerani R, Beekwilder J, Jongsma MA (2003) Selection by phage display of a variant mustard trypsin inhibitor toxic against aphids. Plant J 33:557–566. https://doi.org/10.1046/j.1365-313x.2003.01645.x

Chang JH, Zhang L, Shen SX, Ma ZY (2008) Correlation analysis of physical and chemical characteristics with resistance to Sorghum aphid (*Melanaphis sacchari*) in different Sorghum genotypes. J Plant Genet Resour 9:55–61

Cherqui A, Tjallingii WF (2000) Salivary proteins of aphids, a pilot study on identification, separation and immunolocalisation. J Insect Physiol 46(8):1177–1186. https://doi.org/10.1016/S0022-1910(00)00037-8

Chhajed S, Misra BB, Tello N, Chen S (2019) Chemodiversity of the glucosinolate-myrosinase system at the single cell type resolution. Front Plant Sci 10:618. https://doi.org/10.3389/fpls.2019.00618

Chhajed S, Mostafa I, He Y, Abou-Hashem M, El-Domiaty M, Chen S (2020) Glucosinolate biosynthesis and the glucosinolate–myrosinase system in plant defense. Agronomy 10:1786. https://doi.org/10.3390/agronomy10111786

Chougule NP, Bonning BC (2012) Toxins for transgenic resistance to hemipteran pests. Toxins 4:405–429. https://doi.org/10.3390/toxins4060405

Chougule NP, Li H, Liu S, Linz LB, Narva KE, Meade T, Bonning BC (2013) Retargeting of the *Bacillus thuringiensis* toxin Cyt2Aa against hemipteran insect pests. Proc Natl Acad Sci U S A 110:8465–8470. https://doi.org/10.1073/pnas.1222144110

Crock J, Wildung M, Croteau R (1997) Isolation and bacterial expression of a sesquiterpene synthase cDNA clone from peppermint (*Mentha* x *piperita*, L.) that produces the aphid alarm pheromone (E)-*β*-farnesene. Proc Natl Acad Sci U S A 94:12833–12838. https://doi.org/10.1073/pnas.94.24.12833

Dalin P, Agren J, Bjorkman C, Huttunen P, Karkkainen K (2008) Leaf trichome formation and plant resistance to herbivory. In: Schaller A (ed) Induced plant resistance to herbivory. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-8182-8_4

Dixon RA (2001) Natural products and plant disease resistance. Nature 411:843–847. https://doi.org/10.1038/35081178

Dogimont C, Chovelon V, Pauquet J, Boualem A, Bendahmane A (2014) The Vat locus encodes for a CC-NBS-LRR protein that confers resistance to *Aphis gossypii* infestation and *A. gossypii*-mediated virus resistance. Plant J 80:993–1004. https://doi.org/10.1111/tpj.12690

Dutta S (2007) Development and characterisation of aphid tolerant *Brassica juncea* chromosome addition lines from *Rorippobrassica* somatic hybrid (*Rorippa indica*+*Brassica juncea*) through plant breeding approach. Ph.D. Thesis, Jadavpur University, Kolkata, India

Dutta I, Majumdar P, Saha P, Ray K, Das S (2005) Constitutive and phloem specific expression of *Allium sativum* leaf agglutinin (ASAL) to engineer aphid (*Lipaphis erysimi*) resistance in transgenic Indian mustard (*Brassica juncea*). Plant Sci 169:996–1007. https://doi.org/10.1016/j.plantsci.2005.05.016

Edger PP, Hall JC, Harkess A, Tang M, Coombs J, Mohammadin S, Schranz ME, Xiong Z, Leebens-Mack J, Meyers BC, Sytsma KJ, Koch MA, Al-Shehbaz IA, Pires JC (2018) Brassicales phylogeny inferred from 72 plastid genes: a reanalysis of the phylogenetic localization of two paleopolyploid events and origin of novel chemical defenses. Am J Bot 105:463–469. https://doi.org/10.1002/ajb2.1040

Elzinga DA, Jander G (2013) The role of protein effectors in plant–aphid interactions. Curr Opin Plant Biol 16(4):451–456

Elzinga DA, De Vos M, Jander G (2014) Suppression of plant defenses by a *Myzus persicae* (green peach aphid) salivary effector protein. Mol Plant Microbe Interact 27(7):747–756. https://doi.org/10.1094/MPMI-01-14-0018-R

Felton GW, Eichenseer H (1999) Herbivore saliva and its effects on plant defense against herbivores and pathogens. In: Agrawal AA, Tuzun S, Bent E (eds) Induced plant defenses against pathogens. APS Press, St. Paul, pp 19–36

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391:806–811

Gatehouse AM, Down RE, Powell KS, Sauvion N, Rahbe Y, Newell CA, Merryweather A, Hamilton WD, Gatehouse J (1996) Transgenic potato plants with enhanced resistance

to the peach-potato aphid *Myzus persicae*. Entomol Exp Appl 79:295–307. https://doi.org/10.1111/j.1570-7458.1996.tb00837.x

Gatehouse A, Ferry N, Edwards MG, Bell HA (2011) Insect-resistant biotech crops and their impacts on beneficial arthropods. Philos Trans R Soc B 366:1438–1452. https://doi.org/10.1098/rstb.2010.0330

Gill RS, Bakhetia DRC (1985) Resistance of some *Brassica napus* and *B. campestris* strains to *Lipaphis erysimi* (Kalt.). J Oilseeds Res 2:227–239

Giordanengo P, Brunissen L, Rusterucci C, Vincent C, van Bel A, Dinant S, Girousse C, Faucher M, Bonnemain JL (2010) Compatible plant–aphid interactions: how aphids manipulate plant responses? C R Biol 333:516–523. https://doi.org/10.1016/j.crvi.2010.03.007

Gray SM, Banerjee N (1999) Mechanisms of arthropod transmission of plant and animal viruses. Microbiol Mol Biol Rev 63:128–148

Gutierrez C, Fereres A, Reina M, Cabrera R, Gonzalez-Coloma A (1997) Behavioral and sublethal effects of structurally related lower terpenes on *Myzus persicae*. J Chem Ecol 23:1641–1650. https://doi.org/10.1023/B:JOEC.0000006428.00568.c5

Halbert SE, Corsini D, Wiebe M, Vaughn SF (2009) Plant-derived compounds and extracts with potential as aphid repellents. Ann Appl Biol 154:303–307. https://doi.org/10.1111/j.1744-7348.2008.00300.x

Hamshou M, Shang C, Smagghe G, van Damme EJM (2016) Ribosome-inactivating proteins from apple have strong aphicidal activity in artificial diet and *in planta*. Crop Prot 87:19–24. https://doi.org/10.1016/j.cropro.2016.04.013

Handley R, Ekbom B, Agren J (2005) Variation in trichome density and resistance against a specialist insect herbivore in natural populations of *Arabidopsis thaliana*. Ecol Entomol 30:284–292. https://doi.org/10.1111/j.0307-6946.2005.00699.x

Hao ZP, Zhan HX, Gao LL, Huang F, Zhu LN, Hou SM (2020) Possible effects of leaf tissue characteristics of oilseed rape *Brassica napus* on probing and feeding behaviors of cabbage aphids, *Brevicoryne brassicae*. Arthropod Plant Interact 14:733–744. https://doi.org/10.1007/s11829-020-09782-5

Hilder VA, Boulter D (1999) Genetic engineering of crop plants for insect resistance—a critical review. Crop Prot 18:177–191. https://doi.org/10.1016/S0261-2194(99)00028-9

Huang DF, Pan YH, Zhang SX, Cao JP, Yang XM, Zhang J, Yi W (1997) The discovery of insecticidal protein against aphids from *Pinellia pedatisecta* and *P. ternata*. Sci Agric Sin 30:94–96

Huber DP, Philippe RN, Godard K, Sturrock RN, Bohlmann J (2005) Characterization of four terpene synthase cDNAs from methyl jasmonate-induced Douglas-fir, *Pseudotsuga menziesii*. Phytochemistry 66:1427–1439. https://doi.org/10.1016/j.phytochem.2005.04.030

Humphrey PT, Gloss AD, Alexandre NM, Villalobos MM, Fremgen MR, Groen SC, Meihls LN, Jander G, Whiteman NK (2016) Aversion and attraction to harmful plant secondary compounds jointly shape the foraging ecology of a specialist herbivore. Ecol Evol 6:3256–3268. https://doi.org/10.1002/ece3.2082

Jaouannet M, Rodriguez PA, Thorpe P, Lenoir CJ, MacLeod R, Escudero-Martinez C, Bos JI (2014) Plant immunity in plant–aphid interactions. Front Plant Sci 5:663. https://doi.org/10.3389/fpls.2014.00663

Jarvis JL (1970) Relative injury to some cruciferous oilseeds by the turnip aphid. J Econ Entomol 63:1498–1502

Jeschke V, Kearney EE, Schramm K, Kunert G, Shekhov A, Gershenzon J, Vassao DG (2017) How glucosinolates affect generalist lepidopteran larvae: growth, development and glucosinolate metabolism. Front Plant Sci 8:1995. https://doi.org/10.3389/fpls.2017.01995

Jiang GH, Xie M, Lv ZX, Wang HR, Wang LP, Wu YJ, Zhang HQ, Huang PL (2006) Mechanism of resistance to aphids in strawberry cultivars. J Fruit Sci 23:728–731

Jin S, Kanagaraj A, Verma D, Lange T, Daniell H (2011) Release of hormones from conjugates: chloroplast expression of β-Glucosidase results in elevated phytohormone levels associated

with significant increase in biomass and protection from aphids or whiteflies conferred by sucrose esters. Plant Physiol 155(1):222–235 https://doi.org/10.1104/pp.110.160754

Jun TH, Mian MR, Michel AP (2012) Genetic mapping revealed two loci for soybean aphid resistance in PI 567301B. Theor Appl Genet 124:13–22. https://doi.org/10.1007/s00122-011-1682-9

Kalra VK, Singh H, Rohilla HR (1987) Influence of various genotypes of *Brassica juncea* on biology of mustard aphid, *Lipaphis erysimi* (Kalt.). Indian J Agric Sci 57:277–279

Kanrar S, Venkateswari J, Kirti PB, Chopra VL (2002) Transgenic Indian mustard (*Brassica juncea*) with resistance to the mustard aphid (*Lipaphis erysimi* Kalt.). Plant Cell Rep 20:976–981. https://doi.org/10.1007/s00299-001-0422-z

Kettles GJ, Kaloshian I (2016) The potato aphid salivary effector Me47 is a glutathione-S851 transferase involved in modifying plant responses to aphid infestation. Front Plant Sci 7:1142. https://doi.org/10.3389/fpls.2016.01142

Kim KS, Hill CB, Hartman GL, Hyten DL, Hudson ME, Diers BW (2010) Fine mapping of the soybean aphid-resistance gene Rag2 in soybean PI 200538. Theor Appl Genet 121:599–610. https://doi.org/10.1007/s00122-010-1333-6

Kim Y, Lim S, Kang K, Jung Y, Lee Y, Choi Y, Sano H (2011) Resistance against beet armyworms and cotton aphids in caffeine producing transgenic Chrysanthemum. Plant Biotechnol 28:393–395. https://doi.org/10.5511/plantbiotechnology.11.0510a

Klingler J, Creasy R, Gao L, Nair RM, Calix AS, Jacob HS, Edwards OR, Singh KB (2005) Aphid resistance in *Medicago truncatula* involves antixenosis and phloem-specific, inducible antibiosis, and maps to a single locus flanked by NBS-LRR resistance gene analogs. Plant Physiol 137:1445–1455. https://doi.org/10.1104/pp.104.051243

Klingler JP, Edwards OR, Singh KB (2007) Independent action and contrasting phenotypes of resistance genes against spotted alfalfa aphid and bluegreen aphid in *Medicago truncatula*. New Phytol 173:630–640. https://doi.org/10.1111/j.1469-8137.2006.01939.x

Klingler JP, Nair RM, Edwards OR, Singh KB (2009) A single gene, AIN, in *Medicago truncatula* mediates a hypersensitive response to both bluegreen aphid and pea aphid, but confers resistance only to bluegreen aphid. J Exp Bot 60(14):4115–4127. https://doi.org/10.1093/jxb/erp244

Kloth KJ, ten Broeke CJM, Thoen MPM, van den Brink MH, Wiegers GL, Krips OE, Noldus LPJJ, Dicke M, Jongsma MA (2015) High-throughput phenotyping of plant resistance to aphids by automated video tracking. Plant Methods 11:4. https://doi.org/10.1186/s13007-015-0044-z

Kopke D, Schroder R, Fischer HM, Gershenzon J, Hilker M, Schmidt A (2008) Does egg deposition by herbivorous pine sawflies affect transcription of sesquiterpene synthases in pine? Planta 228:427–438. https://doi.org/10.1007/s00425-008-0747-8

Kumar S, Atri C, Sangha MK, Banga SS (2011) Screening of wild crucifers for resistance to mustard aphid, *Lipaphis erysimi* (Kaltenbach) and attempt at introgression of resistance gene(s) from *Brassica fruticulosa* to *Brassica juncea*. Euphytica 179:461–470. https://doi.org/10.1007/s10681-011-0351-z

Lal MN, Singh SS, Singh VP (1999) Reaction of mustard aphid, *Lipaphis erysimi* (Kalt.) to morphological characters of mustard, *Brassica juncea*. J Entomol Res 23(3):221–223

Lamb RJ, Smith MAH, Bodnaryk RP (1993) Leaf waxiness and the performance of *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae) on three Brassica crops. Can Entomol 125:1023–1031

Lammerink J (1968) Rangi: new rape that resists aphids. N Z J Agric 117:61

Lattanzio V, Arpaia S, Cardinali A, Di Venere D, Linsalata V (2000) Role of endogenous flavonoids in resistance mechanism of Vigna to aphids. J Agric Food Chem 48:5316–5320. https://doi.org/10.1021/jf000229y

LeCoz C, Ducombs G (2006) Plants and plant products. In: Frosch PJ, Menne T, Lepottevin JP (eds) Contact dermatitis, 4th edn. Springer, Berlin/Heidelberg, pp 751–800

Leszczynski B, Tjallingii W, Dixon A, Swiderski R (1995) Effect of methoxyphenols on grain aphid feeding behaviour. Entomol Exp Appl 76:157–162

Louis J, Singh V, Shah J (2012) *Arabidopsis thaliana*-aphid interaction. Arabidopsis Book 10:e0159. https://doi.org/10.1199/tab.0159

Malka O, Shekhov A, Reichelt M, Gershenzon J, Vassao DG, Morin S (2016) Glucosinolate desulfation by the phloem-feeding insect *Bemisia tabaci*. J Chem Ecol 42:230–235. https://doi.org/10.1007/s10886-016-0675-1

Mandal P (2003) Development and characterisation of somatic hybrids between *Rorippa indica* and *Brassica juncea*. Ph.D. Thesis, Jadavpur University, Kolkata, India

Mao J, Zeng F (2014) Plant-mediated RNAi of a gap gene-enhanced tobacco tolerance 895 against the *Myzus persicae*. Transgenic Res 23(1):145–152. https://doi.org/10.1007/s11248-013-9739-y

Maruyama T, Ito M, Honda G (2001) Molecular cloning, functional expression and characterization of (E)-*β*-farnesene synthase from *Citrus junos*. Biol Pharm Bull 24:1171–1175. https://doi.org/10.1248/bpb.24.1171

Meisner J, Mitchell BK (1984) Phagodeterrency induced by some secondary plant substances in adults of the flea beetle *Phyllotreta striolata*. J Plant Dis Prot 91:301–304

Mello MO, Silva-Filho MC (2002) Plant-insect interactions: an evolutionary arms race between two distinct defense mechanisms. Braz J Plant Physiol 14:71–81. https://doi.org/10.1590/S1677-04202002000200001

Miles PW (1999) Aphid saliva. Biol Rev Camb Philos Soc 74(01):41–85

Miles PW, Oertli JJ (1993) The significance of antioxidants in the aphid-plant interactions: the redox hypothesis. Entomol Exp Appl 67(3):275–283. https://doi.org/10.1111/j.1570-7458.1993.tb01678.x

Mondal H (2020) Aphid saliva: a powerful recipe for modulating host resistance towards aphid clonal propagation. Arthropod Plant Interact 14:547–558. https://doi.org/10.1007/s11829-020-09769-2

Morant AV, Jorgensen K, Jorgensen C, Paquette SM, Perez RS, Moller BL, Bak S (2008) β-Glucosidases as detonators of plant chemical defense. Phytochemistry 69:1795–1813. https://doi.org/10.1016/j.phytochem.2008.03.006

Morkunas I, Mai VC, Gabrys B (2011) Phytohormonal signaling in plant responses to aphid feeding. Acta Physiol Plant 33:2057–2073. https://doi.org/10.1007/s11738-011-0751-7

Muir AD, Gruber MY, Hinks CF, Lees GL, Onyilagha J, Soroka J, Erlandson M (1999) Effect of condensed tannins in the diets of major crop insects. In: Gross G, Hemingway RW, Yoshida T (eds) Plant polyphenols 2: chemistry, biology, pharmacology ecology. Kluwer Academic/Plenum Publ., New York, pp 867–881

Muratori F, Le Ralec A, Lognay G, Hance T (2006) Epicuticular factors involved in host recognition for the aphid parasitoid *Aphidius rhopalosiphi*. J Chem Ecol 32:579–593. https://doi.org/10.1007/s10886-005-9023-6

Mutti NS, Louis J, Pappan LK, Pappan K, Begum K, Chen MS, Park Y, Dittmer N, Marshall J, Reese JC, Reeck GR (2008) A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant. Proc Natl Acad Sci U S A 105(29):9965–9969. https://doi.org/10.1073/pnas.0708958105

Painter RH (1951) Insect resistance in crop plants. Univ Kans Press, Lawrence, p 520

Pan YH, Zhang SX, Cao JP, Yang XM, Zhang J, Ying WZ, Huang DF (1998) The isolation, purification of *Pinellia pedatisecta* lectin and its activity on aphid-resistance. Prog Nat Sci 8:502–505

Panda N, Khush GS (1995) Host plant resistance to insects. Wallingford, p 431

Picaud S, Brodelius M, Brodelius PE (2005) Expression, purification and characterization of recombinant (E)-β-farnesene synthase from *Artemisia annua*. Phytochemistry 66:961–967. https://doi.org/10.1016/j.phytochem.2005.03.027

Pitino M, Hogenhout SA (2013) Aphid protein effectors promote aphid colonization in a plant species-specific manner. Mol Plant Microbe Interact 26(1):130–139. https://doi.org/10.1094/MPMI-07-12-0172-FI

Pitino M, Coleman AD, Maffei ME, Ridout CJ, Hogenhout SA (2011) Silencing of aphid genes by dsRNA feeding from plants. PLoS One 6(10):e25709. https://doi.org/10.1371/journal.pone.0025709

Possamai SJ, Trionnaire GL, Bonhomme J, Christophides GK, Rispe C, Tagu D (2007) Gene knockdown by RNAi in the pea aphid *Acyrthosiphon pisum*. BMC Biotechnol 7(1):63. https://doi.org/10.1186/1472-6750-7-63

Powell G (2005) Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. J Gen Virol 86(2):469–472. https://doi.org/10.1099/vir.0.80632-0

Prosser IM, Adams RJ, Beale MH, Hawkins ND, Phillips AL, Pickett JA, Field LM (2006) Cloning and functional characterisation of a *cis*-muuroladiene synthase from black peppermint (*Mentha* × *piperita*) and direct evidence for a chemotype unable to synthesise farnesene. Phytochemistry 67:1564–1571. https://doi.org/10.1016/j.phytochem.2005.06.012

Rahbe Y, Deraison C, Bonade-Bottino M, Girard C, Nardon C, Jouanin L (2003) Effects of the cysteine protease inhibitor oryzacystatin (OC-I) on different aphids and reduced performance of *Myzus persicae* on OC-I expressing transgenic oilseed rape. Plant Sci 164:441–450. https://doi.org/10.1016/S0168-9452(02)00402-8

Rajan SS (1961) Aphid resistance of autotetraploid toria. Indian Oilseeds J 8:251–255

Ram C, Koramutla MK, Bhattacharya R (2017) Identification and comprehensive evaluation of reference genes for RT-qPCR analysis of host gene-expression in *Brassica juncea*-aphid interaction using microarray data. Plant Physiol Biochem 116:57–67. https://doi.org/10.1016/j.plaphy.2017.05.004

Ratzka A, Vogel H, Kliebenstein DJ, Mitchell-Olds T, Kroymann J (2002) Disarming the mustard oil bomb. Proc Natl Acad Sci U S A 99:11223–11228

Ribeiro ADO, Pereira EJG, Galvan TL, Picanco MC, Picoli EDT, Silva DD, Fari MG, Otoni WC (2006) Effect of eggplant transformed with oryza cystatin gene on *Myzus persicae* and *Macrosiphum euphorbiae*. J Appl Entomol 130:84–90. https://doi.org/10.1111/j.1439-0418.2005.01021.x

Roberts WK, Selitrennikoff CP (1986) Plant proteins that inactivate foreign ribosomes. Biosci Rep 6:19–29. https://doi.org/10.1007/BF01145175

Rodriguez PA, Bos JI (2013) Toward understanding the role of aphid effectors in plant infestation. Mol Plant Microbe Interact 26(1):25–30. https://doi.org/10.1094/MPMI-05-12-0119-FI

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene Mi of tomato confers resistance against the potato aphid. Proc Natl Acad Sci 95:9750–9754. https://doi.org/10.1073/pnas.95.17.9750

Sandeep Raj R, Thakur SV, Hussen VS, Joshi MN, Tyagi SN, Bagatharia SB (2017) Development of PR genes panel for screening aphid-tolerant cultivars in *Brassica juncea*. 3 Biotech 7:129. https://doi.org/10.1007/s13205-017-0785-7

Sarkar P, Jana J, Chatterjee S, Sikdar SR (2016) Functional characterization of *Rorippa indica* defensin and its efficacy against *Lipaphis erysimi*. Springerplus 5:511. https://doi.org/10.1186/s40064-016-2144-2

Shahidi-Noghabi S, Van Damme EJ, Smagghe G (2008) Carbohydrate-binding activity of the type-2 ribosome-inactivating protein SNA-I from elderberry (*Sambucus nigra*) is a determining factor for its insecticidal activity. Phytochemistry 69:2972–2978

Sharma HC, Sharma KK, Seetharama N, Ortiz R (2000) Prospects for using transgenic resistance to insects in crop improvement. Electron J Biotechnol 3:21–22. https://doi.org/10.4067/S0717-34582000000200001

Singh SR, Narain A, Srivastava KP, Siddiqui RA (1965) Fecundity of mustard aphid on different rape and mustard species. Indian Oilseeds J 9:215–219

Smith CM (2005) Plant resistance to arthropods: molecular and conventional approaches. Springer, Dordrecht, p 423

Smith C, Clement S (2010) Molecular bases of plant resistance to arthropods. Annu Rev Entomol 57:309–328. https://doi.org/10.1146/annurev-ento-120710-100642

Stoger E, Williams S, Christou P, Down RE, Gatehouse JA (1999) Expression of the insecticidal lectin from snowdrop (*Galanthus nivalis* agglutinin; GNA) in transgenic wheat plants: effects on predation by the grain aphid *Sitobion avenae*. Mol Breed 5:65–73. https://doi.org/10.1023/A:1009616413886

Tjallingii WF (1988) Electrical recording of stylet penetration activities. In: Minks AK, Harrewijn P (eds) Aphids, their biology, natural enemies and control, vol 2B. Elsevier, Amsterdam, pp 95–108

Trebicki P, Tjallingii WF, Harding RM, Rodoni BC, Powell KS (2012) EPG monitoring of the probing behaviour of the common brown leafhopper *Orosius orientalis* on artificial diet and selected host plants. Arthropod Plant Interact 6:405–415. https://doi.org/10.1007/s11829-012-9192-5

Vandenborre G, Smagghe G, Van Damme EJ (2011) Plant lectins as defence proteins against phytophagous insects. Phytochemistry 72(13):1538–1550. https://doi.org/10.1016/j.phytochem.2011.02.024

Vanetten HD, Mansfield JW, Bailey JA, Farmer EE (1994) 2 classes of antibiotics—phytoalexins vs phytoanticipins. Plant Cell 6:1191–1192. https://doi.org/10.1105/tpc.6.9.1191

Verma SS, Sinha RK, Jajoo A (2015) (E)-β-farnesene gene reduces *Lipaphis erysimi* colonization in transgenic *Brassica juncea* lines. Plant Signal Behav 10(7):e1042636. https://doi.org/10.1080/15592324.2015.1042636

Wang E, Wang R, DeParasis J, Loughrin JH, Gan S, Wagner GJ (2001) Suppression of a P450 hydroxylase gene in plant trichome glands enhances natural-product-based aphid resistance. Nat Biotechnol 19:371–374. https://doi.org/10.1038/86770

Wang ZY, Zhang KW, Sun XF, Tang KX, Zhang JR (2005) Enhancement of resistance to aphids by introducing the snowdrop lectin gene GNA into maize plants. J Biosci 30:627–638. https://doi.org/10.1007/BF02703563

Wang W, Luo L, Lu H, Chen S, Kang L, Cui F (2015) Angiotensin-converting enzymes modulate aphid–plant interactions. Sci Rep 5:8885. https://doi.org/10.1038/srep08885

Wietsma R (2010) The effect of differences in aliphatic glucosinolate concentrations in *Arabidopsis thaliana* on herbivores of different feeding guilds and different levels of specialization. Dissertation, Wageningen University and Research Centre, Wageningen

Will T, van Bel AJ (2006) Physical and chemical interactions between aphids and plants. J Exp Bot 57(4):729–737. https://doi.org/10.1093/jxb/erj089

Will T, Tjallingii WF, Thönnessen A, van Bel AJ (2007) Molecular sabotage of plant defence by aphid saliva. Proc Natl Acad Sci 104(25):10536–10541. https://doi.org/10.1073/pnas.0703535104

Will T, Kornemann SR, Furch AC, Tjallingii WF, van Bel AJ (2009) Aphid watery saliva counteracts sieve-tube occlusion: a universal phenomenon? J Exp Biol 212(20):3305–3312. https://doi.org/10.1242/jeb.028514

Wroblewski T, Piskurewicz U, Tomczak A, Ochoa O, Michelmore RW (2007) Silencing of the major family of NBS-LRR encoding genes in lettuce results in the loss of multiple resistance specificities. Plant J 51:803–818. https://doi.org/10.1111/j.1365-313X.2007.03182.x

Wu ZM, Yan HB, Pan WL, Jiang B, Liu JG, Geng BJ, Sun YT, Wang YH, Dong WQ (2012) Transformation of an ectopically expressed bulb lectin gene from *Pinellia pedatisecta* into tobacco plants conferring resistance to aphids (*Myzus nicotianae*). Aust J Crop Sci 6:904–911

Xu L, Duan X, Lv Y, Zhang X, Nie Z, Xie C, Ni Z, Liang R (2014) Silencing of an aphid carboxylesterase gene by use of plant-mediated RNAi impairs *Sitobion avenae* tolerance of Phoxim insecticides. Transgenic Res 23(2):389–396. https://doi.org/10.1007/s11248-013-9765-9

Yadava JS, Singh NB (1999) Strategies to enhance yield potential of rapeseed-mustard in India. In: Proceedings of the 10th international rapeseed congress, Canberra, Australia, vol 6, p 634

Yang ZL, Kunert G, Sporer T, Kornig J, Beran F (2020) Glucosinolate abundance and composition in brassicaceae influence sequestration in a specialist flea beetle. J Chem Ecol 46:186–197. https://doi.org/10.1007/s10886-020-01144-y

Yu XD, Jones HD, Ma YZ, Wang GP, Xu ZS, Zhang BM, Zhang YJ, Ren GW, Pickett JA, Xia LQ (2012) (E)-β-Farnesene synthase genes affect aphid (*Myzus persicae*) infestation in tobacco (*Nicotiana tabacum*). Funct Integr Genomics 12:207–213

Yu XD, Zhang YJ, Ma YZ, Xu ZS, Wang GP, Xia LQ (2013) Expression of an (E)-β-farnesene synthase gene from Asian peppermint in tobacco affected aphid infestation. Crop J 1:50–60. https://doi.org/10.1016/j.cj.2013.07.005

Yu XD, Wang GP, Huang SL, Ma YZ, Xia LQ (2014) Engineering plants for aphid resistance: current status and future perspectives. Theor Appl Genet 127:2065–2083. https://doi.org/10.1007/s00122-014-2371-2

Yu XD, Liu ZC, Huang SL, Chen ZQ, Sun YW, Duan PF, Ma YZ, Xia LQ (2016) RNAi-mediated plant protection against aphids. Pest Manag Sci 72(6):1090–1098. https://doi.org/10.1002/ps.4258

Zhang G, Gu C, Wang D (2010) A novel locus for soybean aphid resistance. Theor Appl Genet 120:1183–1191. https://doi.org/10.1007/s00122-009-1245-5

Chapter 9
Genomic Technology in Insect Pest Resistance for Sustainable Rice Production

Dharminder Bhatia and Renu Khanna

9.1 Introduction

Rice (*Oryza sativa* L.) is attacked by several insect pests which affect its production. Of these, planthoppers and leafhoppers cause significant damage to rice (Denno and Perfect 1994; Dupo and Barrion 2009). Among planthoppers, brown planthopper (BPH), *Nilaparvata lugens* (Stål); small brown planthopper (SBPH) and white-backed planthopper (WBPH), *Sogatella furcifera* (Horvath) are economically important pests in Asia (Brar et al. 2009). Being piercing-sucking pests, these suck the sap from rice phloem with their stylet and cause significant yield loss by direct feeding of rice plant. Among these, BPH in general or combination of BPH and WBPH are the most damaging insect pests hampering the rice production. Heavy infestations of both BPH and WBPH lead to the condition referred to as 'hopper-burn' which is the result of complex interaction of toxins released during insect feeding and plant response thereof. It leads to gross discolouration and dehydration of the rice plant, where the infested patch of rice field seems reddish brown in colour (Backus et al. 2005). More significantly, BPH, WBPH and SBPH also transmit major viral diseases. BPH serves as a vector of grassy stunt virus and rugged stunt virus (Rivera et al. 1966; Ling 1977). WBPH is a vector of southern rice black-streaked dwarf virus (Zhou et al. 2008) and SBPH transmits rice stripe virus and rice black-streaked dwarf virus (Zhang et al. 2011; Wang et al. 2010). In addition, a number of leafhoppers, which hardly reach high densities in the field, cause yield losses in rice to a variable extent and at various growth stages. These include the green leafhopper (GLH), *Nephotettix virescens*; green rice leafhopper (GRH),

D. Bhatia (✉) · R. Khanna
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India
e-mail: d.bhatia@pau.edu; renukhanna-pbg@pau.edu

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_9

Nephotettix cincticeps; zigzag leafhopper (ZLH), *Recilia dorsalis* and potentially other leafhoppers (Abo and Sy 1997).

However, the management of these pests is possible with the regular monitoring of the crop, but this is very time-consuming and laborious. Many insecticides have been recommended to control the infestation of planthoppers but prove ineffective since the pests feed at the base of the plant and the farmers are unable to notice and spray effectively (Sarao et al. 2017). Besides, widespread application of these insecticides may lead to the development of insecticide resistance in hoppers by affecting behavioural and physio-biochemical aspects (Matsumura et al. 2009). Host–plant resistance, therefore, is an ecological and economical means of controlling damage due to hoppers by reducing both direct feeding and transmit of viral diseases (Brar et al. 2009). According to Deutsch et al. (2018), crop losses caused by insects are expected to be further exacerbated by global warming, which increases the population growth and metabolic rates of insects. Development of resistant varieties of rice should be the main priority of plant breeders in order to substitute traditional BPH management strategies (Ghaffar et al. 2011). Over the past several decades, great progress has been made in the screening of insect-resistant rice germplasm, identifying resistance genes, and uncovering the molecular mechanisms of host resistance (Du et al. 2020). In the present book chapter, we provide an update on the genetic and molecular advances of different planthoppers in rice.

9.2 Genetics of Resistance to Planthoppers

Identification of donors, mapping genes/QTLs for resistance to planthoppers and their transfer into elite susceptible cultivars have been considered as an economical and environmental friendly approach. With the prevalence of several biotypes and emergence of new biotypes, single resistance gene introgressed in the background of susceptible cultivar will not sustain for long. Therefore, several diverse genes for resistance to planthoppers are required to manage with the development of new biotype populations (Brar et al. 2009; Sarao et al. 2017). In addition, region-specific deployment of different resistance genes is required to combat region-specific prevalence of biotypes. With the development of molecular markers (SSR, InDel, SNPs) and emergence of several genomic tools, the genetic studies of planthopper resistance particularly BPH in rice have intensified (Hu et al. 2016). A number of genes for resistance to hoppers have been identified: 38 genes for BPH, 9 for WBPH, 14 for GLH, 6 for GRH and 3 for ZLH.

9.2.1 Brown Planthopper (BPH)

The first germplasm donor that showed resistance to BPH was identified in 1967 (Pathak et al. 1969). Since then, many donors for resistance to different BPH biotypes have been identified. Some of the resistant donors are Mudgo, ASD 7, Rathu

Heenati, Ptb33, Babawee, ARC10550, Swarnalata, T12, Chin Saba, Balamawee and several introgression lines derived from *Oryza officinalis*, *O. minuta*, *O. latifolia* and *O. australiensis*. The earliest information on the genetics of BPH resistance was reported in 1970 (Athwal et al. 1971). Currently, 38 major genes designated from *Bph1* to *Bph38* for resistance to BPH have been identified from wild and cultivated rice germplasm (Table 9.1). Many of the resistance genes/QTLs such as *Bph1*, *bph2*, *Bph3*, *bph4*, *Bph6*, *bph7*, *Bph9*, *Bph17*, *Bph19*, *Bph25*, *Bph26*, *Bph27*, *Bph28*, *Bph31 Bph32*, *Bph33* and *Bph38* have been identified from *O. sativa* accessions and *Bph10*, *bph11*, *bph12*, *Bph13*, *Bph14*, *Bph15*, *Bph18*, *Bph20*, *Bph21*, *Bph22*, *Bph27*, *bph29*, *bph30*, *Bph31(t)*, *Bph34*, *Bph35* and *Bph36* from the wild species of rice.

9.2.1.1 BPH-Resistant Locus/Genes Identified from *Oryza sativa* Germplasm

The *Bph1* (Hirabayashi and Ogawa 1995; Jeon et al. 1999; Sharma et al. 2002; Kim and Sohn 2005; Park et al. 2008; Cha et al. 2008) and *bph2* (Murata et al. 1998; Murai et al. 2001; Sharma et al. 2004; Sun et al. 2006), the first two resistant genes, were mapped on the rice chromosome 12. These two genes had shown resistance to BPH biotypes 1 and 2, prevalent at that time and soon deployed in rice mega varieties. In 1973, the first resistant rice cultivar, IR26, was released that contains *Bph1* (Khush 1971), followed by cultivars IR36, IR38 and IR42 with the *bph2* gene. However, *Bph1* and *bph2* rapidly became obsolete in just 3–5 years because of the development of new BPH biotypes (Brar et al. 2015). This gave rise to the continuous efforts to identify and map novel sources of resistance to BPH to breed broad-spectrum and durable resistant varieties.

A Sri Lankan cultivar 'Rathu Heenati' was harbouring a broad-spectrum BPH resistance gene *Bph3*, which confers resistance to all four biotypes (Lakshminarayana and Khush 1977; Sidhu and Khush 1978; Sidhu et al. 1979), and this gene was assigned to the short arm of chromosome 4 (Sun et al. 2005). Later, this gene was isolated using the map-based cloning approach and encodes a cluster of three membrane-spanning lectin receptor kinases (Liu et al. 2015). Similarly, *BPH4* identified in the variety Babawee provides resistance against BPH biotypes 1–4 (Lakshminarayana and Khush 1977). It was reported to have similar allele or closely linked to a dominant gene *Bph3* (Sidhu et al. 1979). Kawaguchi et al. (2001) assigned *bph4* on the short arm of chromosome 6. These four genes have been used extensively in breeding programs in Southeast Asia (Jairin et al. 2007b), and a large number of BPH-resistant varieties have been released by IRRI since 1976. However, some of them have lost effectiveness with the evolution and subsequent increase of new biotypes. In genetic studies of 20 BPH-resistant varieties of rice, Sidhu and Khush (1978) reported that two genes (*BPH1/BPH3* and *bph2/bph4*) controlled the resistance in three varieties, seven varieties had *BPH3*, and ten varieties possessed the *BPH4*-resistant locus. The dominant gene *Bph6* and recessive gene *bph7*, which were effective against the Bangladesh BPH population (mainly attributed to BPH biotype 4), were identified in a rice variety Swarnalata and *indica* rice cultivar T12, respectively (Kabir and Khush 1988). These two genes were further

mapped using the F_2 and backcross populations and a set of SSR markers, on chromosome 4L and chromosome 12, respectively (Qiu et al. 2012, 2014).

Earlier, *Bph9* gene was reported to be present in three BPH-resistant cultivars, Balamawee, Kaharamana and Pokkali. This gene was mapped on chromosome 12L in Pokkali (Murata et al. 2001) and Kaharamana (Sun et al. 2006). Later, He et al. (2013) showed that the gene in Balamawee was different from the other two BPH-resistant cultivars based on various molecular-physiological characteristics of BPH such as settling behavior including nymph preferences, nymph survival, honeydew and tolerance indices. The new gene was then fine mapped and designated as *Bph27*. Sun et al. (2005) mapped another gene *Bph17* on chromosome 4S from Rathu Heenati, which also contains a major BPH-resistant gene *BPH3*. They reported that *Bph17* is different from *Bph3* and the rest of the genes based on chromosomal location and reaction on different biotypes. The *bph19* was identified as single recessive gene in *indica* cultivar AS20-1 that is effective against biotype 2. The gene was mapped on chromosome 3 using two F_2 populations derived with susceptible *japonica* cultivars 'Aichi Asahi' and 'Lijiangxintuanheigu'. Myint et al. (2012) identified *Bph25* and *Bph26* in the *indica* cultivar ADR52 and mapped them on chromosome 6S and chromosome 12L, respectively. Later *Bph26* was cloned by developing near isogenic lines in the background of Taichung 65. Further, based on sequence analysis and feeding ability of BPH virulent biotype, *Bph26* was found to be allelic to *bph2* present in cultivar ASD7 (Tamura et al. 2014). A major effect QTL (*Qbph11*) resistant to BPH was identified in the *indica* rice cultivar 'DV85' on chromosome 11 (Sun et al. 2005), which was later fine mapped and designated as *Bph28* (Wu et al. 2014).

In a study by Jairin et al. (2007a), *Bph3* locus was identified on chromosome 6S using two backcross populations, including one derived from Rathu Heenati and Ptb33 from which *Bph3* gene was found to an underlying gene (Jairin et al. 2007b). However, *Bph3* locus was transferred in several susceptible cultivars using marker-assisted selection and found to provide durable resistance to BPH (Jairin et al. 2009; Haining et al. 2010; Singh et al. 2011), which indicated that *Bph3* locus may contain another gene of resistance to BPH. Later, a dominant gene, *Bph32*, was cloned from the rice variety Ptb33 on chromosome 6S using bioinformatics analysis and transgenic approach (Ren et al. 2016). The *Bph33* locus has been identified from Sri Lankan rice cultivars 'Koliyal' and 'Poliyal' using an $F_{2:3}$ population derived with susceptible rice cultivar '93-11'. This locus was identified on chromosome 4s and found to be different from other BPH genes present in the same region based on its genetic distance (Hu et al. 2018). Further, based on sequence and expression analysis, leucine-rich repeat gene family locus LOC_Os04g02520 was found to be the most plausible candidate gene of *Bph33* locus. Another similar gene *Bph33*(t) was reported by Naik et al. (2018) on chromosome 1 in a breeding line RP2068-18-3-5 (RP2068) derived from the land race Velluthacheera. This line was found to be resistant to BPH population (mostly biotype 4) across India. QTL mapping in a RIL population of cross TN1 X RP2068 identified two major QTLs and few minor QTLs. Further expression analysis of QTL region governing major QTL identified

heat-shock protein-type gene locus 'LOC_Os01g42190' as the most probable candidate, and the region was also referred as *Bph33*(t) locus.

The mega rice cultivar 'IR64' was reported to contain one major BPH resistance gene, *Bph1*, and other minor resistance QTLs (Alam and Cohen 1998; Soundararajan et al. 2004). Yang et al. (2019) identified *Bph37* on chromosome 1 along with *Bph1* on chromosome 12 in IR64 using $F_{2:3}$ population derived with a susceptible *indica* rice line 'KWQZ'. Further pre-near isogenic lines carrying only *Bph37* also showed significant resistance to BPH, showing that it is a different and effective gene. The BPH-resistant locus *Bph38*(t) was identified in a BC_1F_5 mapping population derived from a cross between a BPH-resistant *indica* variety Khazar and an elite BPH-susceptible line Huang-Huan-Zhan. The locus was mapped on chromosome 1L using 702 high-quality polymorphic single-nucleotide polymorphism (SNP) markers. Further based on the analysis of physical region of *Bph38(t)*, the FBXL class of F-box protein 'LOC_Os01g37260' was predicted as the underlying candidate gene.

9.2.1.2 BPH-Resistant Locus/Genes Identified from Wild *Oryza* Species

Several wild species accessions have been identified as resistant to BPH (Sarao et al. 2016). But only a few of them have been utilized for identification and mapping of different BPH-resistant genes (Sarao et al. 2017). These wild *Oryza* species may contain several novel genes for resistance to BPH, which can expand the list of BPH-resistant genes identified so far. Out of 38, 17 genes/locus have been identified from different wild species, including *O. australiensis*, *O. officinalis*, *O. latifolia*, *O. eichengeri*, *O. minuta*, *O. nivara and O. rufipogon*.

The genes *Bph10*, *bph11*, *Bph13*, *Bph14 and Bph15* were identified from introgression lines derived from *O. officinalis*, which were found to be resistant to BPH on screening (Ishii et al. 1994; Hirabayashi et al. 1998; Huang et al. 2001; Lang and Bu 2003; Yang et al. 2004), whereas *Bph12* earlier designated as *Bph12(t)* was identified from the rice line 'B14' derived from *O. latifolia* (Yang et al. 2002). Molecular marker-assisted introgression of *Bph14* and *Bph15* was performed to improve the BPH resistance of Minghui 63 and its derived hybrids such as Shanyou 63 by Hu et al. (2012). The *Bph13* gene was identified in two separate studies from *O. eichengeri* and *O. officinalis*. Liu et al. (2001) reported identification and mapping of *Bph13* using *O. eichengeri*-derived introgression line on chromosome 2, whereas Renganayaki et al. (2002) mapped the *Bph13(t)* gene on chromosome 3 in *O. officinalis*-derived introgression line. However, it seems that both of these genes designated as same will be different. The *Bph18* was identified in an introgression line derived from the wild species *O. australiensis* (Jena et al. 2006). Genetic analysis revealed *Bph18* as a single dominant gene in the introgression line and also found to be non-allelic to another gene, *Bph10*, earlier reported from *O. australiensis*. The locus was then mapped on the long arm of rice chromosome 12.

Two major quantitative trait loci (QTLs) were identified for BPH resistance in *O. minuta* acc. IRGC101141 using an F_2 population derived from a cross between resistant introgression line, 'IR71033-121-15', and a susceptible Korean japonica variety, 'Junambyeo'. One QTL named as *Bph20(t)* was mapped on the chromosome 4, and the other QTL designated as *Bph21(t)* was mapped on chromosome 12. The two QTLs showed additive effect for resistance to BPH. In a *O. rufipogon*-derived introgression line, RBPH54. Two recessive loci *bph20(t)* and *bph21(t)* were identified by developing the BC_2F_2 population (Yang et al. 2012), which were renamed as *bph29* and *bph30* upon fine mapping these loci (Wang et al. 2015). A single dominant locus *Bph34* was identified from *O. nivara* accession IRGC104646 by screening the F_3 population developed with susceptible cultivar PR122 with biotype 4 (Kumar et al. 2018). The locus was mapped on chromosome 4L and found to be different from other loci present in the same region based on sequence analysis and screening diverse accessions. *Bph35* and *Bph36* were identified from introgression lines derived from *O. rufipogon* using advanced bulked segregant analysis on chromosome 4 (Li et al. 2019; Yuexiong et al. 2020). A novel resistance gene *Bph38* derived from the wild rice species *Oryza rufipogon* Griff. was identified by Yang et al. (2020) that conferred high resistance to both BPH and WBPH. The locus was identified using BC_1F_2 and near-isogenic lines (NILs) in the 93-11 (*indica*) and BR54 (*japonica*) genetic backgrounds.

9.3 Genes/QTLs for Resistance to White-Backed Planthopper (WBPH)

Till date, about 12 major WBPH resistance genes have been identified in diverse 67 cultivars or wild rice species (Du et al. 2020; Table 9.1). Out of these, two have been introgressed from the wild rice—*Oryza officinalis*—using backcross and screening, while nine gene have been mapped with linked markers (Ramesh et al. 2014; Du et al. 2020). *Wbph1* was first detected in the rice variety N22 (Sidhu et al. 1979); and further studies indicated that *Wbph1* co-segregated with the RFLP markers RG146 and RG445 in variety IR36 (McCouch et al. 1991). Recently, Cheng et al. (2021) mapped *Wbph1* on chromosome 2 of N22. *Wbph2* was detected in variety ARC10239 and mapped to chromosome 6, where it was found to be associated with RFLP markers RZ667, RG264 and RG64 (Angeles et al. 1981; Liu et al. 2002). *Wbph3* and *wbph4* were identified in varieties ADR52 and Podiwi A8, respectively (Hernandez and Khush 1981). *Wbph5* was detected in variety N' Diang Marie (Wu and Khush 1985), and *Wbph6*(*t*) was found to be associated with RM167 on chromosome 11 (Li et al. 1990, 2004). *Wbph7* and *Wbph8* were derived from introgressed lines of *O. officinalis* and were mapped to the same chromosomal regions as the BPH resistance genes *Bph14* and *Bph15*, respectively. Further study indicated that *Bph14* confers resistance to both the BPH and WBPH (Tan et al. 2004). *Wbph3*,

Table 9.1 Genes for resistance to WBPH tagged with molecular markers in rice

Gene	Chromosome[#]	Donor[$]	Marker	Marker type used	Population type	Reference(s)
Wbph 1	2	N22	RM13650 and RM13478	SSR	F_2	Cheng et al. (2021)
Wbph 2	6	ARC 10239	RZ667, RG264, and RG64	RFLP markers	–	Liu et al. (2002)
Wbph 3	–	ADR52	–	–	–	Hernandez and Khush (1981)
Wbph 4	–	Podiwi A8, ARC 6650 ARC5984	–	–	–	Hernandez and Khush (1981), Padmavathi et al. (2007)
Wbph 5	–	N' Diang Marie	–	–	–	Wu and Khush (1985)
Wbph 6(t)	11	Giu-yi-gu	RM167	SSLP	F_3	Li et al. (2004)
Wbph7(t)	3	B5	R1925 and G1318	RFLP	RIL	Tan et al. (2004)
Wbph8(t)	4	B5	R288 and S11182	RFLP	RIL	Tan et al. (2004)
WbphM1	–	Mudgo	–	–	–	Sidhu et al. (2005)
WbphM2	–	Mudgo	–	–	–	Sidhu et al. (2005)
wbphAR	–	ARC11367	–	–	–	Sidhu et al. (2005)
WbphN	–	NCS2041	–	–	–	Sidhu et al. (2005)
WbphO	–	MO1	–	–	–	Sidhu et al. (2005)
Ovc	6	Asominori	S1520 L688	–	NIL	Yamasaki et al. (2003)
wbph9(t)	6	Sinna Sivappu	RM589-RM539	SSR	$F_{2:3}$	Ramesh et al. (2014)
wbph10(t)	12	Sinna Sivappu	SSR12–17.2-RM28487	SSR	$F_{2:3}$	Ramesh et al. (2014)
wbph11(t)	4	Sinna Sivappu	RM3643-RM1223	SSR	$F_{2:3}$	Ramesh et al. (2014)
WBPH12(t)	4	Sinna Sivappu	RM16592-RM16649	SSR	$F_{2:3}$	Ramesh et al. (2014)

(continued)

Table 9.1 (continued)

Gene	Chromosome[#]	Donor[$]	Marker	Marker type used	Population type	Reference(s)
qWPH2	2	*O. rufipogon*	RM1285-RM555	SSR	BC_3F_3	Chen et al. (2010)
qWBPH5	5	*O. rufipogon*	RM3870-RZ70	–	BC_3F_3	Chen et al. (2010)
qWBPH9	9	*O. rufipogon*	RG451-RM245	–	BC_3F_3	Chen et al. (2010)
qWL6	6	Chunjiang 06	M3-M5	Indel	BC_6F_2	Yang et al. (2014)
qWBPH3.2	3	IR54751	InDel3–23–InDel3–26	Indel	F_2	Fan et al. (2018)
qWBPH11	11	IR54751	DJ53973-SNP56	–	F_2	Fan et al. (2018)
Wbph9	3	OB677	RM3513 and RM3525	SSR	F_2	Cheng et al. (2021)
F3H	4	Cheongcheong/ Nagdong doubled haploid	RM280-RM6909	SSR	DH	Kim et al. (2021)
Small brown planthopper						
Sbph(t)	–	IR50	–	–	–	Nemoto et al. (1994)
qSBPH2b	2	Mudgo	RM29-RM5791	SSR	–	Duan et al. (2009)
qSBPH3d	3	Mudgo	RM 5442-RM3199	SSR	–	Duan et al. (2009)
qSBPH12a	12	Mudgo	I12-17-RM3331	–	–	Duan et al. (2009)
qSBPH2	2	Kasalath	R712-R1843	–	BC_1F_9	Duan et al. (2010)
qSBPH3	3	Kasalath	C1135-C80	–	BC_1F_9	Duan et al. (2010)
qSBPH8	8	Kasalath	R1943-C390	–	BC_1F_9	Duan et al. (2010)
qSBPH11	11	Kasalath	G257-S2260	–	BC_1F_9	Duan et al. (2010)
qSBPH2	2	N22	RM263-RM1385	SSR	RIL	Wang et al. (2013)
qSBPH3	3	N22	RM22-RM545	SSR	RIL	Wang et al. (2013)
qSBPH5	5	N22	RM153-RM413	SSR	RIL	Wang et al. (2013)
qSBPH7	7	N22	RM234-RM429	SSR	RIL	Wang et al. (2013)

(continued)

Table 9.1 (continued)

Gene	Chromosome[#]	Donor[$]	Marker	Marker type used	Population type	Reference(s)
qSBPH11	11	N22	RM209-RM21	SSR	RIL	Wang et al. (2013)
qSBPH3d	3	*O. officinalis*	RM218-RM745	SSR	–	Zhang et al. (2014)
qSBPH7a	7	*O. officinalis*	RM7012-RM6338	SSR	–	Zhang et al. (2014)
qSBPH12b	12	*O. officinalis*	RM463-RM6256	SSR	–	Zhang et al. (2014)
qSBPH1	1	9194	RM3738-RM8236	SSR	$F_{2:3}$	Sun et al. (2017)
qSBPH5	5	9194	RM18452-RM163	SSR	$F_{2:3}$	Sun et al. (2017)
qSBPH8	8	9194	RM210-RM3845	SSR	$F_{2:3}$	Sun et al. (2017)
qSBPH9	9	9194	RM257-RM160	SSR	$F_{2:3}$	Sun et al. (2017)
qSBPH5	5	WR24	INDEL5-11-RM3664	Indel	$F_{2:3}$	Xu et al. (2018)
qSBPH7	7	WR24	RM6403-RM234	SSR	$F_{2:3}$	Xu et al. (2018)
qSBPH10	10	WR24	RM25664-RM228	SSR	$F_{2:3}$	Xu et al. (2018)

Updated from Du et al. (2020)

wbph4 and *Wbph5* were not assigned to any chromosomes, and *Wbph2* and *Wbph6* were only roughly mapped on to the chromosomes. Six more genes have been tentatively identified as *wbphM1* and *wbphM2* in Mudgo, *wbphAR* in ARC 11367, *wbphN* in NCS 2014, *wbphO* in MO1 (Sidhu et al. 2005) and *Ovc* in Asominori (Yamasaki et al. 2003). Several quantitative trait loci (QTLs) associated with WBPH resistance were also detected and mapped from the variety Chunjiang 06 and Dongxiang wild species (Sogawa et al. 2005; Chen et al. 2010). Chen et al. (2010) detected three QTLs, *qWph2*, *qWph5* and *qWph9*, located on the short arm of chromosome 2, the long arm of chromosomes 5 and the long arm of chromosome 9, respectively. Four WBPH resistance genes in Sinna Sivappu, designated as *wbph9*(t), *wbph10*(t), *wbph11*(t) and *WBPH12*(t), were mapped on chromosomes 4, 6 and 12 by molecular markers, respectively (Ramesh et al. 2014). The *qWL6* was identified as a major QTL in the rice response to infestation by WBPH by Yang et al. (2014) in Chunjiang 06, and this QTL was delimited to a 122 kb region on chromosome 6. The *qWBPH11* from IR54751 was found in a 450 kb region between markers

DJ53973 and SNP56 on chromosome 6 (Yang et al. 2014; Fan et al. 2018). Cheng et al. (2021) identified a novel resistance gene *Wbph9* in OB677, which was mapped on chromosome 3.

9.4 Genes/QTLs for Resistance to Small Brown Planthopper (SBPH)

Recently, few studies have been conducted to identify genes/QTLs for SBPH resistance in rice. More than 30 QTLs for SBPH (Duan et al. 2007a, b, 2008, 2009, 2010; Zhang et al. 2014) have been identified from cultivated and wild species using SSST, MSST, antixenosis and antibiosis tests (Table 9.1). Through resistance screening, Duan et al. (2009) detected 25 rice accessions with different levels of resistance to SBPH. Further, three QTLs were identified on chromosomes 2, 3 and 12 in the cultivar Mudgo related to SBPH resistance, i.e. *qSBPH2b*, *qSBPH3d* and *qSBPH12a*, respectively. Additionally, *indica* variety Kasalath contained several QTL alleles for SBPH resistance, and three QTLs conferring antixenosis against SBPH and two QTLs expressing antibiosis to SBPH were detected on chromosomes 2, 3, 8 and 11, respectively (Duan et al. 2010). Additional QTLs, namely, *qSBPH2*, *qSBPH3*, *qSBPH5*, *qSBPH7* and *qSBPH11* for SBPH resistance were identified on chromosomes 2, 3, 5, 7 and 11 in N22, respectively; *qSBPH1*, *qSBPH5*, *qSBPH8* and *qSBPH9* on chromosome 1, 5, 8 and 9 in 9194, respectively; and *qSBPH5*, *qSBPH7* and *qSBPH10* on chromosome 5, 7 and 10 in WR24, respectively (Wang et al. 2013; Zhang et al. 2014; Sun et al. 2017; Xu et al. 2018).

9.5 Genes/QTLs for Resistance to Green Rice Leafhopper (GRH)

The green rice leafhopper (GRH), *Nephotettix cincticeps* Uhler, is a major insect pest of cultivated rice and is distributed mostly in the temperate regions of East Asia (Ghauri 1971). The genetics of resistance to GRH is relatively well understood. Seven major genes for GRH resistance have been identified and mapped on rice chromosomes. The genes *Grh1*, *Grh2*, *Grh3*, *Grh4* and *Grh6* have been identified from *O. sativa*, while *Grh5* was derived from the wild species *O. rufipogon* and *qGRH9* from *O. glaberrima.* The *Grh6* was first identified from the Surinam cultivar SML17 in 1999 (Tamura et al. 2004). Further, Fujita et al. (2004) identified a GRH resistance gene in *O. nivara* at the same position as *Grh6*, so they named it as Grh6-nivara. Pyramided lines containing *Grh2* and *Grh4* showed strong resistance against GRH, but monogenic lines with the *Grh2* or *Grh4* only were susceptible to

GRH (Wang et al. 2004). Mai et al. (2015) identified *qGRH5* in ASD7, which co-segregated with SSR markers RM6082 and RM3381. Moreover, they revealed that *qGRH5* is identical to *GRH1*. The GRH resistance genes, *GRH1*, have been localized on chromosome 5 in IR24, *GRH2* on chromosome 11 in DV85 (Kadowaki et al. 2003), *GRH3* on chromosome 6 in Rantaj emas 2 (Saha et al. 2006), *GRH4* on chromosome 3 in DV85 (Kadowaki et al. 2003), *GRH5* on chromosome 8 in W1962 (*O. rufipogon*) (Fujita et al. 2006), *GRH6* on chromosome 4 in SML17 and IRGC105715 (Fujita et al. 2004; Tamura et al. 2004) and *qGRH9* on chromosome 9 in IRGC104038 (*O. glaberrima*) (Fujita et al. 2010).

9.6 Green Leaf Hopper (GLH)

Although, the green leafhopper—*Nephotettix virescens* (distant)—is spread throughout Asia, it is a more severe pest in the tropics and subtropics. GLH causes yield losses by direct feeding as well as by acting as a vector for viruses causing tungro disease. According to Brar et al. (2009), the attention given to resistance against GLH is primarily due to its capacity to transmit tungro viruses. Till date, 14 GLH resistance loci (11 dominant and three recessive genes) have been identified (*Glh1*, *Glh2*, *Glh3*, *glh4*, *Glh5*, *Glh6*, *Glh7*, *glh8*, *Glh9*, *glh10*, *Glh11*, *Glh12*, *Glh13*, *Glh14*), of which only one, *Glh14*, has RFLP markers. The earliest reported genes were from the 1970s, beginning with Athwal et al. (1971): *Bph1*, *bph2*, *Glh1*, *Glh2* and *Glh3*; however, it is only since the development of DNA-based genetic markers during the 1970s and QTL analysis in the 1980s (Ruane and Sonnino 2007) that clear associations could be drawn between specific regions of the rice genome and resistance to planthoppers and leafhoppers. Scientists at the International Rice Research Institute (IRRI) have identified many leafhopper resistance genes, including GLH1 to GLH5 from Pankhari 203, ASD7, IR8, PTB8 and ASD8, respectively (Athwal et al. 1971; Siwi and Khush 1977). Genetic studies done by Avesi and Khush (1984) indicated presence of *Glh1* in two, *Glh2* in three, *Glh3* in and *glh4* in one variety. IR varieties of rice carry *Bph1*, *bph2* and *Bph3* genes and *Glh3*, *glh4*, *Glh9* and *glh10* (Brar et al. 2009). Two more genes were identified and analysed by Siwi and Khush (1977); the one recessive gene was designated as *glh4* and a dominant gene as *Glh5*. Two dominant genes (*Glh6* and *Glh7*) and a recessive gene (*glh8*) were identified by Rezaul Karim and Pathak (1982) and Ghani and Khush (1998), respectively. *GLH1*, *GLH2*, *GLH3*, *glh4*, *GLH5* and *GLH6* are present on chromosome 5, 11, 6, 3, 8 and 5, respectively (Du et al. 2020). Angeles and Khush (2000) identified *glh10t* in IR36 and *glh11t* in IR20965-11-3-3. GLH14 has been located on chromosome 4 using molecular markers Y3635 and RZ262 in ARC11554 (Sebastian et al. 1996).

9.7 Genes for Resistance to Zigzag Leafhopper

The zigzag leafhopper (ZLH), *Recilia dorsalis*, is prevalent in the tropics and subtropics of Asia. However, this leafhopper, which also transmits tungro viruses, has not been paid much attention (Fujita et al. 2013). A single study by Angeles et al. (1986) identified three loci (*Zlh1*, *Zlh2* and *Zlh3*) associated with resistance to this leafhopper. All the three genes showed dominant reaction and segregated independently of each other. *Zlh1 was* identified from Rathu Heenati, while *Zlh2 and Zlh3* were identified from Ptb21 and Ptb33, respectively. Allelic tests indicated that that *Zlh1*, *Zlh2* and *Zlh3* are independent of *Wbph3* and segregated independently of *bph2* and *Bph3*.

9.8 Cloning of Genes for Resistance to BPH in Rice

After gene identification and mapping, major resistance genes await cloning which helps in understanding underlying molecular mechanisms. To date, a total of 14 BPH-resistant genes have been cloned and functionally characterized (Table 9.2) but no WBPH or other insect particularly small brown planthoppers, green rice leafhopper and green leafhopper resistance genes have been cloned yet. Advances in high-throughput genomics and bioinformatics have facilitated cloning of at least eight BPH resistance genes (*BPH14*, *BPH3*, *BPH26*, *BPH29*, *BPH15*, *BPH9*, *BPH32* and *BPH6*) in the past ten years (Zhao et al. 2016; Guo et al. 2018). Most of

Table 9.2 Cloned BPH resistance genes in rice

Gene	Encoded protein	Plant defense response	Reference
Bph1	CC-NB-NB-LRR	Antibiosis and antixenosis	Zhao et al. (2016)
bph2	CC-NB-NB-LRR	Antibiosis	Tamura et al. (2014)
Bph3	Lectin Receptor Kinases	Antibiosis	Liu et al. (2015)
Bph6	Atypical LRR	PAMPs	Guo et al. (2018)
bph7	CC-NB-NB-LRR	Antibiosis and antixenosis	Zhao et al. (2016)
Bph9	CC-NB-NB-LRR	Antibiosis and antixenosis	Zhao et al. (2016)
Bph10	CC-NB-NB-LRR	Antibiosis and antixenosis	Zhao et al. (2016)
Bph14	CC-NBS-LRR	Antibiosis	Du et al. (2009)
Bph15	Lectin receptor		Cheng et al. (2013)
Bph18	CC-NBS-LRR	Antibiosis and antixenosis	Ji et al. (2016)
Bph21	CC-NB-NB-LRR	Antibiosis and antixenosis	Zhao et al. (2016)
Bph26	CC-NBS-LRR	Antibiosis	Tamura et al. (2014)
bph29	B3 DNA binding domain	Antibiosis	Wang et al. (2015)
Bph32	SCR domain	Antibiosis	Ren et al. (2016)

Updated from Brar et al. (2015)

these genes encode for coiled-coil nucleotide binding and leucine-rich repeat (CC-NB-LRR) protein (Table 9.2). The NBS-LRR class of genes plays an important role in resistance to plant diseases. During disease infestation, these genes induce the downstream disease resistance reactions by recognizing the effectors delivered by pathogens referred as Effector Triggered Immunity (ETI) (Yue et al. 2012). Since large number of genes for BPH resistance have been identified as NBS-LRR type, it can be emphasized that there is much similarity in the molecular mechanism of resistance against diseases and insects in rice. It also suggests that proteins encoded by these BPH resistance genes may interact with BPH effectors in a gene-for-gene manner, and there is a presence of BPH *avr* (avirulence) genes for each BPH *R* gene in rice.

BPH14 was the first BPH resistance gene cloned through map-based cloning (Du et al. 2009). This gene was initially identified on chromosome 3L in B5 rice, an introgression line derived from the wild rice species *O. officinalis* (Huang et al. 2001). *Bph14* was localized within a 120 kb physical region on chromosome 3 flanked by the markers RM570 and G1318. Fine mapping with 5,000 F_5 plants delimited the *Bph14* gene to a 34 kb region which contained two candidate genes, named as *Ra* and *Rb*. The candidate genes were transferred into the BPH-susceptible *indica* variety Kasalath, respectively, and the T2 families were examined for BPH resistance. Only the transgenic lines expressing *Bph14* gene encodes a coiled-coil nucleotide binding and leucine-rich repeat (CC-NB-LRR) protein.

The *Bph26* has been identified as CC-NBLRR class of R gene. It is quite similar to *bph2* based on DNA sequence and feeding behaviour (Tamura et al. 2014). Based on map-based cloning and complementation tests, *BPH18* has been found to co-localize in the DNA region of *BPH26*. *BPH18* is a CC-NBS class of R gene but lacks LRR domain (Ji et al. 2016). *BPH9* is also a CC-NBNB-LRR class of R gene and shows both antixenosis and antibiosis as resistant mechanisms to BPH. The eight BPH resistance genes (*Bph1, bph2, bph7, Bph9, Bph10, Bph18, Bph21, Bph26*) have been identified on chromosome 12L (Table 9.3). These genes are present as a clustered region on chromosome 12L and can be classified into four allelotypes showing different degrees of resistance to BPH to different BPH biotypes (Zhao et al. 2016).

The BPH resistance gene, *BPH6* (Guo et al. 2018) which was previously mapped on chromosome 4L (Qiu et al. 2010), results in exocytosis and cell wall reinforcement. It also induces the coordinated signals of salicylic acid, cytokinin and jasmonic acid. *BPH15* is a lectin receptor kinase gene, OsLecRK, (Cheng et al. 2013) which plays an important role in seed germination in plant and innate immunity. The gene, *BPH3*, identified more than 40 years ago in Rathu Heenati (Laksminarayana and Khush 1977) was initially reported as *BPH17* (Hu et al. 2016). It is a cluster of three genes encoding lectin receptor kinases (OsLecRK1, OsLecRK2 and OsLecRK3) localized in plasma membrane. The plants having co-expression of all the three genes displayed wide-spectrum resistance to both BPH and WBPH (Liu et al. 2015). *The bph29* was identified in *O. rufipogon,* a wild relative of cultivated rice which encodes for B3 DNA-binding domain (Wang et al. 2015). The *BPH32* encodes for short consensus repeat domain-containing protein and was identified in rice variety PTB33 on chromosome 6S (Ren et al. 2016).

Table 9.3 Genes for resistance to BPH tagged with molecular markers in rice

Gene	Chromosome[a]	Donor[b]	Marker	Marker type used	Population type	Reference(s)
Bph1	12L	IR28 (1)	XNpb248, XNpb336	RFLP	F_2/F_3	Hirabayashi and Ogawa (1995)
	12L	Gayabyeo (1)	RRD7, RG457, RG634	RAPD, RFLP, SSR	F_2/F_3	Jeon et al. (1999)
	12L	Mudgo (1)	em5814N, em2802N, R2708	AFLP, RFLP	F_2/F_3	Sharma et al. (2002)
	12L	Samgangbyeo (1)	BpE18-3	RAPD, STS	DH, F_2/F_3	Kim and Sohn (2005)
	12L	Samgangbyeo (1)	OsBphi 252	RDA clones, CAPS	NILs	Park et al. (2008)
	12L	Cheongcheongbyeo (1)	pBPH4, pBPH14	RAPD, SCAR, STS	RILs	Cha et al. (2008)
bph2	12L	NorinPL4	G2140	RFLP	F_2/F_3	Murata et al. (1998)
	12L	NorinPL4	KAM3, KAM4, KAM5	AFLP	F_4/F_5	Murai et al. (2001)
	12L	NorinPL4 (1)	KAM2, KAM3, KAM4	AFLP	F_5	Sharma et al. (2004)
	12L	ASD7 (1, 2)	RM463, RM7102	SSR	F_2/F_3	Sun et al. (2006)
Bph3	6S	PTB33, Rathu Heenati, IR71033-121-15 (2)	RM589, RM588, RM586	SSR	BC_1F_2/BC_3F_2, F_2	Jairin et al. (2007a, b, c), Liu et al. (2015)
bph4	6S	Babawee	RM217, C76A	SSR	F_2/F_3	Kawaguchi et al. (2001)
	6S	Babawee (4)	RM586-RM589	SSR	F_2/F_3	Jairin et al. (2010)
Bph6	4L	Swarnalata	RM6997-RM5742	SSR, STS	F_2/F_3, BC_2F_2	Qiu et al. (2010)

(continued)

bph7	12L	T12	RM28295-RM313	SSR	F_2/F_3, BC_2F_1	Qiu et al. (2014)
Bph9	12L	Pokkali	OPR04, S2545	RAPD, RFLP	F_2, F_3/F_4	Murata et al. (2001)
	12L	Kaharamana (1)	RM463, RM5341	SSR	F_2/F_3	Su et al. (2006)
Bph10	12L	IR65482-4-136-2-2 (*O. australiensis IRGC100882*)	RG457	RFLP	F_2/F_3	Ishii et al. (1994)
		IR54742 (*O. officinalis*) (1, 2, 3)	RG457L-B, RM260	STS, SSR	F_2/F_3	Lang and Bu (2003)
bph11	3L	IR54742-23-19-12-3-54 (*O. officinalis*) (1)	G1318	RFLP	F_2/F_3, RILs	Hirabayashi et al. (1998)
bph12	4S	GSK185-2 (*O. officinalis*)	G271, R93	RFLP	F_2/F_3	Hirabayashi et al. (1999)
Bph12	4S	B14 (*O. latifolia*) (1, 2)	RM261	SSR	F_2/F_3, RILs	Yang et al. (2002)
	4S	B14 (*O. latifolia*) (1, 2)	RM16459-RM1305	SSR	F_2/F_3, $BC_2F_{2:3}$	Qiu et al. (2012)
Bph13	2L	*960044-112* (*O. eichingeri* acc. no. 105159)	RM250, RM240	SSR	–	Liu et al. (2001)
	3S	IR54745-2-21-12-17-6 (*O. officinalis*) (4)	AJ09b230, AJ09c	RAPD	RILs	Renganayaki et al. (2002)
Bph14	3L	B5 (*O. officinalis*)	SM1-G1318	SSR, STS	F_2, RILs	Du et al. (2009)
Bph15	4S	B5 (*O. officinalis*) (1, 2)	C820, S11182	RFLP, AFLP	F_2, F_5	Yang et al. (2004)
Bph17	4S	Rathu Heenati (1, 2)	RM8213-RM5953	SSR	F_2/F_3	Sun et al. (2005)

(continued)

Table 9.3 (continued)

Gene	Chromosome[a]	Donor[b]	Marker	Marker type used	Population type	Reference(s)
Bph18	12L	IR65482-7-216-1-2 (*O. australiensis.* acc. no. 100882) (Korean)	RM1022	SSR, STS	F_2/F_3	Jena et al. (2006), Ji et al. (2016)
bph19	3S	AS20-1 (2)	RM6308-RM3134	SSR	F_2/F_3	Chen et al. (2006)
Bph20	4S	IR71033-121-15 (*O. minuta* acc. no 101141)/Korean Bio1	MS10-RM5953	SSR, STS	F_2/F_3	Rahman et al. (2009)
Bph21	12L	IR71033-121-15 (*O. minuta* acc. no. 101141)/(1, Korean)	RM3726-RM5479	SSR, STS	F_2/F_3	Rahman et al. (2009)
Bph22	6S	IR71033-62-24 (*O. minuta*)	RM19429, RM584, RM585	SSR	F_6	Harini et al. (2010)
Bph25	6S	ADR52/Bio Chikugo-89	S00310	SSR	F_2, BC_3F_2	Myint et al. (2012)
Bph26	12L	ADR52/Bio Chikugo-89	RM5479	SSR	F_2, BC_3F_2	Myint et al. (2012), Tamura et al. (2014)
Bph27	4L	*O. rufipogon*, *acc no. 2183* (2)	RM16853-RM16846	SSR	BC_1F_2	Huang et al. (2013)

	4L	Balamawee	Q5, Q20	SSR, Indels	F_2/F_3	He et al. (2013)
	4L	*O. rufipogon*	RM16766 and RM17033	SSR	F_2	Li et al. (2019)
qBph11, Bph28	11L	DV85 (1, 2)	RM26656-RM26725	SSR, Indels	F_2/F_3	Su et al. (2005), Wu et al. (2014)
bph20(t), bph29	6S	RBPH54 (*O. rufipogon*) (2)	RM435, RM540, BYL7, BYL8	SSR, STS, Indels	NILs	Yang et al. (2012), Wang et al. (2015)
bph21(t), bph30	10S	RBPH54 (*O. rufipogon*) (2)	RM222, RM244	SSR, STS, Indels	NILs	Yang et al. (2012), Wang et al. (2015)
Bph31	3	CR2711-76	PA26 and RM2334	Indel	F_2 BC_2F_2	Prahalada et al. (2017)
Bph32	6S	PTB33	RM19291, RM8072	SSR	–	Ren et al. (2016)
Bph 33	4S	KOLAYAL and POLIYAL	H14 and H84	Indel	$F_{2:3}$ BC_3F_2	Hu et al. (2018)
Bph33(t)	1	RP2068-18-3-5 (RP2068)	RM488 and RM11522	SSR	RIL	Naik et al. (2018)
Bph34	4L	*O. nivara* acc. IRGC104646	RM16994 and RM17007	SSR	$F_{2:3}$	Kumar et al. (2018)
Bph35	4	*O. rufipogon*	PSM16 and R4M13	Indel	$F_{2:3}$	Yuexiong et al. (2020)

(continued)

Table 9.3 (continued)

Gene	Chromosome[a]	Donor[b]	Marker	Marker type used	Population type	Reference(s)
Bph36	4S	*O. rufipogon*	S13 and X48	Indel	F_2 $BC_1F_{2:3}$	Li et al. (2019)
Bph37	1	IR64	RM302, YM35	SSR	$F_{2:3}$	Yang et al. (2019)
Bph38(t)	1L	Khazar	693,369, id 10,112,165	SNP	–	Balachiranjeevi et al. (2019)
Bph38	4	*O. rufipogon*	RM16563 and RM16763	SSR	$F_{2:3}$ BC_1F_2 BC_2F_2	Yang et al. (2020)

Updated from Sarao et al. (2017)

[a] L and S = long and short arm of chromosome respectively

[b] Biotypes used for screening for resistance are given in parenthesis

Currently, only very few WBPH resistance genes have been detected and finely mapped. However, there are several cloned BPH resistance genes, such as *Bph6*, *Bph14* and *Bph3*, that simultaneously confer resistance to BPH and WBPH (Tan et al. 2004; Liu et al. 2016; Guo et al. 2018).

9.9 Pyramiding of Genes for Durable Resistance to Planthoppers Using MAS

Marker-assisted selection (MAS) comprises indirect selection of traits with the molecular marker linked with the desired gene of interest. One of the prerequisite of MAS is the availability of molecular marker tightly linked with the trait of interest. MAS is an important strategy for transferring resistance in the background of elite cultivars The use of cost-effective DNA markers derived from the fine mapped position of the genes overcomes the limitation of tedious methods of phenotypic screening of large number of segregating progenies and is helpful to identify the segregants in the early generation of plant development. Pyramiding different resistance genes using MAS provides opportunities to breeders to develop broad-spectrum and durable resistance for diseases and insects. For achieving the sustained control of insects in rice, it is desirable to use different resistance genes in breeding or pyramiding multiple insect resistance genes into a given rice variety. A study by Horgan et al. (2015) showed that very few of the available BPH resistance genes showed durable resistance in monogenic rice lines; however, pyramiding of two or more genes with strong to weak resistance could improve resistance strength and durability as apparent with the most resistant, traditional varieties. A number of improved BPH-resistant genotypes have been developed through conventional breeding and phenotypic selection for multiple BPH-resistant genes. However. it has limitations for combining multiple genes with same level of resistance. With the mapping and cloning of different genes for resistance to BPH and availability of tightly linked markers, MAS has been utilized successfully in several studies. Identifying suitable combination of genes is also important in MAS for developing durable resistance. The genes governed by different molecular mechanism of resistance will be suitable for developing broad-spectrum and durable resistance. Pyramiding of the three different genes *xa5*, *xa13* and *Xa21*, with different molecular mechanisms for resistance to bacterial blight (BB), is the model example on enhancing the level and spectrum of resistance to various pathotypes (Huang et al. 1997; Sanchez et al. 2000; Singh et al. 2001). This gene combination is still providing resistance to BB at global level.

MAS has been used to introgress the favourable alleles for BPH resistance into elite rice lines (Sun et al. 2005; Jena et al. 2006; Fujita et al. 2013; Brar et al. 2015; Sarao et al. 2017). A large number of the BPH genes have been fine mapped, and few genes have been cloned, which are suitable for marker-assisted selection for BPH resistance, albeit with varying levels of BPH virulence in different parts of Asia. Of the various resistant sources identified, the varieties Rathu Heenati, Ptb33,

MO1, IR71033-121-15, Balamawee and ADR52 in South Asia and Swarnalata in South East Asia have been indicated as potential donors for MAS, since these contain multiple genes for hoppers, and most of them have been cloned and tagged with tightly linked molecular markers (Horgan et al. 2015). In the earlier instances, *Bph1* and *Bph2* were pyramided in the background of japonica line, which showed higher level of resistance than *Bph2* alone but equivalent resistance to *Bph1* (Sharma et al. 2004). Later in China, a number of parental lines of hybrid rice breeding programme were pyramided with *Bph14* and *Bph15* through MAS, which had a higher level of resistance to BPH than the lines carrying single gene (Li et al. 2006). In a study by Fujita et al. (2009) and Myint et al. (2012), pyramided lines with *Bph25* and *Bph26* were found to be resistant to several BPH strains indicating a higher level and durable resistance with combination of two genes. Similarly, lines carrying the combination of *Bph14 + Bph15 and Bph3 + Bph27* were more resistant than the monogenic lines (Hu et al. 2012; Liu et al. 2016). Likewise, pyramided line with *Bph12* and *Bph6* had lower nymph settling and survival and slower population growth and caused less damage than the monogenic lines (Qiu et al. 2012). MAS was also used to pyramid three BPH resistance genes, *Bph14*, *Bph15* and *Bph18*, in the background of elite restorer line, 9311 and its hybrids. The results showed that the *Bph15* has a higher level of resistance than *Bph14* and *Bph18*, whereas *Bph14* was found slightly higher or similar as *Bph18* in resistance response against BPH (Hu et al. 2012).

MAS studies for other planthoppers is lagging behind due to limited genetic information and availability of suitable markers. Very few WBPH-resistant genes have been fine mapped, and no reports are available for pyramiding these genes, while six genes seem to be appropriate for MAS for resistance to GRH with few reports of pyramiding (Fujita et al. 2006, 2010). Although pyramided lines can enhance resistance to hoppers, care should be taken whether pyramided lines could lead to a more rapid adaptation of hoppers if the genes were sequentially deployed in a similar background variety.

9.10 Biotechnological Approaches: Transgene and RNAi

With the availability of biotechnological tools from 1990 onwards and well-established transgenic technology, transgenic rice has been developed for several traits of interest. However, few studies reported transgenics against planthoppers in rice. Plant lectins belonging to the family Amaryllidaceae have been reported to affect fecundity, growth and development of sap sucking insects like planthoppers and show low or no toxicity towards higher animals. Among the Amaryllidaceae lectins, snowdrop lectin, *Galanthus nivalis* L. agglutinin (GNA) and 'garlic leaf lectin' (*Allium sativum* agglutinin from leaf, ASAL) have been found to be non-toxic to mammals and toxic to planthoppers. These lectins are probably involved in the binding to receptors present on the midgut epithelial cells (Powell et al. 1998). The bound lectins inhibit absorption of nutrients or disrupt endocytosis of midgut

cell lectins and other toxic metabolites (Eisemann et al. 1994), causing the insecticidal effect. These genes producing these plant lectins have been used in various studies for controlling planthoppers in rice (Powell et al. 1995; Majumder et al. 2004). The *GNA* or *ASAL* transgene-based rice plants had shown substantial resistance to BPH, WBPH and GRH in terms of increased insect mortality, retarded development and decreased fecundity (Rao et al. 1998; Sudhakar et al. 1998; Foissac et al. 2000; Tang et al. 2001; Sun et al. 2002; Nagadhara et al. 2003, 2004; Saha et al. 2006; Yarasi et al. 2008). Similarly, transgenic plants generated with *Dioscorea batatas* tuber lectin1 gene under the control of phloem-specific promoter of rice sucrose synthase-1 gene showed up to 30% reduced survival rate of BPH as compared to the wild type (Yoshimura et al. 2012). The transgenic rice plants generated with the introduction of ASAL showed interaction with NADH-quinone oxidoreductase (NQO), a key player in electron transport chain, which resulted in toxicity and loss of fecundity during BPH feeding (Bala et al. 2013).

RNAi (RNA interference) is an important approach for meeting the challenges imposed by crop insects with careful secretion of key enzymes/proteins (Gordon and Waterhouse 2007; Price and Gatehouse 2008; Agarwal et al. 2012; Rao Kola et al. 2015). According to Du et al. (2020), the plant-mediated RNA interference (RNAi) is a favourable strategy involving the expression of double-stranded (ds) insect RNA in crops for insect control. This technique has a better mode of action and specificity than the use of protein toxins. In majority of studies on RNAi for insect control, enzymes/proteins of the insect midgut have been considered as the most effective target for the gene silencing. In one of the studies, the transgenic plants were generated using three genes, the hexose transporter gene *NlHT1*, the carboxy peptidase gene *Nlcar* and the trypsin-like serine protease gene *Nltry*, by introducing dsRNA that is expressed in the midgut of the BPH (Zha et al. 2011). It was observed that upon feeding the transgenic plants, the expression of BPH genes were reduced by 40–70% in the third instar nymphs by day 4; however, no lethal phenotypic effect was observed.

The interaction of plants with different insects results in the release of complex blend of volatile compounds. Manipulation of these volatile compounds could be an important strategy for controlling insect pests. Rice plant induces one of the most abundant volatile compounds, that is, 'S-linalool', by feeding of BPH. Similarly, another constitutive produced volatile compound in rice, (*E*)-beta-caryophyllenes, is induced by feeding of chewing herbivores but not by sucking pests like BPH. Both these compounds have been reported to attract BPH parasitoid, *Anagrus nilaparvatae*, in the laboratory (Cheng et al. 2007). RNAi-mediated silencing of S-linalool attracted parasitoid and chewing herbivores but repelled BPH. However, the constitutively produced (*E*)-beta-caryophyllene attracted both parasitoid and BPH, resulting in increased herbivore load (Xiao et al. 2012).

The role of tyrosine hydroxylase, an indispensable survival gene in holometabolous insects, playing key roles in cuticle tanning and immunity, was studied in controlling BPH population in rice (Liu et al. 2020). Introducing dsRNA of this gene by microinjection or feeding caused rapid death of BPH, showing that RNAi-mediated knockdown of this gene could play an important role in controlling BPH. Shangguan

et al. (2018) demonstrated that the expressing dsNlMLP in rice impaired salivary sheath formation and significantly reduced the rate of weight gain and survival of BPHs fed on these plants.

In another study by Shen et al. (2020), three ferritin genes, ferritin 1 heavy chain (*NlFer1*), ferritin 2 light chain (*NlFer2*) and soma ferritin (*Nlsoma-Fer*), were identified from BPH, which showed high expression in the gut. RNAi-mediated silencing of these genes showed <14% mortality with *Nlsoma-Fer*; however, knockdown of *NlFer1* or *NlFer2* led to retarded growth and 100% mortality in young nymphs. This study suggested that *NlFer1* and *NlFer2* are essential for BPH development and reproduction, and the two gut highly expressed genes are promising candidates for application in RNAi-based control of this destructive pest. The identification of suitable candidate genes to be used as targets is the primary requirement to use for this technology. On the other hand, RNAi pathway in the planthoppers needs to be elucidated in order to efficiently use this technology to generate resistance against hoppers.

9.11 Potential of CRSPR/Cas9 in Resistance to Planthoppers

The CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR associated protein) system has emerged as a revolutionary technology, which is showing immense potential in plants to bring beneficial changes in the genome and is making the way for precision crop trait improvement (Voytas and Gao 2014). This technology harnesses the DNA repair pathway by generating double-strand breaks (DSB) at the specified site in the genome (Puchta 2017). The DSB follows the predominant NHEJ (non-homologous end joining) repair pathway, in the absence of a homologous partner in the genome. The broken chromosomes are rejoined imprecisely, thereby introducing indels at the desired site. The introduced changes at the target site can knock out the function of a gene by a frameshift mutation. The technology can be used in replacement of RNAi for the identification of candidate genes in planthoppers that inhibit the survival of insects, development of gene drives for controlling planthoppers in the field and manipulation of host genes governing resistance and susceptibility. In one of the studies by Xue et al. (2018), eye pigmentation genes were knock out by using this technology, showing feasibility for genetic manipulation in this insect. Since the outcome of such modifications can lead to the development of potential gene drives for controlling planthoppers, it faces lot of limitations, constraints and biosafety concerns. One of the potential applications of this technology would be genetic modification of host genes for developing resistance in rice plant against planthoppers. The technology has demonstrated its potential by modifying three bacterial blight susceptibility genes to develop durable resistance in rice (Oliva et al. 2019). In one of the cases of the use of this technology in planthoppers, Lu et al. (2018) reported that there was

increased SA levels and decreased serotonin levels in rice by knocking out CYP71A1 (encoding tryptamine 5-hydroxylase) by CRISPR/Cas9, thereby increasing resistance to BPH. However, CRISPR/Cas9-mediated genome editing has immense potential in developing durable resistance in rice against planthoppers, once our knowledge of resistance or susceptibility genes against these insect pests will increase.

9.12 Future Prospects

Rice insect pests pose a major challenge to rice production and sustainability, particularly with changing climatic conditions. However, recent advances in the cutting-edge science of genomics including next-generation sequencing technologies, high-throughput genotyping and genome editing, along with advances in high-throughput phenotyping technologies, offer several opportunities to meet the challenges of developing pest-resistant varieties. The following are the important points that rice entomologists, geneticist and breeders should take into account as future priorities to breed rice varieties resistant to insect pests:

- A large number of donors for resistance to BPH have been identified; however, there are limited studies to identify resistant sources/donors from diverse germplasm-primitive cultivars, landraces, traditional varieties and wild species of *Oryza* for other insect pests, which should be emphasized.
- Identified resistant donors and genes should be screened against available biotypes globally, and suitable gene combinations for each biotypes should be selected and utilized.
- With the availability of diverse sources of resistance and advanced genotyping technologies, there is a need to identify novel genes/QTLs governing resistance to different insect pests preferably with different modes of resistance.
- It has been observed that monogenic resistance to rice insect pests is not durable, so priority should be given on marker-assisted pyramiding of genes/QTL to different biotypes/insect populations and combine multiple resistance to BPH, GLH and WBPH.
- There is a need to emphasize allele/haplotype mining to identify and introgress desirable alleles/haplotypes for resistance.
- Besides BPH, more efforts should be made to study other insect pests, since these pests also have the probability to become major pests in the future.
- Isogenic lines, particularly for resistance to BPH, WBPH and GLH, should be developed, and such lines should be tested in different areas, regions and countries to deploy target genes in respective areas of rice cultivation.
- RNAi and gene editing particularly CRISPR/Cas9 should be accommodated as a long-term approach in developing germplasm resistant to hoppers.

References

Abo ME, Sy AA (1997) Rice virus diseases: epidemiology and management strategies. J Sustain Agric 11:113–134. https://doi.org/10.1300/J064v11n02_09

Agarwal S, Mohan M, Mangrauthia SK (2012) RNAi: machinery and role in pest and disease management. In: Venkateshwarlu B, Shankar AK, Shankar C, Maheshwari M (eds) Crop stress and its management: perspectives and strategies. Springer, New York, pp 447–469. https://doi.org/10.1007/978-94-007-2220-0_13

Alam SN, Cohen MB (1998) Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population. Theor Appl Genet 97:1370–1379. https://doi.org/10.1007/s001220051031

Angeles ER, Khush GS (2000) Genetic analysis of resistance to green leafhopper, *Nephotettix virescens* (Distant), in three varieties of rice. Plant Breed 119:446–448. https://doi.org/10.1046/j.1439-0523.2000.00513.x

Angeles ER, Khush GS, Heinrichs EA (1981) New genes for resistance to whitebacked planthopper in rice. Crop Sci 21:47–50. https://doi.org/10.2135/cropsci1981.0011183X002100010014x

Angeles ER, Khush GS, Heinrichs EA (1986) Inheritance of resistance to planthoppers and leafhoppers in rice. In: Rice genetics. International Rice Research Institute, Manila, Philippines, pp 537–549. https://doi.org/10.1142/9789812814265_0048

Athwal DS, Pathak MD, Bacalangco EH, Pura CD (1971) Genetics of resistance to brown plant hopper and green leaf hoppers in *Oryza sativa* L. Crop Sci 11:747–750. https://doi.org/10.2135/cropsci1971.0011183X001100050043x

Avesi GM, Khush GS (1984) Genetic analysis for resistance to the green leafhopper, *Nephotettix virescens* (Distant), in some cultivars of rice, *Oryza sativa* L. Crop Prot 3:41–51. https://doi.org/10.1016/0261-2194(84)90005-X

Backus EA, Serrano MS, Ranger CM (2005) Mechanisms of hopperburn: an overview of insect taxonomy, behavior and physiology. Annu Rev Entomol 50:125–151. https://doi.org/10.1146/annurev.ento.49.061802.123310

Bala A, Roy A, Behura N, Hess D, Das S (2013, 2013) Insight to the mode of action of Allium sativum leaf agglutinin (ASAL) expressing in T_3 rice lines on brown planthopper. Am J Plant Sci:28457. https://doi.org/10.4236/ajps.2013.42A052

Balachiranjeevi CH, Prahalada GD, Mahender A et al (2019) Identification of a novel locus, *BPH38*(t), conferring resistance to brown planthopper (*Nilaparvata lugens* Stal.) using early backcross population in rice (*Oryza sativa* L.). Euphytica 215:185. https://doi.org/10.1007/s10681-019-2506-2

Brar DS, Virk PS, Jena KK, Khush GS (2009) Breeding for resistance to planthoppers in rice. In: Heong KL, Hardy B (eds) Planthoppers: new threats to the sustainability of intensive rice production systems in Asia. International Rice Research Institute, Los Baños, Philippines, pp 401–428

Brar DS, Sarao PS, Singh K, Jena KK, Fujita D (2015) Biotechnological approaches for enhancing resistance to planthoppers in rice. In: Singh B, Arora R, Gosal SS (eds) Biological and molecular approaches in pest management. Scientific Publishers, Jodhpur, pp 13–38

Cha Y-S, Ji H, Yun D-W, Ahn B-O, Lee MC, Suh S-C, Lee CS, Ahn EK, Jeon Y-H, Jin I-D, Sohn J-K, Koh H-K (2008) Fine mapping of the rice *Bph1* gene, which confers resistance to the brown planthopper (*Nilaparvata lugens* Stal) and development of STS markers for marker-assisted selection. Mol Cells 26:146–151

Chen J, Wang L, Pang X, Pan Q (2006) Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph19*(t). Mol Gen Genomics 275:321–329. https://doi.org/10.1007/s00438-005-0088-2

Chen J, Huang D, Wang L et al (2010) Identification of quantitative trait loci for resistance to whitebacked planthopper, *Sogatella furcifera*, from an interspecific cross *Oryza sativa* X *O. rufipogon*. Breed Sci 60:153–159. https://doi.org/10.1270/jsbbs.60.153

Cheng AX, Xiang CY, Li JX et al (2007) The rice (E)-beta caryophyllene synthase (OsTPS3) accounts for the major inducible volatile sesquiterpenes. Phytochemistry 68:1632–1641. https://doi.org/10.1016/j.phytochem.2007.04.008

Cheng X, Wu Y, Guo J, Du B, Chen R, Zhu L, He G (2013) A rice lectin receptor-like that is involved in innate immune responses also contributes to seed germination. Plant J 76:687–698. https://doi.org/10.1111/tpj.12328

Cheng L, Nong B, Xu A, He W, Wu B, Qiu Y (2021) Genetic analysis and gene mapping of two whitebacked planthopper resistance genes from rice varieties. (Preprint). https://doi.org/10.21203/rs.3.rs-448706/v1

Denno RF, Perfect TJ (1994) Planthoppers: their ecology and management. Chapman and Hall, New York, p 787

Deutsch CA, Tewksbury JJ, Tigchelaar M, Battisti DS, Merrill SC, Huey RB, Naylor RL (2018) Increase in crop losses to insect pests in a warming climate. Science 361:916–919. https://doi.org/10.1126/science.aat3466

Du B, Zhang W, Liu B, Hu J, Wei Z, Shi Z, He R, Zhu L, Chen R, Han B, He G (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. Proc Natl Acad Sci U S A 106:22163–22168. https://doi.org/10.1073/pnas.0912139106

Du B, Chen R, Guo J, He G (2020) Current understanding of the genomic, genetic, and molecular control of insect resistance in rice. Mol Breed 40:24. https://doi.org/10.1007/s11032-020-1103-3

Duan CX, Wan JM, Zhai HQ, Chen Q, Wang JK, Su N, Lei CL (2007a) Quantitative trait loci mapping of resistance to *Laodelphax striatellus* (Homoptera: Delphacidae) in rice using recombinant inbred lines. J Econ Entomol 100:1450–1455. https://doi.org/10.1603/0022-0493(2007)100[1450:qtlmor]2.0.co;2

Duan CX, Zhang SX, Chen Q, Cheng ZJ, Zhai HQ, Wan JM (2007b) Evaluation of rice germplasm for resistance to the small brown planthopper (*Laodelphax striatellus* Fallen) and analysis on resistance mechanism. Chin J Rice Sci 21:425–430

Duan CX, Zhang SX, Lei CL, Cheng ZJ, Chen Q, Zhai HQ, Wan JM (2008) Evaluation of rice germplasm for resistance to the small brown planthopper (*Laodelphax striatellus*) and analysis of resistance mechanism. Rice Sci 15:36–42. https://doi.org/10.1016/S1672-6308(08)60017-7

Duan CX, Cheng ZL, Lei CL, Zhai HQ, Wan JM (2009) Analysis of QTLs for resistance to small brown planthopper (*Laodelphax striatellus* Fallen) in rice (*Oryza sativa* L.) using an F_2 population from a cross between Mudgo and Wuyujing. Acta Agric Sin 35:388–394. https://doi.org/10.1016/S1875-2780(08)60065-6

Duan CX, Su N, Cheng ZJ, Lei CL, Wang JL, Zhai HQ, Wan JM (2010) QTL analysis for the resistance to small brown planthopper (*Laodelphax striatellus* Fallen) in rice using backcross inbred lines. Plant Breed 129:63–67. https://doi.org/10.1111/j.1439-0523.2009.01648.x

Dupo ALB, Barrion AT (2009) Taxonomy and general biology of delphacid planthoppers in rice agroecosystems. In: Heong KL, Hardy B (eds) Planthoppers: new threats to the sustainability of intensive rice production systems in Asia. International Rice Research Institute, Los Banos, Philippines, pp 3–156

Eisemann CH, Donaldson RA, Pearson RD, Cadogan LC, Vuocolo T, Tellam RL (1994) Larvicidal action of lectins on *Lucilia cuprina*; mechanism of action. Entomol Exp Appl 7:2–11. https://doi.org/10.1111/j.1570-7458.1994.tb01796.x

Fan D, Liu Y, Zhang H, He J, Huang F, Huang S, Wu B, Liu D, Wen P, Liu L, Jiang L, Cheng X, Wan J (2018) Identification and fine mapping of *qWBPH11* conferring resistance to whitebacked planthopper (*Sogatella furcifera* Horvath) in rice (*Oryza sativa* L.). Mol Breed 38:96. https://doi.org/10.1007/s11032-018-0846-6

Foissac X, Loc NT, Christou P, Gatehouse AMR, Gatehouse JA (2000) Resistance to green leafhopper *Nephotettix virescens* and brown planthopper *Nilaparvata lugens* in transgenic rice expressing snowdrop lectin *Galanthus nivalis* agglutinin. J Insect Physiol 46:573–583. https://doi.org/10.1016/S0022-1910(99)00143-2

Fujita D, Doi K, Yoshimura A, Yasui H (2004) Introgression of a resistance gene for green rice leafhopper from *Oryza nivara* into cultivated rice, *Oryza sativa* L. Rice Genet Newslett 21:64–66

Fujita D, Doi K, Yoshimura A, Yasui H (2006) Molecular mapping of a novel gene, *Grh5*, conferring resistance to green rice leaf hopper (*Nephotettix cincticeps* Uhler) in rice, *Oryza sativa* L. Theor Appl Genet 113:567–573. https://doi.org/10.1007/s00122-006-0270-x

Fujita D, Myint KKM, Matsumura M, Yasui H (2009) The genetics of host-plant resistance to rice planthopper and leafhopper. In: Heong KL, Hardy B (eds) Planthoppers: new threats to the sustainability of intensive rice production systems in Asia. International Rice Research Institute, Los Baños, pp 389–400

Fujita D, Doi K, Yoshimura A, Yasui H (2010) A major QTL for resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) derived from African rice (*Oryza glaberrima* Steud.). Breed Sci 60:336–341. https://doi.org/10.1270/jsbbs.60.336

Fujita D, Kohli A, Horgan F (2013) Rice resistance to hoppers and leafhoppers. Crit Rev Plant Sci 32:162–191. https://doi.org/10.1080/07352689.2012.735986

Ghaffar ABMB, Pritchard J, Ford-Lloyd B (2011) Brown planthopper (*N. lugens* Stal) feeding behaviour on rice germplasm as an indicator of resistance. PLoS One 6:e22137. https://doi.org/10.1371/journal.pone.0022137

Ghani MU, Khush GS (1998) A new gene for resistance to green leafhopper *Nephotettix virescens* (distant) in rice. J Genet 67:151–159. https://doi.org/10.1007/BF02927826

Ghauri MSK (1971) Revision of the genus *Nephotettix* Matsumura (Homoptera: Cicadelloidea: Euscelidae) based on the type material. Bull Entomol Res 60:481–512

Gordon KHJ, Waterhouse PM (2007) RNAi for insect-proof plants. Nat Biotechnol 25:1231–1232. https://doi.org/10.1038/nbt1107-1231

Guo J, Xu C, Wu D, Zhao Y, Qiu Y, Wang X, Ouyang Y, Cai B, Liu X, Jing S, Shangguan X, Wang H, Ma Y, Hu L, Wu Y, Shi S, Wang W, Zhu L, Xu X, Chen R, Feng Y, Du B, He G (2018) Bph6 encodes an exocyst-localized protein and confers broad resistance to planthoppers in rice. Nat Genet 50:297–306

Haining Y, Improvement GC, Shaoli W, Xiaoqiong L (2010) Pyramiding lines conferring resistances to brown planthopper and bacterial leaf blight by marker-assisted selection. Mol Plant Breed 8:11–19

Harini AS, Lakshmi SS, Kumar SS, Sivaramakrishnan S, Kadirvel P (2010) Validation and fine-mapping of genetic locus associated with resistance to brown plant hopper [*Nilaparvata lugens* (Stål.)] in rice (*Oryza sativa* L.). Asian J Bio Sci 5:32–37

He J, Liu Y, Liu Y, Jiang L, Wu H, Kang H, Liu S, Chen L, Liu X, Cheng X, Wan J (2013) High-resolution mapping of brown planthopper (BPH) resistance gene *Bph27*(t) in rice (*Oryza sativa* L.). Mol Breed 31:549–557. https://doi.org/10.1007/s11032-012-9814-8

Hernandez JE, Khush GS (1981) Genetics of resistance to whitebacked planthopper in some rice (*Oryza sativa* L.) varieties. Oryza 18:44–50

Hirabayashi H, Ogawa T (1995) RFLP mapping of *Bph-1* (brown planthopper resistance gene) in rice. Breed Sci 45:369–371. https://doi.org/10.1023/A:1021514829783

Hirabayashi H, Angeles ER, Kaji R et al (1998) Identification of brown planthopper resistance gene derived from *O. officinalis* using molecular markers in rice. Breed Sci 48(Suppl):82

Hirabayashi H, Kaji R, Angeles ER et al (1999) RFLP analysis of a new gene for resistance to brown planthopper derived from *O. officinalis* on rice chromosome 4. Breed Res 1:48. Supplement 1 (in Japanese)

Horgan FG, Ramal AF, Bentur JS et al (2015) Virulence of brown planthopper (*Nilaparvata lugens*) populations from South and South East Asia against resistant rice varieties. Crop Protect 78:222–231. https://doi.org/10.1016/j.cropro.2015.09.014

Hu J, Li X, Wu C, Hua H, Gao G, Xiao J, He Y (2012) Pyramiding and evaluation of the brown planthopper resistance genes Bph14 and Bph15 in hybrid rice. Mol Breed 29:61–69. https://doi.org/10.1007/s11032-010-9526-x

Hu J, Xiao C, He Y (2016) Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. Rice 9:30. https://doi.org/10.1186/s12284-016-0099-0

Hu J, Chang X, Zou L, Tang W, Wu W (2018) Identification and fine mapping of Bph33, a new brown planthopper resistance gene in rice (*Oryza sativa* L.). Rice 11:55. https://doi.org/10.1186/s12284-018-0249-7

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J, Khush GS (1997) Pyramiding of bacterial blight resistance genes in rice: marker assisted selection using RFLP and PCR. Theor Appl Genet 95:313–320. https://doi.org/10.1007/s001220050565

Huang Z, He G, Shu L, Li X, Zhang Q (2001) Identification and mapping of two brown planthopper genes in rice. Theor Appl Genet 102:929–934. https://doi.org/10.1007/s001220000455

Huang D, Qiu Y, Zhang Y, Huang F, Meng J, Wei S, Li R, Chen B (2013) Fine mapping and characterization of *BPH27*, a brown planthopper resistance gene from wild rice (*Oryza rufipogon* Griff.). Theor Appl Genet 126:219–229. https://doi.org/10.1007/s00122-012-1975-7

Ishii T, Brar DS, Multani DS, Khush GS (1994) Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, O. sativa. Genome 37:217–221. https://doi.org/10.1139/g94-030

Jairin J, Phengrat K, Teangdeerith S, Vanavichit A, Toojinda T (2007a) Mapping of a broad-spectrum brown plant hopper resistance *gene*, *Bph3*, on rice chromosome 6. Mol Breed 19:35–44. https://doi.org/10.1007/s11032-006-9040-3

Jairin J, Teangdeerith S, Leelagud P et al (2007b) Detection of brown planthopper resistance genes from different rice mapping populations in the same genomic location. Sci Asia 33:347–352. https://doi.org/10.2306/scienceasia1513-1874.2007.33.347

Jairin J, Teangdeerith SN, Leelagud P et al (2007c) Physical mapping of *Bph3*, a brown planthopper resistance locus in rice. Maejo Int J Sci Technol 1:166–177

Jairin J, Teangdeerith S, Leelagud P et al (2009) Development of rice introgression lines with brown planthopper resistance and KDML105 grain quality characteristics through marker-assisted selection. Field Crops Res 110:263–271. https://doi.org/10.1016/j.fcr.2008.09.009

Jairin J, Sansen K, Wongboon W, Kothcharerk J (2010) Detection of a brown planthopper resistance gene *bph4* at the same chromosomal position of *Bph3* using two different genetic backgrounds of rice. Breed Sci 60:71–75. https://doi.org/10.1270/jsbbs.60.71

Jena KK, Jeun GM, Lee JH et al (2006) High-resolution mapping of a new brown planthopper (BPH) resistance gene, *Bphl8(t)*, and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theor Appl Genet 112:288–297. https://doi.org/10.1007/s00122-005-0127-8

Jeon YH, Ahn SN, Choi HC et al (1999) Identification of a RAPD marker linked to a brown planthopper resistance gene in rice. Euphytica 107:23–28. https://doi.org/10.1023/A:1003506830735

Ji H, Kim SR, Kim YH et al (2016) Map-based cloning and characterization of the *Bph18* gene from wild rice conferring resistance to brown planthopper (BPH). Insect Pest Sci Rep 6:34376. https://doi.org/10.1038/srep34376

Kabir MA, Khush GS (1988) Genetic analysis of resistance to brown planthopper in rice, *Oryza sativa* L. Plant Breed 100:54–58

Kadowaki M, Yoshimura A, Yasui H (2003) RFLP mapping of antibiosis to rice green leafhopper. In: Khush GS, Brar DS, Hardy B (eds) Advances in rice genetics. International Rice Research Institute, Los Banos, Philippines, pp 270–271

Kawaguchi M, Murata K, Ishii T et al (2001) Assignment of a brown planthopper (*Nilaparvata lugens* Stal) resistance gene *bph4* to the rice chromosome 6. Breed Sci 51:13–18. https://doi.org/10.1270/jsbbs.51.13

Khush GS (1971) Rice breeding for disease and insect resistance at IRRI. Oryza 8:111–119

Kim SM, Sohn JK (2005) Identification of rice gene (*Bph*1) conferring resistance to brown planthopper (*Nilaparvata lugens* Stal) using STS markers. Mol Cells 20:30–34

Kim EG, Yun S, Park JR, Kim KM (2021) Identification of *F3H*, major secondary metabolite-related gene that confers resistance against whitebacked planthopper through QTL mapping in rice. Plants 10:81. https://doi.org/10.3390/plants10010081

Kumar K, Sarao PS, Bhatia D, Neelam K, Kaur A, Mangat GS, Brar DS, Singh K (2018) High-resolution genetic mapping of a novel brown planthopper resistance locus, Bph34 in *Oryza sativa* L. X *Oryza nivara* (Sharma & Shastry) derived interspecific F_2 population. Theor Appl Genet 131(5):1163–1171. https://doi.org/10.1007/s00122-018-3069-7

Lakshminarayana A, Khush GS (1977) New genes for resistance to the brown planthopper in rice. Crop Sci 17:96–100. https://doi.org/10.2135/cropsci1977.0011183X001700010028x

Lang NT, Bu BC (2003) Genetic and physical maps of gene *Bph-10* controlling brown plant hopper resistance in rice (*Oryza sativa* L.). Omonrice 11:35–41

Li XM, Xiong ZM, Min SK, Hu GW (1990) Genetical analysis of resistance to whitebacked planthopper Sogatella furcifera (Horvath) in four rice varieties (*Oryza sativa* L.) of Yunan province. Chin J Rice Sci 4:113–116

Li X, Zhai H, Wan J, Ma L, Zhuang J, Liu G, Yang C (2004) Mapping of a new gene Wbph6(t) resistant to the whitebacked planthopper, *Sogatella furcifera*, in rice. Rice Sci 11:86–90

Li JB, Xia MY, He GC et al (2006) The evaluation and utilization of new genes for brown planthopper resistance in common wild rice (*Oryza rufipogon* Griff.). Mol Plant Breed 4:365–371

Li Z, Xue Y, Zhou H et al (2019) High-resolution mapping and breeding application of a novel brown planthopper resistance gene derived from wild rice (*Oryza. rufipogon* Griff). Rice 12(41). https://doi.org/10.1186/s12284-019-0289-7

Ling KC (1977) Transmission of rice grassy stunt by the planthopper. (Abstract). In: The rice brown planthopper. Food and Fertilizer Technology Center for Asian and Pacific Region, Taipei (Taiwan), pp 73–82

Liu GQ, Yan HH, Fu Q et al (2001) Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from *Oryza eichingeri*. Chin Sci Bull 46:1459–1462

Liu Z, Liu G, Sogawa K, Zhuang J, Chen S, Zheng K (2002) Mapping the gene Wbph2 in ARC10239 resistant to the whitebacked planthopper *Sogatella furcifera* in rice. Chin J Rice Sci 16:311–314

Liu Y, Wu H, Chen H, Liu Y, He J, Kang H, Sun Z, Pan G, Wang Q, Hu J, Zhou F, Zhou K, Zheng X, Ren Y, Chen L, Wang Y, Zhao Z, Lin Q, Wu F, Zhang X, Guo X, Cheng X, Jiang L, Wu C, Wang H, Wan J (2015) A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. Nat Biotechol 33:301–305. https://doi.org/10.1038/nbt.3069

Liu Y, Chen L, Liu Y et al (2016) Marker assisted pyramiding of two brown planthopper resistance genes, *Bph3* and *Bph27*(t), into elite rice cultivars. Rice 9:27. https://doi.org/10.1186/s12284-016-0096-3

Liu HS, Yang BJ, Wang AY, Luo J, Tang J (2020) RNA interference of tyrosine hydroxylase caused rapid mortality by impairing cuticle formation in *Nilaparvata lugens* (Hemiptera: Delphacidae). Pest Manag Sci 76:2225–2232. https://doi.org/10.1002/ps.5760

Lu HP, Luo T, Fu HW, Wang L, Tan YY, Huang JZ, Shu QY (2018) Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. Nat Plants 4:338–344. https://doi.org/10.1038/s41477-018-0152-7

Mai VT, Fujita D, Matsumura M, Yoshimura A, Yasui H (2015) Genetic basis of multiple resistance to the brown planthopper (*Nilaparvata lugens* Stål) and the green rice leafhopper (*Nephotettix cincticeps* Uhler) in the rice cultivar 'ASD7' (*Oryza sativa* L. ssp. indica). Breed Sci 65(5):420–429. https://doi.org/10.1270/jsbbs.65.420

Majumder P, Banerjee S, Das S (2004) Identification of receptors responsible for binding of the mannose specific lectin to the gut epithelial membrane of the target insects. Glycoconj J 20:525–530. https://doi.org/10.1023/B:GLYC.0000043288.72051.7c

Matsumura M, Takeuchi H, Satoh M, Sanada-Morimura S, Otuka A, Watanabe T, Thanh DV (2009) Current status of insecticide resistance in rice hoppers. In: Heong KL, Hardy B (eds) Planthoppers: new threats to the sustainability of intensive rice production systems in Asia. International Rice Research Institute, Los Baños, the Philippines, pp 233–244

McCouch SR, Khush GS, Tanksley SD (1991) Tagging genes for disease and insect resistance via linkage to RFLP markers. In: Banta SJ, Argosino GS (eds) Rice genetics II. Proceedings of the second international rice genetics symposium, Manila, Philippines, pp 443–449

Murai H, Hashimoto Z, Sharma P et al (2001) Construction of a high resolution linkage map of a rice brown planthopper (*Nilaparvata lugens* Stal) resistance gene *bph2*. Theor Appl Genet 103:526–532. https://doi.org/10.1007/s001220100598

Murata K, Fujiwara M, Kaneda C et al (1998) RFLP mapping of a brown planthopper (*Nilaparvata lugens* Stal) resistance gene *bph2* of *indica* rice introgressed into a *japonica* breeding line "Norin-PL4". Genes Genet Syst 73:359–364. https://doi.org/10.1266/ggs.73.359

Murata K, Fujiwara M, Murai H et al (2001) Mapping of a brown planthopper (*Nilaparvata lugens* Stal) resistance gene *Bph9* on the long arm of chromosome 12. Cereal Res Commun 29:245–250. https://doi.org/10.1007/BF03543667

Myint KKM, Fujita D, Matsumura M et al (2012) Mapping and pyramiding of two major genes for resistance to the brown planthopper (*Nilaparvata lugens* Stål) in the rice cultivar ADR52. Theor Appl Genet 124:495–504. https://doi.org/10.1007/s00122-011-1723-4

Nagadhara D, Ramesh S, Pasalu IC et al (2003) Transgenic *indica* rice resistant to sap sucking insects. Plant Biotechnol J1:231–240. https://doi.org/10.1046/j.1467-7652.2003.00022.x

Nagadhara D, Ramesh S, Pasalu IC et al (2004) Transgenic rice plants expressing the snowdrop lectin gene *gna* exhibit high level resistance to the white backed planthopper. Theor Appl Genet 109:1399–1405. https://doi.org/10.1007/s00122-004-1750-5

Naik SB, Divya D, Sahu N, Sundaram RM, Sarao PS, Singh K, Lakshmi VJ, Bentur JS (2018) A new gene Bph33(t) conferring resistance to brown planthopper (BPH), *Nilaparvata lugens* (Stål) in rice line RP2068-18-3-5. Euphytica 214(3):53. https://doi.org/10.1007/s10681-018-2131-5

Nemoto H, Ishikawa K, Shimura E (1994) The resistances to rice stripe virus and small brown planthopper in rice variety IR50. Breed Sci 44:13–18

Oliva R, Ji C, Atienza-Grande G, Huguet-Tapia JC, Perez-Quintero A, Li T, Yan B (2019) Broad-spectrum resistance to bacterial blight in rice using genome editing. Nat Biotechnol 37:1344–1350. https://doi.org/10.1038/s41587-019-0267-z

Padmavathi G, Ram T, Ramesh K, Rao Y, Pasalu IC, Viraktamath BC (2007) Genetics of the whitebacked planthopper, *Sogatella furcifera* (Horvath), resistance in rice. SABRAO J Breed Genet 39:99–105

Park DS, Song MY, Park SK et al (2008) Molecular tagging of the *Bph1 locus* for resistance to brown planthopper (*Nilaparvata lugens* Stal) through representational difference analysis. Mol Genet Genomics 280:163–172. https://doi.org/10.1007/s00438-008-0353-2

Pathak MD, Cheng CH, Furtono ME (1969) Resistance to *Nephotettix cincticeps* and *Nilaparvata lugens* in varieties of rice. Nature 223:502–504

Powell KS, Gatehouse AMR, Hilder VA et al (1995) Different antimetabolic effects of related plant lectin towards nymphal stages of *Nilaparvata lugens*. Entomol Exp Appl 75:61–65. https://doi.org/10.1111/j.1570-7458.1995.tb01910.x

Powell KS, Spence J, Bharathi M et al (1998) Immuno-histochemical and development studies to elucidate the mechanism of action of the snowdrop lectin on the rice brown planthopper. J Insect Physiol 44:529–539. https://doi.org/10.1016/s0022-1910(98)00054-7

Prahalada GD, Shivakumar N, Lohithaswa HC, Sidde Gowda DK, Ramkumar G, Kim SR, Ramachandra C, Hittalmani S, Mohapatra T, Jena KK (2017) Identification and fine mapping of a new gene, BPH31 conferring resistance to brown planthopper biotype 4 of India to improve rice, *Oryza sativa* L. Rice 10:41. https://doi.org/10.1186/s12284-017-0178-x

Price DRG, Gatehouse JA (2008) RNAi-mediated crop protection against insects. Trends Biotechnol 26:393–400. https://doi.org/10.1016/j.tibtech.2008.04.004

Puchta H (2017) Applying CRISPR/Cas for genome engineering in plants: the best is yet to come. Curr Opin Plant Biol 36:1–8. https://doi.org/10.1016/j.pbi.2016.11.011

Qiu YF, Guo JP, Jing SL et al (2010) High-resolution mapping of the brown planthopper resistance gene *Bph6* in rice and characterizing its resistance in the 9311 and Nipponbare near isogenic backgrounds. Theor Appl Genet 121:1601–1611. https://doi.org/10.1007/s00122-010-1413-7

Qiu Y, Guo J, Jing S, Zhu L, He G (2012) Development and characterization of *japonica* rice lines carrying the brown planthopper-resistance gene *BPH12* and *BPH6*. Theor Appl Genet 124:485–494. https://doi.org/10.1007/s00122-011-1722-5

Qiu Y, Guo J, Jing S et al (2014) Fine mapping of the rice brown planthopper resistance gene *BPH7* and characterization of its resistance in the 93-11 background. Euphytica 198:369–379. https://doi.org/10.1007/s10681-014-1112-6

Rahman ML, Jiang W, Chu SH, Qiao Y, Ham TH, Woo MO, Lee J, Khanam MS, Chin JH, Jeung JU, Brar DS, Jena KK, Koh HJ (2009) High-resolution mapping of two brown planthopper resistance genes, Bph20(t) and Bph21(t), originating from *Oryza minuta*. Theor Appl Genet 119:1237–1246. https://doi.org/10.1007/s00122-009-1125-z

Ramesh K, Padmavathi G, Deen R, Pandey MK, Lakshmi VJ, Bentur JS (2014) Whitebacked planthopper *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae) resistance in rice variety Sinna Sivappu. Euphytica 200:139–148. https://doi.org/10.1007/s10681-014-1175-4

Rao Kola VS, Renuka P, Madhav MS, Mangrauthia SK (2015) Key enzymes and proteins of crop insects as candidate for RNAi based gene silencing. Front Physiol 6:119. https://doi.org/10.3389/fphys.2015.00119

Rao KV, Rathore KS, Hodges TK et al (1998) Expression of snow drop lectin (GNA) in transgenic rice plants confers resistance to rice brown planthopper. Plant J 15:469–477. https://doi.org/10.1046/j.1365-313x.1998.00226.x

Ren J, Gao F, Wu X et al (2016) *Bph32*, a novel gene encoding an unknown SCR domain-containing protein, confers resistance against the brown planthopper in rice. Sci Rep 6:37645. https://doi.org/10.1038/srep37645

Renganayaki K, Fritz AK, Sadasivam S et al (2002) Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, O. sativa. Crop Sci 42:2112–2117. https://doi.org/10.2135/cropsci2002.2112

Rezaul Karim ANM, Pathak MD (1982) New genes for resistance to green leafhopper, *Nephotettix virescens* (distant) in rice *Oryza sativa* L. Crop Prot 1:483–490. https://doi.org/10.1016/0261-2194(82)90029-1

Rivera CT, Ou SH, Iida TT (1966) Grassy stunt disease of rice and its transmission by the planthopper *Nilaparvata lugens* Stål. Plant Dis Rep 50:453–456

Ruane J, Sonnino A (2007) Marker-assisted selection as a tool for genetic improvement of crops, livestock, forestry and fish in developing countries: an overview of the issues. In: Guimaraes EP, Ruane J, Scherf BD, Sonnino A, Dargie JD (eds) Marker assisted selection: current status and future perspectives in crops, livestock, forestry and fish. FAO, Agriculture and Consumer Protection Dept., Rome, pp 3–13

Saha P, Majumder P, Datta I et al (2006) Transgenic rice expressing *Allium sativum* leaf lectin with enhanced resistance against sap sucking insect-pests. Planta 223:1329–1343. https://doi.org/10.1007/s00425-005-0182-z

Sanchez AC, Brar DS, Huang N et al (2000) Sequence tagged site marker assisted selection for three bacterial blight resistance genes in rice. Crop Sci 40:792–797. https://doi.org/10.2135/cropsci2000.403792x

Sarao PS, Sahi KG, Neelam K et al (2016) Donors for resistance to brown planthopper Nilaparvata lugens (Stål) from wild rice species. Rice Sci 23:219–224. https://doi.org/10.1016/j.rsci.2016.06.005

Sarao PS, Bhatia D, Brar DS (2017) Advances in breeding for resistance to hoppers in rice. In: Arora R, Sandhu S (eds) Breeding insect resistant crops for sustainable. Springer Nature Singapore Pte Ltd., Singapore, pp 101–130

Sebastian LS, Ikeda R, Huang N, Imbe T, Coffman WR, McCouch SR (1996) Molecular mapping of resistance to rice spherical virus and green leafhopper. Phytopathology 86:25–30. https://doi.org/10.1094/Phyto-86-25

Shangguan X, Zhang J, Liu B, Zhao Y, Wang H, Wang Z et al (2018) A mucin-like protein of planthopper is required for feeding and induces immunity response in plants. Plant Physiol 176:552–565. https://doi.org/10.1104/pp.17.00755

Sharma PN, Ketipearachchi Y, Murata K et al (2002) RFLP/AFLP mapping of a brown planthopper (*Nilaparvata lugens* Stat) resistance gene *Bph1* in rice. Euphytica 129:109–117. https://doi.org/10.1023/A:1021514829783

Sharma PN, Torii A, Takumi S et al (2004) Marker-assisted pyramiding of brown planthopper (*Nilaparvata lugens* Stat) resistance genes *Bph1* and *Bph2* on rice chromosome 12. Hereditas 136:39–43. https://doi.org/10.1111/j.1601-5223.2004.01726.x

Shen Y, Chen Y-Z, Zhang C-X (2020) RNAi-mediated silencing of ferritin genes in the brown planthopper *Nilaparvata lugens* affects survival, growth and female fecundity. Pest Manag Sci 77:365–377. https://doi.org/10.1002/ps.6026

Sidhu GS, Khush GS (1978) Genetic analysis of brown planthopper resistance in twenty varieties of rice, Oryza *sativa*. Theor Appl Genet 53:199–203. https://doi.org/10.1007/BF00277368

Sidhu GS, Khush GS, Medrano FG (1979) A dominant gene in rice for resistance to white-backed planthopper and its relationship to other plant characteristics. Euphytica 28:227–232

Sidhu N, Bansal UK, Shukla KK, Saini RG (2005) Genetics of resistance to white-backed planthopper in five rice stocks. SABRAO J 37:43–49

Singh S, Sidhu JS, Huang N et al (2001) Pyramiding three bacterial blight resistance genes (*xa*5, *xa*13 and *Xa*21) using marker assisted selection into *indica* cultivar PR106. Theor Appl Genet 102:1011–1015. https://doi.org/10.1007/s001220000495

Singh A, Gopalakrishnan S, Singh VP et al (2011) Marker assisted selection: a paradigm shift in Basmati breeding. Indian J Genet Plant Breed 71:120–128

Siwi BH, Khush GS (1977) New genes for resistance to green leafhopper in rice. Crop Sci 17:17–20. https://doi.org/10.2135/cropsci1977.0011183X001700010006x

Sogawa K, Qian Q, Zeng D, Hu J, Zeng LJ (2005) Differential expression of whitebacked planthopper resistance in the *Japonica*/*Indica* doubled haploid rice population under field evaluation and seedbox screening test. Rice Sci 12(1):63–67

Soundararajan RP, Kadirvel P, Gunathilagaraj K, Maheswaran M (2004) Mapping of quantitative trait loci associated with resistance to brown planthopper in rice by means of a doubled haploid population. Crop Sci 44:2214–2220. https://doi.org/10.2135/cropsci2004.2214

Su CC, Wan J, Zhai HQ et al (2005) A new locus for resistance to brown planthopper identified in *indica* rice variety DV85. Plant Breed 124:93–95. https://doi.org/10.1111/j.1439-0523.2004.01011.x

Su CC, Zhai HQ, Wang CM et al (2006) SSR mapping of brown plant hopper resistance gene *Bph9* in Kaharamana, an *indica* rice (*Oryza sativa* L.). Acta Genet Sin 33:262–268. https://doi.org/10.1016/S0379-4172(06)60049-8

Sudhakar D, Fu X, Stoger E et al (1998) High level of expression and immune-localization of the snowdrop lectin insecticidal protein GNA in transgenic rice plants. Transgenic Res 7:371–378. https://doi.org/10.1023/a:1008856703464

Sun X, Wu A, Tang K (2002) Transgenic rice lines with enhanced resistance to the small brown planthopper. Crop Prot 21:511–514. https://doi.org/10.1016/S0261-2194(01)00127-2

Sun L, Su C, Wang C et al (2005) Mapping of a major resistance gene to brown plant hopper in the rice cultivar Rathu Heenati. Breed Sci 55:391–396. https://doi.org/10.1270/jsbbs.55.391

Sun LH, Wang CM, Su CC et al (2006) Mapping and marker-assisted selection of a brown planthopper resistance gene *bph2* in rice (*Oryza sativa* L.). Acta Genet Sin 33:717–723. https://doi.org/10.1016/S0379-4172(06)60104-2

Sun Z, Liu Y, Xiao S, Hu J, Pan G, He J, Xu T, Huang J, Qiu Z, Fan D, Zhang L, Liu L, Jiang L, Cheng X, Zhao H, Wan J (2017) Identification of quantitative trait loci for resistance to rice black-streaked dwarf virus disease and small brown planthopper in rice. Mol Breed 37:72

Tamura K, Fukuta Y, Hirae M et al (2004) RFLP mapping of a new resistance gene for green rice leafhopper in Kanto PL10. Rice Genet Newsl 21:62–64

Tamura Y, Hattori M, Yoshioka H et al (2014) Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa* L. ssp. *indica* cultivar ADR52. Sci Rep 4:5872. https://doi.org/10.1038/srep05872

Tan GX, Wang QM, Ren X et al (2004) Two whitebacked planthopper resistance genes in rice share the same loci with those for brown planthopper resistance. Heredity 92:212–217. https://doi.org/10.1038/sj.hdy.6800398

Tang K, Hu Q, Sun X et al (2001) Development of transgenic rice homozygous lines with enhanced resistance to rice brown planthopper. In Vitro Cell Dev Biol Plant 37:334–340. https://doi.org/10.1002/1521-3846(200105)21:2%3C117::AID-ABIO117%3E3.0.CO;2-Y

Voytas DF, Gao C (2014) Precision genome engineering and agriculture: opportunities and regulatory challenges. PLoS Biol 12(6):e1001877. https://doi.org/10.1371/journal.pbio.1001877

Wang CM, Yasui H, Yoshimura A, Zhai HQ, Wan JM (2004) Inheritance and QTL mapping of antibiosis to green leafhopper in rice. Crop Sci 44:389–393. https://doi.org/10.2135/cropsci2004.3890

Wang Q, Yang JA, Zhou GH, Zhang HM, Chen JP, Adams MJ (2010) The complete genome sequence of two isolates of Southern rice black-streaked dwarf virus, a new Fijivirus. J Phytopathol 158:733–737. https://doi.org/10.1111/j.1439-0434.2010.01679.x

Wang Q, Liu Y, Hu J, Zhang Y, Xie K, Wang B, Tuyen le Q, Song Z, Wu H, Liu Y, Jiang L, Liu S, Cheng X, Wang C, Zhai H, Wan J (2013) Detection of quantitative trait loci (QTLs) for resistances to small brown planthopper and rice stripe virus in rice using recombinant inbred lines. Int J Mol Sci 14:8406–8421. https://doi.org/10.3390/ijms14048406

Wang Y, Cao L, Zhang Y et al (2015) Map-based cloning and characterization of *BPH29*, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice. J Exp Bot 66:6035–6045. https://doi.org/10.1093/jxb/erv318

Wu CF, Khush GS (1985) A new dominant gene for resistance to whitebacked planthopper in rice. Crop Sci 25:505–509. https://doi.org/10.2135/cropsci1985.0011183X002500030017x

Wu H, Liu Y, He J et al (2014) Fine mapping of brown planthopper (*Nilaparvata lugens* Stal) resistance gene *Bph28*(t) in rice (*Oryza sativa* L.). Mol Breed 33:909–918. https://doi.org/10.1007/s11032-013-0005-z

Xiao Y, Wang Q, Erb M et al (2012) Specific herbivore-induced volatiles defend plants and determine insect community composition in the field. Ecol Lett 15:1130–1139. https://doi.org/10.1111/j.1461-0248.2012.01835.x

Xu T, Liu Y, Zhang L, Liu L, Wang C, Hu J, Sun Z, Pan G, Xiao S, He J, Huang J, Qiu Z, Fan D, Jiang L, Cheng X, Zhai H, Wan J (2018) Mapping of quantitative trait loci associated with rice black-streaked dwarf virus disease and its insect vector in rice (*Oryza sativa* L.). Plant Breed 137:698–705. https://doi.org/10.1111/pbr.12620

Xue W-H, Xu N, Yuan X-B, Chen H-H, Zhang J-L, Fu S-J, Zhang C-X, Xu H-J (2018) CRISPR/Cas9-mediated knockout of two eye pigmentation genes in the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). Insect Biochem and Mol Biol 93:19–26. https://doi.org/10.1016/j.ibmb.2017.12.003

Yamasaki M, Yoshimura A, Yasui H (2003) Genetic basis of ovicidal response to whitebacked planthopper *Sogatella furcifera* (Horvath) in rice (*Oryza sativa* L.). Mol Breed 12:133–143. https://doi.org/10.1023/A:1026018821472

Yang HY, Ren X, Weng QM et al (2002) Molecular mapping and genetic analysis of a rice brown planthopper (*Nilarparvata lugens* Stal) resistance gene. Hereditas 136:39–43. https://doi.org/10.1034/j.1601-5223.2002.1360106.x

Yang H, You A, Yang Z et al (2004) High resolution genetic mapping at the *Bph15* locus for brown plant hopper resistance in rice (*Oryza sativa* L.). Theor Appl Genet 110:182–191. https://doi.org/10.1007/s00122-004-1844-0

Yang L, Li RB, Li YR et al (2012) Genetic mapping of *bph20(t)* and *bph21(t)* loci conferring brown planthopper resistance to *Nilaparvata lugens* Stål in rice (*Oryza sativa* L.). Euphytica 183:161–171. https://doi.org/10.1186/1471-2229-14-145

Yang Y, Xu J, Leng Y, Xiong G, Hu J, Zhang G, Huang L, Wang L, Guo L, Li J, Chen F, Qian Q, Zeng D (2014) Quantitative trait loci identification, fine mapping and gene expression profiling for ovicidal response to whitebacked planthopper (*Sogatella furcifera* Horváth) in rice (*Oryza sativa* L.). BMC Plant Biol 14:145. https://doi.org/10.1186/1471-2229-14-145

Yang M, Cheng L, Yan L et al (2019) Mapping and characterization of a quantitative trait locus resistance to the brown planthopper in the rice variety IR64. Hereditas 156:22. https://doi.org/10.1186/s41065-019-0098-4

Yang M, Lin J, Cheng L, Zhou H, Chen S, Liu F, Li R, Qiu Y (2020) Identification of a novel planthopper resistance gene from wild rice (*Oryza rufipogon* Griff.). Crop J 8(6):1057–1070. https://doi.org/10.1016/j.cj.2020.03.011

Yarasi B, Vijaya KS, Pasalu IC et al (2008) Transgenic rice expressing *Allium sativum* leaf agglutinin ASAL exhibits high level resistance against major sap sucking pests. BMC Plant Biol 8:102. https://doi.org/10.1186/1471-2229-8-102

Yoshimura S, Komatsu M, Kaku K et al (2012) Production of transgenic rice plants expressing Dioscorea batatas tuber lectin 1 to confer resistance against brown planthopper. Plant Biotechnol 29:501–504. https://doi.org/10.5511/plantbiotechnology.12.0726b

Yue JX, Meyers BC, Chen JQ et al (2012) Tracing the origin and evolutionary history of plant nucleotide-binding site leucine- rich repeat (NBS-LRR) genes. New Phytol 193:1049–1063. https://doi.org/10.1111/j.1469-8137.2011.04006.x

Yuexiong Z, Gang Q, Qianqian M, Minyi W, Xinghai Y, Zengfeng M, Haifu L, Chi L et al (2020) Identification of major locus Bph35 resistance to brown planthopper in rice. Rice Sci 27(3):237–245. https://doi.org/10.1016/j.rsci.2020.04.006

Zha W, Peng X, Chen R et al (2011) Knockdown of midgut genes by dsRNA-transgenic plant-mediated RNA interference in the Hemipteran insect *Nilaparvata lugens*. PLoS One 65:e20504. https://doi.org/10.1371/journal.pone.0020504

Zhang YX, Wang Q, Jiang L, Liu LL, Wang BX, Shen YY, Cheng XN, Wan J (2011) Fine mapping of qSTV11KAS, a major QTL for rice stripe disease resistance. Theor Appl Genet 122:1591–1604. https://doi.org/10.1007/s00122-011-1557-0

Zhang W, Dong Y, Yang L et al (2014) Small brown planthopper resistance loci in wild rice (*Oryza officinalis*). Mol Genet Genomics 289:373–382. https://doi.org/10.1007/s00438-014-0814-8

Zhao Y, Huang J, Wang Z, Jing S, Wang Y, Ouyang YD, Cai BD et al (2016) Allelic diversity in an NLR gene BPH9 enables rice to combat planthopper variation. Proc Natl Acad Sci U S A 113:12850–12855. https://doi.org/10.1073/pnas.1614862113

Zhou GH, Wen JJ, Cai DJ, Li P, Xu DL, Zhang SG (2008) Southern rice black-streaked dwarf virus: a new proposed Fijivirus species in the family Reoviridae. Chin Sci Bull 53:3677–3685. https://doi.org/10.1007/s11434-008-0467-2

Chapter 10
Biogenetically Engineered Insect-Resistant Crops in Integrated Pest Management Programs

Amarjit S. Tanda

10.1 Introduction

The integrated technology control blending idea was described, amalgamating agrochemical and biocontrol methods knowing bio-environmental and used aspects for insect resistance in pest management strategies. After the overuse of chemicals, in 1970, when ravages on the human health and environment were noticed, the new concept of integrated pest management (IPM) was designed. IPM was given as a "careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the multiplication of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment" by FAO in 2018. Undoubtedly, IPMT is a part of what IPM already plans; the first IPMT plan proposed is innovative, designed newly, and original scientifically. The basic principle is the integration of technology and not just management for the prevention of harmful organism populations (reduction of pest control need probability). This IPMT program may or may not be possible in all farmland situations due to the following: (1) increasing agro-bio-ecosystem complexity/stability with suitable rotations. We will have to deal with ecosystems made up of several elements that continuously vary genetically and interact with one another, tillage, fertilization, irrigation, and pesticide application, which may trigger new interactions between ecosystem components. Scientific studies also suggest that the more complex the ecosystem is, the more stable it is. Additionally, we will have to deal with several ecosystems because of soil type and landscape; however, this very new design or model may be beneficial in a particular farmland or may be a failure in

A. S. Tanda (✉)
School of Horticulture, University of Western Sydney, Richmond, NSW, Australia

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_10

another location or needs to be modified technically. So, a new combination of technology decides the outcome of a holistic approach using various procedures, (2) implementing innovative agronomic practices integrating GE-resistant/tolerant cultivars, and lower risk sowing/harvesting date. Here, GE-resistant/tolerant cultivars are one of the most important parts of IPMT by prevention, to reduce the risk from pests. GE insect-resistant cultivars are agronomic plans and practices that IPMT strategies exploit in the framework of a holistic approach including prevention plans and IPMT principles: pest monitoring, identification, collection and sampling, and forecasting/multiplication levels. Other treatments are adopted only after assessing that the economic damage threshold has been exceeded. Harmful pest must be monitored by adequate methods and tools, before any pest management technology is designed, and treatments may then be undertaken only where pest levels were found above the economic threshold levels, with sustainable biological agents or other nonchemical treatments protecting pollinators for higher crop production.

10.2 IPM to IPMT

Yes, it should now be named in a technical way, as the present time is not of a simple management but of technology or biotechnology which is being achieved by selecting and blending of various suitable techniques beneficial in biological fields for pest protection, conservation of pollinators, and enhancement of crop yields with minimum damage to the farmland ecosystem. It is a holistic process that integrates a range of practices for economic control of pests. IPM is just a management; however, IPMT is a step further and a new biotechnological model of combination of suitable methods/strategies or procedures. IPMT reduces the pest populations below the economic injury level (EIL) protecting the pollinators and their habitats using GE cultivars with minimum or zero chemicals which rather utilize biological operators and manage insect pests, plant pathogens, and weeds if designed with innovative technology without any psychological or cultural harm. IPMT shall not be stressing the role of GE only but shall always adopt the holistic plan using all possible opportunities/pillars of IPMT available, suitable to a specific pest problem. Please be aware that GE cultivars or IPMT model should be least affected in adoption depending on the bio-ecosystem structures ranging from small to large scale to avoid any damages. A farmer may design his own most suitable IPMT "flexible" package in the specific conditions, modulating it according to pest population dynamics using pest monitoring techniques. So we propose that the idea of integrated pest management (IPM) should be termed as the integrated pest management technology (IPMT) because it is actually an amalgamation technology of various compatible techniques in different ways or innovative ideas of new and applied approaches holistically and their integration of all possible pest control measures to slow down the development of insect pest populations reducing risks to the human health and the bio-ecological system with minimum use of agrochemicals (Fig. 10.1) (Wijnands et al. 2012; FAO 2018). Presently, IPMT is used as a well-judged

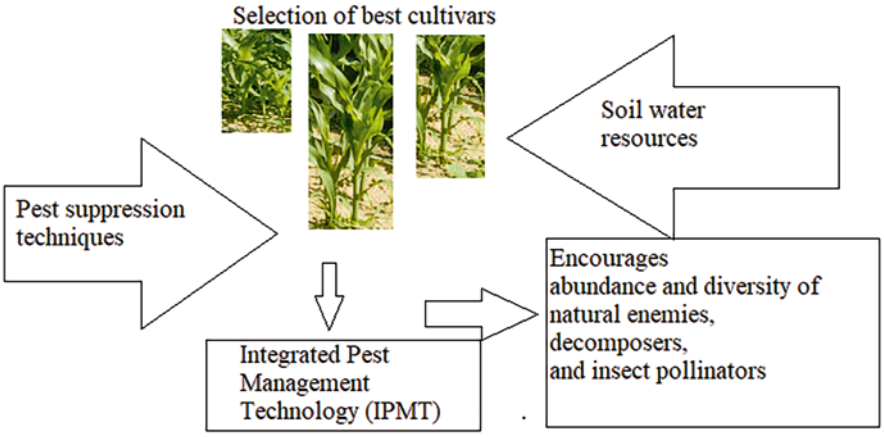

Fig. 10.1 IPMT (IPM is proposed to be named as IPMT) as it is an integration technology of various insect pest management methods

technology for the protection of various field crops globally (FAO 2018; OECD 2018; Kaur et al. 2021; Tanda 2020, 2021a, b). IPMT feasibility and adaptability can further be ameliorated to assimilate available approaches and GE crop experiences, with the limitations of biotechnology, and strategies for various field crops keeping in mind the failures and successes of GE traits in IPMT systems. The applications of information technology (IT) and its utilization in IPMT is the future necessity. Two IT technologies, the World Wide Web (the Web) and databases, will focus within the context of their applications to IPMT. Few applications have been highlighted to illustrate the potential impacts of IT technologies in IPMT in the near future (see Chap. 1).

An IPMT plan may be framed, comprehending the crop environment, biology of insect pest and natural enemies, and best utility of agricultural practices to control crop pests. Natural bio-enemies and crop pollinator services comprise regional crop cultivar selection, soil, nutrients, and water management, pest suppression method usage, and their implementation for beneficial species' abundance and biological diversity. To augment when desired the inhibitory procedures, the blending of biotechnological, biotic, cultural, mechanical, and other physical control strategies may be successful for the safety of surroundings, social well-being, and economic protection. Among all these procedures could be (1) natural enemies or sterile insects' release techniques, (2) use of pheromone traps, (3) nets or tillage utilization, which are the major elements for developing a functional, long-lasting, and pliable IPMT strategy that may suit most environmental-social requirements. For insect pest protection, when insecticides are used, from broad-spectrum products, only selective ones are picked. We strongly recommend to use the discriminating insecticides only when badly needed, using fitting equipment, optimal dosage, and at a suitable timing (Ervin and Jussaume 2014; Owen 2016) when the crop is not in peak blooming (Tanda 2019, 2021a, b) for the safety of bee pollinators.

For other insect pest control procedures, insect resistance of host plant, evolved through genetic engineering technology (GET) methods, is an IPMT foundation and a harmonizing practice in enhanced food production. The field area of GE crops has touched 190 million hectares worldwide (ISAAA 2017). Many GE plants offer resistance to lepidopteran or coleopteran pests. Insecticidal proteins obtained from *Bacillus thuringiensis* (Bt) for insect resistance are found in cotton, soybean against lepidopteran pests, and in maize against lepidopteran and coleopteran insect pests. In Bangladesh, a Bt trait in eggplant working against a lepidopteran borer is also available. Using RNAi, other insect active traits and non-Bt insecticidal proteins are being evolved to expand GE crop folder of HT traits (ISAAA 2019) that offer a good tolerance level to insect pest biodiversity.

GE cultivars offer additional protection to complement IPMT programs and improve their tolerance sustainability, better economics, and environmental-social assistance such as crop pest management resolutions affect neighboring farmers and social groups (Ervin and Jussaume 2014; Ervin and Frisvold 2016). Comprehending plant traits, inserted new GE characteristics, fruit production processes, and socio-economic environment is analytical to firmly amalgamating GE crops into IPMT systems, which are greatly in demand (Meissle 2016). The aim of this chapter is to discuss the present status and future scope of blending biogenetically engineered cultivars in the integrated pest management technology programs for the management of insect pests for a critical assessment and to contribute to the existing literature in (1) redesigning or restructuring new IPMT models for the best use of fundamental principles, (2) key role of socioeconomic elements, (3) availability to farms, and (4) adoption regulations for IPMT programs, and finally, (5) the main benefits of GE crop technology to the growers. We also direct attention to the chances and threats in both developed and developing countries for flourishing

Table 10.1 Biogenetic engineered crops and integrated pest management technology

Biogenetic engineered cultivars	Economic value of crop	Integrated pest management technology applied against disease/pest
Cotton	Cotton seed oil, fiber source, animal feed	Cotton bollworm resistance
Maize	High-fructose corn syrup, corn starch, animal feed	Corn borer resistance
Eggplant	Human vegetable food	Insect resistance
Bean	Human food	Virus disease resistance
Alfalfa	Animal feed	Insect resistance
Potato	Human vegetable food	Insect resistance
Soybean	Soybean oil, animal feed	Soybean dwarf virus and *Phytophthora* fungus resistance/insect resistance
Cowpea	Human food	Insect resistance (pod borer) (*Maruca vitrata*)
Plum	Human fruit	Virus (plum pox virus) resistance
Rice	Human cereal food	Stem borer resistance
Tomato	Human vegetable food	Insect resistance

integrating GE crop practices into an IPMT strategy to manage major insect pests, in important field crops (Table 10.1).

10.3 GE Cultivars in IPMT

GE cultivars and newly designed IPMT holistic approach programs in the sustainability of agroindustry worldwide play a crucial role by inserting attributes and developing new GE varieties having one mode of action in resisting one insect group, or against various insects, with multiple modes of action. GE cultivars have also moved from one insect protection indicating proteins from Bt to new attributes depending on the RNAi or expressing proteins from non-Bt origins (ISAAA 2019) for insect resistance. There are several advantages in using GE cultivars for insect management to minimize the spray of less efficient insecticides, poor environmentally friendly, highly pest specific, and best suitable insect control process for the farming community (Brookes and Barfoot 2013, 2016). An advantage was found in such systems widely, to lessen the insect pest stress and loaded crop expenses for farmers adopting Bt stuff, as in the USA using Bt maize-resistant cultivars (Hutchison et al. 2010; Dively et al. 2018) and in China with tolerant Bt cotton (Wu et al. 2008) and in the USA, in controlling the target insect. Still for numerous Bt crops in different areas, for sustainable use of the IPMT approach technology, many challenges are there for its flourishing execution (Alemu 2020; Kaur et al. 2021) (Fig. 10.2 and Table 10.2).

For the success of this IPMT system, still insect resistance evolution and integration is the biggest threat for entomologists and plant biotechnologists. In the adoption of many instances, without appropriate IRM or IPMT procedures have resulted into the development of insect resistance with overdependence on Bt cultivars (Gassmann et al. 2014; Tabashnik and Carrière 2017).

In South Africa, resistance development to Cry1Ab shows maize against the African stalk borer *Busseola fusca* (Fuller) (Lep.: Noctuidae); in Puerto Rico, Brazil, and Argentina and in the mainland USA, in the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Fuller) (Lep.: Noctuidae), resistance to Cry1F-in maize, (Storer et al. 2010; Farias et al. 2014; Huang et al. 2014); in India, in the pink bollworm *Pectinophora gossypiella* (Saunders) (Lep.: Gelechiidae), development of resistance to Cry1Ac-expressing cotton (Dhurua and Gujar 2011); and in the USA, defiance to Cry3Bb1-expressing maize in the western corn rootworm *Diabrotica virgifera* LeConte (Col.: Chrysomelidae) (Gassmann et al. 2011, 2014) are the main field examples of insect resistance development, challenging the new Bt cultivar sustainability.

In the USA, Canada, Australia, the EU, the Philippines and South Africa where IRM or IPMT procedures being obligatory, to reduce the possibility of resistance to insects, in commercial Bt crops, IRM processes have been executed providently (Matten et al. 2008). The refuge concept, where Bt protein is absent, assist in Bt-susceptible insect production, is the main process to the IRM strategy (Gould

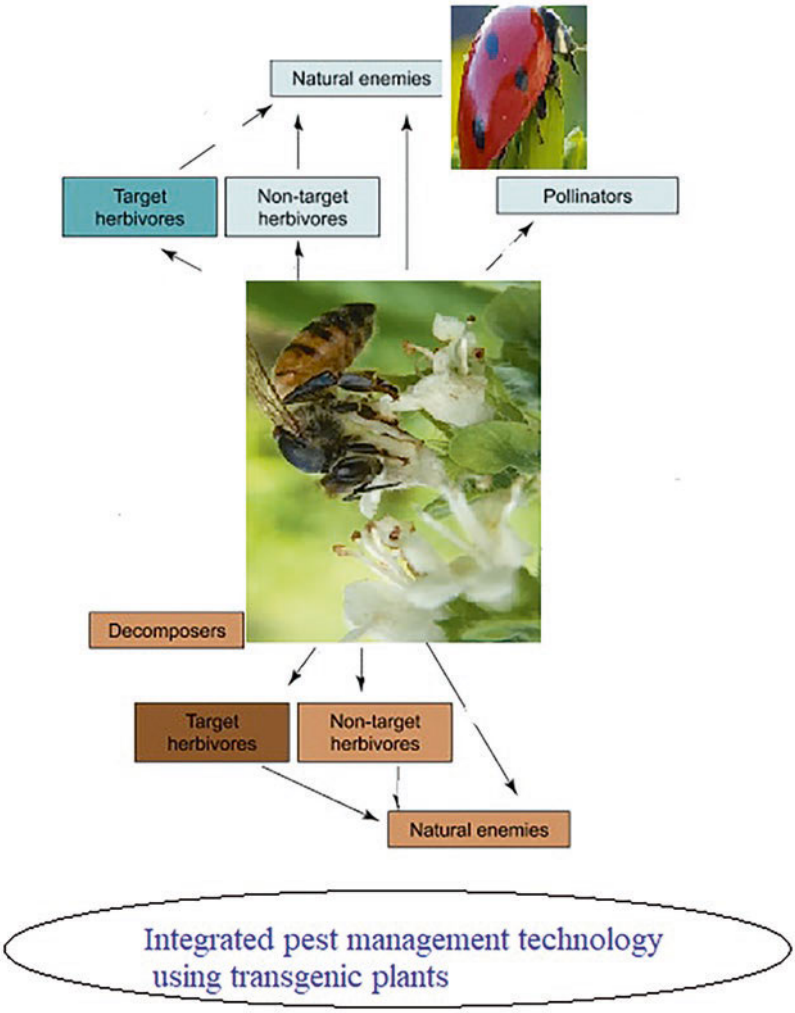

Fig. 10.2 Successfully fitting GE crops into an IPMT holistic approach

et al. 2016). In Australia, Canada, and the US, countries where IRM is a government need, refuges act as a small cost to the farmers because they suffer maximum insect losses and need an extra process management, and thus refuge acceptance by farmers is maximum. The IRM adoption program in the industry of cotton in Australia depicts a flourishing example. Australian cotton growers in 1990 encountered disastrous levels of resistance to insecticides in Lepidopteran borers (Wilson et al. 2018). Consistently in Australia, full refuge adoption, for IRM farmers, is of high awareness, with various refuge choices and suitable education and training. Thorough training coupled with farmer inspections have assisted the system in Canada for about 91% (CCPC 2018) and, to a small extent, in the Corn Belt of the US (68–72%)

Table 10.2 Biotechniques for developing resistant cultivars against major insect pests and diseases in crop breeding program

Biotechnological breeding tools	Crop and resistance to insect-pests	Crop and resistance to diseases
Gene stacking	Insect resistance in rice cultivars	Resistance enhancement to crop diseases
Gene pyramiding	Pyramiding of two insect resistance genes in rice	Durable resistance to diseases
Quantitative resistance (QR)	Resistance to leaf hoppers and brown plant hopper	Dissection and adoption in maize
Marker-assisted selection (MAS) and marker-assisted breeding (MAB) technology	MAS insects resistance in rice	MAS in disease resistance breeding
Multiple resistance	Multiple insect resistance in maize	Multiple disease resistance in barley and rice and implications in MAS
Durable resistance	Durable management of insect pests	Durable management of diseases

(ABSTC 2016), to adopt refuge in a wider area. To be low refuge compliance, one of the primary reasons was resolved, and the truancy of IRM processes can have crucial results as in all the cases of field-developed resistance mentioned before (Tabashnik et al. 2013). Countries, like Argentina, Brazil, and China, are where IRM practices are not instructed (Choudhary and Gaur 2008). Promoting an integrated insect resistance process to high insect attack in refuges and for others of GE practices has more advantages, describing the significance of IRM in IPMT, showing how refugia and GE crops can help natural enemies and pollinators greatly, which is a reply to IPM scheme in a new agro environment (Lu et al. 2012). In northern China, to test the concept that Bt cultivars can aid in biocontrol system, aphid abundance and insect predator in Bt cotton fields were evaluated in field environment (Lu et al. 2012). It was observed that Bt cotton with minimum insecticide spray supported more populations of predators and reduced the aphid abundance (Romeis et al. 2018), which helped the theory that extensive adoption of Bt cotton may encourage several advantages at field conditions.

Another threat for Bt varieties may be more infestations of a secondary pest that was under check by the broad-spectrum insecticides but is not managed by the selective GE developed cultivars. In China, enlarged abundance of mirid bugs (Hemiptera: Miridae) was observed in fields (Lu et al. 2010), with the adoption of Bt cotton in large area, and less use of insecticides. If supplementary measures are undertaken, and IPMT plan is improved, growers may be benefited greatly (Naranjo and Ellsworth 2009a, 2009b; Ellsworth et al. 2017). For all problems, just GE technology or resistance developed by the host plant through conventional methods, gene bio-engineering is not a magical weapon, and the crop production model will not become a long-lasting IPMT master plan automatically. For future field crops,

knowledge, training tools, and designing of strong, durable IPMT programs and trait technology, understanding the threats for each commercial crop, pest complex system, and regional climatic conditions as the key limitations of GE crops are also significantly important (Tanda 2019, 2021a, b).

10.4 IPMT and Cotton Bt

Using an amalgamation of cultural and chemical applications from Arizona, the boll weevil *Anthonomus grandis* Boheman (Col.: Curculionidae) has been eliminated victoriously. In southern California and Arizona, a whitefly cryptospecies of *Bemisia tabaci* (Gennadius) (Hem.: Aleyrodidae) [*B. argentifolii* Bellows and Perring] became a major pest in the cotton industry, in vegetables and melons (Ellsworth et al. 2017). Reducing the number of fruiting bodies of the plant, mirid bug *Lygus hesperus* (Knight) (Hem.: Miridae) is a threat to cotton production. Because of its enigmatic feeding habits inside the bolls, the pink bollworm *P. gossypiella* is another challenge to cotton industry.

In the beginning, salt marsh caterpillars [*Estigmene acrea* (Drury), aphids (Hem.: Aphididae), Lep.: Erebidae], cotton leaf perforators (*Bucculatrix thurberiella* Busck, Lep.: Bucculatricidae), or cabbage loopers [*Trichoplusia ni* (Hübner), Lep.: Noctuidae] were the familiar insect pests; later on resistance developed in mites resulting in secondary outbreaks with exhaustive 10–13 per season statewide crop applications (Naranjo and Ellsworth 2009b).

After the pink bollworm eradication program, the GE cotton cultivars' successful adoption rates were increased exceptionally, with a maximum of 98% of acreage in Bt cotton in 2008 (Naranjo and Ellsworth 2010; Tabashnik et al. 2010). With the introduction of flonicamid, the outcome was encouraging for the conservation of natural enemies, danger to the flower pollinators, and decrease in the use of foliar sprays (Naranjo and Ellsworth 2009a; Naranjo et al. 2015; Ellsworth et al. 2017).

The use of insect-resistant cultivars in the IPMT process of Arizona cotton has rescued farmers over $500 million in fiber production using pest protection and crop management expenses ($274/ha/year), by avoiding 25 million pounds of pesticides in the surroundings (Ellsworth et al. 2017). Applied refuges can be implemented using novel sterile insect technique (SIT) and mating disruption pheromone practices. Refuges were provided by pink bollworm sterile male moth releases in Bt and non-Bt cultivars and disturbing the breeding cycle (Naranjo and Ellsworth 2010; Tabashnik et al. 2010, 2012). Assisted by IPMT practices, this modern pest elimination crusade executed in all affected states of the US and in the north of Mexico, with poor pink bollworm control and cotton quarantine boosting (USDA 2018).

The protection of natural enemies authorized by effective biotechnological measures was helpful to Arizona farmers with 42% profit (Ellsworth et al. 2017). Moreover in Arizona, biocontrol approach was more significant possibly to eradicate the pink bollworm in Bt cotton.

In the future, the integration of various pest management tactics, identifying more resistant alleles for the eradication of *P. gossypiella* and *A. grandis*, and monitoring of insect resistance to Cry toxins in approved cultivars should be pursued beyond the current knowledge. For cotton growers, there are financial benefits in using biotechnology in IPMT strategies.

10.5 IPMT and Eggplant Bt

For maize, cotton, and soybean, GE cultivars were a life-changing fairy tale, as Bt crops were restricted to wide areas (Shelton et al. 2017). Fruits and vegetables were oversprayed with toxic chemicals because they are attacked by many insect pests, with more market merit, and limited use in beautifying products (Shelton et al. 2008) called as the food paradox (Palumbo and Castle 2009). Losses by the eggplant fruit and shoot borer (EFSB) can be 80% in the Philippines, and the management mainly depends on regular insecticidal sprays (Francisco 2009). With high pest losses, absence of any control measure, and great market vegetable value, there is a big scope for exploiting GE biotechnology as a drive for an IPMT program as EFSB is similar to the European corn borer which was so strongly managed by Bt maize (Anderson et al. 2019).

The *cry1Ac* gene indicates the Cry1Ac protein, which bestows resistance against the attack of some lepidopteran insect pests as well as EFSB. Studies on the evolution of the Bt eggplant with effectiveness tests and management of EFSB were described in greenhouse experiments (Choudhary and Gaur 2008). Because of the advantages of Bt brinjal for EFSB suppression, in Bangladesh, the integration of GE technology in IPMT has enhanced all the time, and more than 27,000 farmers cultivated Bt brinjal (Shelton et al. 2018). Research confirmed that Bt brinjal offers full management of EFSB and minimizes the insecticidal costs along with an immense profit to humans and providing ecological gains to the growers (Shelton et al. 2018). In Brazil Bt maize and in Arizona and Mexico Bt cotton adoption have widely helped the natural biocontrol agents to suppress primary and secondary insects, including sucking pests. The research has revealed that in the Philippines the main natural enemies of the crop have been protected by growing Bt eggplant (Navasero et al. 2016; Tanda 2019, 2021a, b), and others have reported that preservation of natural enemies adopting Bt plants can aid in checking secondary insect pests too (Tian et al. 2015). Reports have also demonstrated that biological agents can help in dallying the development of insect resistance to Bt cultivars, which is another benefit to the growers (Liu et al. 2014).

The Agriculture Ministry has been honest in helping biotechnological research, and this has been an important element in its acceptance in Bangladesh agroindustry (Shelton et al. 2017). In India, where studies on Bt eggplant was first initiated and the Genetic Engineering Committee of India accepted in 2009, Bt eggplant is still not cultivated because of political coercion on the Minister of the Environment and Forests (Shelton 2010).

In February 2018, the scientific and technical team carried out a 4-day workshop and training program at BARI on gene equivalency and maintaining line purity (Cornell University 2018; Hossain and Menon 2018). Reports confer the adoption of Bt biotechnology and insect resistance integration in IPMT for the management of chief insect pests in minor crops too in all the developing nations cutting down on the toxic agrochemicals for human health (Anderson et al. 2019; Tanda 2019, 2021a, b). To develop the ideas, research should continue on the new strategies of BT blending in IPMT, isolating new insect-resistant toxins in vegetable germ plasma for the management of other tissue borers.

10.6 IPMT and Maize Bt

In Brazil, maize is the main grain crop, and fall armyworm *S. frugiperda* is the key pest (Blanco et al. 2016). This offers for *S. frugiperda* to finish 8–10 generations on maize a year (Storer et al. 2012). The genetically engineered maize cultivar manufactures Cry1Ab, Cry1F, Cry1A.105, Cry2Ab, and Vip3Aa, which are Bt proteins that are poisonous showing resistance to *S. frugiperda*. Though the resistance to Bt was established before, in 4 years, it has also further developed to Cry1A and Cry1F, which are Bt proteins too (Farias et al. 2014; Omoto et al. 2016). For high-dose/refuge resistance strategy, few suppositions such as pest species recessive inheritance resistance, low initial resistance allele frequency, and abundant refuges of non-Bt near Bt plants encouraging random mating should be considered (Tabashnik et al. 2013). In Brazil, all GE cultivars used have contravened one or the other major preconditions (Tabashnik et al. 2013). To have been relatively high with *S. frugiperda* populations leading to quick evolution of resistance (Farias et al. 2016; Omoto et al. 2016), minimal industry and grower adoption of refuges contributed to the accelerated resistance evolution observed with *S. frugiperda*. Against Cry1A and Cry1F, proteins also showed resistance allele frequency. Finally, not to be high-dose against *S. frugiperda*, proteins like the Cry1As and Cry1F are known (Vélez et al. 2016).

To these Bt resistance problems have been the introduction of Bt pyramids to affected geographies like Brazil one proposed resistance management solution. At least two proteins that are effective against the same target insect GE pyramid products express. The effectiveness of the pyramid strategy in Brazil as a resistance management tool has been limited due to cross-resistance among similar Bt proteins (Bernardi et al. 2015). Where multiple crops share similar Bt proteins, cross-crop resistance is another concern in diverse crop landscapes. The selection period for cross-crop insects will be extended and thus accelerate resistance evolution. However, research results suggest that if cross-crop resistance occurs among different Bt crops, landscapes like Brazil where corn, cotton, and soybean share similar Bt proteins (Yang et al. 2016). Therefore, rapid resistance evolution with pests like *S. frugiperda* is likely linked to multiple factors described in this study.

If GE products like those described above are not placed into a well-understood IPM framework capable of sustaining the value of these technologies, resistance management has a limited likelihood of success. The potential utility and contribution of IPM tactics need to be better understood, including cultural and biological controls. To drive the implementation of refuges and best management practices (BMPs) with growers, the industry has developed several initiatives. To develop BMPs for maize, soybean, and cotton, farmer's industry alignment meetings led by the Insecticide Resistance Action Committee (IRAC) were initiated in 2015. To educate and provide incentives for adopting refuge, though these have resulted in minimal uptake up to this point, the industry also developed several pilot programs with growers. For tropical geographies like in Brazil, to use with GE and non-GE refuge crops that harbor pests like *S. frugiperda* will challenge IPM and IRM strategies although research continues to refine management tactics. To develop an industry framework that drives the adoption of key IPM and IRM practices, socioeconomic factors should be combined with agricultural systems knowledge. Tactics like planting of refuges should be pursued in addition to the regulation that requires critical resistance management. Deploying new GE technologies in countries like Brazil should proceed with caution until either or both of these approaches are further developed.

More biotechnological studies should be perused on the modern plans amalgamating BT varieties in IPMT programs, and segregating alleles toxins resistant to pests for the control of major insect pests of corn and other cereals through innovative thinking.

10.7 Virus IPMT and Bean Golden Mosaic

In Brazil and other countries in Latin America, common bean (*Phaseolus vulgaris* L.) is an important staple food. For managing bean golden mosaic virus (BGMV) similar to brinjal, common bean is an orphan crop that can utilize GE technology to complement the IPM approach. Of the most destructive viral diseases of common beans in Brazil, BGMV is the causal agent. Especially in tropical areas for this and several other crops, it is efficiently vectored by the whitefly *B. tabaci*, which is also an important insect pest. BGMV causes stunted growth, yellowing and flower abortion, and high yield losses (Anderson et al. 2016, 2019). Chemical pesticide application and overuse of pesticides on common beans are common problems leading to environmental effects and insect resistance problems, and traditional pest control tactics for the insect vector are limited (Bonfim et al. 2007).

Using RNAi technology to develop a BGMV-resistant variety by the Brazilian Agricultural Research Corporation (Embrapa) (De Faria et al. 2016), GE common bean was modified. Registered and protected as cultivar BRS FC401 RMD by the Brazilian Ministry of Agriculture, Livestock, and Food Supply in 2016 (Souza et al. 2018), for BGMV-resistant common bean in Brazil, commercial approval was granted in 2011 (Comissão Técnica Nacional de Biossegurança (CTNBio) 2011).

For farmers to control this viral pathogen without chemicals, GE common bean offers an opportunity. For successful integration of this technology into a sustainable IPM plan, there remain several key challenges. The current challenge is to successfully insert this GE trait into commercial varieties that are optimized for the different regions (Souza et al. 2018) following regulatory approval. To ensure sustainable use and durability of the trait additionally, IPM and farm management practices are being optimized, and farmer training is being offered. Of the emerging IPM plan, practices are all valuable components, including management plans implementing a whitefly host-free period, where common bean fields are planted designating sentinel areas early in the season to screen for the presence and abundance of viruliferous whitefly populations, chemical control, and optimizing planting time. Due to direct feeding as well as deposition of honeydew to reduce damage by whitefly on which mold fungi can grow and reduce photosynthesis, these tactics are important. While BGMV is the most devastating virus, as a disease vector because it is not the only whitefly-transmitted virus to common beans (Brown et al. 2015), it is important to reduce the area with wide presence of whitefly. In northeastern Brazil, new geminiviruses [*Macroptilium* yellow spot virus—MaYSV, *Macroptilium* yellow vein virus—MaYVV, and soybean chlorotic spot virus—SoCSV (Sobrinho et al. 2014)] are a threat to common beans, and the flexivirus of common beans cowpea mild mottle virus is a destructive disease (De Faria et al. 2016).

To quickly identify if a threshold for pest population or viral pathogen load is being exceeded will also be critical to success, building professional capacity through farmer training and developing an alert system. This work to optimize management practices and increase farmer training is being conducted with growers on small plots (up to a half hectare) because the GE common bean varieties have not yet been commercialized. Using an alert system to evaluate the real need for chemical control and implementing whitefly host-free periods yielded encouraging results. To maximize income with lower risks of crop losses going forward, the use of monitoring system for whitefly and the sentinel areas will help growers to make the correct decision about common beans or switch to an alternative crop. For achieving agricultural and environmental sustainability, food security, and grower profitability, GE common bean with resistance to BGMV will help to diversify the tool box in Brazil for IPM, and pest management of whitefly is an essential integrated approach. Sentinel areas and pest-free periods, including whitefly monitoring, must be continued and leveraged to enable decision-making and successful integration of a sustainable IPM plan and IPM practices.

10.8 GE Cultivars, IPMT, and Cost-Benefits

GE cultivars have been developed, basically with “market-led” traits, some of which have become commercially successful with enhanced shelf-life of fruits and vegetables, and resistance to insect pests or viruses including tolerance to some herbicides. All these traits have had benefits for growers, including a reduced price to

consumers owing to reduced cost and increased ease of production. GE cultivars delay ripening of fruit and vegetables, thus permitting an enhanced length of storage. Growers benefit from more flexibility in crop production and harvesting. Consumers would benefit by the accessibility of fruits and vegetables as GE tomato cultivars soften much more slowly than traditional varieties, resulting in improved shelf-life, reduced cost of raising, greater quality, and lower cost.

The following examples show how GE cultivars can be applied to some of the specific problems of agro-industry, showing the potential for benefits.

10.9 Insect Pest Resistance

GE cultivars containing insect resistance genes from *Bacillus thuringiensis* have made it possible to minimize significantly the amount of insecticides sprayed on cotton in the USA. There is a need for more research on GE cultivars that have been made resistant to local pests and diseases to assess their sustainability in the face of increased selection pressures for ever more serious pests.

10.10 Crop Productivity

The chief technologies of "Green Revolution" was the evolution of high-yielding semidwarf wheat cultivars. The genes in these cultivars offered two benefits: they developed a shorter and stronger plant that responded to more fertilizer, and they enhanced production directly by reducing cell elongation in the vegetative plant parts and invested more in the reproductive plant parts for grain formation.

10.11 Biotic and Abiotic Resistance

GE cultivars have a built-in tolerance to biotic and abiotic stresses which assist to stabilize crop yield. In Africa, rice yellow mottle virus (RYMV) destroyed rice, and breeders have used a novel technique that mimics "genetic immunization" by developing GE rice cultivars that are resistant to RYMV. GE cultivars developed to manage papaya ringspot virus, blight-resistant potatoes. and varieties created to overproduce citric acid in roots and provide better tolerance to aluminum in acidic soils.

10.12 Environmental Influence

The availability of water and its efficient usage have become a big issue worldwide. Regional variations in farmland systems and the potential effect of substituting a traditional crop with a new GE cultivars require careful evaluation.

10.13 Crop Production Costs and Yield

GE cultivars have benefited several growers with decreased production costs and great yields. Crop pest resistance genes were carefully incorporated in cultivars to avoid selecting for future pest resistance, offering alternative opportunities to decrease the use of insecticides in many major crops.

10.14 Development of Pharmaceuticals

Researchers are currently investigating the potential for GM technology to develop vaccines and pharmaceuticals in plants. Vaccines against infectious diseases of the gastrointestinal tract have been developed in potato and bananas. Nonetheless, to produce therapeutic agents, the GE cultivars have immense potential to control diseases in the developing countries.

About one-third of the medicines used are developed from plants, for instance, aspirin (the acetylated form of a natural plant product, salicylic acid). About 10% of medicinal plants have been identified, and GM technology is being used to boost the yields of these medicinal substances once identified. Currently, there is an intensive research in progress to investigate the potential of GM technology to enhance the yields of active compounds.

So we recommend that GE cultivars' research and development should target on plants that will (1) enhance and sustain food production; (2) provide nutritional benefits to the human world; (3) decrease the climatic effect in agro-industry; and (4) enhance the availability of pharmaceuticals and vaccines; while (5) designing the protocols and regulations that ensure that GE cultivars developed for purposes other than food, such as pharmaceuticals and industrial chemicals, and do not disperse or mix with either transgenic or nontransgenic food crops.

10.15 Conclusions

Earlier GE cultivar IPMT holistic approaches, models, and strategies were designed from the scientific perspective with a focus on ecological, environmental, and evolutionary aspects of insect pest management and diseases to reduce or prevent

economic losses in crop production. There was a limited scope to include the human, social, business, and communication aspects of the total equation in the previous processes or models that may be deficient in effective promotion and implementation of GE cultivar IPMT holistic approaches. Several examples discussed in this chapter showed the influence of these factors on development, outreach, and successful implementation of IPMT technologies worldwide. Since IPMT is an integral part of agro-industry, which is a consumer-oriented enterprise, and agriculture is a part of global trade, which is influenced by several other factors, IPMT is redefined for the modern times where advanced agricultural technologies and communication tools play a critical role in food production and consumption. Although the two outer layers in the new model can be applicable to more than pest management, they do have a significant influence on IPMT within the entire crop production and are the driving force for farming operations. Agricultural researchers, educators, sociologists, economists, business analysts, managers, growers, pest management professionals, agricultural input manufacturers, retailers, and consumers play a critical role in food production globally. By reconfiguring the components and including various factors that influence them, the new IPMT model provides a template for focusing on different areas of the paradigm and to encourage collaboration among different disciplines. This new model of GE cultivar IPMT holistic approaches is expected to guide IPMT strategies around the world to develop and implement sustainable agricultural practices to ensure profitability for the growers, affordability to consumers, and food security to the growing world population.

Minimizing environmental impacts attributable to pest management practices, the idea of designing new-term IPMT instead of IPM, the goal of IPMT strategy to support the sustainable high quality of crop production, has been proposed for the first time integrating resistant genomic biotechniques. The implementation of GE cultivar IPMT holistic approaches can be very challenging for several reasons, while the benefits of using an IPMT approach are evident (Meissle 2016; Tanda 2020, 2021a, b). To control pests in the short run, durability and sustainable use requires a long-term vision; GE cultivars should not be viewed as a silver bullet, while their success may seem like an infallible solution. Using Bt and HT traits, insects will inevitably develop resistance after some time, so go for isolating more durable resistant alleles to diversify the research systems. It is equally critical that comparable IPMT practices are developed, optimized, and maintained for various crops and pests just as it is crucial for IPMT practices in Brazil including sentinel areas, whitefly monitoring, and pest-free periods to be continued for whitefly control in common beans. Due to insufficient technology and GE cultivar IPMT holistic approach system, we should know how to integrate in the best way as technology durability may fail, so knowledge and understanding of the technology, pest, crop, region, alternative tools, and even social circumstances are critical for the success of an IPMT program. To demonstrate the short-term and long-term benefits of implementing a sustainable approach, incentives may be needed to gain producer compliance with best management and resistance management requirements, and often grower training is needed.

To encourage research, enlarge implementation, and highlight the significance IPMT program, a coaction by governments, the seed and pesticide manufacturers, farmer associations, and social organizations is critically required (Meissle 2016; Anderson et al. 2019; Tanda 2020, 2021a, b). Over the past five decades, major successes in getting farmer cooperation for pest resistance management were led by economic failures, and the solutions involved a healthy cooperation among government, industry, and the crop growers. Innovative solutions and IPMT strategies aimed at pest resistance durability and sustainability must continue to be developed particularly for important crop plants and areas where there is more pest resistance risk areas or farmer adoption of resistance program was unsuccessful. The advantages of a new IPMT practice, using minimum sprays of broad-spectrum pesticides, long durable pest management in bio-ecologically balanced cropping processes, and least risks to human and the surroundings, should be clearly described. Successful, durable, sustainable, and eco-friendly IPMT plans depend on a wide portfolio of biotechniques, of which GE cultivars represent a valuable methodology. By leveraging the experiences gained with GE crops, understanding the limitations of the technology, and considering the successes of GE traits in IPMT plans for different crops and regions, we can enhance the durability and versatility of IPMT plans for future crops (Anderson et al. 2019; Tanda 2021a, b). To meet the future global requirements for food, feed, and fiber in a sustainable and responsible way, the future is very promising for GM biotechnology to intensify new research ideas and endeavors to go beyond literature. Conventional plant breeding procedures, especially with the invention of genomic biotechnology, are planned to both develop and utilize genetic variations to cloister effective alleles helpful in enhanced production and resistance to pest and disease, adding new ideas and thinking. These innovative biotechniques may be further useful in organic agro-industry beyond the current practices. Despite long research studies on plant-insect interactions, few examples available where using molecular techniques are well-characterized, and even infrequent are cases where this understanding has been successfully applied to control crop pests. In consequence, the field seems to be stable and urgently requires changes in strategies to identify novel biotechniques by which insects attack crops and plants resist insects. Thus, advance holistic molecular procedures should be developed that involve intricated host plant-insect interactions. So studies on modern and untapped strategies in host plant-insect interactions exploring molecular tools into pest management technology, embracing microbial partnerships, and identifying unsuitable host plant, insect damage tolerance, and agronomic practices to increase pest virulence must be undertaken.

References

Agricultural Biotechnology Stewardship Technical Committee (ABSTC) (2016) ABSTC/EPA compliance assurance program report presentation. AIS Subcommittee

Alemu M (2020) Trends of biotechnology applications in pest management: a review. Int J Appl Sci Biotechnol 8(2):108–131

Anderson JA, Gipmans M, Hurst S, Layton R, Nehra N, Pickett J (2016) Emerging agricultural biotechnologies for sustainable agriculture and food security. J Agric Food Chem 64:383–393

Anderson JA, Ellsworth PC, Faria JC, Head GP, Owen MDK, Pilcher CD, Shelton AM, Meissle M (2019) Genetically engineered crops: importance of diversified integrated pest management for agricultural sustainability. Front Bioeng Biotechnol 7:24

Bernardi D, Salmeron E, Horikoshi RJ, Bernardi O, Dourado PM, Carvalho RA (2015) Cross-resistance between Cry1 proteins in fall armyworm (*Spodoptera frugiperda*) may affect the durability of current pyramided Bt maize hybrids in Brazil. PLoS One 10:e0140130

Blanco C, Chiaravalle W, Dalla-Rizza M, Farias J, García-Degano M, Gastaminza G (2016) Current situation of pests targeted by Bt crops in Latin America. Curr Opin Insect Sci 15:131–138

Bonfim K, Faria JC, Nogueira EO, Mendes ÉA, Aragão FJ (2007) RNAi-mediated resistance to Bean golden mosaic virus in genetically engineered common bean (*Phaseolus vulgaris*). Mol Plant-Microbe Interact 20:717–726

Brookes G, Barfoot P (2013) The global income and production effects of genetically modified (GM) crops 1996–2011. GM Crops Food 4:74–83

Brookes G, Barfoot P (2016) Global income and production impacts of using GM crop technology 1996–2014. GM Crops Food 7:38–77

Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC (2015) Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Arch Virol 160:1593–1619

Canadian Corn Pest Coalition (CCPC) (2018) Are Canadian growers following IRM? https://www.cornpest.ca/resistance-management/are-canadian-growers-following-irm/. Accessed 5 Feb 2019

Choudhary B, Gaur K (2008) The development and regulation of Bt brinjal in India (eggplant/Aubergine). ISAAA, Ithaca, NY

Comissão Técnica Nacional de Biossegurança (CTNBio) (2011) Extrato de Parecer N° 3024/2011. http://ctnbio.mcti.gov.br/documents/566529/686135/Extrato+de+Parecer+n%C2%BA%203024.2011.pdf/af87fca4-9b8c-48b4-834c cb890ca258d9?version=1.0. Accessed 16 Mar 2016

Cornell University (2018) Feed the Future South Asia Eggplant Improvement Partnership. https://bteggplant.cornell.edu/. Accessed 5 Feb 2019

De Faria J, Aragao F, Souza T, Quintela E, Kitajima E, Ribeiro SG (2016) Golden mosaic of common beans in Brazil: management with a transgenic approach. APS Features-10. https://doi.org/10.1094/APSFeature-2016-10. Accessed 5 Feb 2019

Dhurua S, Gujar GT (2011) Field-evolved resistance to *Bt* toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Manag Sci 67:898–903

Dively GP, Venugopal PD, Bean D, Whalen J, Holmstrom K, Kuhar TP (2018) Regional pest suppression associated with widespread Bt maize adoption benefits vegetable growers. Proc Natl Acad Sci U S A 115:3320–3325

Ellsworth PC, Fournier A, Frisvold G, Naranjo SE (2017) Chronicling the socio-economic impact of integrating biological control, technology, and knowledge over 25 years of IPM in Arizona. In: Mason PG, Gillespie DR, Vincent C (eds) Proceedings of the 5th international symposium on biological control of arthropods. CABI, Langkawi, pp 214–216

Ervin DE, Frisvold GB (2016) Community-based approaches to herbicide-resistant weed management: lessons from science and practice. Weed Sci 64:602–626

Ervin D, Jussaume R (2014) Integrating social science into managing herbicide-resistant weeds and associated environmental impacts. Weed Sci 62:403–414

FAO (2018) AGP-integrated pest management. www.FAO.org/agriculture/crops/thematic-sitemap/theme/pests/ipm. Accessed 5 Feb 2019

Farias JR, Andow DA, Horikoshi RJ, Sorgatto RJ, Fresia P, Santos AC (2014) Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Crop Protect 64:150–158

Farias JR, Andow DA, Horikoshi RJ, Bernardi D, Ribeiro RDS (2016) Frequency of Cry1F resistance alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Pest Manag Sci 72:2295–2302

Francisco S (2009) Costs and benefits of UPLB Bt eggplant with resistance to fruit and shoot borer in the Philippines, projected impacts of agricultural biotechnologies for fruits and vegetables in the Philippines and Indonesia. International Services for the Acquisition of Agri-Biotech Applications and the Southeast Asian Ministers of Education Organization-Southeast Asia Regional Center for Graduate Study and Research in Agriculture, Ithaca, NY

Gassmann AJ, Petzold-Maxwell JL, Keweshan RS, Dunbar MW (2011) Field-evolved resistance to Bt maize by western corn rootworm. PLoS One 6:e22629

Gassmann AJ, Petzold-Maxwell JL, Clifton EH, Dunbar MW, Hoffmann AM, Ingber DA (2014) Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proc Natl Acad Sci U S A 111:5141–5146

Gould F, Amasino RM, Brossard D, Buell CR, Dixon RA, Falck-Zepeda JB (2016) Genetically engineered crops: experiences and prospects. The National Academies Press, Washington, DC

Hossain A, Menon S (2018) Stewardship efforts through capacity building initiatives for seed quality testing. https://bteggplant.cornell.edu/content/news/blog/stewardship-efforts-through-capacity-building-initiatives-seed-quality-testing. Accessed 29 Mar 2018

Huang F, Qureshi JA, Meagher RL Jr, Reisig DD, Head GP, Andow DA (2014) Cry1F resistance in fall armyworm *Spodoptera frugiperda*: single gene versus pyramided Bt maize. PLoS One 9:e112958

Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ (2010) Area-wide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330:222–225

ISAAA (2017) Global status of commercialized biotech/GM crops: 2017. ISAAA brief no. 53. ISAAA, Ithaca, NY

ISAAA (2019) ISAAA's GM approval database. www.isaaa.org/gmapprovaldatabase. Accessed 5 Feb 2019

Kaur R, Bharti U, Tanda AS (2021) Concept of CRISPR-CAS9 system and its application on insect genome: a preliminary review. In: Tanda AS (ed) Molecular advances in insect resistance of field crops. Springer, New York

Liu X, Chen M, Collins HL, Onstad DW, Roush RT, Zhang Q et al (2014) Natural enemies delay insect resistance to Bt crops. PLoS One 9:e90366

Lu Y, Wu K, Jiang Y, Xia B, Li P, Feng H et al (2010) Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. Science 328:1151–1154

Lu Y, Wu K, Jiang Y, Guo Y, Desneux N (2012) Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature 487:362

Matten SR, Head GP, Quemada HD (2008) How governmental regulation can help or hinder the integration of Bt crops within IPM programs. In: Romeis J, Shelton AM, Kennedy GG (eds) Integration of insect-resistant genetically modified crops within IPM programs. Springer, Dordrecht, pp 27–39

Meissle M (2016) How to assess the role of genetically engineered crops in integrated plant production? IOBC-WPRS Bull 114:23–29

Naranjo SE, Ellsworth PC (2009a) The contribution of conservation biological control to integrated management of *Bemisia tabaci* in cotton. Biol Control 51:458–470. https://doi.org/10.1016/j.biocontrol.2009.08.006

Naranjo SE, Ellsworth PC (2009b) Fifty years of the integrated control concept: moving the model implementation forward in Arizona. Pest Manag Sci 65:1267–1286

Naranjo SE, Ellsworth PC (2010) Fourteen years of Bt cotton advances IPM in Arizona. Southwestern Entomol 35:437–444

Naranjo SE, Ellsworth PC, Frisvold G (2015) Economic value of biological control in IPM of managed plant systems. Annu Rev Entomol 60:621–645

Navasero MV, Candano RN, Hautea DM, Hautea RA, Shotkoski FA, Shelton AM (2016) Assessing potential impact of Bt eggplants on non-target arthropods in the Philippines. PLoS One 11:e0165190

OECD (2018) Integrated pest management hub. https://www.oecd.org/chemicalsafety/integrated-pest-management/. Accessed 5 Feb 2019

Omoto C, Bernardi O, Salmeron E, Sorgatto RJ, Dourado PM, Crivellari A et al (2016) Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. Pest Manag Sci 72:1727–1736

Owen MDK (2016) Diverse approaches to herbicide-resistant weed management. Weed Sci 64:570–584

Palumbo JC, Castle SJ (2009) IPM for fresh-market lettuce production in the desert southwest: the produce paradox. Pest Manag Sci 65:1311–1320

Romeis J, Naranjo SE, Meissle M, Shelton AM (2018) Genetically engineered crops help support conservation biological control. Biol Control 130:136–154

Shelton A (2010) The long road to commercialization of Bt brinjal (eggplant) in India. Crop Protect 29:412–414

Shelton AM, Fuchs M, Shotkowski F (2008) Transgenic vegetables and fruits for control of insect and insect-vectored pathogens. In: Romeis J, Shelton AM, Kennedy GG (eds) Integration of insect-resistant, genetically modified crops within IPM Programs. Springer, Dordrecht, pp 249–272

Shelton AM, Hokanson KE, Hautea DM, Hossain MJ, Hossain MA, Paranjape V. et al. (2017) ISB news report-August 2017-Bt eggplant: a genetically engineered 'minor' crop comes of age in Bangladesh and the Philippines. ISB Report. http://hdl.handle.net/10919/78874. Accessed 5 Feb 2019

Shelton A, Hossain M, Paranjape V, Azad A (2018) Bt eggplant project in Bangladesh: history, present status, and future direction. Front Bioeng Biotechnol 6:106

Sobrinho RR, Xavier CAD, De Barros Pereira HM, De Andrade Lima GS, Assunção IP, Mizubuti ESG et al (2014) Contrasting genetic structure between two begomoviruses infecting the same leguminous hosts. J Gen Virol 95:2540–2552

Souza TLP, Faria JC, Aragão FJ, Del Peloso MJ, Faria LC, Wendland A et al (2018) Agronomic performance and yield stability of the RNA interference-based Bean golden mosaic virus-resistant Common Bean. Crop Sci 58:1–13

Storer NP, Babcock JM, Schlenz M, Meade T, Thompson GD, Bing JW et al (2010) Discovery and characterization of field resistance to *Bt* maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. J Econ Entomol 103:1031–1038

Storer NP, Kubiszak ME, King JE, Thompson GD, Santos AC (2012) Status of resistance to Bt maize in *Spodoptera frugiperda*: lessons from Puerto Rico. J Inverteb Pathol 110:294–300

Tabashnik BE, Carrière Y (2017) Surge in insect resistance to transgenic crops and prospects for sustainability. Nat Biotechnol 35:926

Tabashnik BE, Sisterson MS, Ellsworth PC, Dennehy TJ, Antilla L, Liesner L et al (2010) Suppressing resistance to Bt cotton with sterile insect releases. Nat Biotechnol 28:1304–1307

Tabashnik BE, Morin S, Unnithan GC, Yelich AJ, Ellers-Kirk C, Harpold VS et al (2012) Sustained susceptibility of pink bollworm to Bt cotton in the United States. GM Crops Food 3:194–200

Tabashnik BE, Brévault T, Carrière Y (2013) Insect resistance to Bt crops: lessons from the first billion acres. Nat Biotechnol 31:510

Tanda AS (2019) Entomophilous crops get better fruit quality and yield: an appraisal. Indian J Entomol 81(2):227–234

Tanda AS (2020) Biogenetic engineering in developing insect resistant crops: constraints and applications. 5th Edition of Global Congress on Plant Biology and Biotechnology (GPB 2020), 11–13 Nov 2020, Valencia, Spain

Tanda AS (2021a) Insect pollinators matter in sustainable world food production. Indian J Entomol. Accepted for publication

Tanda AS (2021b) Integrated pest management technology and biogenetic engineered crops: challenges and solutions. Int J Pest Manage. Accepted for publication

Tian J-C, Yao J, Long L-P, Romeis J, Shelton AM (2015) Bt crops benefit natural enemies to control non-target pests. Sci Rep 5:16636

USDA (2018) USDA announces pink bollworm eradication significantly saving cotton farmers in yearly control costs. https://www.usda.gov/media/press-releases/2018/10/19/usda-announces-pink-bollworm-eradication-significantly-saving. Accessed 5 Feb 2019

Vélez AM, Vellichirammal NN, Jurat-Fuentes JL, Siegfried BD (2016) Cry1F resistance among lepidopteran pests: a model for improved resistance management? Curr Opin Insect Sci 15:116–124

Wijnands FG, Baur R, Malavolta C, Gerowitt B (2012) Integrated pest management – design and application of feasible and effective strategies. In: IOBC-WPRS 2012: integrated pest management: the way forward to sustainable agricultural production; Proceedings of the conference on reducing pesticide dependency, commemorating the 50th anniversary of Rachel Carson's "Silent Spring". IOBC/WPRS Bulletin, Lelystad

Wilson LJ, Whitehouse ME, Herron GA (2018) The management of insect pests in Australian cotton: an evolving story. Annu Rev Entomol 63:215–237

Wu K-M, Lu Y-H, Feng H-Q, Jiang Y-Y, Zhao J-Z (2008) Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. Science 321:1676–1678

Yang F, Kerns DL, Brown S, Kurtz R, Dennehy T, Braxton B (2016) Performance and cross-crop resistance of Cry1F-maize selected *Spodoptera frugiperda* on transgenic Bt cotton: implications for resistance management. Sci Rep 6:28059

Chapter 11
Current Understanding of the Plant Defense Mechanism and Available Genetic Resources for Aphid Resistance in Wheat

Beant Singh, Deepika Narang, and Parveen Chhuneja

11.1 Introduction

Wheat is the most widely grown crop in the world and is a staple food for one third of the world population. Every year, the annual monetary loss of Rs. 413.68 billion has been reported due to insect pests in wheat from India (Dhaliwal et al. 2010). Among the various insect pests, aphids are considered as one of the major biotic threats to food production of the country. Eleven different aphid species are reported to attack wheat, out of which five species, viz., corn leaf aphid (CLA) (*Rhopalosiphum maidis*), bird cherry-oat aphid (BCOA) (*Rhopalosiphum padi*), greenbug (GB) (*Schizaphis graminum*), English grain aphid (EGA) (*Sitobion avenae*), and Russian wheat aphid (RWA) *Diuraphis noxia* cause considerable economic damage to wheat crop (Dixon 1987; Deol et al. 1987). Aphids suck sap from tender plant parts and secrete honey dew on which black sooty mold grows. This saprophytic fungus reduces the photosynthetic efficiency of plants (Rabbinge et al. 1981). They cause 20–30% yield losses in cereal crops (Voss et al. 1997; Singh and Deol 2003). Apart from direct loss by sucking sap from foliage, it also injects toxins via saliva and transmits barley yellow dwarf virus (Leather et al. 1989). Because of short life span and high dispersal rates, aphid management is a challenging job, and large amounts of pesticides are being used for their control in wheat. It leads to destruction of non-targeted beneficial natural enemies and problems of insecticide resistance and resurgence (Singh and Kaur 2017). Host plant resistance (HPR) is an eco-friendly

B. Singh (✉)
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India
e-mail: beant19@pau.edu

D. Narang · P. Chhuneja
School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_11

approach and forms an integral component of Integrated Pest Management (IPM) programs. It is defined as heritable trait in a plant of a population, or a race, or a variety of certain species, resulting in less damage than in other individuals which lack these genetic characteristics. In this way, HPR is conditioned by certain genes which express the presence or absence of certain morphological or biochemical traits that interferes with the ability of an insect pest to utilize plant as a host.

Since the beginning of agriculture, the importance of varietal improvement is well known. In the ancient time, selection and introduction were commonly used methods, since knowledge about use of hybridization, mutation, and polyploidy was not in practice. The earliest documented report on plant resistance to insects was the study of Hessian fly biology on different wheat cultivars by farmers in the USA (Havens 1801). The first *Hessian fly*-resistant wheat (cv. Underhill) was cultivated by farmers in the eighteenth and nineteenth centuries. The first review on the status of the knowledge of plant resistance to insects was published by Snelling (1941). However, the era of breeding of insect-resistant crops was established by the work of Painter (1951). Despite the growing interest in HPR during the twentieth century, the importance of HPR as insect control method remained under the shadow of chemical control. Insecticides such as DDT showed spectacular results during post-World War II period, and research strategies slowly shifted from HPR to this approach. However, Carson (1962) in her book *Silent Spring* first highlighted the detrimental effects of pesticides to the environment and human beings. This book was important to again tilt the balance toward HPR and started the new era of modern environment-friendly methods of pest control.

Over the past 70 years, breeding crops for pest resistance has gained momentum, and several insect-resistant crops have been developed. With the advent and use of molecular tools in the last 30 years, the field of plant resistance to arthropods offered enormous opportunities for continued development of new crop cultivars with genes for durable insect resistance. Marker-assisted selection (MAS) using DNA markers linked to the insect resistance genes can aid in developing resistant varieties. All these developments have represented enormous steps forward for plant resistance to become a solid interdisciplinary activity that contributes in improving pest management and consequently food production in a sustainable way.

The chapter summarizes the information related to categories of HPR, availability of genetic resources for aphid resistance in wheat, breeding/molecular techniques employed for introgression of aphid resistance in cultivated wheat, RNA interference technologies employed for knocking out susceptible genes from wheat, and challenges and future prospects in aphid resistance program.

11.2 Types of Plant Defense Mechanisms Against Aphids in Wheat

Painter (1941) classified HPR to insects into three categories, i.e., Non-preference/Antixenosis, Antibiosis, and Tolerance. The host selection process in aphids is divided into six stages: (a) pre-alighting behavior, (b) assessment of surface cues before stylet insertion, (c) probing epidermis, (d) stylet pathway activity, (e) sieve element puncture and salivation, and (f) phloem acceptance and sustained ingestion (Powell et al. 2006). At every stage, plant tries to protect itself from aphid infestation by operating one or other types of HPR mechanism.

11.2.1 Non-preference/Antixenosis

Antixenosis is considered as the first line of defense of plants against insect damage. It makes the plants less suitable for insect/aphid colonization and adversely affects their host finding ability. The host finding process in insects consists of pre- and post-alighting phases and involves olfactory, visual, gustatory, and thigmotactic responses (Smith 2005). In aphids, host selection is mainly based on chemical cues (Powell and Hardie 2001), but visual signals may also play a role (Doering and Chittka 2007).

11.2.1.1 Pre-alighting Responses

Visual cues: Visual cues during host searching process depend upon the spectral quality of light and color, size, shape, and dimensions of the plants (Smith 2005). The aphids usually prefer yellow-colored surfaces (Pettersson et al. 2007). However the BCOA, *R. padi* (L.), shows a higher response to green than yellow color as compared to other aphid species infesting wheat crop (Kieckhefer et al. 1976). The size of the green-/yellow-colored area (plant density) is another important factor which determines the landing rate of aphids on plant (Ahman et al. 1985). Moharramipour et al. (1997) also reported that yellow and non-waxy leaves of barley are preferred by cereal aphids for feeding or have additive effect on aphid resistance. The co-evolution theory of color preference on *Prunus padus* also revealed strong preference of *R. padi* toward green leaves (Archetti and Leather 2005).

Olfactory cues: All plants release volatiles which may act as repellents or attractants to insects. These volatiles are received by olfactory structures of insects, and primary olfactory structures are located in the last two segments of the insect antennae (Gillot 2005). Many volatiles are common to all plants, whereas others are specific to certain plant genera or species or cultivars/varieties (Bruce et al. 2005). Some volatiles are mainly released by plants upon damage. Methyl

salicylate and cis-jasmone are such compounds released by plants during aphid feeding and act as repellent to the BCOA, EGA (*Sitobion miscanthi*), and rose grain aphid (RGA), (*Metopolophium dirhodum*) (Hardie et al. 1994; Pettersson et al. 1994; Pickett and Glinwood 2007; Birkett et al. 2000). The direct spraying of these compounds on wheat plants at seedling stage exhibited negative effect on aphid growth and positive effects on some natural enemies such as ladybird beetles and parasitoids (Bruce et al. 2003; Birkett et al. 2000).

11.2.1.2 Post-alighting Response

Once aphids land on plants, their behavior is further influenced by a wide range of characters associated with plant morphology and chemistry (Pettersson et al. 2007). The most important factor for aphid decision to reject or accept a plant as host is information received at stylet insertion (Powell et al. 2006). It is believed that aphids suck up small sap samples that are rapidly transported to the pharyngeal organ. Plant penetration can be divided into three phases: (1) pathway phase, the phase where brief cell punctures occur; (2) xylem phase, drinking phase to relieve water stress; and (3) phloem phase, where the main feeding takes place (Pettersson et al. 2007). It is at the phloem level where the final decision to accept or reject a plant is made (Pettersson et al. 2007). A number of papers have reported significant differences in feeding behavior of aphid when compared on resistant and susceptible wheat genotypes (Singh et al. 2020; Greenslade et al. 2016; Pereira et al. 2010).

Antixenosis tests measure the differential response of insects among different plant genotypes. It can be expressed as relative amount of feeding or oviposition among different genotypes. The most common type of antixenosis test with aphids is the free-choice test, in which each genotype is equidistantly planted in a circular pattern, then aphids are released in the center of the circle, and counting of aphid feeding/oviposition is made after a particular interval of time (Hesler et al. 1999; Hesler 2005; Webster et al. 1994). Later on, a slight modification is made in this free-choice test, and a leaf disc from different plant genotypes is placed in glass vials with distilled water and is held in a testing platform. Nowadays, the volatiles collected from the plants are placed on the different arms of olfactometer for antixenosis tests. Light orientation must be managed properly, since aphids are attracted to light sources, possibly giving false resistance/susceptibility results. Antixenosis reduces the initial infestation and is considered as an important component of HPR. However, importance of antixenosis decreases in current agriculture ecosystem where monoculture predominates which deprives the insect of their preferred host and eventually insect starts accepting even a less preferred host.

11.2.2 Antibiosis

Antibiosis is another resistance mechanism which negatively affects the physiology of an insect. Antibiosis may lead to higher mortality, smaller body size/weight, reduced fecundity, or prolonged periods of insect development (Smith 2005). This type of resistance has been found in several wheat and barley genotypes against aphids (Aradottir et al. 2016; Singh et al. 2020; Hesler et al. 1999; Hesler 2005). In this type of mechanism, the allelochemicals or non-nutritional chemicals (hydroxamic acids, DIMBOA, etc.) produced by plants usually affect the biology or behavior of aphids. Givovich and Niemeyer (1996) reported that hydroxamic acid present in some wheat genotypes adversely affects the biology of RWA (*Diuraphis noxia*). Similarly Ni and Quisenberry (2000) showed that the genes *Dn5* and *Dn1* conferring antibiosis to RWA might be related to concentrations of secondary metabolites. However, Macaulay et al. (2020) also reported that QTL for gramine content is not linked to aphid resistance in barley. Gramine is an indole alkaloid present in barley responsible for host plant defense against insects. Aphids are also known to alter the chemistry of their hosts and sometimes enhance nutritional status of plants (Telang et al. 1999). The nymphs of RWA resulted in increase of essential amino acids in infested plants. Similarly, Castro et al. (2007) reported significant increases in protein content with GB (*Schizaphis graminum*)-infested wheat plants. Although chemicals are the most common causes for antibiosis effects, plant structures like trichomes may also directly affect the physiology of insects in a negative way.

Procedures for identifying antibiosis effects are more laborious than antixenosis tests since they must give information related to relative developmental, reproduction, and mortality of insects on different plant genotypes. Life table consisting of data about insect longevity, mortality, fecundity/female/unit time, and intrinsic rate of increase (*rm*) on different genotypes needs to be developed for such studies. However this method is time-consuming, and alternative procedures such as mean relative growth rate (MRGR) have been proposed for aphid screening (Leather and Dixon 1984).

11.2.3 Tolerance

Tolerance is the ability of plants to withstand or recover from an insect attack equal to the attack caused in a susceptible genotype, and it is determined by the genetic characteristics that enable plants to continue growing, recover, or add new growth after and/or during insect damage (Smith 2005). Tolerant plants tend to produce more biomass and involve plant traits related to biomass production. Rosenthal and Kotanen (1994) reported that compensation, seen as regrowth, depends upon the storage capacity, photosynthetic rate, allocation patterns, and nutrient uptake of plants. These traits vary according to extrinsic (environment, insect species, spatial

distribution) and intrinsic (plant genetics) factors. The ability of plants to tolerate insect damage has been widely reported, and it is known to be frequently interacting with the other mechanisms of resistance. For example, in wheat and barley, tolerance to aphids has been reported (Hesler 2005; Hesler et al. 1999; Lage et al. 2004; Smith and Starkey 2003; Zhu et al. 2005). It has been reported that Russian aphid-tolerant plants often possess higher photosynthetic rates and resulted in higher growth rates and stored root carbon (Heng-Moss et al. 2003). Photosystem and chlorophyll genes associated with photosynthesis are highly expressed in foliage of aphid-tolerant plants (Marimuthu and Smith 2012). Boyko et al. (2006) suggested that the molecular basis for tolerance to the RWA in plants carrying the *Dnx* gene involves the upregulation of transcription sequences similar to those that regulate photosynthesis, photorespiration, protein synthesis, antioxidant production, and detoxification. Ni et al. (2002) showed that non-damaged leaf areas of plants infested with RWA increased their concentrations of chlorophylls and helps the plants to compensate the loss of photosynthetic capacity by increasing metabolic activity in non-damaged areas.

Since tolerance is related to plant responses to insect damage, its measurement greatly depends on the aphid species that is being evaluated. The tolerance against RWA and GB can be measured by estimating chlorophyll loss (Lage et al. 2003, 2004; Sotelo et al. 2009). Alternatively, plant growth and biomass measurements after exposure of genotypes for particular interval of time can be used for tolerance studies (Dunn et al. 2007; Hesler 2005; Hesler et al. 1999).

11.3 Breeding for Aphid Resistance

Host plant resistance is an economical and ecologically sound strategy and forms an integral part of an IPM program. The first step for successful aphid breeding program is the selection of germplasm to be screened against aphid. Wild relatives of wheat and landraces are the most important potential sources for insect resistance. Probability of success in finding aphid resistance could be increased if the germplasm is selected from the aphids' center of origin or where the wild relatives/landraces have historically co-evolved with the aphids.

11.3.1 Identification of Resistant Donors

Correct identification of resistant donor is the most important step for the aphid resistance breeding program. The screening methods for identification of sources of resistance should be based on the symptoms of attack and biology/behavior of aphids. A number of protocols are developed to rapidly screen the germplasm and identify resistant genotypes against aphids in wheat (Anonymous 2004; Berzonsky et al. 2003; Dunn et al. 2007). The chlorophyll content can also be used as an

indirect method (tolerance) for identification of resistant germplasm (Franzen et al. 2008). Some methods to measure antibiosis and antixenosis are already discussed in earlier section and can be used to screen large germplasm or segregating populations under field/laboratory conditions. Sometimes, all three categories of resistance interact in a single plant genotype, and it becomes difficult to distinguish if reduced performance of aphids is due to antibiosis or antixenosis effects. The techniques including a combination of different resistance mechanisms should be used for identification of resistant germplasm. Another consideration for wheat breeding is the genetic diversity of aphid population. One should consider the target region/area for which wheat is bred and have information related to the aphid dynamics and prevalent aphid biotypes of the region.

11.3.1.1 Available Genetic Resources for Resistance to Aphids in Wheat

The polyploid nature of wheat allows introgression of a large number of genetic variations from related species. The choice of breeding method to be used for introgression of genetic resistance from related species in wheat depends upon the evolutionary distance between the species (Friebe et al. 1996). The resistance from primary and secondary gene pool (*Triticum turgidum*, *T. dicoccoides*, *T. monococcum*, and *Ae. tauschii*) can be achieved by direct hybridization, homologous recombination, and backcrossing, while homoeologous recombination can be used for transferring resistance from tertiary gene pool (*Aegilops* species, *Secale* species, *Agropyron elongatum*, *A. intermedium*, and *A. trichophorum*). Introgression from distant species is very difficult however; techniques such as centric breakage-fusion of univalents and radiation treatment to induce chromosome breaks can be used to transfer resistance from such species (Friebe et al. 1996). Chromosome 1R from rye has been widely used to introgress GB resistance in wheat (Kim et al. 2004; Lu et al. 2010; Mater et al. 2004). Similarly EGA, RWA, and BCOA resistance has been introgressed from *Aegilops* species and *T. araraticum* (Smith et al. 2004). Migui and Lamb (2003) found that the ploidy level plays an important role in resistance to aphids and genotypes with low ploidy level were more frequently resistant to aphids. In general, *T. boeoticum*, *Ae. tauschii*, and *T. araraticum* had the higher levels of antibiosis to BCOA, whereas *Ae. tauschii* and *T. turgidum* had the higher levels of overall resistance to GB, while *T. araraticum* and *T. dicoccoides* presented the higher levels of overall resistance to EGA (Migui and Lamb 2003). Singh et al. (2006) and Singh and Singh (2009) also identified confirmed sources of aphid (*R. maidis*) in barley. Aphid species-wise availability of genetic sources of resistance in wheat and related species is shown in Table 11.1. The list of aphid resistance genes identified in wheat and its progenitors is given in Table 11.2.

Table 11.1 List of resistant wheat germplasm against different aphid species

Variety/Accession No.	Plant species	Aphid species	References
DS28A	*Triticum aestivum*	***Schizaphis graminum***	Tyler et al. (1987), Porter et al. (1994)
Amigo; TAM107 and TAM200	*Secale cereale*	-do-	Tyler et al. (1987), Lu et al. (2010)
Largo	*Aegilops tauschii*	-do-	Tyler et al. (1987), Azhaguvel et al. (2012)
CI 17959	*Ae. tauschii*	-do-	Tyler et al. (1987)
CI 17882; CI 17884 and CI 17885	*Ae. speltoides*	-do-	Crespo-Herrera et al. (2013), Tyler et al. (1987), Triebe et al. (1991)
GRS1201	*S. cereale*	-do-	Porter et al. (1994), Lu et al. (2010)
W7984	*Ae. tauschii*	-do-	Weng et al. (2005), Tan et al. (2017)
PI 595379	*Ae. tauschii*	-do-	Xu et al. (2020)
CETA/*Ae. tauschii* Wx1027	*Ae. tauschii*	-do-	Zhu et al. (2005), Crespo-Herrera et al. (2019a)
CROC 1/Ae. *tauschii* Wx224	*Ae. tauschii*	-do-	Zhu et al. (2005)
68111/Rugby//Ward//*Ae. tauschii* TA2477	*Ae. tauschii*	-do-	Zhu et al. (2005)
Altar 84/*Ae. tauschii* TA2841	*Ae. tauschii*	-do-	Zhu et al. (2005)
Sokoll	*Ae. tauschii*	-do-	Crespo-Herrera et al. (2019a)
Wichita/ TA1695//2*Wichita	*Ae. tauschii*	-do-	Zhu et al. (2005), Boina et al. (2005)
Sando's 4040	*T. aestivum*	-do-	Boyko et al. (2004)
KSU97-85-3	*Ae. tauschii*	-do-	Zhu et al. (2005)
CWI76364	*Ae. tauschii*	-do-	Crespo-Herrera et al. (2014)
NARC-09	*T. aestivum*	-do-	Akhtar et al. (2013)
Momal-2002	*T. aestivum*	-do-	Akhtar et al. (2013)
TD-1	*T. aestivum*	-do-	Akhtar et al. (2013)
Lassani-08	*T. aestivum*	-do-	Iqbal et al. (2018)
CWI76364	*Triticum dicoccum*	***Rhopalosiphum padi***	Crespo-Herrera et al. (2014)
Pioneer (S) 25R40	*T. aestivum*	-do-	Girvin et al. (2017)
Limagrain LCS Mint	*T. aestivum*	-do-	Girvin et al. (2017)
MFA (S) 2248	*T. aestivum*	-do-	Girvin et al. (2017)
Pioneer (S) 25R77	*T. aestivum*	-do-	Girvin et al. (2017)
Limagrain LS Wizard	*T. aestivum*	-do-	Girvin et al. (2017)
V-5, PR-83, NR-241, SN-128	*T. aestivum*	-do-	Akhtar et al. (2009)
NIAW 917	*T. aestivum*	-do-	Patil et al. (2018)

(continued)

Table 11.1 (continued)

Variety/Accession No.	Plant species	Aphid species	References
8TA5L, H7089-52, Stniism 3, MV4	*Triticale*	-do-	Crespo-Herrera et al. (2019b)
C273	*T. turgidum* spp. Durum	***Sitobion avenae* (Fabricius)**	Liu et al. (2011)
XN98-10-35	*Synthetic wheat*	-do-	Wang et al. (2015)
Amigo	*T. aestivum*	-do-	Hu et al. (2016)
Batis	*T. aestivum*	-do-	Hu et al. (2016)
Xanthus	*T. aestivum*	-do-	Hu et al. (2016)
Astron, Ww2730	*T. aestivum*	-do-	Hu et al. (2016)
98-10-30	*Synthetic wheat*	-do-	Hu et al. (2016)
Tm	*T. monococcum*	-do-	Hu et al. (2016)
RSP-561	*T. aestivum*	-do-	Mir et al. (2017)
Zhong 4 wumang, Jibao No.1	*T. aestivum*	-do-	Li et al. (2006)
PI 137739	*T. aestivum*	***Diuraphis noxia* (Mordvilko)**	Liu et al. (2001)
PI 262660	*T. aestivum*	-do-	Liu et al. (2001), Ma et al. (1998)
SQ24	*Ae. tauschii*	-do-	Nkongolo et al. (1991)
PI 372129	*T. aestivum*	-do-	Liu et al. (2002), Ma et al. (1998)
PI 294994	*T. aestivum*	-do-	Liu et al. (2001)
PI 243781	*T. aestivum*	-do-	Liu et al. (2002)
Turkey 77	*S. cereale*	-do-	Lapitan et al. (2007), Marais et al. (1994)
PI 294994	*T. aestivum*	-do-	Liu et al. (2001)
PI 220127	*T. aestivum*	-do-	Liu et al. (2001)
1881	*T. turgidum*	-do-	Navabi et al. (2004)
1RSam.1AL	Wheat-rye translocation	-do-	Crespo-Herrera et al. (2019b)
MA1S.1RLe(1B)	Wheat-rye substitution	-do-	Crespo-Herrera et al. (2019b)

Bird Cherry-Oat Aphid (*Rhopalosiphum padi*)

According to Blackman and Eastop (2007), the origin of this aphid is difficult to trace because it is currently distributed worldwide and its sexual phase takes part on various *Prunus* species. This aphid species can reduce yield by 31–62% (Voss et al. 1997; Riedell et al. 2003). *A. elongatum*, *A. intermedium*, *A. repens*, and *Elymus angustus* and their introgressed wheat lines were first found to show antibiosis type of resistance (Tremblay et al. 1989). Thereafter, rye-derived wheat lines and triticale were identified which posses all three categories of resistance to BCOA (Hesler 2005; Hesler and Tharp 2005; Hesler et al. 2007). Recently, Singh et al. (2018) identified BCOA resistance in some of the *Ae. tauschii* lines. However, so far no

Table 11.2 List of aphid resistance genes in wheat

Gene/ QTL	Aphid species	Variety/Accession No.	Plant species	References
Gb1	*Schizaphis graminum*	DS28A	*Triticum aestivum*	Tyler et al. (1987), Porter et al. (1994)
Gb2	-do-	Amigo; TAM107 and TAM200	*Secale cereale*	Tyler et al. (1987), Lu et al. (2010)
Gb3	-do-	Largo	*Aegilops tauschii*	Tyler et al. (1987), Azhaguvel et al. (2012)
Gb4	-do-	CI 17959	*Ae. tauschii*	Tyler et al. (1987)
Gb5	-do-	CI 17882; CI 17884 and CI 17885	*Ae. speltoides*	Crespo-Herrera et al. (2013), Tyler et al. (1987), Triebe et al. (1991)
Gb6	-do-	GRS1201	*S. cereale*	Porter et al. (1994), Lu et al. (2010)
Gb7/ Gbx2	-do-	W7984	*Ae. tauschii*	Weng et al. (2005), Tan et al. (2017)
Gb8	-do-	PI 595379	*Ae. tauschii*	Xu et al. (2020)
Gba	-do-	CETA/*Ae. tauschii* Wx1027	*Ae. tauschii*	Zhu et al. (2005), Crespo-Herrera et al. (2019a)
Gbb	-do-	CROC 1/*Ae. tauschii* Wx224	*Ae. tauschii*	Zhu et al. (2005)
Gbc	-do-	68111/Rugby//Ward//*Ae. tauschii* TA2477	*Ae. tauschii*	Zhu et al. (2005)
Gbd	-do-	Altar 84/*Ae. tauschii* TA2841	*Ae. tauschii*	Zhu et al. (2005)
GbSkll	-do-	Sokoll	*Ae. tauschii*	Crespo-Herrera et al. (2019a)
Gbx1	-do-	Wichita/ TA1695//2*Wichita	*Ae. tauschii*	Zhu et al. (2005), Boina et al. (2005)
Gby	-do-	Sando's 4040	*T. aestivum*	Boyko et al. (2004)
Gbz	-do-	KSU97-85-3	*Ae. tauschii*	Zhu et al. (2005)
QGb. slu-2DL	-do-	CWI76364	*Ae. tauschii*	Crespo-Herrera et al. (2014)
QRp. slu-4BL	*Rhopalosiphum padi*	CWI76364	*Triticum dicoccum*	Crespo-Herrera et al. (2014)
QRp. slu-5AL	-do-	CWI76364	*T. dicoccum*	Crespo-Herrera et al. (2014)
QRp. slu-5BL	-do-	CWI76364	*T. dicoccum*	Crespo-Herrera et al. (2014)
RA1	*Sitobion avenae* (Fabricius)	C273	*T. turgidum* spp. durum	Liu et al. (2011)
Sa2	-do-	*XN98-10-35*	*Synthetic wheat*	Wang et al. (2015)

(continued)

Table 11.2 (continued)

Gene/ QTL	Aphid species	Variety/Accession No.	Plant species	References
Dn1	*Diuraphis noxia* (Mordvilko)	PI 137739	*T. aestivum*	Liu et al. (2001)
Dn2	-do-	PI 262660	*T. aestivum*	Liu et al. (2001), Ma et al. (1998)
Dn3	-do-	SQ24	*Ae. tauschii*	Nkongolo et al. (1991)
Dn4	-do-	PI 372129	*T. aestivum*	Liu et al. (2002), Ma et al. (1998)
Dn5	-do-	PI 294994	*T. aestivum*	Liu et al. (2001)
Dn6	-do-	PI 243781	*T. aestivum*	Liu et al. (2002)
Dn7	-do-	Turkey 77	*S. cereale*	Lapitan et al. (2007), Marais et al. (1994)
Dn8	-do-	PI 294994	*T. aestivum*	Liu et al. (2001)
Dn9	-do-	PI 294994	*T. aestivum*	Liu et al. (2001)
Dnx	-do-	PI 220127	*T. aestivum*	Liu et al. (2001)
Dn1881	-do-	1881	*T. turgidum*	Navabi et al. (2004)

resistance genes to BCOA have been properly identified or introgressed into elite wheat cultivars (Porter et al. 2009).

English Grain Aphid (*Sitobion avenae*)

This aphid originates in Europe and currently it is distributed in Africa, India and Nepal, North America, and South America (Blackman and Eastop 2007). Normally, populations of EGA have the highest reproductive rate at heading stage and cause 3–21% yield losses in spring by feeding at booting stage (Watt 1979; Voss et al. 1997; Singh and Deol 2003). However, the damage caused by the EGA is less deleterious than GB and BCOA at same population density (Kieckhefer and Kantack 1980; Voss et al. 1997). So far only one resistance gene (RA-1 located on 6 AL chromosome) linked to EGA resistance has been mapped in the wheat line C273. This gene is reported to be linked with SSR markers *Xwmc179*, *Xwmc553*, and *Xwmc201* (Liu et al. 2011). Resistance to EGA has been also identified in some wheat relatives such as *T. monococcum*, *T. boeticum*, *T. araraticum*, *T. dicoccoides*, and *T. urartu* (Migui and Lamb 2003, 2004; Di Pietro et al. 1998).

Greenbug (*Schizaphis graminum*)

Greenbug is widely distributed in Asia, southern Europe, Africa, and North and South America (Blackman and Eastop 2007). GB can cause 35–40% damage to winter wheat (Kieckhefer and Gellner 1992). The durum wheat "DS 28A" was identified as a resistance source in the 1950s (Porter et al. 1997). However, biotype

"B" of GB developed the ability to damage GB-resistant DS 28A genotype in 1961 (Porter et al. 1997). Successively, a number of biotypes were recognized in GB, and presently there are 11 biotypes designated from letter A to K (Berzonsky et al. 2003; Porter et al. 1997). Since these different GB populations were designated according to their capability to injure plant genotypes with certain resistance genes, the "biotype" concept is related to a phenotypic expression that does not totally reflect aphid genetic diversity (Blackman and Eastop 2007). Weng et al. (2010) found that biotypes E, I, and K are genetically related, whereas biotype H is distant from all of the other biotypes. Host association may have a significant role in this genetic differentiation, since different biotypes were found on different hosts, viz., I and K biotypes was first identified on sorghum, biotype E on wheat, biotype G on *Agropyron* species, and biotype H on *Ae. cylindrica* and *A. intermedium* (Burd and Porter 2006; Weng et al. 2010). Contrary to the common thought that the evolution of GB biotypes resulted from the deployment of resistant cultivars, Porter et al. (1997) demonstrated that GB biotypes were already present in nature before resistant cultivars were widely released. Fourteen GB resistance genes (*Gb1*, *Gb2*, *Gb3*, *Gb4*, *Gb5*, *Gb6*, *Gb7/Gbx2*, *Gba*, *Gbb*, *Gbc*, *Gbd*, *Gbx1*, *Gby*, and *Gbz*) are reported in wheat and mostly originated from its related plant species *Ae. tauschii*. Genes *Gba*, *Gbb*, *Gbc*, *Gbd*, and *Gbx1* are located in the same region of chromosome 7D and linked with *Xgwm671* SSR marker (Zhu et al. 2005). All these genes (except *Gbx1*) are either allelic or linked (Zhu et al. 2005). SSR markers *Xbcd98* and *Xwmc157* are tightly linked to *Gby* and *Gbz* genes, respectively. These *Gby* and *Gbz* genes are located on chromosomes 7A and 7D, respectively (Boyko et al. 2004; Zhu et al. 2004).

Russian Wheat Aphid (*Diuraphis noxia*)

This aphid species injects a toxin into plants while feeding resulting in a characteristic leaf rolling symptoms; however feeding at the ear head stage results in bending of ear heads (Blackman and Eastop 2007). It is widely distributed in East Asia, South Africa, and North and South America, but not reported in India and adjoining countries. RWA can cause up to 40% yield losses in winter wheat (Kieckhefer and Gellner 1992). Currently, 11 genes are reported to confer resistance to RWA, designated from *Dn1* to *Dn9*, *Dnx*, and *Dn1881*. All these genes are single dominant genes except for *Dn3* which is recessive, and most of them are located on the D genome except one on the B genome and another one on 1RS from rye. Liu et al. (2001) showed that *Dn1*, *Dn2*, and *Dn5* resistance genes are (located on 7DS) either allelic or tightly linked to one another. All these genes are linked to the same SSR marker *Xgwm111* (Liu et al. 2001). Unlike the development of GB biotypes, it is believed that the occurrence of new genetic variation in RWA with the ability to harm wheat is due to the deployment of resistant cultivars (Weiland et al. 2008). Until 2003, only one biotype was reported in the USA however; Haley et al. (2004) identified a new biotype RWA-2 and *Dn7* gene from rye which was found to be effective against this aphid biotype (Haley et al. 2004). In 2006, three new RWA

biotypes were identified, RWA-3, RWA-4, and RWA-5, of which RWA-3 is virulent to all known resistance sources, including *Dn7* (Burd et al. 2006). Weiland et al. (2008) identified three more biotypes in Colorado state, RWA-6, RWA-7, and RWA-8, to which *Dn7* gene and the wheat genotypes Stars 02RWA2414-11, CO03765, and CI2410 are resistant.

11.4 Challenges in Breeding for Aphid Resistance

Presently, most of the identified resistant genes in wheat crop interact with aphids in a gene for gene fashion. Combining resistance genes would be a suitable option in the absence of resistance genes with broad effects. Porter et al. (2000) pyramided *Gb2* and *Gb3* resistance genes in wheat against GB, but pyramided genes had no stronger effects on aphid performance compared to the parents carrying the single genes. So, a careful selection of genes to be combined is crucial. Sometimes, two or more aphid species inhabit the same field and sometimes even on the same plant, e.g., BCOA and GB coexist in Europe and North and South America. The insects compete for resources, and usually one species predominates over the others. Therefore, constantly growing resistant varieties imparting resistance to a single specie may lead to the predominance of that specie that was previously not problematic. Finding genetic resources resistant to multiple species is the most desirable solution. As mentioned in previous sections, resistance to two or three aphid species has been found in wild relatives of wheat. Unraveling the genetic basis of such resistance sources is important, since the number of genes and their interaction are important aspects for plant breeding procedures.

One of the challenges for big breeding programs is that protocols to evaluate aphid resistance are difficult to implement on a large scale. Another problem is that sometimes there is no correlation between seedling and adult plant resistance (Migui and Lamb 2004). Thus screening techniques and phenotypic selection should be employed at later plant stages.

11.5 Potential of Gene Editing and Transgenic Technology in Aphid Resistance Breeding Program

Aphids manipulate the plants to make them more suitable as hosts, i.e., more susceptible. The knowledge of host plant susceptibility (S) genes can also be exploited for HPR by using new techniques such as CRISPR/Cas9 or RNAi, to knock out S genes. S genes may be involved in reducing functional plant defense or increasing plant factors that favor aphid growth (e.g., improved nutritional quality). Approximately 40% of the omics studies reviewed by Åhman et al. (2019) indicate that aphids modify the host for their advantage. The susceptible genes related to

following functions/activities can be knocked out to make plants less susceptible to aphids.

11.5.1 Genes Related to Host Plant Defense Pathways

Some receptors in plants can detect insect attack and induce many defense signaling pathways, viz., salicylate (SA) and jasmonate (JA) pathways. Chewing insects predominantly induce the JA pathway genes, whereas aphids commonly induce genes related to the SA pathway (Thompson and Goggin 2006; Walling 2008). In general, aphids are more sensitive to plant defense involving the JA signaling pathway, SA induction is considered to be a way for aphids to deceive the plant because crosstalk between hormonal pathways hinders the SA-induced plant to fully induce the JA pathways. Indications of SA-JA antagonism have been found in GB on susceptible sorghum (Zhu-Salzman et al. 2004) and EGA on susceptible wheat (Ferry et al. 2011). The SA defense pathway leads to local cell death hindering the plant pathogen to infest and proliferate in the plant. However, such a hypersensitive response is not always seen when aphids upregulate the SA pathway in resistant plants (Thompson and Goggin 2006). In wheat, susceptible to EGA, a JA-regulated agglutinin was downregulated initially in both infested and systemic tissues (away from the site of infestation), but after 8 days, JA-regulated pathway was downregulated only in the infested tissue (Ferry et al. 2011). The genes which downregulate the JA-pathway need be knocked out using newly emerged technologies.

11.5.2 Modification of Plant Genes Related to Food Accessibility

To successfully feed on phloem sap, aphids need to penetrate the plant tissue with their mouth parts, prevent sieve tube clogging, and maintain leaf water potential in the vascular bundles. In order for aphids to reach the phloem, their stylets first penetrate the epidermis in the cell wall between two cells and then proceed deeper between other cells in the plant tissues. Aphids also penetrate the cell walls when they probe cells along the pathway to the vascular bundles, as well as when they feed from phloem or drink from xylem (Pettersson et al. 2007). Thus there is a reason to believe that the structure of the cell walls may influence the ease with which the phloem and xylem can be reached, especially by the small, newborn nymphs in a colony. The main component of the cell walls is cellulose which is combined with other polysaccharides: various hemicelluloses, pectin (galacturons) and callose (β-1,3-glucans), and glycoproteins (arabinogalactan proteins and extensins). The primary cell wall is flexible, whereas the secondary cell wall that is deposited after cessation of cell expansion is rigid, strengthened by the polyphenol lignin (Zhong

et al. 2019). Some of the studies infer cell wall manipulations by aphids. Reddy et al. (2013) found some pectinase genes upregulated by *D. noxia* in susceptible compared to resistant wheat, indicating aphid modification of cell walls to its favor. Such pectinase or expansin genes might be potential S genes to target by CRISPR/Cas9 or RNAi (Reddy et al. 2013; Bricchi et al. 2012). The silencing of such pectinase genes could help in developing aphid resistance breeding program. Aphids (*M. persicae*) took longer time to penetrate the epidermis and mesophyll and reach the vascular bundles of the *Arabidopsis* mutants (wrky22), possibly due to the downregulation of the cell wall loosening genes for pectin lyases and expansins (Kloth et al. 2016). Singh et al. (2020) reported that size of the vascular bundle, number of layers of mesophyll cells, and leaf thickness also influence the ability of *R. padi* to penetrate the plant cells. The silencing of genes related to the functions of these cells could help in aphid resistance breeding program.

11.5.3 Aphid Modification of Food Quality

Phloem sap is imbalanced as aphid food since it is rich in sugars and relatively low in amino acids (Dinant et al. 2010). However, nutrient-focused studies have shown that *D. noxia* and *S. graminum* increased phloem concentrations of essential amino acids in susceptible barley and wheat (Telang et al. 1999; Sandström et al. 2000). Apart from nutrients, there is a large array of secondary compounds in the phloem sap, which might negatively affect feeding of aphids (Dinant et al. 2010; Foyer et al. 2015). However, aphid modifies the quality of their food by different mechanisms. *M. persicae* downregulated many genes related to flavonoid biosynthesis as well as transporter genes related to secondary metabolites in *Arabidopsis* (Bricchi et al. 2012) and potato (Alvarez et al. 2014), while *S. avenae* downregulated a lectin-like protein in wheat, previously inferred to have a role in resistance to Hessian fly (Ferry et al. 2011). Genes involved in nutrient transport such as certain sugar and nitrogen transporters may be target genes for silencing. Based on the imbalanced N/C ratio of phloem sap, nitrogen-related genes are possibly more important to target for reducing aphid performance than carbohydrate-related genes. Genes for turgor-regulating proteins, aquaporins offer another possibility for gene editing in order to indirectly reduce the aphid access to nutrients in the phloem.

11.6 Potential of Transgenic in Aphid Resistance Program

Insect pheromones also offer potential for management of aphids in wheat. Bruce et al. (2015) first developed transgenic wheat by deploying the genes responsible for the biosynthesis of alarm pheromones, (*E*)-β-farnesene (*E*βf), in the crop. It was achieved by using a synthetic gene based on a sequence from peppermint with a plastid targeting amino acid sequence, with or without a gene for biosynthesis of the

precursor farnesyl diphosphate. In laboratory, behavioral assays with these transgenic wheat plants, three cereal aphids species were repelled, while foraging of a parasitic natural enemy was increased. Although these studies show considerable potential for aphid control, field trials employing the single and double constructs showed no reduction in aphids or increase in parasitism of natural enemies. Apart from social acceptance in public, the impacts of climatic conditions, insect density, and inter- and intraspecific competition need further investigations for success of transgenic technology in wheat.

11.7 Conclusion and Future Prospects

There is a large variation of resistance traits in wild relatives of wheat and wheat landraces that can be successfully exploited in wheat breeding programs. However, the pre-breeding process is a crucial step in which efforts must be made before transferring resistance from less adapted germplasm. If aphid resistance is exclusively targeted, breeding would be more feasible and relatively easier to handle with small population sizes. However, this is usually not the case, and aphid resistance is considered as only one among several desired characteristics for its incorporation into cultivated wheat such as grain yield. Hence, ways to easily implement aphid resistance in wheat breeding programs are necessary, without sacrificing efficiency of breeding for other traits. Even though phenotyping (selection methods) for aphid resistance breeding can be a challenging issue, they can also be well fitted into the current wheat breeding methods, and advantage of new breeding technologies such as marker-assisted selection or genomic selection or RNAi needs to be incorporated in the breeding program.

References

Ahman I, Weibull J, Pettersson J (1985) The role of plant size and plant density for host finding in *Rhopalosiphum padi* (L.) (Hem.: Aphididae). Swed J Agric Res 15(1):19–24

Åhman I, Kim SY, Zhu LH (2019) Plant genes benefitting aphids-potential for exploitation in resistance breeding. Front Plant Sci 10:1452

Akhtar N, Moin N, Jilani G, Mohsin A, Yasmin S, Tashfeen A, Goraya M, Begum I (2009) Evaluation of resistance in wheat against *Rhopalosiphum padi* (l.) (homoptera: aphididae) under laboratory conditions. Pak J Agric Res 22(1–2):67–72

Akhtar N, Iqbal A, Javed HI, Khan J, Riaz M, Gillani WA, Mahmood T, Rasool A, Yasmin T (2013) Evaluation of recommended wheat varieties for resistance against *Schizaphis graminum* (rondani) (aphididae: homoptera) under laboratory conditions. Pak J Agric Res 26(4):321–327

Alvarez AE, D'Amato AMA, Tjallingii WF, Dicke M, Vosman B (2014) Response of Solanum tuberosum to Myzus persicae infestation at different stages of foliage maturity. Insect Sci 21:727–740. https://doi.org/10.1111/1744-7917.12072

Anonymous (2004) Progress report of all India Coordinated Wheat and Barley Improvement Project 2004–05, Project Director's Report. pp 1–66

Aradottir GI, Martin JL, Clark SJ, Pickett JA, Smart LE (2016) Searching for wheat resistance to aphids and wheat bulb fly in the historical Watkins and Gediflux wheat collections. Ann Appl Biol 70:179–188. https://doi.org/10.1111/aab.12326

Archetti M, Leather SR (2005) A test of the co-evolution theory of autumn colours: colour preference of *Rhopalosiphum padi* on *Prunus padus*. Oikos 110:339–343

Azhaguvel P, Rudd JC, Ma Y, Luo MC, Weng Y (2012) Fine genetic mapping of greenbug aphid-resistance gene Gb3 in *Aegilops tauschii*. Theor Appl Genet 124:555–564

Berzonsky WA, Ding H, Haley SD, Harris MO, Lamb RJ, McKenzie RIH, Ohm HW, Patterson FL, Peairs FB, Porter DR, Ratcliffe RH, Shanower TG (2003) Breeding wheat for resistance to insects. Plant Breed Rev 22:221–296

Birkett MA, Campbell CAM, Chamberlain K, Guerrieri E, Hick AJ, Martin JL, Matthes M, Napier JA, Pettersson J, Pickett JA, Poppy GM, Pow EM, Pye BJ, Smart LE, Wadhams GH, Wadhams LJ, Woodcock CM (2000) New roles for cis-jasmone as an insect semiochemical and in plant defense. Proc Natl Acad Sci USA 97(16):9329–9334

Blackman RL, Eastop VF (2007) Taxonomic issues. In: Van Emden HF et al (eds) Aphids as crop pests. CAB International, Oxford, pp 1–29

Boina D, Prabhakar S, Smith CM, Starkey S, Zhu L, Boyko E, Reese JC (2005) Categories of resistance to biotype I greenbugs (Homoptera: Aphididae) in wheat lines containing the greenbug resistance genes Gbx and Gby. J Kansas Entomol Soc 78:252–260

Boyko E, Starkey S, Smith M (2004) Molecular genetic mapping of Gby, a new greenbug resistance gene in bread wheat. Theor Appl Genet 109:1230–1236

Boyko EV, Smith CM, Thara VK, Bruno JM, Deng Y, Starkey SR, Klaahsen DL (2006) Molecular basis of plant gene expression during aphid invasion: wheat Pto- and Pti-like sequences are involved in interactions between wheat and Russian wheat aphid (Homoptera: Aphididae). J Econ Entomol 99(4):1430–1445

Bricchi I, Bertea CM, Occhipinti A, Paponov IA, Maffei ME (2012) Dynamics of membrane potential variation and gene expression induced by Spodoptera littoralis, *Myzus persicae*, and Pseudomonas syringae in Arabidopsis. PLoS One 7:e46673. https://doi.org/10.1371/journal.pone.0046673

Bruce TJA, Martin JL, Pickett JA, Pye BJ, Smart LE, Wadhams LJ (2003) cis Jasmone treatment induces resistance in wheat plants against the grain aphid, Sitobion avenae (Fabricius) (Homoptera: Aphididae). Pest Manag Sci 59(9):1031–1036

Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trends Plant Sci 10(6):269–274

Bruce TJA, Aradottir GI, Smart LE, Martin JL, Caulfield JC, Doherty A, Sparks CA, Woodcock CM, Birkett MA, Napier JA, Jones HD, Pickett JA (2015) The first crop plant genetically engineered to release an insect pheromone for defence. Sci Rep 5:118–112

Burd JD, Porter DR (2006) Biotypic diversity in greenbug (Hemiptera: Aphididae): characterizing new virulence and host associations. J Econ Entomol 99(3):959–965

Burd JD, Porter DR, Puterka GJ, Haley SD, Peairs FB (2006) Biotypic variation among north American Russian wheat aphid (Homoptera: Aphididae) populations. J Econ Entomol 99(5):1862–1866

Carson R (1962) The silent spring, 4th edn. Mariner Books, New York

Castro AM, Clua AA, Gimenez DO, Tocho E, Tacaliti MS, Collado M, Worland A, Bottini R, Snape JW (2007) Genetic resistance to greenbug is expressed with higher contents of proteins and non-structural carbohydrates in wheat substitution lines. In: Buck HT et al (eds) Wheat production in stressed environments. Springer, New York, pp 139–147

Crespo-Herrera LA, Smith CM, Singh RP, Åhman I (2013) Resistance to multiple cereal aphids in wheat–alien substitution and translocation lines. Arthropod Plant Interact 7:535–545

Crespo-Herrera LAA, Akhunov E, Garkava-Gustavsson L, Jordan KWW, Smith CMM, Singh RPP, Ahman I (2014) Mapping resistance to the bird cherry-oat aphid and the greenbug in wheat using sequence-based genotyping. Theor Appl Genet 127:1963–1973

Crespo-Herrera L, Singh RP, Reynolds M, Huerta-Espino J (2019a) Genetics of Greenbug resistance in synthetic Hexaploid wheat derived germplasm. Front Plant Sci 10:782

Crespo-Herrera LA, Singh RP, Sabraoui A, El-Bouhssini M (2019b) Resistance to insect pests in wheat—rye and *Aegilops speltoides* Tausch translocation and substitution lines. Euphytica 215:123. https://doi.org/10.1007/s10681-019-2449-7

Deol GS, Gill KS, Brar JS (1987) Aphid out break on wheat and barley in Punjab. Newslett Aphid Soc India 6:7–9

Dhaliwal GS, Jindal V, Dhawan AK (2010) Insect pest problems and crop losses: changing trends. Indian J Ecol 37:1–7

Di Pietro JP, Caillaud CM, Chaubet B, Pierre JS, Trottet M (1998) Variation in resistance to the grain aphid, *Sitobion avenae* (Sternorhynca: Aphididae), among diploid wheat genotypes: multivariate analysis of agronomic data. Plant Breed 117(5):407–412

Dinant S, Bonnemain JL, Girousse C, Kehr J (2010) Phloem sap intricacy and interplay with aphid feeding. C R Biol 333:504–515

Dixon AFG (1987) Cereal aphids as an applied problem. Agric Zool Rev 2:1–57

Doering TF, Chittka L (2007) Visual ecology of aphids-a critical review on the role of colours in host finding. Arthropod Plant Interact 1(1):3–16

Dunn BL, Carver BF, Baker CA, Porter DR (2007) Rapid phenotypic assessment of bird cherry-oat aphid resistance in winter wheat. Plant Breed 126(3):240–243

Ferry N, Stavroulakis S, Guan W, Davison GM, Bell HA, Weaver RJ et al (2011) Molecular interactions between wheat and cereal aphid (*Sitobion avenae*): analysis of changes to the wheat proteome. Proteomics 11:1985–2002

Foyer CH, Verrall SR, Hancock RD (2015) Systematic analysis of phloem-feeding insect-induced transcriptional reprogramming in Arabidopsis highlights common features and reveals distinct responses to specialist and generalist insects. J Exp Bot 66:495–512

Franzen LD, Gutsche AR, Heng-Moss TM, Higley LG, Macedo TB (2008) Physiological responses of wheat and barley to Russian wheat aphid, *Diuraphis noxia* (Mordvilko) and bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae). Arthropod-Plant Interact 2(4):227–235

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91(1):59–87

Gillot C (2005) Entomology. Springer, Dordrecht, The Netherlands

Girvin J, Whitworth RJ, Aguirre Rojas LM, Smith CM (2017) Resistance of select winter wheat (*Triticum aestivum*) cultivars to *Rhopalosiphum padi* (Hemiptera: Aphididae). J Econ Entomol 110(4):1886–1889

Givovich A, Niemeyer HM (1996) Role of hydroxamic acids in the resistance of wheat to the Russian wheat aphid, Diuraphis noxia (Mordvilko) (Hom, Aphididae). J Appl Entomol 120(9):537–539

Greenslade AFC, Ward JL, Martin JL, Corol DI, Clark SJ, Smart LE, Aradottir GI (2016) *Triticum monococcum* lines with distinct metabolic phenotypes and phloem-based partial resistance to the bird cherry–oat aphid *Rhopalosiphum padi*. Ann Appl Biol 168(3):435–449

Haley SD, Peairs FB, Walker CB, Rudolph JB, Randolph TL (2004) Occurrence of a new Russian wheat aphid biotype in Colorado. Crop Sci 44(5):1589–1592

Hardie J, Isaacs R, Pickett JA, Wadhams LJ, Woodcock CM (1994) Methyl salicylate and (—)-(1R,5S)-myrtenal are plant-derived repellents for black bean aphid, Aphis fabae Scop. (Homoptera: Aphididae). J Chem Ecol 20:2847–2855

Havens JN (1801) Observations on the Hessian fly. In: Charles R et al (eds) Transactions of the society for the promotion of agriculture, arts and manufactures, 2nd edn. Society for the Promotion of Useful Arts, New York, pp 71–86

Heng-Moss T, Ni X, Macedo T, Markwell JP, Baxendale FP, Quisenberry S, Tolmay V (2003) Comparison of chlorophyll and carotenoid concentrations among 30 Russian wheat aphid (Homoptera: Aphididae)-infested wheat isolines. J Econ Entomol 96(2):475–481

Hesler LS (2005) Resistance to *Rhopalosiphum padi* (Homoptera: Aphididae) in three triticale accessions. J Econ Entomol 98(2):603–610

Hesler LS, Tharp CI (2005) Antibiosis and antixenosis to *Rhopalosiphum padi* among triticale accessions. Euphytica 143(1–2):153–160

Hesler LS, Riedell WE, Kieckhefer RW, Haley SD, Collins RD (1999) Resistance to *Rhopalosiphum padi* (Homoptera: Aphididae) in wheat germplasm accessions. J Econ Entomol 92(5):1234–1238

Hesler LS, Haley SD, Nkongolo KK, Peairs FB (2007) Resistance to *Rhopalosiphum padi* (Homoptera: Aphididae) in triticale and triticale-derived wheat lines resistant to *Diuraphis noxia* (Homoptera: Aphididae). J Entomol Sci 42(2):217–227

Hu XS, Liu YJ, Wang YH, Wang Z, Xl Y (2016) Resistance of wheat accessions to the English grain aphid *Sitobion avenae*. PLoS One 11(6):e0156158. https://doi.org/10.1371/journal.pone.0156158

Iqbal M, Waqar H, Rehman HM, Aslam M, Jamil M (2018) Evaluation of different wheat varieties for resistance against aphid, *Schizaphis graminum* R. (Homoptera: Aphididae) under laboratory conditions. Asian J Agric Biol 6(4):549–555

Kieckhefer RW, Gellner JL (1992) Yield losses in winter-wheat caused by low-density cereal aphid populations. Agron J 84(2):180–183

Kieckhefer R, Kantack B (1980) Losses in yield in spring wheat in South Dakota caused by cereal aphids. J Econ Entomol 73(4):582–585

Kieckhefer RW, Dickmann DA, Miller EL (1976) Color responses of cereal aphids. Ann Entomol Soc Am 69(4):721–724

Kim W, Johnson JW, Baenziger PS, Lukaszewski AJ, Gaines CS (2004) Agronomic effect of wheat-rye translocation carrying rye chromatin (1R) from different sources. Crop Sci 44(4):1254–1258

Kloth KJ, Wiegers GL, Busscher-Lange J, van Haarst JC, Kruijer W, Bouwmeester HJ (2016) AtWRKY22 promotes susceptibility to aphids and modulates salicylic acid and jasmonic acid signalling. J Exp Bot 67:3383–3396

Lage J, Skovmand B, Andersen SB (2003) Characterization of greenbug (Homoptera: Aphididae) resistance in synthetic hexaploid wheats. J Econ Entomol 96(6):1922–1928

Lage J, Skovmand B, Andersen S (2004) Resistance categories of synthetic hexaploid wheats resistant to the Russian wheat aphid (*Diuraphis noxia*). Euphytica 136(3):291–296

Lapitan NLV, Peng J, Sharma V (2007) A high-density map and PCR markers for Russian wheat aphid resistance gene Dn7 on chromosome 1RS/1BL. Crop Sci 47(2):811–820

Leather SR, Dixon AFG (1984) Aphid growth and reproductive rates. Entomol Exp Appl 35(2):137–140

Leather SR, KFA Walters, Dixon AFG (1989) Factors determining the pest status of the bird cherry-oat aphid Rhopalosiphum padi (L.) (Homoptera: Aphididae), in Europe: a study and review. Bull Entomol Res 79:345Ð360

Li XQ, Guo XR, Li KB, Yin J, Cao YZ (2006) Resistance of wheat varieties (lines) to *Sitobion miscanthi* (Takahashi) (Aphidoidea: Aphididae). Acta Entomol Sin 49:963–968

Liu XM, Smith CM, Gill BS, Tolmay V (2001) Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. Theor Appl Genet 102(4):504–510

Liu XM, Smith CM, Gill BS (2002) Identification of microsatellite markers linked to Russian wheat aphid resistance genes Dn4 and Dn6. Theor Appl Genet 104(6–7):1042–1048

Liu XL, Yang XF, Wang CY, Wang YJ, Zhang H, Ji WQ (2011) Molecular mapping of resistance gene to English grain aphid (*Sitobion avenae* F.) in Triticum durum wheat line C273. Theor Appl Genet 124:1–7

Lu H, Rudd JC, Burd JD, Weng Y (2010) Molecular mapping of greenbug resistance genes Gb2 and Gb6 in T1AL.1RS wheat-rye translocations. Plant Breed 129(5):472–476

Ma ZQ, Saidi A, Quick JS, Lapitan NLV (1998) Genetic mapping of Russian wheat aphid resistance genes Dn2 and Dn4 in wheat. Genome 41(2):303–306

Macaulay M, Ramsay L, Åhman I (2020) Quantitative trait locus for resistance to the aphid Rhopalosiphum padi L. in barley (Hordeum vulgare L.) is not linked with a genomic region for gramine concentration. Arthropod-Plant Interact 14:57–65

Marais GF, Horn M, Dutoit F (1994) Intergeneric transfer (rye to wheat) of a gene(s) for Russian wheat aphid resistance. Plant Breed 113(4):265–271

Marimuthu M, Smith MC (2012) Barley tolerance of Russian wheat aphid (Hemiptera: Aphididae) biotype 2 herbivory involves expression of defense response and developmental genes. Plant Signal Behav 7(3):382–391

Mater Y, Baenziger S, Gill K, Graybosch R, Whitcher L, Baker C, Specht J, Dweikat I (2004) Linkage mapping of powdery mildew and greenbug resistance genes on recombinant IRS from 'Amigol' and 'Kavkaz' wheat-rye translocations of chromosome 1RS.1AL. Genome 47(2):292–298

Migui SM, Lamb RJ (2003) Patterns of resistance to three cereal aphids among wheats in the genus Triticum (Poaceae). Bull Entomol Res 93(4):323–333

Migui SM, Lamb RJ (2004) Seedling and adult plant resistance to Sitobion avenae (Hemiptera: Aphididae) in *Triticum monococcum* (Poaceae), an ancestor of wheat. Bull Entomol Res 94(1):35–46

Mir IA, Ahmad H, Sharma D, Ganai SA, Sharma S (2017) Screening for resistance in wheat against aphid *Sitobion avenae* F. Indian J Entomol 79(1):59–61

Moharramipour S, Tsumuki H, Sato K, Murata S, Kanehisa K (1997) Effects of leaf color, epicuticular wax and gramine content in barley hybrids on aphid populations. Appl Entomol Zool 32:1–8

Navabi Z, Shiran B, Assad MT (2004) Microsatellite mapping of a Russian wheat aphid resistance gene on chromosome 7B of an Iranian tetraploid wheat line: preliminary results. Cereal Res Commun 32(4):451–457

Ni X, Quisenberry SS (2000) Comparison of DIMBOA concentrations among wheat isolines and corresponding plant introduction lines. Entomol Exp Appl 96(3):275–279

Ni XZ, Quisenberry SS, Heng-Moss T, Markwell J, Higley L, Baxendale F, Sarath G, Klucas R (2002) Dynamic change in photosynthetic pigments and chlorophyll degradation elicited by cereal aphid feeding. Entomol Exp Appl 105(1):43–53

Nkongolo KK, Quick JS, Limin A, Fowler DB (1991) Sources and inheritance of resistance to Russian wheat aphid in *Triticum* species amphiploids and *Triticum tauschii*. Can J Plant Sci 71(3):703–708

Painter RH (1941) The economic value and biologic significance of insect resistance in plants. J Econ Entomol 34(3):358–367

Painter RH (1951) Insect resistance in crop plants. Macmillan, New York. http://dx.doi.org/10.1097/00010694-195112000-00015

Patil SD, Padhye AP, More PE, Kharbade SB, Dodak SS (2018) Varietal resistance in various wheat varieties against wheat aphid (*Rhopalosiphum padi L*). Chem Sci Rev Lett 7(25):146–152

Pereira RRC, Moraes JC, Prado E, Dacosta RR (2010) Resistance inducing agents on the biology and probing behaviour of the greenbug in wheat. Sci Agric 67(4):430–434

Pettersson J, Pickett J, Pye B, Quiroz A, Smart L, Wadhams L, Woodcock C (1994) Winter host component reduces colonization by bird-cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera, Aphididae), and other aphids in cereal fields. J Chem Ecol 20(10):2565–2574

Pettersson J, Tjallingii WF, Hardie J (2007) Host-plant selection and feeding. In: VanEmden HF et al (eds) Aphids as crop pests. CABI Publishing, Wallingford, UK, pp 87–113

Pickett JA, Glinwood RT (2007) Chemical ecology. In: VanEmden HFHR (ed) Aphids as crop pests. CABI Publishing, Wallingford, UK, pp 235–260

Porter DR, Webster JA, Friebe B (1994) Inheritance of greenbug biotype-G resistance in wheat. Crop Sci 34:625–628

Porter DR, Burd JD, Sufran KA, Webster JA, Teetes GL (1997) Greenbug (Homoptera: Aphididae) biotypes: selected by resistant cultivars or preadapted opportunists? J Econ Entomol 90:1055–1065

Porter DR, Burd JD, Shufran KA, Webster JA (2000) Efficacy of pyramiding greenbug (Homoptera: Aphididae) resistance genes in wheat. J Econ Entomol 93(4):1315–1318
Porter DR, Harris MO, Hesler LS, Puterka GJ (2009) Insects which challenge global wheat production. In: Carver BF (ed) Wheat science and trade. Wiley-Blackwell, Iowa, pp 189–201
Powell G, Hardie J (2001) The chemical ecology of aphid host alternation: how do return migrants find the primary host plant? Appl Entomol Zool 36(3):259–267
Powell G, Tosh CR, Hardie J (2006) Host plant selection byaphids: behavioral, evolutionary, and applied perspectives. Annu Rev Entomol 51:309–330
Rabbinge R, Drees EM, Van der Graaf M, Verberne FCM, Wesselo A (1981) Damage effects of cereal aphids in wheat. Neth J Plant Pathol 87:217–232
Reddy SK, Weng Y, Rudd JC, Akhunova A, Liu S (2013) Transcriptomics of induced defense responses to greenbug aphid feeding in near isogenic wheat lines. Plant Sci 212:26–36. https://doi.org/10.1016/j.plantsci.2013.08.002
Riedell WE, Kieckhefer RW, Langham MAC, Hesler LS (2003) Root and shoot responses to bird cherry oat aphids and barley yellow dwarf virus in spring wheat. Crop Sci 43:1380–1386
Rosenthal J, Kotanen P (1994) Terrestrial plant tolerance to herbivory. Trends Ecol Evol 9(4):145–148
Sandström J, Telang A, Moran NA (2000) Nutritional enhancement of host plants by aphids – a comparison of three aphid species on grasses. J Insect Physiol 46:33–40. https://doi.org/10.1016/S0022-1910(99)00098-0
Singh B, Deol GS (2003) Quantative grain yield losses caused by aphid complex in wheat. Crop Res 26(3):501–504
Singh B, Kaur J (2017) Compatibility of different pesticides used for aphid and stripe rust control in wheat. J Wheat Res 9(1):54–59
Singh B, Singh S (2009) Screening and identification of source of resistance against corn leaf aphid (*Rhopalosiphum maidis* Fitch.) in barley. Indian J Entomol 71(3):255–258
Singh B, Dhindsa GS, Singh H (2006) Screening of barley (*Hordeum vulgare*) germplasm against corn leaf aphid, *Rhopalosiphum maidis* (Fitch.). Crop Improv 33(1):58–61
Singh B, Kaur S, Chunneja P (2018) Evaluation of plant resistance in progenitors of wheat against foliage feeding aphids (*Rhopalosiphum padi* L. and *R. maidis* Fitch.) in Punjab. Agric Res J 55(1):117–121
Singh B, Simon A, Kirstie H, Kurup S, Clark S, Aradottir G (2020) Characterization of bird cherry-oat aphid (*Rhopalosiphum padi* L.) behaviour and aphid host preference in relation to partially resistant and susceptible wheat landraces. Ann Appl Biol. https://doi.org/10.1111/aab.12616
Smith CM (2005) Plant resistance to arthropods: molecular and conventional approaches. Springer, Aa Dordrecht, Netherlands
Smith CM, Starkey S (2003) Resistance to greenbug (Heteroptera:Aphididae) biotype I in *Aegilops tauschii* synthetic wheats. J Econ Entomol 96(5):1571–1576
Smith CM, Havlickova H, Starkey S, Gill BS, Holubec V (2004) Identification of Aegilops germplasm with multiple aphid resistance. Euphytica 135(3):265–273
Snelling RO (1941) Resistance of plants to insect attack. Bot Rev 7(10):543–586
Sotelo P, Starkey S, Voothuluru P, Wilde GE, Smith CM (2009) Resistance to Russian wheat aphid biotype 2 in CIMMYT synthetic hexaploid wheat lines. J Econ Entomol 102(3):1255–1261
Tan CT, Yu H, Yang Y, Xu X, Chen M, Rudd JC, Xue Q, Ibrahim AMH, Garza L, Wang S (2017) Development and validation of KASP markers for the greenbug resistance gene Gb7 and the Hessian fly resistance gene H32 in wheat. Theor Appl Genet 130:1867–1884
Telang A, Sandström J, Dyreson E, Moran NA (1999) Feeding damage by *Diuraphis noxia* results in a nutritionally enhanced phloem diet. Entomol Exp Appl 91(3):403–412
Thompson GA, Goggin FL (2006) Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. J Exp Bot 57:755–766. https://doi.org/10.1093/jxb/erj135
Tremblay C, Cloutier C, Comeau A (1989) Resistance to the bird cherry-oat aphid, *Rhopalosiphum padi* L. (Homoptera: Aphididae), in perennial Gramineae and wheat × perennial Gramineae hybrids. Environ Entomol 18(6):921–923

Triebe B, Mukai Y, Dhaliwal HS, Martin TJ, Gill BS (1991) Identification of alien chromatin specifying resistance to wheat streak mosaic and greenbug in wheat germ plasm by C-banding and in situ hybridization. Theor Appl Genet 81:381–389

Tyler JM, Webster JA, Merkle OG (1987) Designations for genes in wheat germplasm conferring greenbug resistance. Crop Sci 27:526–527

Voss TS, Kieckhefer RW, Fuller BW, McLeod MJ, Beck DA (1997) Yield losses in maturing spring wheat caused by cereal aphids (Homoptera: Aphididae) under laboratory conditions. J Econ Entomol 90(5):1346–1350

Walling LL (2008) Avoiding effective defenses: strategies employed by phloem feeding insects. Plant Physiol 146:859–866. https://doi.org/10.1104/pp.107.113142

Wang C, Luo K, Wang L, Zhao H-Y, Zhang G (2015) Molecular mapping of resistance gene to the English grain aphid, Sitobion avenae, in a Chinese wheat line XN98-10-35. Mol Breed. https://doi.org/10.1007/s11032-015-0395-1

Watt A (1979) The effect of cereal growth stages on the reproductive activity of Sitobion avenae and *Metopolophium dirhodum*. Ann Appl Biol 91(2):147–157

Webster J, Inayatullah C, Hamissou M, Mirkes K (1994) Leaf pubescence effects in wheat on yellow sugarcane aphids and greenbugs (Homoptera: Aphididae). J Econ Entomol 87(1):231–240

Weiland AA, Peairs FB, Ranpolph TL, Rudolph JB, Haley SD, Puterka GJ (2008) Biotypic diversity in Colorado Russian wheat aphid (Hemiptera: Aphididae) populations. J Econ Entomol 101(2):569–574

Weng Y, Li W, Devkota RN, Rudd JC (2005) Microsatellite markers associated with two Aegilops tauschii-derived greenbug resistance loci in wheat. Theor Appl Genet 110:462–469

Weng Y, Perumal A, Burd JD, Rudd JC (2010) Biotypic diversity in greenbug (Hemiptera: Aphididae): microsatellite-based regional divergence and host-adapted differentiation. J Econ Entomol 103(4):1454–1463

Xu X, Li G, Carver BF, Armstrong JS (2020) Gb8, a new gene conferring resistance to economically important greenbug biotypes in wheat. Theor Appl Genet 133:615–622

Zhong R, Cui D, Ye ZH (2019) Secondary cell wall biosynthesis. New Phytol 221:1703–1723. https://doi.org/10.1111/nph.15537

Zhu LC, Smith CM, Fritz A, Boyko EV, Flinn MB (2004) Genetic analysis and molecular mapping of a wheat gene conferring tolerance to the greenbug (*Schizaphis graminum* Rondani). Theor Appl Genet 109(2):289–293

Zhu LC, Smith CM, Fritz A, Boyko E, Voothuluru P, Gill BS (2005) Inheritance and molecular mapping of new greenbug resistance genes in wheat germplasms derived from *Aegilops tauschii*. Theor Appl Genet 111(5):831–837

Zhu-Salzman K, Salzman RA, Ahn JE, Koiwa H (2004) Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. Plant Physiol 134:420–431. https://doi.org/10.1104/pp.103.028324

Chapter 12
Concept of CRISPR-CAS9 Technology and Its Application in Crop Improvement Systems

Ravneet Kaur, Umesh Bharti, and Amarjit S. Tanda

Abbreviations

Cas	CRISPR-associated (Cas) proteins
CRISPR	Clustered regularly interspaced short palindromic repeats
SgRNA	Single guide RNA
TALEN	Transcription activator-like effector nuclease
ZFN	Zinc finger nuclease

12.1 Introduction

Insects comprise more than half of all living organisms on earth making up more than 58% of the known global biodiversity. They are known to inhabit diverse and extreme habitat types and play major role in the function and stability of terrestrial and aquatic ecosystems (Foottit and Adler 2009). They play pivotal ecological and evolutionary role in the world's ecosystems (Condamine et al. 2016). They are nutrient cyclers, pollinators, herbivores, and predators; maintain soil structure and fertility; play roles as natural enemies of other pest organisms and as biological control agents; are good food source for other organisms; and have evolved into a hyper-diverse fauna making them the world's most diverse group of animals (Grimaldi and Engel 2005).

Most insects are major agricultural pests such as *Pyrilla perpusilla*, diamond-back moth, cotton pink bollworm (*Pectinophora gossypiella*), red cotton bug, *Hieroglyphus banian*, etc. that feed on foliage and damage crops, with an estimated loss of global crop production by 10–16% (Chakraborty and Newton 2011). Insects also act as vectors of several human diseases, for example, *Anopheles* species are vectors of malaria, an infectious and deadly disease threatening nearly half of the

R. Kaur (✉) · U. Bharti
Department of Zoology, Post Graduate Govt. College for Girls, Chandigarh, India

A. S. Tanda
School of Horticulture, University of Western Sydney, Richmond, NSW, Australia

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_12

world's population (World Health Organization 2014). Some insects are also beneficial economically such as silkworm, lac insect, and honeybees that are also important to human society. Social insects (specially honeybees) are ideal for studying social behavior. Considering the important roles of insects in ecology and human life, insect research has gained great biological and practical significance. Nowadays various genome sequencing technologies are being used to decode the genomes of many insects. This technology has published genomes of model organisms (*Drosophila*), has now also covered non-drosophilid insects like moths, butterflies, beetles, and various Hymenopteran including social insects. The information not only provide the raw material for further comparative and evolutionary genomic studies but additionally achieve identification of several unknown genes and their functions through recently emerged genome altering technologies. This has brought about advancements in the hereditary control of model and non-model organisms. Traditional gene knockin and knockout experiments in insects were initially limited to transposon-based transgenic innovations, while the use of gene knockouts was confined to *Drosophila* species only. However, recent genome editing approaches such as ZFN, TALEN, and CRISPR/Cas9 have permitted proficient gene alteration of non-model organisms and have additionally opened the functional testing of domesticated genes, as well as target genes in life environment interactions.

CRISPR/Cas9 promisingly affects transformative and environmental exploration by clarifying the gap between DNA arrangements and phenotypic expression. In this manner, the blend of relative genomics and CRISPR/Cas9 innovation is an integral asset for finding novel genes and uncovering the behavioral mechanisms of non-model organisms. Recently, gene editing technology has advanced CRISPR/Cas9-mediated methodology and is being utilized in many insects. This is another, ecological amicable and promising instrument for control of pests in the fields. Some of the butterflies of major lepidopteran families (Nymphalidae, Danaidae, Papilionidae) have been a decent model to study insect genetics, and CRISPR/Cas9-mediated technique has also been utilized to analyze various phenotypic expressions and insect behaviors. The CRISPR/Cas9 framework provides a more in-depth and stable strategy for examining functional genes. The procedure is an advancement over other gene editing tools in different insect species. Therefore, the chapter is an attempt to examine different techniques that have been used by means of CRISPR/Cas9 system in insects and its application in future insect studies.

12.2 The CRISPR-Cas9 Biotechnology

CRISPR known as clustered regularly interspaced short palindromic repeats of genetic information is used by bacterial species as a part of their natural antiviral system. Jinek et al. (2012) explored this system to be used as a gene editing tool. CRISPR/Cas9 system edits and modifies genes by precise cutting of DNA and allows natural DNA repair systems to take over. This system consists of two parts: the Cas9 enzyme and a guide RNA.

- Cas9: It is a CRISPR-associated (Cas) endonuclease that acts as molecular scissors to splice DNA at a precise location that is specified by a guide RNA.
- Guide RNA (gRNA): It is a type of RNA molecule that binds to Cas9. The specification of the binding is based on the sequence of the gRNA, at which Cas9 will cut DNA.

12.3 Stages of Modern Biotechnique Development

Until recently, genetic manipulations have been done by inserting desired DNA using transposons into fly embryos. However, recently improvements have been made in these transgenic techniques, particularly with respect to integration of DNA at specific sites in the genome. According to the history of technologies, the modern technologies are classified into three stages of development on the basis their function, mechanism, efficiency, and accuracy.

12.3.1 Stage 1

The first stage was transposon-based transgenesis: Rubin and Spradling (1982) effectively made an initial endeavor to transfer exogenous genes into *Drosophila melanogaster* through P-element intervened transposon. Nonetheless, due to host explicitness for co-factors, the P-element could not be applied in non-drosophilid insects (Rio and Rubin 1988). Later, four distinct transposons were investigated for heredity controls of non-drosophilid insects. These transposons were *mariner*, *Minos*, *Hermes*, and *piggyBac*. Out of these, the common vector *piggyBac* has been successfully utilized and applied in the transfer of genes in numerous insect species. Tamura et al. (2000) developed a framework for stable transgenesis in the silkworm *Bombyx mori* L. utilizing a transposon, *piggyBac*. The DNA investigations of GFP-positive G1 stage silkworms showed successive and numerous independent additions, and this transgene was moved to the next generation in a steady form through Mendelian inheritance. The presence of the characteristic TTAA sequence and inverted terminal repeats of *piggyBac* at the restrictions of all the studied inserts set up the precise transposition events. Marcus et al. (2004) reported the effective germline change with two distinctive transposable component vectors, *Hermes* and *piggyBac*, each conveying *EGFP* coding sequences driven by the *3XP3* synthetic enhancer that drives gene expression in the eyes of the African satyrid butterfly *Bicyclus anynana*. The transformation rates accounted for 5% in *piggyBac gene* and 10.2% in *Hermes gene*. This new generated information permitted the study of developmental genetics of diversity of color patterns on the wings of butterflies. Martins et al. (2012) dealt with the germline of diamond back moth, *Plutella xylostella* (one of the most economically important agricultural pests) whose larvae harm the foliage of cruciferous vegetables like cabbage, broccoli, cauliflower, etc.

by voracious feeding. It is typically constrained by *synthetic substances* (*pesticides*); however, it is notable for its rapid advancement of resistance from pesticides. Biological control has not been successful against this pest. The study reported the first germline change of the diamondback moth, using transposable element (*piggyBac*) by means of embryo microinjection. The experiment generated four distinct constructs with the mutation rates as 0.48–0.68%. Venken and Bellen (2007) dealt with *Drosophila melanogaster* for the investigation of different biological enquiries related to development, neuroscience, genetics, cell biology, and disease. The transposons have remained insufficient in their viability, due to its random integration, low transformation frequency, unstable coordinated sequence, and limited carrying capacity, due to which the transgenesis technologies have been updated that have grouped GAL4/UAS framework as well as site-specific recombinases and integrases like *Cre*, *FLP*, and *ΦC31*.

12.3.2 Stage 2

Bibikova et al. (2003) and Bogdanove and Voytas (2011) classified the second advancement stage of gene editing technology by including zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) that are made of a particular DNA recognition protein and DNA excision protein (*FokI*). This extended the genetic modifications past model organisms. ZFN and TALEN are known to improve the productivity, adequacy, and precise modification of target genes as compared to transposon-based technologies, and these have been successfully applied in *Drosophila* (Gratz et al. 2013). Wang et al. (2013) examined ZFN- and TALEN-mediated gene mutagenesis in *Bombyx mori* that focused on *BmBLOS2 which* is a homolog of the human biogenesis of lysosome-related organelles complex 1 (subunit 2 gene), which prompts synthesis of urate granules in larval epidermis of this moth.

The deficiency of *BmBLOS2* gene brought about the change of the larval integument from opaque to clear, producing oily skin phenotype (OSP). Thus, *BmBLOS2* exemplified a brilliant target gene for testing the development of novel genome engineering strategies. The authors utilized the Cas9-sgRNA framework to target *BmBLOS2* in *Bombyx mori*. They screened the *BmBLOS2* open reading frame (ORF) and recognized two 23-bp sgRNA-targeting sites (S1 and S2). Each sgRNA was independently blended with Cas9 mRNA and infused into the preblastodermal embryos. The result was mosaic development with lustrous integument phenotype in most of the larvae. The mutant frequency was 95.6% (152 for sgRNA-1) and 94.0% (94 for sgRNA-2). Ninety-five percent of the mutants with severe oily skin phenotype were recorded. The genomic DNA with severe oily skin phenotype was extracted from the second instar larvae, and fragments spanning the S1 and S2 targeting sites were separated and amplified by PCR and sequenced.

The induction by Cas9-sgRNA system was like the outcomes acquired by ZFN and TALEN technique. The insertions and deletions were acquired at the target site

due to non-homologous end-joining (NHEJ) repair method. This trial demonstrated the effective use of Cas9-sgRNA system to initiate targeted mutagenesis in *B. mori*. Dong et al. (2015) worked on *Aedes aegypti* that described the use of CRISPR/Cas9 technique to disrupt a gene-of-interest. They tested the efficacy of the CRISPR/Cas9 framework in a transgenic mosquito line that expressed two distinct eye markers, which permitted them to take advantage of a simple visual screening system for knockout mutant. They also tested different CRISPR/Cas9 constructs to disrupt the coding sequence of the enhanced cyan fluorescent protein (ECFP) gene in these transgenic mosquito lines that expressed both ECFP and Dsred under eye-specific 3xP3 promoter. They also successfully disrupted the marker gene that demonstrated the functionality of CRISPR/Cas9 system as a genetic tool for targeted gene disruption in *Aedes aegypti*. However, zinc finger nuclease (ZNF) has certain inherent drawbacks that have confined their wide utility as they are costly and hard to assemble and are restricted to target sites due to the three-nucleotide recognition mode, while the system with TALEN involves cumbersome methodology and a large protein that is difficult to efficiently deliver into all cells.

12.3.3 Stage 3

The third advancement of this innovation has been the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 system, and it is the most promising stage of development in genome editing. Nishimasu et al. (2014) recommended the mechanism of CRISPR/Cas9 system via ribonucleoprotein (RNP) complex, where the target recognition lobe of Cas9 guides specific binding of target DNA by interacting with homologous sgRNA causing the splicing of the target DNA. It is viewed as a progressive innovation with uncommon viability, effectiveness, accuracy, applicability, and relevance for a variety of species. Various researchers (Gaj et al. 2013; Hsu et al. 2014; Shalem et al. 2015) have reported its application, assessment, and advancement over other known gene editing tools. Gratz et al. (2013) reported the first knockout application of CRISPR/Cas9 in *Drosophila melanogaster*. The mutations were introduced in the *yellow* gene of the fly. Depending on the delivery methods of Cas9 and sgRNA, the researchers have proposed four strategies to set up CRISPR/Cas9 procedure in *Drosophila*. The initial step is to develop Cas9 and sgRNA into vectors (plasmids). Two expression plasmids were constructed with Cas9 protein regulated by *Hsp70* promoter and sgRNA by a U6 promoter, respectively. The proficiency of mutagenesis was found to be very low (5.9%) due to inefficient and error-prone non-homologous end-joining (NHEJ) recombination. Gokcezade et al. (2014) suggested merger of Cas9 and sgRNA into one single vector instead of two, which increased mutagenesis rate to more than 10%. Bassett et al. (2013) and Yu et al. (2013) adopted another strategy in which transcribed Cas9 mRNA and sgRNA were co-injected into early-stage embryos in vitro. The results indicated more than 80% of injected flies with mosaic expression of the *yellow*

gene, and another six genes showed 36–80% mutagenesis that showed increased efficiency.

Bassett et al. (2013) suggested that this high mutation rate was related to high concentrations of Cas9/sgRNA, resulting in an adult survival rate of less than 3%. Conflicting this, the reduction in concentrations of Cas9/sgRNA mRNA resulted in the unusual decrease in the proportion of mosaic adults from 86% to 10%. Kondo and Ueda (2013) set up the third strategy in which they crossed the two strains of transgenic fly, one expressed Cas9 protein specifically in germline cells (driven by the nanos promoter) and the other ubiquitously expressed sgRNA (regulated by U6 promoter). The system showed an expansion in mutagenesis rate from 57% to 90% with high proficiency as compared to other two strategies, but it had a restriction of the time spent in establishing sgRNA transgenic fly stocks.

12.3.4 Stage 4

The fourth strategy involved the insertion of sgRNA expression vectors into early-stage embryos of germline expressing Cas9 transgenic strains. This methodology reduced the complexity of the two-component injection scheme to one component. The earlier studies used different promoters to construct Cas9 transgenic strains either driven by promotor vasa (Sebo et al. 2014) or by promotor nanos (Ren et al. 2013). Sebo et al. (2014) observed a high mutagenesis rate of 71% in injected flies harboring gene mutations, but in their studies significant proportion of 68% mutants was infertile. Studies by Ren et al. (2013) showed a higher mutagenic rate of 93.3% and fertility rate up to 81% in G0 stage flies. Port et al. (2014) used different *U6* promoters to drive sgRNA in which *U6*: 3 showed strongest ability to induce mutagenesis in germ cells and somatic cells. Xue et al. (2014) used a new promoter (*CR7T* promoter) to drive sgRNA efficiently and to accomplish high transformation rates.

12.3.5 Other Strategic Biotechniques

CRISPR/Cas9-mediated knockin procedures have expanded the CRISPR technique by modifying complex genomes of organisms. Exogenous DNA can be incorporated precisely via homology-directed repair (HR) through donor DNA templates. Port et al. (2014) suggested the successful injection of donor DNA into transgenic embryos harboring both Cas9 and sgRNA cascade while insertion of donor DNA combined with sgRNA-encoding plasmids into transgenic Cas9 embryos (Gratz et al. 2014; Ren et al. 2013; Xue et al. 2014; Zhang et al. 2014). Gokcezade et al. (2014) reported the insertion of donor plasmids along with a plasmid encoding Cas9 and sgRNA into non-transgenic embryos. Yu et al. (2013) also targeted the *yellow* gene and demonstrated increased efficiency. CRISPR/Cas9 technology was also

used to introduce site-specific mutations into *white* (*w*) and *Sex lethal* (*Sxl*) genes of *Drosophila suzukii*. The mutant phenotype of white eyes was obtained at low efficiency because the flies were injected with plasmid DNA encoding Cas9 and the sgRNA other than mRNA. It may also have been due to the specificity of the *white* gene and *Drosophila* species. Li and Scott (2016) revealed that mutant flies for the *Sxl* gene resulted in abnormal genitalia and reproductive tissues in female individuals. Another study revealed the role of succinyl-CoA synthetase/ligase associated with metabolism in *Drosophila*. Quan et al. (2017) generated a mutation in the alpha subunit of SCS enzyme (*Scs*α) using the CRISPR/Cas9 technique and observed that *Scs*α-deficient individuals exhibited abnormalities in developmental patterns, reduced locomotor activity, and increased mortality rate under starvation conditions. Asaoka et al. (2016) utilized the CRISPR/Cas9 system to establish linear ubiquitin E3 ligase-deficient flies, which revealed reduced survival rate and defective climbing in response to heat. A sex-specific gene was also studied in *Drosophila* using CRISPR/Cas9 system. The transcriptional activity of the male X chromosome is equivalent to two female X chromosomes, the male-specific lethal (MSL) complex is recruited to regions of the X chromosome, and this is dependent on the chromatin-linked adapter for MSL proteins (CLAMP) zinc finger protein. Urban et al. (2016) created mutations in the *clamp* gene in flies and found different mortality rates of *clamp* null males and females at different developmental stages utilizing CRISPR/Cas9 system. Chechenova et al. (2017) observed the association of troponin C (TpnC) gene with muscle formation using CRISPR/Cas9 technology in *Drosophila*. Mendoza-Garcia et al. (2017) reported the use of CRISPR/Cas9 technology in mutant transcription factor binding sites in enhancer regions of the *Alk* locus in *Drosophila*, thereby mediating the expression of the *Alk* gene. This revealed that the expression of a transcription factor was also altered in a sex-specific manner.

Xia et al. (2014) suggested the progress of functional genomics research in *Bombyx mori* after the launch and achievement of the silkworm genome project. In Lepidoptera, there are many other agricultural pests of crops and vegetables that are quite devastating. *Bombyx mori* is the first agricultural lepidopteran that has provided the efficacy of CRISPR/Cas9 as a genome editing tool for functional genes. The frequent use of chemical pesticides for pest management has developed high resistance in the major lepidopteran pests which had made them more difficult to control. Some of the examples of lepidopteran pests include *Helicoverpa armigera*, *Plutella xylostella*, *Spodoptera littoralis*, and *Spodoptera litura*. *Bombyx mori* is an important, economically beneficial species as well as a good model organism to study a function of a gene in vitro. Daimon et al. (2014) suggested that out of all the known genome editing tools (ZFNs, TALENs), CRISPR/Cas9 is the most convenient and effective method to verify the application of CRISPR/Cas9 (Wang et al. 2013). When this gene was mutated, the larval integument is changed from opaque to translucent. CRISPR/Cas9 system also enables the production of multiple-gene mutations and large fragment simultaneously in *B. mori* (Ma et al. 2014). Wei et al. (2014) studied the screening of a notable homozygous mutant phenotype of the *Bm-ok* gene and the mutation efficiency via CRISPR/Cas9 technology. Yamaguchi et al. (2013) studied the role of *Wnt1* gene in the formation of spot patterns; Yamaguchi

et al. (2011) studied its role in formation of normal abdominal segments in *B. mori*. Thus, this *Wnt1* signaling pathway is significant in embryonic development in insects. Zhang et al. (2015) manipulated the *Wnt1* signaling pathway via CRISPR/Cas9 system by screening the *BmWnt1* gene and observed knockout mutants for this gene that exhibited defects in body segmentation and pigmentation in a dose-dependent manner, and it was also revealed that the Hox homologous genes are downregulated in *BmWnt1*-deficient individuals. Liu et al. (2017a, b) utilized CRISPR/Cas9 system to observe the knockout mutants for *BmOrco* gene that showed the disruption of olfactory system. The homozygous mutants for this gene were not able to respond to sex pheromones. Some studies have also suggested that CRISPR/Cas9 system can be used to integrate donor DNA into the genome of *Bombyx mori*. The absence of NHEJ-related factors (*BmKu70*, *BmKu80*, *BmLigIV*, *BmXLF*, and *BmXRCC4*) is known to increase homologous recombination efficiency mediated by CRISPR/Cas9 in *B. mori* (Ma et al. 2014; Zhu et al. 2015). Wei et al. (2014) worked on four more genes (Bm-ok, BmKMO, BmTH, and Bmtan) by the direct microinjection of specific sgRNA and Cas9 mRNA into embryos, with mutation frequencies of 16.7–35.0%. Ma et al. (2014) set up an arrangement of two articulation vectors for Cas9 and sgRNA independently, creating the heritable site-coordinated release of Bmku70 in *B. mori.* They suggested the expansion of homologous recombination recurrence in Bmku70 knockout *Bombyx mori.* Liu et al. (2014) utilized this framework to activate multiplex genome altering of six qualities in BmNs cell lines all the while. Also, Zhu et al. (2015) took out the components Ku70, Ku80, Lig IV, XRCC4, and XLF in non-homologous end-joining utilizing the CRISPR/Cas9 framework, which expanded the exercises of homologous recombination up to seven times in silkworm cells. Further endeavors have additionally been made to improve the CRISPR/Cas9 system (Zeng et al. 2016). A *U6* promoter from *Bombyx mori* appeared to successfully drive sgRNA initiated with nucleotide bases to induce mutations in vitro and in vivo. Ling et al. (2015) applied CRISPR/Cas9 framework in gene functional analysis of the miR-2 cluster by knockout of miR-2 targeted *Bmawd* and *Bmfng* genes in silkworm. Zhang et al. (2015) determined the functional analysis of the BmWnt1 gene using the CRISPR/Cas9 system that generated a large deletion of 18 kb, resulting in severe developmental defects during embryogenesis as compared to previously reported interference RNA studies.

Li et al. (2015) studied the somatic mutation *BmEO* employing transgenic CRISPR/Cas9 system and expanded the duration of the final instar larval stage of silkworm. CRISPR/Cas9 in combination with Gal4/UAS overexpression and RNA-seq analysis provided insights into *BmEO* ecdysone hormonal regulation of tissue degeneration during metamorphosis. Xin et al. (2015) studied the germline mutation of *Bmsage* gene using the Cas9/sgRNA system that resulted in poorly developed silk glands and absence of middle and posterior silk glands. The outcomes inferred the job of *Bmsage* gene in the development of these glands at the embryonic stage.

12.4 CRISPR/Cas9 as a Therapeutic Technology (Gene Therapy)

CRISPR/Cas9 technology has also given fruitful results in gene therapy. There is no approach to eliminate a viral genome in insects. However some investigations have accomplished this by disrupting a viral gene such as HIV-1 or hepatitis B genes (Ebina et al. 2013; Seeger and Sohn 2014; Liao et al. 2015; Pellagatti et al. 2015). Dong et al. (2016) used *Bombyx mori* as the host of *BmNPV* to construct a virus-induced CRISPR/Cas9 system in which the viral infection activated the Cas9 nuclease. Chen et al. (2017) targeted and introduced mutations in two genes (*BmNPV ie-1* and *me53*). This technique has proved to be significant in improvisation of modern sericulture and has opened new avenues for antiviral therapies. Zhang et al. (2017a, b) utilized the information on the mutants created by loss of function of Juvenile hormone (JH) and 20-hydroxyecdysone (20E) in *Bombyx mori* using the CRISPR/Cas9 system. These two genes are responsible for insect growth and development and extend the larval stage to increase silk production.

Spodoptera litura is a cosmopolitan, an omnivorous, and a destructive pest. Bi et al. (2016) targeted *Slabd-A* gene in *Spodoptera litura* by utilizing CRISPR/Cas9 system and obtained *Slabd-A*-deficient mutants that exhibited abnormal body segmentation and pigmentation. Huang et al. (2016) knocked out the same gene (*abd-A*) in *Plutella xylostella* in which the eggs were injected with Cas9 mRNA using CRISPR/Cas9 method that resulted in 91% transformation for *Pxabd-A* gene in G0 stage. The results suggested an essential role of *Pxabd-A* gene in promoting segmentation and gonad development in *Plutella xylostella*.

12.5 CRISPR/Cas9 in Pest Management

12.5.1 Control of Lepidopteran Pests (Butterflies and Moths)

Bt transgenic crops and *Bacillus thuringiensis* (Bt) insecticides are broadly utilized for the control of agricultural pests (Bravo et al. 2011). The defense against these Bt toxins has evolved in insects and threatened the process of pest management. RNA interference and other genetic manipulation experiments have proposed that cadherin is involved in Cry1Ac resistance in several lepidopteran pests. Wu (2014) recognized a cadherin-like receptor of the Bt Cry1A toxin in some Lepidoptera insects. This receptor is known to interact with Cry1Ab protoxin, leading to proteolytic cleavage and formation of a pre-pore oligomeric structure in *Manduca sexta* (Gómez et al. 2002). Wang et al. (2016) infused a mixture of *Cas*9 mRNA and an sgRNA into the eggs of *Helicoverpa armigera* and targeted the ninth exon of the cadherin gene. CRISPR/Cas9 tool created *HaCad* gene mutant individuals, which displayed 549 times higher resistance to Cry1Ac compared with a control strain.

The outcome suggested that *HaCad* is a key receptor for Cry1Ac and is responsible for Cry1Ac resistance.

Khan et al. (2017) used this system to mutate four pigment genes (*white*, *brown*, *scarlet*, and *ok*) in *Helicoverpa armigera* that caused various physiological phenotypic expressions. *Dendrolimus punctatus* (pine caterpillar moth), a devastating forest pest prevalent in China and Southeast Asia, induces severe defoliation and reduced resin production. Liu et al. (2017a) suggested the use of CRISPR/Cas9 technology to manipulate the genome of this pest and provided an effective strategy for its management. The study targeted *Wnt-1* gene to modify gene expression in *Dendrolimus punctatus* in a precise and an efficient way. They noticed multiple mutant phenotypes such as distorted head, abnormal posterior segmentation, defective legs, etc. that confirmed the role of *Wnt-1* gene being associated with segmentation and development. Monteiro (2015) observed the evolutionary mechanism of transformation of function of eye spots on wings of nymphalid butterflies from ventral hindwing to dorsal wing surfaces. This behavior is responsible for predation, courtship, and sexual dimorphism in these butterflies. Reed and Serfas (2004) and Oliver et al. (2012) proposed that this eyespot pattern is directed by a progression of homologous genes (*Spalt*, *North*, *Dll*, and *en*) that regulates through a single origin of expression. Zhang and Reed (2016) used CRISPR/Cas9 framework to study these eyespot color patterns in two nymphalid butterflies *Vanessa cardui* and *Junonia coenia*. The study uncovered that transcription factor genes (*Spalt* and *Distal-less* (*Dll*)) help in promoting and repressing the formation of eyespot development, respectively. Li et al. (2016) used CRISPR/Cas9 to modify the genome of *Papilio machaon* in which four *Abd-B* genes were targeted and sgRNA/Cas9 mixtures were injected into fresh eggs of *P. machaon*. The mutants obtained showed variant phenotypes with four pairs of extra prolegs from abdominal segments A7–A9, which were absent in wild-type individuals. This exploration provided a valuable genomic and genetic innovation for examining butterflies and other insects. Before long, Zhang et al. (2017a, b) recognized eight additional genes associated with melanin pigmentation in four butterfly species and noticed knockout mutants for these genes to manipulate their function using the CRISPR/Cas9 system. Li et al. (2015) observed mutations (in somatic cells) in *Abd-B* gene with high rate (92.5%) in swallowtail butterfly, *Papilio Xuthus*. Later, a highly efficient, heritable gene knockout at two clock gene loci, *cry2* and *clk*, was reported in the monarch butterfly, *Danaus plexippus*, by Markert et al. (2016) in which 50% of larvae presented mutation rates ranging from 3% to 28%. The knockout for *clk* gene characterized its critical role during migration of these monarch butterflies that encoded a transcriptional activator of the circadian clock.

12.5.2 Control of Coleopteran Pests (Beetles)

Tribolium castaneum (the red flour beetle) is a major coleopteran pest of stored grains that feeds on maize, wheat, and rice and brings the nutritional value of food to its lowest quality. Moreover, their internal secretions contain the cancer-causing chemicals such as benzoquinone (carcinogen). This pest is normally controlled with traditional fumigation methods. The advent of a *T. castaneum* genomic database has urged scientists to eradicate it through genetic-based models (Tribolium Genome Sequencing Consortium 2008; Kim et al. 2010) that could further improve pest management and also reduce the damage to the environment. Analysts have used various techniques like transgenesis (Pavlopoulos et al. 2004; Berghammer et al. 2009), RNA interference technology (Gilles and Averof 2014), heat shock-mediated mis-expression of genes (Schinko et al. 2012), the GAL4/UAS system (Schinko et al. 2010), and the targeting of compensation mechanisms that are activated after disruption (Perkin et al. 2017). Gilles et al. (2015) used the CRISPR/Cas9 system in *T. castaneum* and revealed that mutations in the *E-cadherin* gene caused severe defects in dorsal closure.

12.5.3 Order Hymenoptera (Bees, Ants, Wasps)

The order Hymenoptera (bees, ants, wasps, sawflies) is the third largest order of insects after Coleoptera and Lepidoptera (Davis et al. 2010). Most hymenopterans are pollinators or natural enemies as predator of pests. *Nasonia vitripennis* (the parasitoid wasp) is a natural enemy of various pests and an ideal insect model for experimental studies. Li et al. (2017) utilized CRISPR/Cas9 system by targeting the eye pigmentation gene *cinnabar* in *Nasonia vitripenni* by injecting sgRNA and Cas9 mixtures into eggs that resulted in effective and heritable mutants. These findings helped to study haplo-diploid sex determination, axis pattern formation, and other biological behaviors in *N. vitripennis*.

12.5.4 Order Diptera (Mosquitoes, True Flies)

Among dipterans, mosquitoes are vectors of viruses causing severe diseases in humans, such as Zika, malaria, dengue, chikungunya, and filariasis (Gabrieli et al. 2014; Reegan et al. 2017). Numerous engineered synthetic insecticides have been used to control these vectors for decades. These insecticides affect the natural environment (Bayen 2012) and have induced resistance in vector mosquitoes (Tikar et al. 2009). *Aedes aegypti* is a significant vector for important arboviruses such as yellow fever, dengue, and chikungunya viruses, which cause critical health impacts on humans. Dong et al. (2015) depicted the use of CRISPR/Cas9 system in *Aedes*

aegypti in which the target gene was disrupted, i.e., Dsred and enhanced cyan fluorescent protein (ECFP) from the eye tissue-specific 3xP3 promoter which is a powerful tool to study gene function in ECFP transgenic *Aedes aegypti* lines. They obtained 5.5% knockout efficiency by injection in vitro transcribed Cas9 mRNA and sgRNA into mutant embryos.

Kistler et al. (2015) expanded the CRISPR/Cas9 mutation rate up to 24% by distinguishing active sgRNAs and proper Cas9 mRNA concentrations in five *Aedes aegypti* genes. Basu et al. (2015) assessed a large group of sgRNAs in early embryos that promoted the viability of editing in *Aedes aegypti* up to 90%. Basu et al. (2015) additionally obtained a transformation rate of 2.1% through homologous recombination-based incorporation of a transgene in *A. aegypti* using the CRISPR/Cas9 system. Since only the female mosquitoes feed on blood and transmit pathogens (Papathanos et al. 2009), converting female mosquitoes into harmless male mosquitoes is a new promise as a new vector control management strategy. Male determination in *Aedes aegypti* is controlled by the M factor, a dominant male-determining factor on the Y chromosome in the M locus. Hall et al. (2015) utilized the CRISPR/Cas9 system in the functional investigation of *Nix* gene, the first insect M factor. Knockout of *Nix* gene resulted in largely feminized genetic males, providing an opportunity to depict gene interactions in the sex-determination pathway of *A. aegypti*.

Weaver and Barrett (2004) attempted to comprehend the rearing mechanism to control the *Aedes aegypti* population. Aryan et al. (2013) and Smidler et al. (2013) recommended in vivo targeting genes of interest in mosquito using genome editing tools (ZFNs and TALENs). Zhang et al. (2016) utilized CRISPR/Cas9 system to study the functional verification of a microRNA by targeting a microRNA-309 gene. The mutants resulted in a loss of functioning of ovaries, significant decrease in follicle number, reduction in growth, and a reduced rate of egg hatching in mutant *lines of Aedes aegypti.* The homeobox 4 (*SIX4*) gene was identified as a direct target of miR-309. Hammond et al. (2016) utilized CRISPR/Cas9 technology and revealed three genes related to a female-sterility phenotype in malarial vector *Anopheles gambiae*. Itokawa et al. (2016) examined the connection between detoxifying enzymes and insecticide resistance in *Culex quinquefasciatus* through the CRISPR/Cas9 technique in a resistant strain of this mosquito. The target gene was cytochrome P450 gene *CYP9M10*, and the individuals lacking functional *CYP9M10* copy displayed an approximate 110-fold decrease in permethrin resistance, suggesting that *CYP9M10* to be an essential factor related with pyrethroid resistance. By and large, these examinations showed that the CRISPR/Cas9-mediated tool can be utilized adequately to modify the insect genome and possibly to control the population and disease transmission. The biosafety dangers of this innovation related to the release of CRISPR/Cas9-edited insects into the environment should be considered when selecting the most suitable, environmentally friendly method of implementing the gene driven framework (Esvelt et al. 2014; Oye et al. 2014; Alphey 2016; Champer et al. 2016; Taning et al. 2017). The CRISPR/Cas9 system has shown extraordinary potential within the genetic editing of non-model species. CRISPR/

Cas9 has additionally been used in functional characterization of the SlitPBP3 gene in *S. litura* (Zhu et al. 2016). Besides, the *Dop1* gene was knocked out in non-model *Gryllus bimaculatus* utilizing the CRISPR/Cas9 system that resulted in mutants defective in aversive learning with sodium chloride punishment, but not appetitive learning with water or sucrose reward (Awata et al. 2015).

12.6 Future Challenges

The CRISPR/Cas9 system has become a revolutionary tool for gene manipulation in both prokaryotic and eukaryotic genetics. It has established, developed, and shown a great potential as an efficient editing tool in model (*Drosophila* sp. and *Bombyx mori*) and non-model insects (butterfly, mosquito, and beetle). The system works efficiently and successfully in knockin, knockout, deletion, and insertion experiments. This has allowed to study the functionality of some very important genes, regulatory elements, genome tracking systems, etc. Based on CRISPR/Cas9 technology, the genome manipulations have exerted a significant impact on functional studies and pest control. It has the advantage of requiring less time and effort and easy to design and use in insects. However, the size of the CRISPR/Cas9 framework is generally huge; thus it isn't reasonable for packaging into phage vectors. A more modest and smaller-sized CRISPR framework is needed for productive genome editing in crop species. The use of CRISPR/Cas9 technique along with other gene editing tools (RNAi, ZNF, TALEN) allows to surpass some major challenges in functional genomic studies to control some most important and economically relevant agricultural pests. The CRISPR/Cas9 system allows to generate mutant lines by a relatively simple and reasonable method. However, this technique is time-consuming and has presented low efficiency in some species. Recently, Cagliari et al. (2020) demonstrated the use of the CRISPR/Cas9 system in a non-model insect, *Euschistus heros* (Neotropical stink bug) for the first time in which they used both RNAi and CRISPR/Cas9 as complementary tools in the gene manipulation. There is also a need to expand the proficiency of knockin homologous recombination which requires new procedures and frameworks to improve homology-directed repair (HDR) and viral vector efficiencies and to inhibit endogenous non-homologous end-joining recombination/associated transposons. It is additionally important to evade off-target impacts and to detect specific targets by CRISPR/Cas9 that require progressive procedures. This efficient technology has also been used in many species of insects to study developmental pattern, sexual behavior, agriculture, forestry, fishes, public health, and pest control strategies. These emerging systems suggest that gene editing will become more proficient and helpful in the future, the space for advancement in arthropods, particularly in insect species, is broad, and more noteworthy achievements can be accomplished in the coming years.

References

Alphey L (2016) Can CRISPR-Cas9 gene drives curb malaria? Nat Biotechnol 34(2):149–150

Aryan A, Anderson MAE, Myles KM, Adelman ZN (2013) TALEN-based gene disruption in the dengue vector *Aedes aegypti*. PLoS One 8(3):e60082. https://doi.org/10.1371/journal.pone.0060082

Asaoka T, Almagro J, Ehrhardt C, Tsai I, Schleiffer A, Deszcz L (2016) Linear ubiquitination by LUBEL has a role in *Drosophila* heat stress response. EMBO Rep 17(11):1624–1640. https://doi.org/10.15252/embr.201642378. Epub 2016 Oct 4

Awata H, Watanabe T, Hamanaka Y, Mito T, Noji S, Mizunami M (2015) Knockout crickets for the study of learning and memory: dopamine receptor Dop1 mediates aversive but not appetitive reinforcement in crickets. Sci Rep 5:15885. https://doi.org/10.1038/srep15885

Bassett AR, Tibbit C, Ponting CP, Liu JL (2013) Highly efficient targeted mutagenesis of *Drosophila* with the CRISPR/Cas9 system. Cell Rep 4(1):220–228. https://doi.org/10.1016/j.celrep.2013.06.020

Basu S, Aryan A, Overcash JM, Samuel GH, Anderson ME, Dahlem TJ, Myles KM, Adelman ZN (2015) Silencing of end-joining repair for efficient site-specific gene insertion after TALEN/CRISPR mutagenesis in Aedes aegypti. Proc Natl Acad Sci U S A 112(13):4038–4043. https://doi.org/10.1073/pnas.1502370112

Bayen S (2012) Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: a review. Environ Int 48:84–101. https://doi.org/10.1016/j.envint.2012.07.008. Epub 2012 Aug 9

Berghammer AJ, Weber M, Trauner J, Klingler M (2009) Red flour beetle (*Tribolium*) germline transformation and insertional mutagenesis. Cold Spring Harb Protoc 2009:pdb.prot5259. https://doi.org/10.1101/pdb.prot5259

Bi HL, Xu J, Tan AJ, Huang YP (2016) CRISPR/Cas9-mediated targeted gene mutagenesis in *Spodoptera litura*. Insect Sci 23(3):469–477. https://doi.org/10.1111/1744-7917.12341. Epub 2016 May 13

Bibikova M, Beumer K, Trautman JK, Carroll D (2003) Enhancing gene targeting with designed zinc finger nucleases. Science 300(5620):764. https://doi.org/10.1126/science.1079512

Bogdanove AJ, Voytas DF (2011) TAL effectors: customizable proteins for DNA targeting. Science 333(6051):1843–1846. https://doi.org/10.1126/science.1204094

Bravo A, Likitvivatanavong S, Gill SS, Soberón M (2011) *Bacillus thuringiensis*: a story of a successful bioinsecticide. Insect Biochem Mol Biol 41(7):423–431. https://doi.org/10.1016/j.ibmb.2011.02.006. Epub 2011 Mar 2

Cagliari D, Smagghe G, Zotti M, Taning CNT (2020) RNAi and CRISPR/Cas9 as functional genomics tools in the neotropical stink bug, Euschistus heros. Insects 11(12):838. https://doi.org/10.3390/insects11120838

Chakraborty S, Newton AC (2011) Climate change, plant diseases and food security: an overview. Plant Pathol 60(1):2–14. https://doi.org/10.1111/j.1365-3059.2010.02411.x

Champer J, Buchman A, Akbari OS (2016) Cheating evolution: engineering gene drives to manipulate the fate of wild populations. Nat Rev Genet 17(3):146–159. https://doi.org/10.1038/nrg.2015.34. Epub 2016 Feb 15

Chechenova MB, Maes S, Oas ST, Nelson C, Kiani KG, Bryantsev AL (2017) Functional redundancy and nonredundancy between two Troponin C isoforms in *Drosophila* adult muscles. Mol Biol Cell 28:760–770. https://doi.org/10.1091/mbc.E16-07-0498. Epub 2017 Jan 11

Chen S, Hou C, Bi H, Wang Y, Xu J, Li M (2017) Transgenic CRISPR/Cas9-mediated viral gene targeting for antiviral therapy of *Bombyx mori* nucleopolyhedrovirus (BmNPV). J Virol 91:e02465–e02516

Condamine F, Clapham M, Kergoat G (2016) Global patterns of insect diversification: towards a reconciliation of fossil and molecular evidence? Sci Rep 6:19208. https://doi.org/10.1038/srep19208

Daimon T, Kiuchi T, Takasu Y (2014) Recent progress in genome engineering techniques in the silkworm, *Bombyx mori*. Develop Growth Differ 56:14–25. https://doi.org/10.1111/dgd.12096. Epub 2013 Nov 1

Davis RB, Baldauf SL, Mayhew PJ (2010) The origins of species richness in the Hymenoptera: insights from a family-level supertree. BMC Evol Biol 10:109. https://doi.org/10.1186/1471 2148-10-109

Dong SZ, Lin JY, Held NL, Clem RJ, Passarelli AL, Franz AWE (2015) Heritable CRISPR/ Cas9-mediated genome editing in the yellow fever mosquito, Aedes aegypti. PLoS One 10(3):e0122353. https://doi.org/10.1371/journal.pone.0122353

Dong ZQ, Chen TT, Zhang J, Hu N, Cao MY, Dong FF (2016) Establishment of a highly efficient virus-inducible CRISPR/Cas9 system in insect cells. Antivir Res 130:50–57. https://doi.org/10.1016/j.antiviral.2016.03.009. Epub 2016 Mar 12

Ebina H, Misawa N, Kanemura Y, Koyanagi Y (2013) Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. Sci Rep 3:2510. https://doi.org/10.1038/srep02510

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) Concerning RNA-guided gene drives for the alteration of wild populations. eLife 3:e03401. https://doi.org/10.7554/eLife.03401

Foottit RG, Adler PH (2009) Insect biodiversity: science and society. Wiley-Blackwell, West Sussex, UK, p 632. https://doi.org/10.21829/azm.2011.272770

Gabrieli P, Smidler A, Catteruccia F (2014) Engineering the control of mosquito-borne infectious diseases. Genome Biol 15:535. https://doi.org/10.1186/s13059-014-0535-7

Gaj T, Gersbach CA, Barbas CF (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31(7):397–405. https://doi.org/10.1016/j.tibtech.2013.04.004. Epub 2013 May 9

Gilles AF, Averof M (2014) Functional genetics for all: engineered nucleases, CRISPR and the gene editing revolution. EvoDevo 5:1–13. https://doi.org/10.1186/2041-9139-5-43

Gilles AF, Schinko JB, Averof M (2015) Efficient CRISPR-mediated gene targeting and transgene replacement in the beetle *Tribolium castaneum*. Development 142:2832–2839. https://doi.org/10.1242/dev.125054. Epub 2015 Jul 9

Gokcezade J, Sienski G, Duchek P (2014) Efficient CRISPR/Cas9 plasmids for rapid and versatile genome editing in *Drosophila*. G3 4:2279–2282. https://doi.org/10.1534/g3.114.014126

Gómez I, Sánchez J, Miranda R, Bravo A, Soberón M (2002) Cadherin-like receptor binding facilitates proteolytic cleavage of helix α-1 in domain I and oligomer pre-pore formation of *Bacillus thuringiensis* Cry1Ab toxin. FEBS Lett 513:242–246. https://doi.org/10.1016/s0014-5793(02)02321-9

Gratz SJ, Cummings AM, Nguyen JN, Hamm DC, Donohue LK, Harrison MM, Wildonger J, O'Connor-Giles KM (2013) Genome engineering of *Drosophila* with the CRISPR RNA-guided Cas9 nuclease. Genetics 194(4):1029–1035. https://doi.org/10.1534/genetics.113.152710. Epub 2013 May 24

Gratz SJ, Ukken FP, Rubinstein CD, Thiede G, Donohue LK, Cummings AM, O'Connor-Giles KM (2014) Highly specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in *Drosophila*. Genetics 196(4):961–971. https://doi.org/10.1534/genetics.113.160713. Epub 2014 Jan 29

Grimaldi D, Engel MS (2005) Evolution of the insects. Cambridge University Press, Cambridge, p xv + 755. ISBN 0 521 82149 5

Hall AB, Basu S, Jiang XF, Qi YM, Timoshevskiy VA, Biedler JK, Sharakhova MV, Elahi R, Anderson ME, Chen XG, Sharakhov IV, Adelman ZN, Tu ZJ (2015) A male-determining factor in the mosquito Aedes aegypti. Science 348(6240):1268–1270. https://doi.org/10.1126/science.aaa2850. Epub 2015 May 21

Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker D, Marois E, Russell S, Burt A, Windbichler N, Crisanti A, Nolan T (2016) A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nat Biotechnol 34(1):78–83. https://doi.org/10.1038/nbt.3439. Epub 2015 Dec 7

Hsu PD, Lander ES, Zhang F (2014) Development and applications of CRISPR-Cas9 for genome engineering. Cell 157(6):1262–1278. https://doi.org/10.1016/j.cell.2014.05.010

Huang Y, Chen Y, Zeng B, Wang Y, James AA, Gurr GM et al (2016) CRISPR/Cas9 mediated knockout of the *abdominal-A* homeotic gene in the global pest, diamondback moth (*Plutella xylostella*). Insect Biochem Mol Biol 75:98–106. https://doi.org/10.1016/j.ibmb.2016.06.004. Epub 2016 Jun 16

Itokawa K, Komagata O, Kasai S, Ogawa K, Tomita T (2016) Testing the causality between *CYP9M10* and pyrethroid resistance using the TALEN and CRISPR/Cas9 technologies. Sci Rep 6:24652. https://doi.org/10.1038/srep24652

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. Aug 17;337(6096):816–21. https://doi.org/10.1126/science.1225829. Epub 2012 Jun 28. PMID: 22745249; PMCID: PMC6286148.

Khan SA, Reichelt M, Heckel DG (2017) Functional analysis of the ABCs of eye color in *Helicoverpa armigera* with CRISPR/Cas9-induced mutations. Sci Rep 7:40025. https://doi.org/10.1038/srep40025

Kim HS, Murphy T, Xia J, Caragea D, Park Y, Beeman RW (2010) BeetleBase in 2010: revisions to provide comprehensive genomic information for *Tribolium castaneum*. Nucleic Acids Res 38:D437–D442. https://doi.org/10.1093/nar/gkp807

Kistler KE, Vosshall LB, Matthews BJ (2015) Genome engineering with CRISPR-Cas9 in the mosquito *Aedes aegypti*. Cell Rep 11:51–60. https://doi.org/10.1016/j.celrep.2015.03.009. Epub 2015 Mar 26

Kondo S, Ueda R (2013) Highly improved gene targeting by germline-specific Cas9 expression in *Drosophila*. Genetics 195(3):715–721. https://doi.org/10.1534/genetics.113.156737

Li F, Scott MJ (2016) CRISPR/Cas9-mediated mutagenesis of the white and Sex lethal loci in the invasive pest, *Drosophila suzukii*. Biochem Biophys Res Commun 469:911–916. https://doi.org/10.1016/j.bbrc.2015.12.081. Epub 2015 Dec 22

Li X, Fan D, Zhang W, Liu G, Zhang L, Zhao L (2015) Outbred genome sequencing and CRISPR/Cas9 gene editing in butterflies. Nat Commun 6:8212. https://doi.org/10.1038/ncomms9212

Li XY, Liu GC, Sheng WJ, Dong ZW, Chen L, Zhao RP (2016) Genome editing in the butterfly type-species *Papilio machaon*. Insect Sci 24:708–711. https://doi.org/10.1111/1744-7917.12421. Epub 2017 Feb 1

Li M, Au LYC, Douglah D, Chong A, White BJ, Ferree PM (2017) Generation of heritable germline mutations in the jewel wasp *Nasonia vitripennis* using CRISPR/Cas9. Sci Rep 7:901. https://doi.org/10.1038/s41598-017-00990-3

Liao HK, Gu Y, Diaz A, Marlett J, Takahashi Y, Li M (2015) Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. Nat Commun 6:6413. https://doi.org/10.1038/ncomms7413

Ling L, Ge X, Li Z, Zeng B, Xu J, Chen X, Shang P, James AA, Huang Y, Tan A (2015) MiR-2 family targets awd and fng to regulate wing morphogenesis in Bombyx mori. RNA Biol. 12(7):742–8. https://doi.org/10.1080/15476286.2015.1048957. PMID: 26037405; PMCID: PMC4615647

Liu YY, Ma SY, Wang XG, Chang JS, Gao J, Shi R, Zhang JD, Lu W, Liu Y, Zhao P, Xia QY (2014) Highly efficient multiplex targeted mutagenesis and genomic structure variation in Bombyx mori cells using CRISPR/Cas9. Insect Biochemistry and Molecular Biology, 49: 35–42

Liu H, Liu Q, Zhou X, Huang Y, Zhang Z (2017a) Genome editing of *Wnt-1*, a gene associated with segmentation, via CRISPR/Cas9 in the pine caterpillar moth, *Dendrolimus punctatus*. Front Physiol 7:666. https://doi.org/10.3389/fphys.2016.00666

Liu Q, Liu W, Zeng B, Wang G, Hao D, Huang Y (2017b) Deletion of the *Bombyx mori* odorant receptor co-receptor (*BmOrco*) impairs olfactory sensitivity in silkworms. Insect Biochem Mol Biol 86:58–67. https://doi.org/10.1016/j.ibmb.2017.05.007. Epub 2017 May 31

Ma SY, Chang JS, Wang XG, Liu YY, Zhang JD, Lu W, Gao J, Shi R, Zhao P, Xia QY (2014) CRISPR/Cas9 mediated multiplex genome editing and heritable mutagenesis of BmKu70 in *Bombyx mori*. Sci Rep 4:4489. https://doi.org/10.1038/srep04489

Marcus JM, Ramos DM, Monteiro A (2004) Germline transformation of the butterfly Bicyclus anynana. Proc R Soc Lond B Biol Sci 271:S263–S265. https://doi.org/10.1098/rsbl.2004.0175

Markert MJ, Zhang Y, Enuameh MS, Reppert SM, Wolfe SA, Merlin C (2016) Genomic access to monarch migration using TALEN and CRISPR/Cas9-mediated targeted mutagenesis. G3 6:905–915. https://doi.org/10.1534/g3.116.027029

Martins S, Naish N, Walker AS, Morrison NI, Scaife S, Fu G, Dafa'alla T, Alphey L (2012) Germline transformation of the diamondback moth, Plutella xylostella L. using the piggyBac transposable element. Insect Mol Biol 21(4):414–421. https://doi.org/10.1111/j.1365-2583.2012.01146.x. Epub 2012 May 24

Mendoza-Garcia P, Hugosson F, Fallah M, Higgins ML, Iwasaki Y, Pfeifer K (2017) The Zic family homologue Odd-paired regulates Alk expression in *Drosophila*. PLoS Genet 13:e1006617. https://doi.org/10.1371/journal.pgen.1006617

Monteiro A (2015) Origin, development, and evolution of butterfly eyespots. Annu Rev Entomol 60:253–271. https://doi.org/10.1146/annurev-ento-010814-020942. Epub 2014 Oct 17

Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, Ishitani R, Zhang F, Nureki O (2014) Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell 156(5):935–949. https://doi.org/10.1016/j.cell.2014.02.001. Epub 2014 Feb 13

Oliver JC, Tong XL, Gall LF, Piel WH, Monteiro A (2012) A single origin for nymphalid butterfly eyespots followed by widespread loss of associated gene expression. PLoS Genet 8:e1002893. https://doi.org/10.1371/journal.pgen.1002893. Epub 2012 Aug 16

Oye KA, Esvelt K, Appleton E, Catteruccia F, Church G, Kuiken T (2014) Regulating gene drives. Science 345:626–628. https://doi.org/10.1126/science.1254287. Epub 2014 Jul 17

Papathanos PA, Bossin HC, Benedict MQ, Catteruccia F, Malcolm CA, Alphey L (2009) Sex separation strategies: past experience and new approaches. Malar J 8(Suppl. 2):S5. https://doi.org/10.1186/1475-2875-8-S2-S5

Pavlopoulos A, Berghammer AJ, Averof M, Klingler M (2004) Efficient transformation of the beetle *Tribolium castaneum* using the Minos transposable element: quantitative and qualitative analysis of genomic integration events. Genetics 167:737–746. https://doi.org/10.1534/genetics.103.023085

Pellagatti A, Dolatshad H, Valletta S, Boultwood J (2015) Application of CRISPR/Cas9 genome editing to the study and treatment of disease. Arch Toxicol 89:1023–1034. https://doi.org/10.1007/s00204-015-1504-y. Epub 2015 Apr 1

Perkin LC, Elpidina EN, Oppert B (2017) RNA interference and dietary inhibitors induce a similar compensation response in *Tribolium castaneum* larvae. Insect Mol Biol 26:35–45. https://doi.org/10.1111/imb.12269

Port F, Chen HM, Lee T, Bullock SL (2014) Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in *Drosophila*. Proc Natl Acad Sci U S A 111(29):E2967–E2976. https://doi.org/10.1073/pnas.1405500111. Epub 2014 Jul 7

Quan X, Sato-Miyata Y, Tsuda M, Muramatsu K, Asano T, Takeo S (2017) Deficiency of succinyl-CoA synthetase alpha subunit delays development, impairs locomotor activity and reduces survival under starvation in *Drosophila*. Biochem Biophys Res Commun 483:566–571. https://doi.org/10.1016/j.bbrc.2016.12.105. Epub 2016 Dec 23

Reed RD, Serfas MS (2004) Butterfly wing pattern evolution is associated with changes in a Notch/Distal-less temporal pattern formation process. Curr Biol 14:1159–1166. https://doi.org/10.1016/j.cub.2004.06.046

Reegan AD, Ceasar SA, Paulraj MG, Ignacimuthu S, Al-Dhabi N (2017) Current status of genome editing in vector mosquitoes: a review. Biosci Trends 10:424–432. https://doi.org/10.5582/bst.2016.01180. Epub 2016 Dec 18

Ren XJ, Sun J, Housden BE, Hu YH, Roesel C, Lin SL, Liu LP, Yang ZH, Mao DC, Sun LZ, Wu QJ, Ji JY, Xi JZ, Mohr SE, Xu J, Perrimon N, Ni JQ (2013) Optimized gene editing technol-

ogy for *Drosophila melanogaster* using germ line-specific Cas9. Proc Natl Acad Sci U S A 110(47):19012–19017. https://doi.org/10.1534/g3.114.013821

Rio DC, Rubin GM (1988) Identification and purification of a *Drosophila* protein that binds to the terminal 31-base-pair inverted repeats of the P transposable element. Proc Natl Acad Sci U S A 85(23):8929–8933. https://doi.org/10.1073/pnas.85.23.8929

Rubin GM, Spradling AC (1982) Genetic transformation of *Drosophila* with transposable element vectors. Science 218(4570):348–353

Schinko JB, Weber M, Viktorinova I, Kiupakis A, Averof M, Klingler M (2010) Functionality of the GAL4/UAS system in *Tribolium* requires the use of endogenous core promoters. BMC Dev Biol 10:53. https://doi.org/10.1186/1471-213X-10-53

Schinko JB, Hillebrand K, Bucher G (2012) Heat shock-mediated misexpression of genes in the beetle *Tribolium castaneum*. Dev Genes Evol 222:287–298. https://doi.org/10.1007/s00427-012-0412-x. Epub 2012 Jul 20

Sebo ZL, Lee HB, Peng Y, Guo Y (2014) A simplified and efficient germline-specific CRISPR/Cas9 system for *Drosophila* genomic engineering. Fly 8(1):52–57. https://doi.org/10.4161/fly.26828. Epub 2013 Oct 18

Seeger C, Sohn JA (2014) Targeting hepatitis B virus with CRISPR/Cas9. Mol Ther Nucleic Acids 3:e216. https://doi.org/10.1038/mtna.2014.68

Shalem O, Sanjana NE, Zhang F (2015) High-throughput functional genomics using CRISPR-Cas9. Nat Rev Genet 16(5):299–311. https://doi.org/10.1038/nrg3899. Epub 2015 Apr 9

Smidler AL, Terenzi O, Soichot J, Levashina EA, Marois E (2013) Targeted mutagenesis in the malaria mosquito using TALE nucleases. PLoS One 8:e74511. https://doi.org/10.1371/journal.pone.0074511

Tamura T, Thibert C, Royer C, Kanda T, Abraham E, Kamba M, Komoto N, Thomas JL, Mauchamp B, Chavancy G, Shirk P, Fraser M, Prudhomme JC, Couble P (2000) Germline transformation of the silkworm *Bombyx mori* L. using a piggyBac transposon-derived vector. Nat Biotechnol 18(1):81–84. https://doi.org/10.1186/1471-2156-2-11

Taning CNT, Van Eynde B, Yu N, Ma S, Smagghe G (2017) CRISPR/Cas9 in insects: applications, best practices and biosafety concerns. J Insect Physiol 98:245–257. https://doi.org/10.1016/j.jinsphys.2017.01.007. Epub 2017 Jan 18

Tikar SN, Kumar A, Prasad GB, Prakash S (2009) Temephos-induced resistance in *Aedes aegypti* and its cross-resistance studies to certain insecticides from India. Parasitol Res 105:57–63. https://doi.org/10.1007/s00436-009-1362-8. Epub 2009 Feb 20

Tribolium Genome Sequencing Consortium (2008) The genome of the model beetle and pest *Tribolium castaneum*. Nature 452:949–955. https://doi.org/10.1038/nature06784. Epub 2008 Mar 23

Urban JA, Doherty CA, Jordan WT III, Bliss JE, Feng J, Soruco MM (2016) The essential *Drosophila* CLAMP protein differentially regulates non-coding roX RNAs in male and females. Chromosom Res 25:101–113. https://doi.org/10.1007/s10577-016-9541-9

Venken KJT, Bellen HJ (2007) Transgenesis upgrades for *Drosophila melanogaster*. Development 134(20):3571–3584. https://doi.org/10.1242/dev.005686

Wang YQ, Li ZQ, Xu J, Zeng BS, Ling L, You L, Chen YZ, Huang YP, Tan AJ (2013) The CRISPR/Cas system mediates efficient genome engineering in *Bombyx mori*. Cell Res 23(12):1414–1416

Wang J, Zhang H, Wang H, Zhao S, Zuo Y, Yang Y (2016) Functional validation of cadherin as a receptor of Bt toxin Cry1Ac in *Helicoverpa armigera* utilizing the CRISPR/Cas9 system. Insect Biochem Mol Biol 76:11–17. https://doi.org/10.1016/j.ibmb.2016.06.008. Epub 2016 Jun 22

Weaver, S. C., and A. D. Barrett (2004) Transmission cycles, host range, evolution and emergence of arboviral disease. Nat. Rev. Microbiol.2:789–801

Wei W, Xin HH, Roy B, Dai JB, Miao YG, Gao GJ (2014) Heritable genome editing with CRISPR/Cas9 in the silkworm, Bombyx mori. PLoS One 9(7):e101210. https://doi.org/10.1371/journal.pone.0101210

World Health Organization (2014) World Malaria Report 2014

Wu YD (2014) Detection and mechanisms of resistance evolved in insects to Cry toxins from *Bacillus thuringiensis*. Adv Insect Physiol 47:297–342. https://doi.org/10.1016/B978-0-12800197-4.00006-3

Xia Q, Li S, Feng Q (2014) Advances in silkworm studies accelerated by the genome sequencing of *Bombyx mori*. Annu Rev Entomol 59:513–536. https://doi.org/10.1146/annurev-ento-011613-161940. Epub 2013 Oct 25

Xin HH, Zhang DP, Chen RT, Cai ZZ, Lu Y, Liang S, Miao YG (2015) Transcription factor bmsage plays a crucial role in silk gland generation in silkworm, *Bombyx mori*. Arch Insect Biochem Physiol 90(2):59–69. https://doi.org/10.1002/arch.21244. Epub 2015 Apr 28

Xue ZY, Ren MD, Wu MH, Dai JB, Rong YS, Gao GJ (2014) Efficient gene knock-out and knock-in with transgenic Cas9 in *Drosophila*. G3 4(5):925–929. https://doi.org/10.1534/g3.114.010496

Yamaguchi J, Mizoguchi T, Fujiwara H (2011) siRNAs induce efficient RNAi response in *Bombyx mori* embryos. PLoS One 6:e25469. https://doi.org/10.1371/journal.pone.0025469. Epub 2011 Sep 30

Yamaguchi J, Banno Y, Mita K, Yamamoto K, Ando T, Fujiwara H (2013) Periodic *Wnt1* expression in response to ecdysteroid generates twin-spot markings on caterpillars. Nat Commun 4:1857. https://doi.org/10.1038/ncomms2778

Yu ZS, Ren MD, Wang ZX, Zhang B, Rong YS, Jiao RJ, Gao GJ (2013) Highly efficient genome modifications mediated by CRISPR/Cas9 in *Drosophila*. Genetics 195(1):289–291. https://doi.org/10.1534/genetics.113.153825. Epub 2013 Jul 5

Zeng BS, Zhan S, Wang YQ, Huang YP, Xu J, Liu Q, Li ZQ, Huang YP, Tan AJ (2016) Expansion of CRISPR targeting sites in *Bombyx mori*. Insect Biochem Mol Biol 72:31–40. https://doi.org/10.1016/j.ibmb.2016.03.006. Epub 2016 Mar 24

Zhang L, Reed RD (2016) Genome editing in butterflies reveals that *spalt* promotes and *Distal-less* represses eyespot colour patterns. Nat Commun 7:11769. https://doi.org/10.1038/ncomms11769

Zhang X, Koolhaas WH, Schnorrer F (2014) A versatile two-step CRISPR- and RMCE-based strategy for efficient genome engineering in *Drosophila*. G3 4(12):2409–2418. https://doi.org/10.1534/g3.114.013979

Zhang ZJ, Aslam AFM, Liu XJ, Li MW, Huang YP, Tan AJ (2015) Functional analysis of *Bombyx Wnt1* during embryogenesis using the CRISPR/Cas9 system. J Insect Physiol 79:73–79. https://doi.org/10.1016/j.jinsphys.2015.06.004. Epub 2015 Jun 9

Zhang Y, Zhao B, Roy S, Saha TT, Kokoza VA, Li M (2016) microRNA-309 targets the homeobox gene *SIX4* and controls ovarian development in the mosquito *Aedes aegypti*. Proc Natl Acad Sci U S A 113:E4828–E4836. https://doi.org/10.1073/pnas.1609792113. Epub 2016 Aug 3

Zhang Z, Liu X, Shiotsuki T, Wang Z, Xu X, Huang Y (2017a) Depletion of juvenile hormone esterase extends larval growth in *Bombyx mori*. Insect Biochem Mol Biol 81:72–79. https://doi.org/10.1016/j.ibmb.2017.01.001. Epub 2017 Jan 3

Zhang L, Martin A, Perry MW, Van Der Burg KR, Matsuoka Y, Monteiro A (2017b) Genetic basis of melanin pigmentation in butterfly wings. Genetics 205:1537–1550. https://doi.org/10.1534/genetics.116.196451. Epub 2017 Feb 13

Zhu L, Mon H, Xu J, Lee JM, Kusakabe T (2015) CRISPR/Cas9-mediated knockout of factors in non-homologous end joining pathway enhances gene targeting in silkworm cells. Sci Rep 5:18103. https://doi.org/10.1038/srep18103

Zhu GH, Xu J, Cui Z, Dong XT, Ye ZF, Niu DJ (2016) Functional characterization of *SlitPBP3* in *Spodoptera litura* by CRISPR/Cas9 mediated genome editing. Insect Biochem Mol Biol 75:1–9. https://doi.org/10.1016/j.ibmb.2016.05.006. Epub 2016 May 15

Chapter 13
Multi-omics Approaches in Insect-Plant Interactions

Morthala Shankara Sai Reddy, Somala Karthik, Battu Jabez Raju, and Gummudala Yashaswini

13.1 Introduction

Global farmland is limited, and the world's population is increasing every day (Zhang 2018). As a result, modern food production relies heavily on synthetic pesticides and fertilizers to boost food crop productivity (Aktar et al. 2009; Schreinemachers and Tipraqsa 2012; Davis 2014; Hedlund et al. 2020). Pest infestations cause roughly 45% of annual food production to be lost (Abhilash and Singh 2009). Pesticides are used in two million tonnes around the world each year, and the pesticides used pose a risk to human health and the environment due to their high toxicity, persistence, and bioaccumulation (Sharma et al. 2019; Hedlund et al. 2020). Pesticides are also toxic to non-target beneficial organisms like predators and parasitoids (Stanley and Preetha 2016). They are responsible for pest resurgence and the development of resistance in insects (Metcalf and Luckmann 1994; Sharma and Ortiz 2002).

Rather than using pesticides, plants can take advantage of their natural resistance to insect pests. This natural resistance is the sum of the constitutional and genetically inherited characteristics that make a cultivar or species more resistant to injury than others (Smith 2005). This resistance was first identified in a wheat cultivar "Underhill" which was found to be resistant to Hessian fly (Havens 1792; Smith 2005). The presence of secondary metabolites (Fraenkel 1959) or structural defenses may be responsible for plant insect defenses (Fraenkel 1959) or due to structural defenses (Hanley et al. 2007). Epigenomics, genomics, metagenomics,

M. S. Sai Reddy (✉) · S. Karthik · G. Yashaswini
Department of Entomology, PG College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Samastipur, Bihar, India

B. J. Raju
Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_13

transcriptomics, proteomics, metabolomics, volatilomics, and phenomics are examples of omics that are used to identify the compounds that cause resistance in plants (Tu et al. 2018; Liu et al. 2019; Wu et al. 2019; Wang et al. 2020a, b).

Scientists across the globe use this resistance shown by plants for breeding resistance as a trait in agricultural crops. Traditional resistance breeding methods, on the other hand, are more time-consuming than newer technologies such as genomics, transcriptomics, proteomics, and metabolomics. In this way, a whole plant can be screened in less time for compounds that could be useful in pest management. These technologies make it simple to identify the genes that code for these compounds, which could then be incorporated into agricultural crops to breed insect-resistant cultivars. Multi-omics is a multidisciplinary approach to study the complexity of systems biology. Plant-insect interactions are a continuous dialogue between the offender group, insects, and the defender group, plants, through a network of various signaling pathways. Multi-omics tools help us study and understand these interactions at all levels, from genes to ecosystems. This chapter summarizes the utility of these omics tools in intercepting host plant-insect pest interactions, plant resistance mechanisms developed to defend themselves against insect herbivory, and also how integrating them could transform the scenario of pest management in agricultural systems. Following a brief description of these tools, we present an overview of the progress made in understanding plant defense against phytophagous insect pests using these omics tools.

13.2 The Multi-omics: Genomics, Epigenomics, Metagenomics, Transcriptomics, Proteomics, Metabolomics, Volatilomics, and Phenomics

Multi-omics, integrative omics, or panomics is a biological analytical methodology in which data sets are multiple "omes" such as the genome, epigenome, transcriptome, proteome, metabolome, volatilome, phenome, etc. The process of life at its very existence is a network of pathways and interconnected genome, epigenome, transcriptome, proteome, metabolome, etc. These interactions between them are represented in Fig. 13.1.

13.2.1 Genomics

Genomics is a multidisciplinary tool in studying the systems biology of living organisms with core focus on the mapping of genome and its editing. It also involves the high-throughput sequencing and analysis of genomes and measures the expression of genes (Lockhart and Winzeler 2000). The pomace fly *Drosophila melanogaster* was the first insect whose genome is sequenced completely in 2000 (Palli et al. 2012).

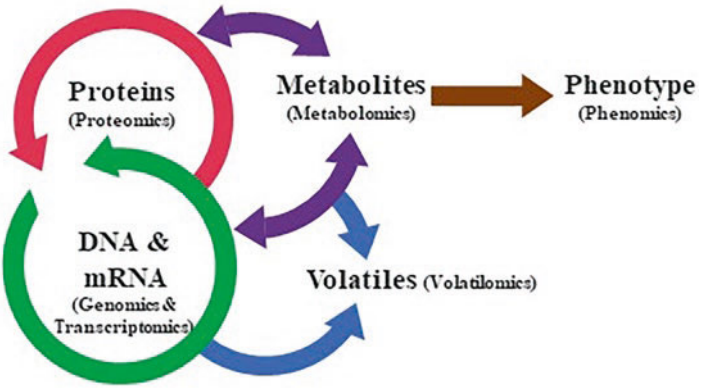

Fig. 13.1 The omics interaction

Genome sequencing can be done using Whole Genome Shotgun (WGS) sequencing and automated Sanger sequencing technologies. However, Next-Generation Sequencing (NGS) is employed nowadays for sequencing the genome (Palli et al. 2012). Topical tools such as Chromatin Immunoprecipitation followed by ChIP-seq analysis (Nakato and Shirahige 2017), Transcription Activator-Like Effector Nucleases (TALENs) (Zhang et al. 2013a, b), and Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR)/Cas systems (CRISPR/Cas9) (Doudna and Charpentier 2014) are developed for editing the genomes (Nejat et al. 2018).

13.2.1.1 Genomics of Host Plant-Insect Interactions

The interaction amid insect pests and host plants is not as simple as it seems to appear. There is a continuous dialogue between these two organisms where the surrounding environment also has a say in it. The feeding by the insect pest triggers a cascade of chemical reactions in its host plant, and a hidden battle occurs between them, which indeed is an arms race and decides the victor. Plants produce a wide multiplicity of chemicals under a variety of signaling pathways which form the defense against the herbivore. During this process the genes are either upregulated or downregulated. To understand this better, studies are to be conducted at a molecular level. Through genomics, high-throughput characterization of the genome of plants can be done, and indeed genes of interest, viz., genes coding for resistance against insect pests, could be exploited for crop improvement (Zheng and Dicke 2008). In response the insect genes are also regulated and expressed under these host defense mechanisms. These interactions can be characterized by sequencing the genome on either side and editing the genome for benefits of host. These intrusions may be done through genomics technologies.

The host plants and insect pests coevolve. Using genomic approaches, it was discovered that shifts in butterfly host plants are linked to both genome-wide

adaptive molecular evolution and ecological changes, contributing to an increase in butterfly diversity (Allio et al. 2021). Similar approach was applied to other insects like *Nilaparvata lugens* (brown plant hopper) which is the most critical pest of rice ecosystem in the world. The resistance gene *Bph1* was introduced in the commercial rice varieties for developing BPH-resistant rice varieties. However, it also incited the emergence of a new virulent biotype of brown plant hopper which overpowered the resistance conferred by *Bph1*. For the brown plant hopper, a high-density linkage map was created covering 96.6% of its genome to find the loci which is responsible for its virulence. Genome-wide scanning and interval mapping showed that the *Qhp7* locus governs predilection for *Bph1* plants (Jing et al. 2014).

Several model systems have shed light on the roles of genes and their networks in the detoxification or sequestration of host allelochemicals and toxins. Insects have specialized detoxification enzymes that help them triumph over chemical defense response in different plant species. An approach aimed at polyphagous pests' detoxification genes could be a promising option. Insect susceptibility was increased when insecticidal detoxification genes like gossypol-inducing cytochrome P450 were knocked out (Hafeez et al. 2019). CRISPR/Cas9 knockdown of the *CYP6AE* gene cluster in the polyphagous pest *Helicoverpa armigera* demonstrated the role of these enzymes in detoxification of various toxic phytochemicals (Wang et al. 2018). *Drosophila sechellia*, endemic to the Seychelles Islands, feeds on the fruits of *Morinda citrifolia* which are toxic to other drosophilids as the fruits have octanoic acid to which *D. sechellia* is resistant. Adults exposed to 0.7% OA were subjected to RNA-seq which showed that 132 genes are differentially expressed. Osiris and Tweedle genes were responsible for resistance to octanoic acid in *D. sechellia* (Etges 2019). *Aphis glycines* resistance in soybean was due to *Rag*. On 18 of the 20 soybean chromosomes, there were associations between SNPs and soybean aphid counts, according to genome-wide association mapping. These SNPs could be used to identify accessions with novel aphid resistance traits that could be used to breed aphid resistance in soybeans (Hanson et al. 2018).

Ecological genomics combines genome and ecologically relevant gene analyses for enhanced understanding of the effects of biotic and abiotic factors on an organism and their interactions. It investigates genomic variation at the genetic, epigenetic, and transcriptional levels. The transcriptomic profiles of galls on *Quercus castanea* (oak) induced by the cynipid wasp *Amphibolips michoacaensis* show that changes in gene regulation associated with metabolism and cell cycle can cause wasp larvae to manipulate the phenotype of the host plant (Betancourt et al. 2020).

Plants have developed a variety of strategies in response to biotic stress factors. While resistance genes (R genes) determine a plant's ability to resist pests/diseases (Rathinam et al. 2019), susceptible genes (S genes) determine how quickly it succumbs to stress. Editing susceptible genes to develop plants resistant to insects is proving to be a viable approach. Insects rely on chemical components from plants for their development, immunity, and behavior. This has been successfully demonstrated in rice (Lu et al. 2018). The *CYP71A1* gene, which encodes tryptamine 5-hydroxylase and catalyzes the conversion of tryptamine to serotonin, was knocked out using CRISPR/Cas9. This resulted in a reduction in plant hopper growth.

Chemical communication and identification of mating partner could be hampered by genome editing. These two are just two of the several factors that contribute to flourishing plant-insect interactions. In insects, olfactory receptors (ORs) are imperative for recognizing host plant and mating partner odorants. The CRISPR/Cas9 knockout of the olfactory receptor coreceptor gene in *Spodoptera litura* resulted in distraction in selection of mating partner and loss of identity toward host plants, resulting in anosmia (Koutroumpa et al. 2016). Adoption of such technologies could be a viable option for preventing insect damage and keeping insect pests away from crops.

13.2.2 Epigenomics

Waddington introduced "epigenetics" for the first time by combining the terms "epigenesis" and "genetics." Epigenomics is the study of complete sets of these heritable alterations (Malhotra et al. 2020) in gene expression that aren't subject to changes in the underlying DNA sequence, viz., a change in phenotype without a change in genotype. Alteration of nucleotide sequences is not only the sole reason for hereditary variations, but there are certain other mechanisms (they are also heritable) that can modulate gene expression resulting into a changed phenotype. DNA chemical modifications or modifications of proteins that are closely associated with DNA, such as chromatin, are used to mediate epigenetic mechanisms.

Previously, fluorescent in situ hybridization was used for epigenome profiling, and more recently, chromosome conformation capture (3C), 3C-on chip (4C), 3C-carbon copy (5C), Hi-C, MEDME (Modeling Enrichment Derived from MeDIP Experiments), and BATMAN (Bayesian Tool for Methylation Analysis), Methylated-CpG Island Recovery Assay (MIRA), Formaldehyde-Assisted Isolation of Regulatory Elements-seq (FAIRE-seq) (Tollefsbol 2011), HiChIP (Mumbach et al. 2016), and A TAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) (Buenrostro et al. 2013) have been used for epigenomics studies. Moreover, epigenome editing can be done using CRISPR/Cas9 system (Hilton et al. 2015).

13.2.2.1 Applications of Epigenomics

Epigenetic processes like inherited histone and DNA methylation modifications are likely the mechanisms in order to pass on stress memory to future generations. Induced resistance was found associated with the epigenetic memory which caused the inheritance of acquired resistant traits in the next generation of pests (Rasmann et al. 2012).

Epigenetics helps us to better understand different patterns of gene expression. Potato aphid salivary protein Me47, discovered by Kettles and Kaloshian in 2016, facilitates aphid infestation in *Solanum lycopersicum* and *Nicotiana benthamiana*.

Me47, on the other hand, inhibits the infestation in *Arabidopsis thaliana*. sRNAs regulate gene expression through epigenetic regulation, posttranscriptional control of transcript abundance, and translational control.

Plants subjected to herbivory were more resistant when they come across similar conditions in the following generation. The priming of jasmonic acid (JA)-related defense responses is part of this phenomenon of transgenerational resistance to herbivores, which is also dependent on siRNA biogenesis (Kotkar and Giri 2020). If the parental plants are subjected to environmental stress, the genome-wide DNA methylation pattern is modified, and the progeny shows modifications of P content, leaf morphology, root/shoot biomass ratio, and stress tolerance relative to the control (Baulcombe and Dean 2014).

In insects, genomic imprinting is the most common form of transgenerational epigenetic inheritance. It is mediated by the formation of different methylation patterns, and the inheritance of these epigenetic marks results in differential gene expression. For example, DNA methylation is involved in genomic imprinting in the mealybug *Planococcus citri*. Paternally inherited chromosomes in males are completely silenced which is allied with DNA hypomethylation, while maternally inherited chromosomes remain hypermethylated and active (MacDonald 2012).

Dendrolimus punctatus whole-transcriptome sequencing was used to identify noncoding RNA (ncRNA) regulators involved in population bursts, such as microRNAs, long noncoding RNAs, and circular RNAs. Long noncoding RNAs (IncRNAs) appear to be the primary ncRNA regulators of *D. punctatus* outbreaks, whereas circular RNAs are mostly involved in synapses and cell junction regulation (Zhang et al. 2020).

13.2.3 Metagenomics

Metagenomics is also known as environmental and community genomics (Nazir 2016). It involves genomic analysis of an assemblage of microbes in most environments and pools the genomes of all the organisms in the community.

Sanger sequencing shotgun sequencing, SMRT (single-molecule real-time sequencing), and NGS platforms such as Illumina, Ion Torrent, HeliScope, Pacific Biosciences (PacBio), 454/Roche, Sequencing by Oligo Ligation Detection (SOLiD), and Oxford Nanopore (ON) were used for metagenomic DNA sequencing (Olson et al. 2019; Chopra et al. 2020).

13.2.3.1 Metagenomics of Host Plant-Insect Interactions

Metagenomics of the gut microbiota of the diamondback moth *Plutella xylostella* revealed that gut bacteria with specific enzymes such as threonine synthase, alanine-synthesizing transaminase, 1,4-beta-cellobiosidase, endoglucanase, beta-glucosidase peroxidases, catalases, and superoxide dismutases played a key role in

the breakdown of plant cell walls, plant phenolic detoxification, and amino acid synthesis (Xia et al. 2017).

Insect gut bacteria are susceptible to antibiotics when they are associated with certain host plants. Ignasiak and Maxwell (2017) reported that vindoline found in *Catharanthus roseus* (Periwinkle) showed antibacterial activity. Metagenomics of the aphid *Pentalonia nigronervosa* suggested that there was symbiosis between its endosymbionts *Buchnera aphidicola* and *Wolbachia* sp. which were involved in providing essential nutrients to their aphid host (De Clerck et al. 2015). *Bacillus cereus*, *Enterobacter cloacae* complex sp., *Streptomyces* sp., and some *Pseudomonas* and *Wolbachia* spp. were found to be more abundant in the resistant strains, indicating a promising role of gut microbiota in the development of insecticide resistance in insects (Wang et al. 2021).

13.2.4 Transcriptomics

The "transcriptome" is defined as the complete complement of mRNA molecules generated by a cell or population of cells. Charles Auffray coined the term in 1996. Transcriptomics refers to the study of transcriptomes and involves everything related to ribose nucleic acids (RNAs) including the structure of transcripts, parent genes involved, splicing pattern, and posttranscriptional modifications, as well as their transcription and expression levels, functions, locations, trafficking, and degradation. Transcriptomics includes messenger RNAs (mRNAs), microRNAs (miRNAs), and various types of long noncoding RNAs (lncRNAs) (Milward et al. 2016). The transcriptome of the host plant is an arsenal from which it draws the weapons which could be used against the herbivores.

Traditionally, transcriptomic studies were conducted by employing complementary deoxyribonucleic acid (cDNA) clones to generate expressed sequence tags (ESTs), which were then analyzed using automated Sanger sequencing techniques or serial study of gene expression (SAGE). However, currently DNA microarray (indirect sequencing) and RNA sequencing (direct sequencing) are being used in transcriptomic studies (Milward et al. 2016).

Specific functions of the cells can also be studied using transcriptomics. Among many factors that influence the functions of the cells, the location and structure of the cells can also reflect their function. In complex living systems, such as mammals, various microenvironments may exist at different locations of the same tissues. To better understand the functions of the cells, very recently single-cell transcriptomic and spatial transcriptomic technologies are being developed. For a long time, the scientific community relied on laser microdissection (LMD) and fluorescence-activated cell sorting (FACS) for studying the transcriptomes of single cells. However, the introduction of droplet-based sequencing technologies like Drop-seq has accelerated single-cell transcriptomics. Drop-seq is a methodology that quantifies mRNA level of transcripts in thousands of single cells using a microfluidics-based technology that encapsulates individual cells in droplets

containing barcoded beads. This permits the screening of single-cell transcriptomes at a high throughput (Rich-Griffin et al. 2020). But for understanding the functions of cells which are specifically found only at certain locations, spatial transcriptomics is used together with single-cell transcriptomics. Technologies like single-molecule fluorescence in situ hybridization (smFISH) (Raj et al. 2008), fluorescent in situ RNA sequencing (FISSEQ) (Moor and Itzkovitz 2017), and Slide-seq (Rodriques et al. 2019) are used in spatial transcriptomics. Similar to Drop-seq which is used for spatial analysis of transcription, Slide-seq is also a novel spatial transcriptomics technology and can be used for the analysis of transcription spatially. RNA is transferred from tissue sections onto a surface covered in DNA-barcoded beads with known positions in Slide-seq, which allows sequencing to infer the RNA's locations. It enables the investigation of spatial gene expression at the level of individual cells (Rodriques et al. 2019).

13.2.4.1 Transcriptomics of Host-Insect Interactions and Host Plant Resistance

Transcriptomics aids in the study of defense-related genes for better understanding and to prevent the formation of biotypes in insect pests. Transcriptomic analysis of whitefly (*Bemisia tabaci*)-resistant cotton plants revealed that defense-related genes such as protein kinases, transcription factors, metabolite synthases, and hormone- and pathogen-related genes exhibit differential expression following whitefly attack (Li et al. 2016). Transcriptomic analysis of *Sitobion avenae* biotypes 1 and 3 revealed that 39 of the 126 and 861 differentially expressed genes observed in biotypes 1 and 3, respectively, were shared by both biotypes. Defense-related genes were found to have undergone extensive expression restructuring in both biotypes 1 and 3, and their functions were found to have functional divergence, indicating that the defensive gene function was altered in a different host (Wang et al. 2020a, b).

Comparative transcriptome analysis is an effective method for comparing gene expression patterns across subjects and providing insights into biological processes. For instance, insulin-like peptides (ILPs) identified in *D. melanogaster* that are similar to insulin and insulin growth factor (IGF) of vertebrates (Leyria et al. 2020) are involved in the regulation of growth, nervous system development, reproduction, and lifespan. Nine hundred seventy messenger RNAs (mRNAs) were differentially expressed in resistant and susceptible alfalfa to thrips, according to a transcriptomic analysis, and five pathways, beta-alanine metabolism, fatty acid degradation, chloroalkane and chloroalkene degradation, flavonoid biosynthesis, and phenylalanine metabolism, were linked to resistance in resistant plants (Tu et al. 2018). Insect succession studies of mustard leaves (*Brassica juncea*) infected with mustard aphid (*Lipaphis erysimi*) and cowpea aphid (*Aphis craccivora*) were conducted to investigate the factors influencing the success of these aphid infestations. When compared to *A. craccivora*, the transcript levels of transcription factors, oxidative homeostasis, defense hormones, and secondary metabolites were either suppressed or only partially activated in *L. erysimi* (Duhlian et al. 2020).

The transcripts in non-hosts consist of differentially expressed genes indicating that the transcriptome is flexible and changes with the host plant, thus enabling insects to adapt to new hosts quickly. The transcriptome of the larval gut of a butterfly *Heliconius melpomene* larvae feeding on a new non-host plant was analyzed, and the results demonstrated that the transcriptome was different from that of the larva feeding on its host plant *Passiflora biflora* indicating that the different transcriptome enabled its adaption to non-host plant (Yu et al. 2016).

13.2.5 Proteomics

Proteins form a major component of biological systems and are composed of polypeptides containing amino acids. The functions they perform are diverse and include catalyzing metabolic reactions (as enzymes), providing structure to the cells and organisms, and transporting molecules (as ion channels). The complete set of proteins produced or modified by an organism or a biological system is called proteome, which is coined by Wilkins. Proteomics is the study of all proteins found in an organism, as well as their quantification, recognition, and functional modifications (Palli et al. 2012). The total proteome of an organism is depicted in its genome. The genome has specific genes coding for different proteins by the processes called transcription and translation. The encoded proteins perform diverse functions in an organism.

Modern proteomics primarily employs mass spectrometry to quantify, identify, sequence, and determine the presence of posttranslational modifications. There are two broad strategies, the bottom-up approach and the top-down approach (Palli et al. 2012). Bottom-up approach generally involves the cleaving of proteins into peptide fragments that are small but distinctive enough to allow protein identification. When bottom-up approach is used on a mixture of proteins, it is called shotgun proteomics (Zhang et al. 2013a, b). However, using this method, it is not possible to investigate site-specific mutations and posttranslational modifications of proteins. This augmented the interest in top-down approach where there is no need for chemical or enzymatic proteolysis. In top-down approach, individual proteins are preferred for analysis by mass spectrometry (Bogdanov and Smith 2005). Also, proteome characterization in plants is often interrupted by the high abundance of proteins such as ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) in shotgun proteomics. To overcome this, Polyethyleneimine Assisted Rubisco Cleanup (PARC) method was developed which improves efficiency of identification of proteins related to plant defense (Zhang et al. 2013a, b).

13.2.5.1 Proteomics of Host-Insect Interaction and Host Plant Resistance

During host plant-insect interactions, proteins that aid in manipulating host plant behavior are produced, which is beneficial to food production systems because it reduces losses caused by insect pest attacks. Proteomics can help analyze these

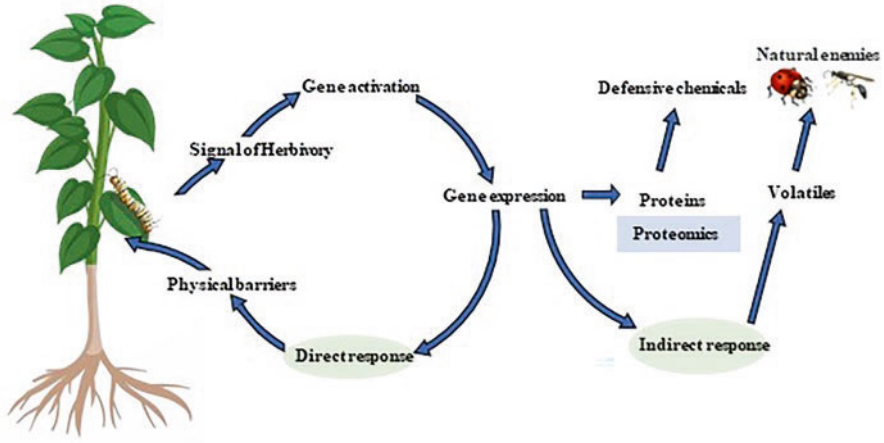

Fig. 13.2 Proteome-based response of a host plant to insect herbivory

plant-insect interactions and also to identify and quantify proteins which could potentially produce resistance to insect pests.

The reaction of a plant to the attack by insect pests can also be seen as changes in its proteome along with its genome and transcriptome. A simple depiction of proteome-based response of a host plant to insect herbivory is represented in Fig. 13.2. Several genes could be differentially expressed as a defense response which indeed is depicted in the end products such as defense-related proteins and other secondary metabolites. The response of a tomato plant compatible with an aphid (*Macrosiphum euphorbiae*) was studied by Coppola et al. (2013). According to the analysis of identified genes and proteins, the response is characterized by increased oxidative stress and production of proteins involved in the detoxification of oxygen radicals. The defense response to aphids is based on salicylic acid, jasmonic acid, ethylene, and brassinosteroids-related signaling pathways. Comparative proteomics of phloem exudates of rice plants infested with BPH and a healthy plant revealed that proteins were associated with defense signal transduction, redox regulation, carbohydrate and protein metabolism, and cell structural proteins. Moreover, the defense-related proteins identified in hopper fed rice plants were also produced in response to plant-pathogen interactions (Du et al. 2015). Proteomic changes in potato leaves caused by mechanical wounding, feeding by Colorado potato beetle (*Leptinotarsa decemlineata*), and aphid (*Macrosiphum euphorbiae*) were studied by Duceppe et al. (2012), and it was found that beetle fed leaves had a decrease in the photosynthesis-related proteins.

Proteomic technology also helps in understanding the tritrophic interactions between the plants, insects, and their endosymbionts. Francis et al. (2010) found that the several proteins differentially regulated during these interactions were originated from the endosymbionts which have a role in aphid adaptation to host plant resistance. The proteomic changes in plant by the feeding of specialist herbivore *Manduca sexta* were studied using Matrix-Assisted Laser Desorption

Ionization-Time of Flight (MALDI-TOF) and LC-MS. The phytophagous beetles mainly from Chrysomeloidea and Curculionoidea were thought to have genes which encode different plant cell wall-degrading enzymes. To illustrate this, larval midgut contents of mustard leaf beetle *Phaedon cochleariae* were analyzed using proteomics approach. Thirteen proteins were identified belonging to the families' xylanases, polygalacturonases, and cellulases which are responsible for the degradation of plant cell wall polysaccharides (Kirsch et al. 2012). The responses of aphids (*Macrosiphum euphorbiae*) to induced plant stress, viz., defoliation by beetle (*L. decemlineata*) and water stress, revealed that symbiosis was prioritized during the stressed conditions (Nguyen et al. 2007).

Polyphagy is accompanied by a change in the proteome of insect pests which helps them to adjust to new host plants. Insect pests change their proteomes conveniently to suite their wide host adaptability. Proteomics can be used to study how the plant defenses work inside the insect body and how the insect counteracts these defense strategies. The wheat plant proteins in the gut of sunn pest (*Eurygaster integriceps*) were identified using proteomics. Six proteins, viz., serpin, α-amylase, α-amylase inhibitor, dehydroascorbate reductase, triticin, and α-L-arabinofuranosidase, were identified. α-Amylase inhibitor interferes with the digestive process of insects, and this could be used as a pest management strategy to produce transgenic crops (Saadati and Toorchi 2017). The emerald ash borer (*Agrilus planipennis*) is an invasive wood-boring pest introduced into North America where all the native ash plants (*Fraxinus* spp.) are susceptible. It was found that an Asian species, viz., Manchurian ash (*F. mandshurica*), was resistant to it. To investigate this, phloem proteomes of the resistant and susceptible species were compared. PR-10 (an aspartic protease), PCBER (Phenylcoumaran Benzylic Ether Reductase), and a thylakoid-bound ascorbate peroxidase were found to be the proteins responsible for the resistance. The genes coding for these proteins could be identified and introduced into susceptible plants for host plant resistance against this pest (Whitehill et al. 2011).

13.2.6 Metabolomics

Living organisms respond to genetic or environmental changes, and these changes are frequently reflected in their metabolic profiles. Different kinds of metabolism associated with different functions are represented in Fig. 13.3. The high-throughput characterization of all small-molecule metabolites including those metabolites produced as a plant response as well as biochemical pathway products on a global scale is called metabolomics (Idle and Gonzalez 2007; Palli et al. 2012; Liu and Locasale 2017). It has three approaches, viz., untargeted metabolomics where metabolites of known and unknown identities are screened (qualification), targeted metabolomics where quantification of metabolites occurs very precisely, and metabolic profiling which collects and analyzes data from crude extracts to categorize them based on all metabolites rather than sorting out them into individual metabolites.

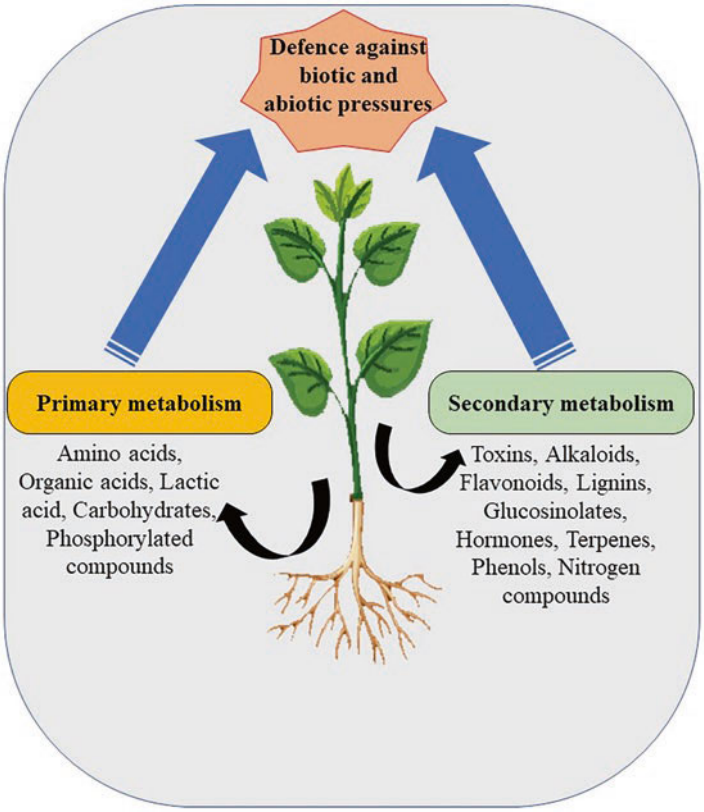

Fig. 13.3 Metabolism associated with different functions related with metabolomics

There are three distinctive techniques used in metabolomics: one is based on gas or liquid chromatography coupled to mass spectrometry (GC- or LC-MS), the second one is Fourier transform-ion cyclotron resonance coupled with mass spectrometry (FT-ICR-MS), and the other is based on nuclear magnetic resonance (NMR). To identify and quantify known metabolites, gas chromatography and LC-MS are used, whereas NMR methods are used to identify unknown metabolites (Palli et al. 2012).

13.2.6.1 Metabolomics as a Tool for Screening Host Plant Resistance

Metabolomics is a comparative tool for screening bioactive compounds in plants which serve as defense against herbivory by insects. The metabolome of a resistant cultivar can be compared with that of a susceptible one, and the compound responsible for resistance can be easily detected. For example, *Barbarea vulgaris*, a brassicaceous species, was found to show natural resistance against

feeding pollen beetles (*Brassicogethes aeneus*) due to secondary plant compounds (glucobarbarin and saponins) in the green flower buds (Austel et al. 2021). Similarly, metabolomics was used to screen defense mechanism against pathogens and pests in cereals. Erb et al. (2009) found out that feeding by Western corn rootworm *Diabrotica virgifera* on roots of maize induced the production of benzoxazinoid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), which is an antifeedant, and it was increased by the feeding of *Spodoptera littoralis*. Metabolomics of resistant wild and susceptible cultivated tomato varieties suggested that resistant ones contained acyl sugars known for their anti-herbivory effects (Mirnezhad et al. 2010). Nuclear magnetic resonance-based metabolomics used for analyzing thrips-resistant cultivars found association of flavonoid luteolin, phenylpropanoid sinapic acid, and amino acid β-alanine. Macel et al. (2019) found that monomer and dimer acyclic diterpene glycosides (capsianosides) were the metabolites in pepper that are linked to resistance.

Metabolomic studies provide an insight into the plant-insect interactions that decide the host range of insect pests based on the metabolites produced by the plant (Sanchez-Arcos et al. 2019). Metabolomics of different legumes infested with host-specific and non-specific races of pea aphid (*Acyrthosiphon pisum*) were analyzed, and the results suggested that the metabolites differed in host-race-specific interaction and non-host-specific interaction.

Most of the time, secondary metabolites of the plants are used against insects in host plant resistance (Mazid et al. 2011). Only a few defensive chemicals have been identified from the plants which are useful for insect resistance. So, metabolomics can help us to search for active compounds in the plants which can be used for plant resistance against insects.

13.2.7 Volatilomics

Volatilomics is a branch of chemistry that studies how biological systems emit Volatile Organic Compounds (VOCs) under controlled conditions. In nature, these molecules are produced by bacteria and fungi (Insam and Seewald 2010). They are also produced by plants (flowers, leaves, fruits, and roots) and animals (humans, insects, etc.). On a broader sense, volatilomics seems to be a subset of metabolomics. However, it is more than a subset of metabolomics as it can help to study chemically mediated interactions between the organisms.

Gas chromatography coupled to mass spectrometry (GC-MS) can be used to differentiate volatile semiochemicals in chemical ecology (Gosset et al. 2009) and biotic interactions between plants, insects, and phytopathogens (Gfeller et al. 2013). Direct infusion mass spectrometry (DI-MS) techniques are a useful tool for monitoring plant volatile organic compound emissions caused by herbivore attacks in real time (Majchrzak et al. 2020).

13.2.7.1 Volatilomics as a Tool for Screening Host Plant Resistance

VOCs are emitted by almost all plants, and the content and composition of these organic compounds vary with different genotypes as well as with the phenotypic plasticity. Airborne VOCs protect plants from herbivory, pathogens, and also attract pollinators, seed dispersers as well as other beneficial microorganisms and animals (Dudareva and Pichersky 2008). Flowers emit VOCs in order to attract pollinators. Insect herbivory activates extra floral nectaries that attract both ants and butterflies. In some cases, oviposition by insects causes plant volatile emission, which parasitoids and predators use to locate hosts and prey. For example, spider mites produce VOCs that attract predators. Plants emit a variety of sesquiterpenes, monoterpenes, and homoterpenes in response to herbivores like *Spodoptera littoralis* chewing on them, which attract predatory wasps. The volatilome is a direct defense mechanism used by some plants by secreting deterrent compounds constitutively in specialized tissues, whereas others produce VOCs as an indirect defense.

Comparing the volatilomes of plants helps us to find out how they respond to different kinds of herbivory. The plants are also known to communicate with each other and share information among them. If a plant attacked by pests can alert the surrounding plants around it, the non-infested plants strengthen their defenses to tackle the incoming infestation. Clancy et al. (2020) using comparative volatilomics and transcriptomics suggested that plant defense against insects varied as the volatile emissions were reduced when fed by aphids and increased when fed with *S. littoralis*. Kalske and Kessler (2020) reported that VOCs emitted by *Solidago altissima* infested with *Trirhabda virgata* can elicit resistance in non-infested plants.

Plants are known to produce chemicals known as herbivore-induced plant volatiles (HIPVs) when attacked by insect pests which are known to attract the insect pests' natural enemies. HIPVs from *Arabidopsis thaliana* infested with *Plutella xylostella* attracted the wasp *Diadegma semiclausum* as compared to the plants which are not infested. Also, *A. thaliana* plants having a high density of the aphid (*Brevicoryne brassicae*) population attracted *D. semiclausum* when compared to the plants having a low density of aphids (Kroes et al. 2017).

13.2.8 Phenomics

Phenomics refers to high-dimensional phenotypic data on a large scale for an organism, whereas phenome refers to the overall phenotype, i.e., how a trait's genome is expressed in a given environment. Traditionally, phenotyping data has been recorded visually or manually, which is both prolonged and labor-intensive. In recent years, there has been a raise in the use of sensing technologies for detecting specific phenotypic reactions that occur during plant-insect interaction as a result of the increased possibility of measurement errors in traits. The application of non-invasive imaging technologies to high-throughput phenotyping is a rapidly developing field (Berger et al. 2010). Color imaging technique is used to estimate biomass, plant

structure, phenology, and leaf health, for example (chlorosis, necrosis). Plant responses to disease-causing pathogens and pests can be identified conveniently prior to economic losses using phenomics approaches such as chlorophyll fluorescent imaging, thermal and hyperspectral imaging, etc. (Shashko et al. 2020).

13.2.8.1 Applications of HTP to Measure Insect Damage

Plant health and productivity, as well as herbivorous insect feeding damage, are measured using high-throughput phenotyping (HTP) systems. RGB cameras or flatbed scanners can be used to quantify caterpillar defoliation, aphid-caused chlorosis and necrosis, and thrips-caused feeding scars with images captured by it digitally (Hebert et al. 2007). Mite infestations on plants can be detected using multicolor fluorescence imaging as mites cause a significant increase in the blue-to-red autofluorescence ratio (Buschmann and Lichtenthaler 1998). Aphids can be detected remotely using multispectral and hyperspectral imaging (Backoulou et al. 2011).

HTP systems can detect symptoms of infestation that aren't apparent to the naked eye like the stomatal conductance and water balance of plants, which are affected by the infestation of insect pests using near-infrared and far-infrared cameras (Nabity et al. 2009). Fluorescence cameras can be used to detect photosynthetic efficiency and chlorophyll content (Kerchev et al. 2012). In phenotyping studies, diagnostic spectral signatures linked to pest damage severity can be used to enumerate development of symptoms and estimate pest abundance. For insect behavior, survival, and development bioassays, which are important for determining host plant resistance, high-throughput imaging has the potential to reduce labor and processing time.

The damage symptoms of insects on their host plants can be quantified digitally using HTP platforms. Physiological aberrations in plants as a result of insect herbivory can also be measured by infrared imaging. Remote sensing of insect pests can be possible in the field of pest monitoring using HTP systems. Phenotyping systems can study plant interactions with pests feeding on the roots.

13.3 Data Repositories

A data repository can be defined as a place that stores data, makes it accessible for use, and organizes it in a logical manner. Modern biology places a strong emphasis on sharing this available data and knowledge. The development of autonomous databases containing genetic information for various insects and plants is underway (Clement and Quisenberry 1998). NCBI (2021) search, for instance, of plant and insect genome projects yielded 793 and 832 records and their associated data. Whole-genomic sequence data has emerged as the main source of information for designing microarrays, tilling arrays, and molecular markers, as well as a significant reference for combining other omics-derived data with genome sequences.

Phytozome (Goodstein et al. 2012) (http://www.phytozome.net/) and Gramene (Monaco et al. 2014) (http://www.gramene.org/) are the two genomics projects among green plants that have established to be successful in accelerating gene discovery and its functional analyses. Some of the insect and plant data repositories are represented in Tables 13.1 and 13.2.

Repositories may expand the scope of comparative in silico analysis to better estimate plant-insect interactions in a variety of non-model insect and plant species (Fukushima et al. 2009). To decipher the systems level biology, physiology, disease mechanisms, insect-plant interaction, insect resistance, and growth and

Table 13.1 A list of insect-related repositories

Taxon/description	URL
Fruit fly, *Drosophila melanogaster*	http://www.flybase.net
Mosquito, *Aedes aegypti*	http://mosquito.colostate.edu/tikiwiki/
Anopheles gambiae	http://www.ensembl.org/Anopheles_gambiae/
Hessian fly	http://agripestbase.org/hessianfly/
Honey bee, *Apis mellifera*	http://www.hgsc.bcm.tmc.edu/projects/honeybee/
Hymenoptera	http://hymenopteragenome.org/
Ant	http://antgenomes.org/
Harpegnathos saltator	http://cib.res.in/
Camponotus floridanus	http://cib.res.in/
Locusta migratoria	http://locustdb.genomics.org.cn/
Aphid	http://w3.rennes.inra.fr/AphidBase/
Acyrthosiphon pisum	http://cib.res.in/
Leaf beetle egg-induced defense genes	http://www.agcol.arizona.edu/pave/elm/
Dendroctonus ponderosae	http://cib.res.in/
Dung beetle	http://flylab.wits.ac.za/EI/est2uni/home.php
Lepidoptera	http://butterflybase.ice.mpg.de/
Heliconius melpomene	http://cib.res.in/
Silkworm moth, *Bombyx mori*	http://www.ab.a.u-tokyo.ac.jp/silkbase/
Wild silk moths	http://www.cdfd.org.in/wildsilkbase/home.php
Diamondback moth	http://iae.fafu.edu.cn/DBM/
Manduca sexta	http://agripestbase.org/manduca/
Spodoptera	http://bioweb.ensam.inra.fr/spodobase/
InsectBase	http://www.insectgenome.com
Fully sequenced insect genomes	http://cdfd.org.in/INSATDB/home.php
Arthropod cuticular proteins	http://bioinformatics.biol.uoa.gr/cuticleDB/
Immune genes in insect genomes	http://bordensteinlab.vanderbilt.edu/IIID/test_immunity.php
Fully sequenced insect genomes	http://cdfd.org.in/INSATDB/home.php
Agricultural pests	http://agripestbase.org/
NCBI (GeneBank & Refseq)	http://www.ncbi.nlm.nih.gov

Table 13.2 A list of plant-related repositories

Crop/group	URL
Oryza sativa Japonica	http://rgp.dna.affrc.go.jp/E/IRGSP/index.html
Oryza sativa indica	http://rice.genomics.org.cn/rice/index2.jsp
Zea mays	http://www.maizegdb.org/
Sorghum bicolor	http://genome.jgi-psf.org/Sorbi1/Sorbi1.home.html
Triticum aestivum	http://www.wheatgenome.org/
Hordeum vulgare	http://www.public.iastate.edu/~imagefpc/IBSC%20Webpage/IBSC%20Template-home.html
Capsella rubella	http://www.jgi.doe.gov/sequencing/why/3066.html
Brassica	http://www.jgi.doe.gov/sequencing/why/3066.html
Solanum lycopersicum	http://solgenomics.net/
Solanum tuberosum	http://www.potatogenome.net/index.php/Main_Page
Manihot esculenta	http://www.phytozome.org/cassava.php
Ricinus communis	http://castorbean.jcvi.org/
Vitis vinifera	http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/
Carica papaya	http://asgpb.mhpcc.hawaii.edu/papaya/
Legumes	legumeinfo.org
Rosaceae crops	rosaceae.org
Salicaceae	Popgenie.org

development of various important insects, molecular biologists use high-throughput genomics, transcriptomics, epigenomics, regulatory genomics, and proteomics methods. This portal will provide a comprehensive overview of insect-specific data resources and application tools, including genomics, transcriptomics, proteomics, regulatory genomics, miRNA profiling, RNA interference studies, and more.

Pooled repositories containing information about plant-herbivore interactions are uncommon, despite the availability of separate plant and insect resources. Currently, "Interaction Web Database" contains data sets published on interaction of species from a variety of communities around the world. It currently contains information for various interaction types, including plant-pollinator, plant-frugivore, plant-herbivore, plant-ant mutualist, and predator-prey interactions. The "BRC–Database of Insects and their Food Plants" has 47,000 interactions between 9300 invertebrate taxa (insects and mites) and their host plants (http://www.brc.ac.uk/dbif/homepage.aspx). HOSTS is a lepidopteran host plant database with 180,000 records containing taxonomically "clean" host plant data for 22,000 Lepidoptera species culled from 1600 published and manuscript sources (Robinson et al. 2010). The leading repositories in molecular field are GenBank, http://www.ncbi.nlm.nih.gov/genbank, and DDBJ, https://www.ddbj.nig.ac.jp; ENA, https://www.ebi.ac.uk/ena.

13.4 Bioinformatics and Its Tools to Integrate Omics Data

The data obtained through multi-omics approaches is available in a variety of formats, most of which are machine-readable. Generated omics data from various platforms typically represents genes, transcripts, proteins, and metabolites that are potentially interconnected in network pathways. Bioinformatics is critical for integrating and intercepting omics data, and the integration of this information aids in a better understanding of insect pest systems biology. Bioinformatics is the use of information technology tools such as databases and mining software to apply mathematical approaches and algorithms to biology. Omics data analysis includes data processing and molecular identification, statistical data analysis, pathway and network analysis, and system modeling. It also enables the use of knowledge management, annotation and text mining tools, pathway identification, and network inference and analysis to integrate heterogeneous high-throughput data sets generated by a study with existing data sets. These efforts are aimed at elucidating the molecular pathways that underpin physiology in order to describe a system that uses a combination of environmental and physiological measures to improve detection and monitoring of a phenomenon, such as insect damage in plant protection research, and to facilitate treatment and management.

Tools and methods for integrating multiple omics data sets must be used to better understand insect-plant interactions. The tools will be chosen based on the following criteria. To begin, the methodology must include an integrative step in which multiple data sets are studied at the same time. Second, the method must include a minimum of two omics data sets derived from samples that are similar in some way. Finally, the method or technique should be easily accessible as a tool or set of tools that can be used with any data set. The tools/methods are classified in the following sections based on their capacity to address various biological case studies (Subramanian et al. 2020).

(a) ***Bayesian Approach***: "Bayesian statistics is a mathematical procedure that applies probabilities to statistical problems. It provides people the tools to update their beliefs in the evidence of new data. A data analysis approach that provides a posterior probability distribution for some parameter (e.g., treatment effect) derived from observed data as well as a prior probability distribution for the parameter. Statistical inference is based on the posterior distribution." It includes tools like Pathway Recognition Algorithm using Data Integration on Genomic Models (PARADIGM), iCluster, iClusterPlus, LRAcluster, Bayesian Consensus Clustering (BCC), Multiple Dataset Integration (MDI), and Bayesian Random Effects Mixture model for joint clustering Single Cell multi-omics data (BREM-SC). For example, the evolution of pierid butterflies and their hosts as they compete for dominance in a serious arms race could be easily studied by integrating network pathway analysis in them using Bayesian approaches.

(b) ***Network Approach***: The use of a network-based approach to integrate and interpret various omics data sets, including metabolomics, is rapidly gaining traction,

e.g., Similarity Network Fusion (SNF). Kantsa et al. (2019) investigated the interaction of plant volatiles and pollinators using a network-based approach.

(c) ***Fusion-Based Approaches***: Data fusion is the process of combining multiple data sources to produce information that is more consistent, accurate, and useful than any single data source can provide. Data fusion processes are frequently classified as low, intermediate, or high, depending on the stage of processing where fusion occurs. Low-level data fusion combines raw data from numerous sources to generate new raw data. Fusional data is estimated to be more informative and synthetic than the original inputs, e.g., Pattern Fusion Analysis (PFA). Lee et al. (2011) integrated the genomic and proteomic data of the rice plant into a network called Rice Net using which they identified pathways associated with response to biotic stress.

(d) ***Similarity-Based Approaches***: Similarity-based methods use similarities or distances between samples to cluster data. The similarities between samples in each omics are computed separately using these methods and then combined in different ways. Only similarity values are used in the integration step. Similarity-based methods have the advantage of being able to support a wide range of omics types, as well as categorical and ordinal data. Only a definition of a similarity measure is required for each omics, e.g., PINSPlus and Neighborhood-based Multi-Omics clustering (NEMO). Muto-Fujita et al. (2017) used similarity-based approach to integrate the omics data for studying the host plant-insect interaction. They reported that plants taxonomically related to the host plant are selected by particular families of butterfly.

(e) ***Other Multivariate Approaches***: mixOmics, moCluster, Multiple Co-inertia Analysis (MCIA), Joint and Individual Variation Explained (JIVE), Multiple Factor Analysis (MFA), rMKL-LPP, and integrative Non-negative Matrix Factorization (iNMF). Comparative metabolomics was performed to analyze the performance of Hepialid moths to exotic plants. The analysis of metabolomic data was performed using mixOmics (Atijegbe et al. 2020).

13.5 Integrated Omics Approach in Insect-Plant Interactions

High-throughput omics techniques like genomic, transcriptomic, metabolomic, proteomic, volatilomic, phenomic, etc. have been widely used in the insect-plant interaction studies. The availability of large-scale omics data sets has resulted in the emergence of integrated multi-omics approach, which helps in understanding the functional complexity of the biological systems ranging from microscopic molecular mechanisms to macroscopic ecological communities. The insect-plant interaction can be chosen as the best model as huge information is available at molecular and ecosystem levels. Some of the studies related to multi-omics approaches in insect-plant interactions were listed in Table 13.3.

To acquire nutrients from their host plants, herbivores have evolved diverse feeding mechanisms. Plants are affected in various ways by different herbivores,

Table 13.3 Multi-omics approaches in insect-plant interactions

	Type of sample	Methods	Multi-omics approach	Tools	Reference
Insects					
Tribolium castaneum	CNS and protein releasing sites	Expressed sequence tags, RT-PCR, MALDI-TOF/MS, ESI-Q-TOF/MS	Genomics, transcriptomics, and peptidomics	BLAST, GENBOREE, NCBI Database and trace archives, ClustalW	Li et al. (2008)
Acyrthosiphon pisum	Salivary glands	EST sequencing, LC-MS/MS, MALDI-TOF/MS	Transcriptomics and proteomics	EGassembler, NCBI EST library, Blast2GO, InterProScan, PhylomeDB, ClustalX	Carolan et al. (2011)
Spodoptera frugiperda	Cells of Sf9 line	qRT-PCR, iTRAQ analysis, LC-MS/MS	Transcriptomics and proteomics	BLAST+, NCBI non-redundant protein database, Swiss-Prot protein database, KEGG database, COG database, Blast2GO	Cui et al. (2020)
Aphis gossypii	Whole body	Gene ontology, qRT-PCR, LC-ESI-MS/MS, RACE-seq, RNA-seq	Transcriptomics and proteomics	TransDecoder, Trinotate, Gene Ontology analysis, and Cluster of Orthologous Group annotation, UniProt/Swiss-Prot Homo database	Chen et al. (2019)
Nilaparvata lugens Sogatella furcifera Laodelphax striatellus	Salivary gland	FT-ICR-MS, qRT-PCR	Transcriptomics and proteomics	SOAP, TGI clustering tool, GenBank databases, Blast2go, InterProScan software, COG database, blastx, Estscan, BGI WEGO, NCBI database	Huang et al. (2018)
Leguminivora glycinivorella	Larvae (both 3rd instar and diapause-destined) and pupae	LC-MS/MS, qRT-PCR, RNA-seq	Transcriptomics and proteomics	UniProt database, KEGG database, NCBI non-redundant protein database, Protein family database, COG database, KO database, Swiss-Prot database, GO database, Blast2GO	Yang et al. (2020)

(continued)

Table 13.3 (continued)

	Type of sample	Methods	Multi-omics approach	Tools	Reference
Phaedon cochleariae	Larvae	2-DE, LC-MS/MS, RNA-seq	Transcriptomics and proteomics	NCBInr database, CLC Genomics Workbench, KEGG, InterProScan, GO analysis, Blast2GO, Mascot	Kirsch et al. (2012)
Bemisia tabaci	Adults	RNA-seq, qRT-PCR, gene ontology, nLC-MS/MS	Transcriptomics and proteomics	NCBInr database, KEGG, Blast2GO, SOAPdenovo, Mascot	Yang et al. (2013)
Acyrthosiphon pisum	Final larval stadium	NMR spectroscopy, DIGE, 2-DE, CID-MS/MS	Proteomics and metabolomics	NCBInr database, *A. pisum* genome assembly, *A. pisum* ESTdatabase, AphidBase, InterProScan, Blast2GO, AcypiCyc database	Wang et al. (2010)
Plants					
Tanacetum vulgare	Leaves	PTR-ToF-MS, GC-MS, RNA-seq, PCR	Volatilomics and transcriptomics	TransDecoder, Trinity, Evigene pipeline, AHRD pipeline, InterProScan, Protein family database, Swiss-Prot database, Trembl database, NCBI invertebrate protein database	Clancy et al. (2020)
Solanum lycopersicum	Leaves	Microarray analysis, RT-PCR, 2-DE, MALDI-TOF/MS, LC-MS/MS, MALDI-TOF PMF	Transcriptomics and proteomics	BlastN, Blast2GO, SGN Tomato Unigene database, NCBI non-redundant protein database	Coppola et al. (2013)
Solanum lycopersicum	Leaves	RNA-seq, LC-ESI-MS, RT-PCR	Transcriptomics and metabolomics	GO and GOslim annotations, KEGG database, Pubchem database, HMD database, Golm Metabolome Database, PlantCyc, SIEVE, GENE-E	Coppola et al. (2019)

(continued)

Table 13.3 (continued)

	Type of sample	Methods	Multi-omics approach	Tools	Reference
Camellia sinensis	Leaves	RNA-seq, PacBio, Iso-Seq, GC-TOF-MS, qPCR, RT-PCR	Transcriptomics and metabolomics	NCBInr database, Swiss-Prot Protein database, KEGG database, COG database, Pfam database, GO database, LECO-Fiehn Rtx5 database	Liu et al. (2021)
Triticum aestivum	Stem	RNA-seq, MALDI-TOF-MS, GC-TOF-MS	Transcriptomics, proteomics, and metabolomics	miRBase, NCBI nr protein database, Swiss-Prot database, Pfam database, TransDecoder, Blast2GO, DeCyder, MASCOT, Triticeae Toolbox, GrainGenes	Biyiklioglu et al. (2018)
Triticum aestivum	Stem	nHPLC-MS/MS, UPLC-MS/MS	Proteomics and metabolomics	Wheat protein database, UniProt database, R package InterpretMSSpectrum, MS-FINDER program v2.40, Human Metabolome Database, METLIN	Lavergne et al. (2020)

ranging from higher trophic levels to the molecular level, when they are attacked (Heil 2008; Mooney et al. 2012). Insect-associated organisms, such as parasites and endosymbionts, can also have an impact on plant-insect interactions (Douglas 2013; Zhu et al. 2014). Plants have evolved a variety of morphological, biochemical, and molecular level defense survival strategies in response to herbivory.

The Hypothesized in Higher in Response to Insect Attack

Recognition of insect oral secretions/structural components of the insect mouth parts

↓

Signals from injured plant cells (signal input), followed by signal transduction (calcium ion fluxes, phosphorylation cascades, and hormonal cross-talk)

↓

Signal processing (reprogramming of the transcriptome, proteome, and metabolome)

↓

Signal responses (production of defense compounds, , and)

↓

Genotypic and/or phenotypic responses (adaptation, selection, and evolution) (Eulgem 2005; Howe and Jander 2008)

Ecologists are mainly interested in the study of genes, metabolites, and pathways and the development of insect pest management in plant-insect interaction models considering the impact of biotic and abiotic factors on insect populations (Tonhasca Jr and Byrne 1994; Adams et al. 2005; Bezemer et al. 2014). Omics data availability has led to increased interest for utility of integrated approaches like combining molecular and ecological approaches to understand the insect-plant interactions from community to gene level (Baldwin 2001; Stam et al. 2014).

High-throughput screening of the insect genomes, epigenomes, and metagenomes helps to identify the plant response to insect herbivory. It is phenotypical change incited in the plant as a response to the herbivory stems from the changes occurring at the genetic level. Comparison of the genomes, epigenomes, and metagenomes of the plants provides a basic idea of the genetic response of the plants to insect herbivory. For example, comparative transcriptomic and biochemical analysis of tomato and brinjal suggested that salicylic acid and jasmonic acid were considerably reduced in tomato after the attack of *Tuta absoluta*, while their concentrations are considerably increased after the infestation in brinjal. The transcriptomics revealed that 1072 and 2834 genes were differentially expressed in tomato and brinjal, respectively. Subjecting the differentially expressed genes to Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that PR1b1, NPR1, NPR3, MAPKs, and ANP1 gene families are triggered as a response to *T. absoluta* infestation (Chen et al. 2021).

Changes in gene expression levels are one of the globally assessable effects of plant responses to herbivores (Reymond et al. 2000). The field of transcriptomics has undergone several changes from the first microarray chip, which detected 45 *Arabidopsis thaliana* transcripts (Schena et al. 1995), to full transcriptome microarrays and RNA sequencing (Gan et al. 2011). Now it is considered as one of the major omics technique to study the response of a plant to insect herbivory (Thompson and Goggin 2006). In response to a variety of insect attacks, comparative transcriptomics can be used to recognize common and attacker-specific gene expression patterns (Dubey et al. 2013). Plant defense is a dynamic process with a regulated transcriptome in both time and space (Windram et al. 2012). Recent technologies like NGS can be used for transcriptomic analysis in identifying plant species consumed by herbivores by characterizing the DNA present in gut or feces of insects (Pompanon et al. 2012) and also aid in the identification of genetic loci for ecologically important traits.

Modern proteomic technologies, such as high-throughput quantitative proteomics, aid in the characterization of proteomes and their differential modulation during plant development as well as biotic and abiotic stresses. Collins et al. (2010) identified physiological factors influencing feeding behavior by *Plutella xylostella* larvae on the proteomes of herbivore-susceptible and resistant *A. thaliana* recombinant inbred lines. Zhang et al. (2013a, b) used Polyethyleneimine Assisted Rubisco Cleanup (PARC) to investigate defense mechanisms during plant-insect interactions. Duceppe et al. (2012) carried out comparative proteomic analysis of 500 leaf proteins and identified potato plant (*Solanum tuberosum* L.) response to mechanical wounding and herbivory by Colorado potato beetle *Leptinotarsa decemlineata* or

potato aphid (*Macrosiphum euphorbiae*) using two-dimensional gel electrophoresis (2-DE). Francis et al. (2010) identified contribution of symbionts to differential adaptation of avirulent and semi-virulent potato aphids to resistant and susceptible tomato lines using 2D-DIGE (Difference Gel Electrophoresis) coupled with protein recognition by MALDI-TOF-MS (Matrix-Assisted Laser Deionization-Time of Flight-Mass Spectrometry).

Secondary metabolites acting as signal molecules or direct defense chemicals in plants, such as alkaloids, terpenoids, cyanogenic glycosides, glucosinolates, and phenolics, have been linked to the evolution of plant chemical defense systems against herbivory (Zebelo and Maffei 2012). Poelman et al. (2008) observed negative correlation between herbivore abundance and *Brassica oleracea* richness due to the presence of secondary metabolite, glucoiberin. Schranz et al. (2009) in *Boechera stricta* found cytochrome P450s variations in QTL produced the glucosinolate compounds like valine and isoleucine contributing resistance to cabbage looper (*Trichoplusia ni*). Metabolic engineering of raffinose family of oligosaccharides in *A. thaliana* phloem resulted in changes in carbon partitioning as well as increased resistance to feeding by the green peach aphid (*Myzus persicae*) (Cao et al. 2013). Jansen et al. (2009) studied metabolic changes in both *Brassica oleracea* and *Pieris rapae* and discovered phenylpropanoids that were induced in plant tissue and found in the insect during feeding. As a result, metabolomic analyses can offer valuable information about defense in plants against insects, or they can be combined with other omics approaches to link phenotype and genotype. Plants emit volatile organic compounds which have a significant role in the host selection process. The odor cues that attract the pests could be analyzed using volatilomic approach. Analyzing the plant volatiles that attract insect pests for oviposition can be used to develop environmentally friendly pest management strategies.

With the advancement of genomics, transcriptomics, proteomics, and metabolomics technology, high-throughput and high-resolution phenomics tools for measuring phenotypic traits of organisms in response to genetic mutation and external factors have been rapidly evolving (Furbank and Tester 2011; Fiorani and Schurr 2013). It needs coordinated skill of non-invasive imaging, spectroscopy, image analysis, robotics, and high-performance computing (Finkel 2009). In large plant collections, Chen et al. (2012) developed high-throughput phenotyping methods to recognize increased resistance to aphids. According to Stiling and Cornelissen (2007), higher levels of CO_2 reduced herbivore abundance; increased relative consumption rates, development time, and total consumption; and reduced relative growth rate, conversion efficiency, and pupal weight. We can now explore relevant correlations and construct mathematical or statistical models describing different biological processes related to plant-insect interaction using high-throughput multi-omics data and robust bioinformatics and data mining tools. New biological hypothesis can be generated, tested, and corrected based on predicted models derived from high-throughput omics data before being used for insect-resistant crop plant synthetic engineering and integrated pest management.

13.6 Potential and Future Directions

Multi-omics approaches hold promise for environment-friendly insect pest management, reduced pollution, and improved human health. The pest and disease resistance traits that are naturally present in crops can be easily exploited to develop resistant crops. As screening the plants in natural habitats takes a lot of time, multi-omics approaches come in handy as they are reliable, quick, and effective tools for identifying the desirable traits in the plants. These omics strategies make breeding for host plant resistance and profiling for plant-based antibiotics, therapeutics, and drugs easier and faster, making it simple to study the complex interactions between insect pests and their hosts. Novel insecticides' modes of action and targets could be screened, and insecticides with unknown modes of action could be understudied by comparing them to known mode of action compounds (Aliferis and Chrysayi-Tokousbalides 2011). These omics tools can also help researchers figure out how insects develop resistance to insecticides (Chen et al. 2019).

These technologies could be used to investigate the molecular changes and signaling pathways that influence and regulate insect growth and development, as well as diapause. For example, the polydnaviruses infecting the parasitoid wasps (Ichneumonidae and Braconidae) alter the immunity of these insect hosts and ensure successful parasitization (Hasegawa et al. 2017; Hasegawa and Turnbull 2014). These technologies could be used to examine the interactions between virus particles and insect immunity, as well as to explore their potential for biological control. The interactions of insects with their endosymbionts and their role in insect development and survival could be studied using technologies like Target-Enriched Endosymbiont Sequencing (TEEseq). Besides this, omics tools could also be applied to interdisciplinary research as well. The process of pesticide development usually taking longer periods could be shortened using these omics tools. Omics approaches are also used in cancer research, drug interactions in living organisms, establishing the phylogeny, and understanding the evolution and other biotechnological aspects.

13.7 Conclusion

The warfare among the plants and insect herbivores decides the successful infestation of the insect pests or the successful defense of the host plants. This war is a complex phenomenon and is a race of armaments against time and evolution as both the pests and insects try to counter each other's defense strategies. Ascertaining the arsenal of both the groups in this war could be a game-changing decision which determines the winner and the loser. As both the groups try to overpower each other, they tend to produce many changes in their structure and physiology. For any given change occurring in an organism, there might be a cascade of chemical reactions triggered and executed to bring about that particular change. These networks of

pathways are complicated and are difficult to comprehend. The changes occurring both in the insects and plants at the molecular level could be understudied for untapping their potential in pest management. Multi-omics technologies prove useful for studying these network pathways. They are helpful to identify the pathways starting from the scratch, i.e., genes involved in the pathways to the end result of their expression leading to the formation of metabolites which are the end result. Besides their use in agricultural sector, they have potential in screening plant-derived compounds which improve the health of human beings. Although a considerable amount of work was done on these technologies and their use in screening defense-related compounds, still their usage is limited due to the lack of knowledge and expertise in these technologies besides its high cost. In the near future, these technologies could be used for understanding the complex interactions between the insect pests and their hosts, screening novel pesticide molecules, the development of insect-resistant transgenic plants and therapeutic drugs in human health, and behavior manipulation technologies useful for ecofriendly pest management.

References

Abhilash PC, Singh N (2009) Pesticide use and application: an Indian scenario. J Hazard Mater 165(1–3):1–12. https://doi.org/10.1016/j.jhazmat.2008.10.061

Adams BM, Banks HT, Banks JE, Stark JD (2005) Population dynamics models in plant–insect herbivore–pesticide interactions. Math Biosci 196(1):39–64. https://doi.org/10.1016/j.mbs.2004.09.001

Aktar MW, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2(1):1–12. https://doi.org/10.2478/v10102-009-0001-7

Aliferis KA, Chrysayi-Tokousbalides M (2011) Metabolomics in pesticide research and development: review and future perspectives. Metabolomics 7(1):35–53. https://doi.org/10.1007/s11306-010-0231-x

Allio R, Nabholz B, Wanke S, Chomicki G, Pérez-Escobar OA, Cotton AM, Clamens AL, Kergoat GJ, Sperling FA, Condamine FL (2021) Genome-wide macroevolutionary signatures of key innovations in butterflies colonizing new host plants. Nat Commun 12(1):1–15. https://doi.org/10.1038/s41467-020-20507-3

Atijegbe SR, Mansfield S, Ferguson CM, Worner SP, Rostás M (2020) Host range expansion of an endemic insect herbivore is associated with high nitrogen and low fibre content in exotic pasture plants. J Chem Ecol 46:544–556. https://doi.org/10.1007/s10886-020-01183-5

Austel N, Böttcher C, Meiners T (2021) Chemical defence in Brassicaceae against pollen beetles revealed by metabolomics and flower bud manipulation approaches. Plant Cell Environ 44(2):519–534. https://doi.org/10.1111/pce.13949

Backoulou GF, Elliott NC, Giles K, Phoofolo M, Catana V, Mirik M, Michels J (2011) Spatially discriminating Russian wheat aphid induced plant stress from other wheat stressing factors. Comput Electron Agric 78(2):123–129. https://doi.org/10.1016/j.compag.2011.06.005

Baldwin IT (2001) An ecologically motivated analysis of plant-herbivore interactions in native tobacco. Plant Physiol 127(4):1449–1458. https://doi.org/10.1104/pp.010762

Baulcombe DC, Dean C (2014) Epigenetic regulation in plant responses to the environment. Cold Spring Harb Perspect Biol 6(9):a019471. https://doi.org/10.1101/cshperspect.a019471

Berger B, Parent B, Tester M (2010) High-throughput shoot imaging to study drought responses. J Exp Bot 61(13):3519–3528. https://doi.org/10.1093/jxb/erq201

Betancourt EK, Soto PH, Cortés NC, Anaya MR, Estrella AH, Oyama K (2020) Ecological genomics of plant-insect interactions: the case of wasp-induced galls. In: Núñez-Farfán J, Valverde P (eds) Evolutionary ecology of plant-herbivore interaction. Springer, Cham, pp 315–341. https://doi.org/10.1007/978-3-030-46012-9_17

Bezemer TM, Harvey JA, Cronin JT (2014) Response of native insect communities to invasive plants. Annu Rev Entomol 59:119–141. https://doi.org/10.1146/annurev-ento-011613-162104

Biyiklioglu S, Alptekin B, Akpinar BA, Varella AC, Hofland ML, Weaver DK, Bothner B, Budak H (2018) A large-scale multiomics analysis of wheat stem solidness and the wheat stem sawfly feeding response, and syntenic associations in barley, Brachypodium, and rice. Funct Integr Genomics 18(3):241–259. https://doi.org/10.1007/s10142-017-0585-5

Bogdanov B, Smith RD (2005) Proteomics by FTICR mass spectrometry: top down and bottom up. Mass Spectrom Rev 24(2):168–200. https://doi.org/10.1002/mas.20015

Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ (2013) Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat Methods 10(12):1213–1218. https://doi.org/10.1038/nmeth.2688

Buschmann C, Lichtenthaler HK (1998) Principles and characteristics of multi-colour fluorescence imaging of plants. J Plant Physiol 152(2–3):297–314. https://doi.org/10.1016/S0176-1617(98)80144-2

Cao T, Lahiri I, Singh V, Louis J, Shah J, Ayre BG (2013) Metabolic engineering of raffinose-family oligosaccharides in the phloem reveals alterations in carbon partitioning and enhances resistance to green peach aphid. Front Plant Sci 4:263. https://doi.org/10.3389/fpls.2013.00263

Carolan JC, Caragea D, Reardon KT, Mutti NS, Dittmer N, Pappan K, Cui F, Castaneto M, Poulain J, Dossat C, Tagu D (2011) Predicted effector molecules in the salivary secretome of the pea aphid (*Acyrthosiphon pisum*): a dual transcriptomic/proteomic approach. J Proteome Res 10(4):1505–1518

Chen X, Vosman B, Visser RG, van der Vlugt RA, Broekgaarden C (2012) High throughput phenotyping for aphid resistance in large plant collections. Plant Methods 8(1):1–7. https://doi.org/10.1186/1746-4811-8-33

Chen X, Xia J, Shang Q, Song D, Gao X (2019) UDP-glucosyltransferases potentially contribute to imidacloprid resistance in *Aphis gossypii* glover based on transcriptomic and proteomic analyses. Pestic Biochem Physiol 159:98–106. https://doi.org/10.1016/j.pestbp.2019.06.002

Chen LM, Li XW, He TJ, Li PJ, Liu Y, Zhou SX, Wu QC, Chen TT, Lu YB, Hou YM (2021) Comparative biochemical and transcriptome analyses in tomato and eggplant reveal their differential responses to *Tuta absoluta* infestation. Genomics 113(4):2108–2121. https://doi.org/10.1016/j.ygeno.2021.05.002

Chopra RS, Chopra C, Sharma NR (2020) Metagenomics: techniques, applications, challenges and opportunities, 1st edn. Springer, Singapore, p 227. https://doi.org/10.1007/978-981-15-6529-8

Clancy MV, Haberer G, Jud W, Niederbacher B, Niederbacher S, Senft M, Zytynska SE, Weisser WW, Schnitzler JP (2020) Under fire-simultaneous volatilome and transcriptome analysis unravels fine-scale responses of tansy chemotypes to dual herbivore attack. BMC Plant Biol 20(1):1–18. https://doi.org/10.1186/s12870-020-02745-1

Clement SL, Quisenberry SS (1998) Global plant genetic resources for insect-resistant crops, 1st edn. CRC Press, Boca Raton, FL, p 320. https://doi.org/10.1201/9780429117855

Collins RM, Afzal M, Ward DA, Prescott MC, Sait SM, Rees HH, Tomsett AB (2010) Differential proteomic analysis of *Arabidopsis thaliana* genotypes exhibiting resistance or susceptibility to the insect herbivore, *Plutella xylostella*. PLoS One 5(4):e10103. https://doi.org/10.1371/journal.pone.0010103

Coppola V, Coppola M, Rocco M, Digilio MC, D'Ambrosio C, Renzone G, Martinelli R, Scaloni A, Pennacchio F, Rao R, Corrado G (2013) Transcriptomic and proteomic analysis of a compatible tomato-aphid interaction reveals a predominant salicylic acid-dependent plant response. BMC Genomics 14(1):1–18. https://doi.org/10.1186/1471-2164-14-515

Coppola M, Diretto G, Digilio MC, Woo SL, Giuliano G, Molisso D, Pennacchio F, Lorito M, Rao R (2019) Transcriptome and metabolome reprogramming in tomato plants by *Trichoderma*

harzianum strain T22 primes and enhances defense responses against aphids. Front Physiol 10:745. https://doi.org/10.3389/fphys.2019.00745

Cui G, Sun R, Veeran S, Shu B, Yuan H, Zhong G (2020) Combined transcriptomic and proteomic analysis of harmine on *Spodoptera frugiperda* Sf9 cells to reveal the potential resistance mechanism. J Proteome 211:103573. https://doi.org/10.1016/j.jprot.2019.103573

Davis FR (2014) Banned: a history of pesticides and the science of toxicology. Yale University Press, New Haven, CT, p 288. https://www.jstor.org/stable/j.ctt13x1tbs

De Clerck C, Fujiwara A, Joncour P, Léonard S, Félix ML, Francis F, Jijakli MH, Tsuchida T, Massart S (2015) A metagenomic approach from aphid's hemolymph sheds light on the potential roles of co-existing endosymbionts. Microbiome 3(1):1–11. https://doi.org/10.1186/s40168-015-0130-5

Doudna JA, Charpentier E (2014) The new frontier of genome engineering with CRISPR-Cas9. Science 346:6213. https://doi.org/10.1126/science.1258096

Douglas AE (2013) Microbial brokers of insect-plant interactions revisited. J Chem Ecol 39(7):952–961. https://doi.org/10.1007/s10886-013-0308-x

Du B, Wei Z, Wang Z, Wang X, Peng X, Du B, Chen R, Zhu L, He G (2015) Phloem-exudate proteome analysis of response to insect brown plant-hopper in rice. J Plant Physiol 183:13–22. https://doi.org/10.1016/j.jplph.2015.03.020

Dubey NK, Goel R, Ranjan A, Idris A, Singh SK, Bag SK, Chandrashekar K, Pandey KD, Singh PK, Sawant SV (2013) Comparative transcriptome analysis of *Gossypium hirsutum* L. in response to sap sucking insects: aphid and whitefly. BMC Genomics 14(1):1–20. https://doi.org/10.1186/1471-2164-14-241

Duceppe MO, Cloutier C, Michaud D (2012) Wounding, insect chewing and phloem sap feeding differentially alter the leaf proteome of potato, *Solanum tuberosum* L. Proteome Sci 10(1):1–14. https://doi.org/10.1186/1477-5956-10-73

Dudareva N, Pichersky E (2008) Metabolic engineering of plant volatiles. Curr Opin Biotechnol 19:181–189. https://doi.org/10.1016/j.copbio.2008.02.011

Duhlian L, Koramutla MK, Subramanian S, Chamola R, Bhattacharya R (2020) Comparative transcriptomics revealed differential regulation of defense related genes in *Brassica juncea* leading to successful and unsuccessful infestation by aphid species. Sci Rep 10(1):1–14. https://doi.org/10.1038/s41598-020-66217-0

Erb M, Flors V, Karlen D, De Lange E, Planchamp C, D'Alessandro M, Turlings TC, Ton J (2009) Signal signature of aboveground-induced resistance upon belowground herbivory in maize. Plant J 59(2):292–302. https://doi.org/10.1111/j.1365-313X.2009.03868.x

Etges WJ (2019) Evolutionary genomics of host plant adaptation: insights from Drosophila. Curr Opin Insect Sci 36:96–102. https://doi.org/10.1016/j.cois.2019.08.011

Eulgem T (2005) Regulation of the Arabidopsis defense transcriptome. Trends Plant Sci 10(2):71–78. https://doi.org/10.1016/j.tplants.2004.12.006

Finkel E (2009) With 'phenomics,' plant scientists hope to shift breeding into overdrive. Science 325:380–381. https://doi.org/10.1126/science.325_380

Fiorani F, Schurr U (2013) Future scenarios for plant phenotyping. Annu Rev Plant Biol 64:267–291. https://doi.org/10.1146/annurev-arplant-050312-120137

Fraenkel GS (1959) The raison d'etre of secondary plant substances. Science 129:1466–1470. https://doi.org/10.1126/science.129.3361.1466

Francis F, Guillonneau F, Leprince P, De Pauw E, Haubruge E, Jia L, Goggin FL (2010) Tritrophic interactions among *Macrosiphum euphorbiae* aphids, their host plants and endosymbionts: investigation by a proteomic approach. J Insect Physiol 56(6):575–585. https://doi.org/10.1016/j.jinsphys.2009.12.001

Fukushima A, Kusano M, Redestig H, Arita M, Saito K (2009) Integrated omics approaches in plant systems biology. Curr Opin Chem Biol 13(5–6):532–538. https://doi.org/10.1016/j.cbpa.2009.09.022

Furbank RT, Tester M (2011) Phenomics–technologies to relieve the phenotyping bottleneck. Trends Plant Sci 16(12):635–644. https://doi.org/10.1016/j.tplants.2011.09.005

Gan X, Stegle O, Behr J, Steffen JG, Drewe P, Hildebrand KL, Lyngsoe R, Schultheiss SJ, Osborne EJ, Sreedharan VT, Kahles A, Bohnert R, Jean G, Derwent P, Kersey P, Belfield EJ, Harberd NP, Kemen E, Toomajian C, Kover PX, Clark RM, Rätsch G, Mott R (2011) Multiple reference genomes and transcriptomes for *Arabidopsis thaliana*. Nature 477(7365):419–423. https://doi.org/10.1038/nature10414

Gfeller A, Laloux M, Barsics F, Kati DE, Haubruge E, Du Jardin P, Verheggen FJ, Lognay G, Wathelet JP, Fauconnier ML (2013) Characterization of volatile organic compounds emitted by barley (*Hordeum vulgare* L.) roots and their attractiveness to wireworms. J Chem Ecol 39(8):1129–1139. https://doi.org/10.1007/s10886-013-0302-3

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res 40(1):1178–1186. https://doi.org/10.1093/nar/gkr944

Gosset V, Harmel N, Göbel C, Francis F, Haubruge E, Wathelet JP, Du Jardin P, Feussner I, Fauconnier ML (2009) Attacks by a piercing-sucking insect (*Myzus persicae* Sultzer) or a chewing insect (*Leptinotarsa decemlineata* Say) on potato plants (*Solanum tuberosum* L.) induce differential changes in volatile compound release and oxylipin synthesis. J Exp Bot 60(4):1231–1240. https://doi.org/10.1093/jxb/erp015

Hafeez M, Liu S, Jan S, Shi L, Fernández-Grandon GM, Gulzar A, Ali B, Rehman M, Wang M (2019) Knock-down of gossypol-inducing cytochrome P450 genes reduced deltamethrin sensitivity in *Spodoptera exigua* (Hübner). Int J Mol Sci 20(9):2248. https://doi.org/10.3390/ijms20092248

Hanley ME, Lamont BB, Fairbanks MM, Rafferty CM (2007) Plant structural traits and their role in anti-herbivore defence. Perspect Plant Ecol Evol Syst 8(4):157–178. https://doi.org/10.1016/j.ppees.2007.01.001

Hanson AA, Lorenz AJ, Hesler LS, Bhusal SJ, Bansal R, Michel AP, Jiang GL, Koch RL (2018) Genome-wide association mapping of host-plant resistance to soybean aphid. Plant Genome 11(3):180011. https://doi.org/10.3835/plantgenome2018.02.0011

Hasegawa DK, Turnbull MW (2014) Recent findings in evolution and function of insect innexins. FEBS Lett 588(8):1403–1410. https://doi.org/10.1016/j.febslet.2014.03.006

Hasegawa DK, Erickson SL, Hersh BM, Turnbull MW (2017) Virus Innexins induce alterations in insect cell and tissue function. J Insect Physiol 98:173–181. https://doi.org/10.1016/j.jinsphys.2017.01.003

Havens JN (1792) Observations on the Hessian fly. Trans N Y Soc Agron Pt 1:89–107

Hebert SL, Jia L, Goggin FL (2007) Quantitative differences in aphid virulence and foliar symptom development on tomato plants carrying the Mi resistance gene. Environ Entomol 36(2):458–467. https://doi.org/10.1093/ee/36.2.458

Hedlund J, Longo SB, York R (2020) Agriculture, pesticide use, and economic development: a global examination (1990–2014). Rural Sociol 85(2):519–544. https://doi.org/10.1111/ruso.12303

Heil M (2008) Indirect defence via tritrophic interactions. New Phytol 178(1):41–61. https://doi.org/10.1111/j.1469-8137.2007.02330.x

Hilton IB, D'ippolito AM, Vockley CM, Thakore PI, Crawford GE, Reddy TE, Gersbach CA (2015) Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nat Biotechnol 33(5):510–517. https://doi.org/10.1038/nbt.3199

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59:41–66. https://doi.org/10.1146/annurev.arplant.59.032607.092825

Huang HJ, Lu JB, Li Q, Bao YY, Zhang CX (2018) Combined transcriptomic/proteomic analysis of salivary gland and secreted saliva in three planthopper species. J Proteomics 172:25–35. https://doi.org/10.1016/j.jprot.2017.11.003

Idle JR, Gonzalez FJ (2007) Metabolomics. Cell Metab 6(5):348–351. https://doi.org/10.1016/j.cmet.2007.10.005

Ignasiak K, Maxwell A (2017) Antibiotic-resistant bacteria in the guts of insects feeding on plants: prospects for discovering plant-derived antibiotics. BMC Microbiol 17(1):1–17. https://doi.org/10.1186/s12866-017-1133-0

Insam H, Seewald MS (2010) Volatile organic compounds (VOCs) in soils. Biol Fert Soils 46(3):199–213. https://doi.org/10.1007/s00374-010-0442-3

Jansen JJ, Allwood JW, Marsden-Edwards E, van der Putten WH, Goodacre R, van Dam NM (2009) Metabolomic analysis of the interaction between plants and herbivores. Metabolomics 5(1):150–161. https://doi.org/10.1007/s11306-008-0124-4

Jing S, Zhang L, Ma Y, Liu B, Zhao Y, Yu H, Zhou X, Qin R, Zhu L, He G (2014) Genome-wide mapping of virulence in brown planthopper identifies loci that break down host plant resistance. PLoS One 9(6):e98911. https://doi.org/10.1371/journal.pone.0098911

Kalske A, Kessler A (2020) Population-wide shifts in herbivore resistance strategies over succession. Ecology 101(11):e03157. https://doi.org/10.1002/ecy.3157

Kantsa A, Raguso RA, Lekkas T, Kalantzi OI, Petanidou T (2019) Floral volatiles and visitors: a meta-network of associations in a natural community. J Ecol 107(6):2574–2586. https://doi.org/10.1111/1365-2745.13197

Kerchev PI, Fenton B, Foyer CH, Hancock RD (2012) Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. Plant Cell Environ 35(2):441–453. https://doi.org/10.1111/j.1365-3040.2011.02399.x

Kirsch R, Wielsch N, Vogel H, Svatoš A, Heckel DG, Pauchet Y (2012) Combining proteomics and transcriptome sequencing to identify active plant-cell-wall-degrading enzymes in a leaf beetle. BMC Genomics 13(1):1–15. https://doi.org/10.1186/1471-2164-13-587

Kotkar H, Giri A (2020) Plant epigenetics and the 'intelligent' priming system to combat biotic stress. In: Epigenetics of the immune system. Academic, Cambridge, pp 25–38

Koutroumpa FA, Monsempes C, François MC, De Cian A, Royer C, Concordet JP, Jacquin-Joly E (2016) Heritable genome editing with CRISPR/Cas9 induces anosmia in a crop pest moth. Sci Rep 6(1):1–9. https://doi.org/10.1038/srep29620

Kroes A, Weldegergis BT, Cappai F, Dicke M, van Loon JJ (2017) Terpenoid biosynthesis in Arabidopsis attacked by caterpillars and aphids: effects of aphid density on the attraction of a caterpillar parasitoid. Oecologia 185(4):699–712. https://doi.org/10.1007/s00442-017-3985-2

Lavergne FD, Broeckling CD, Brown KJ, Cockrell DM, Haley SD, Peairs FB, Pearce S, Wolfe LM, Jahn CE, Heuberger AL (2020) Differential stem proteomics and metabolomics profiles for four wheat cultivars in response to the insect pest wheat stem sawfly. J Proteome Res 19(3):1037–1051. https://doi.org/10.1021/acs.jproteome.9b00561

Lee I, Seo YS, Coltrane D, Hwang S, Oh T, Marcotte EM, Ronald PC (2011) Genetic dissection of the biotic stress response using a genome-scale gene network for rice. Proc Natl Acad Sci 108(45):18548–18553.

Leyria J, Orchard I, Lange AB (2020) Transcriptomic analysis of regulatory pathways involved in female reproductive physiology of *Rhodnius prolixus* under different nutritional states. Sci Rep 10(1):1–16

Li B, Predel R, Neupert S, Hauser F, Tanaka Y, Cazzamali G, Williamson M, Arakane Y, Verleyen P, Schoofs L, Schachtner J (2008) Genomics, transcriptomics, and peptidomics of neuropeptides and protein hormones in the red flour beetle *Tribolium castaneum*. Genome Res 18(1):113–122. https://doi.org/10.1101/gr.6714008

Li J, Zhu L, Hull JJ, Liang S, Daniell H, Jin S, Zhang X (2016) Transcriptome analysis reveals a comprehensive insect resistance response mechanism in cotton to infestation by the phloem feeding insect *Bemisia tabaci* (whitefly). Plant Biotechnol J 14(10):1956–1975. https://doi.org/10.1111/pbi.12554

Liu X, Locasale JW (2017) Metabolomics: a primer. Trends Biochem Sci 42(4):274–284. https://doi.org/10.1016/j.tibs.2017.01.004

Liu Y, Lu S, Liu K, Wang S, Huang L, Guo L (2019) Proteomics: a powerful tool to study plant responses to biotic stress. Plant Methods 15(1):1–20. https://doi.org/10.1186/s13007-019-0515-8

Liu H, Li S, Xiao G, Wang Q (2021) Formation of volatiles in response to tea green leafhopper (*Empoasca onukii* Matsuda) herbivory in tea plants: a multi-omics study. Plant Cell Rep 40(4):753–766. https://doi.org/10.1007/s00299-021-02674-9

Lockhart DJ, Winzeler EA (2000) Genomics, gene expression and DNA arrays. Nature 405(6788):827–836. https://doi.org/10.1038/35015701

Lu HP, Luo T, Fu HW, Wang L, Tan YY, Huang JZ, Wang Q, Ye GY, Gatehouse AM, Lou YG, Shu QY (2018) Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. Nat Plants 4(6):338–344. https://doi.org/10.1038/s41477-018-0152-7

MacDonald WA (2012) Epigenetic mechanisms of genomic imprinting: common themes in the regulation of imprinted regions in mammals, plants, and insects. Genet Res Int 2012:585024. https://doi.org/10.1155/2012/585024

Macel M, Visschers IG, Peters JL, Kappers IF, de Vos RC, van Dam NM (2019) Metabolomics of thrips resistance in pepper (*Capsicum* spp.) reveals monomer and dimer acyclic diterpene glycosides as potential chemical defenses. J Chem Ecol 45(5):490–501. https://doi.org/10.1007/s10886-019-01074-4

Majchrzak T, Wojnowski W, Rutkowska M, Wasik A (2020) Real-time volatilomics: a novel approach for analyzing biological samples. Trends Plant Sci 25(3):302–312. https://doi.org/10.1016/j.tplants.2019.12.005

Malhotra PK, Verma G, Sidhu GS, Duhan N (2020) Epigenomics: role, approaches and applications in plants. J Anim Plant Sci 30(5):1071. https://doi.org/10.36899/JAPS.2020.5.0122

Mazid M, Khan TA, Mohammad F (2011) Role of secondary metabolites in defense mechanisms of plants. Biol Med 3(2):232–249

Metcalf RL, Luckmann WH (1994) Introduction to insect pest management, 3rd edn. Wiley, New York, p 672

Milward EA, Shahandeh A, Heidari M, Johnstone DM, Daneshi N, Hondermarck H (2016) In: Bradshaw RA, Stahl PD (eds) Transcriptomics. Encyclopedia of cell biology, 1st edn. Academic, Cambridge, pp 160–165. https://doi.org/10.1016/B978-0-12-394447-4.40029-5

Mirnezhad M, Romero-González RR, Leiss KA, Choi YH, Verpoorte R, Klinkhamer PG (2010) Metabolomic analysis of host plant resistance to thrips in wild and cultivated tomatoes. Phytochem Anal 21(1):110–117. https://doi.org/10.1002/pca.1182

Monaco MK, Stein J, Naithani S, Wei S, Dharmawardhana P, Kumari S, Amarasinghe V, Youens-Clark K, Thomason J, Preece J, Pasternak S (2014) Gramene 2013: comparative plant genomics resources. Nucleic Acids Res 42(1):1193–1199. https://doi.org/10.1093/nar/gkt1110

Mooney KA, Pratt RT, Singer MS (2012) The tri-trophic interactions hypothesis: interactive effects of host plant quality, diet breadth and natural enemies on herbivores. PLoS One 7(4):e34403. https://doi.org/10.1371/journal.pone.0034403

Moor AE, Itzkovitz S (2017) Spatial transcriptomics: paving the way for tissue-level systems biology. Curr Opin Biotechnol 46:126–133. https://doi.org/10.1016/j.copbio.2017.02.004

Mumbach MR, Rubin AJ, Flynn RA, Dai C, Khavari PA, Greenleaf WJ, Chang HY (2016) HiChIP: efficient and sensitive analysis of protein-directed genome architecture. Nat Methods 13(11):919–922. https://doi.org/10.1038/nmeth.3999

Muto-Fujita A, Takemoto K, Kanaya S, Nakazato T, Tokimatsu T, Matsumoto N, Kono M, Chubachi Y, Ozaki K, Kotera M (2017) Data integration aids understanding of butterfly–host plant networks. Sci Rep 7(1):1–4. https://doi.org/10.1038/srep43368

Nabity PD, Zavala JA, DeLucia EH (2009) Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. Ann Bot 103(4):655–663. https://doi.org/10.1093/aob/mcn127

Nakato R, Shirahige K (2017) Recent advances in ChIP-seq analysis: from quality management to whole-genome annotation. Brief Bioinform 18(2):279–290. https://doi.org/10.1093/bib/bbw023

Nazir A (2016) Review on metagenomics and its applications. Imp J Intersdiscip Res 2(3):10

Nejat N, Ramalingam A, Mantri N (2018) Advances in transcriptomics of plants. Adv Biochem Eng Biotechnol 164:161–185. https://doi.org/10.1007/10_2017_52

Nguyen TT, Michaud D, Cloutier C (2007) Proteomic profiling of aphid *Macrosiphum euphorbiae* responses to host-plant-mediated stress induced by defoliation and water deficit. J Insect Physiol 3(6):601–611. https://doi.org/10.1016/j.jinsphys.2007.02.018

Olson ND, Treangen TJ, Hill CM, Cepeda-Espinoza V, Ghurye J, Koren S, Pop M (2019) Metagenomic assembly through the lens of validation: recent advances in assessing and improving the quality of genomes assembled from metagenomes. Brief Bioinform 20(4):1140–1150. https://doi.org/10.1093/bib/bbx098

Palli SR, Bai H, Wigginton J (2012) Insect genomics. In: Insect molecular biology and biochemistry. Academic, Cambridge, pp 1–29

Poelman EH, Broekgaarden C, Van Loon JJ, Dicke M (2008) Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. Mol Ecol 17(14):3352–3365. https://doi.org/10.1111/j.1365-294X.2008.03838.x

Pompanon F, Deagle BE, Symondson WO, Brown DS, Jarman SN, Taberlet P (2012) Who is eating what: diet assessment using next generation sequencing. Mol Ecol 21(8):1931–1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x

Raj A, van den Bogaard P, Rifkin SA, van Oudenaarden A, Tyagi S (2008) Imaging individual mRNA molecules using multiple singly labeled probes. Nat Methods 5:877–879. https://doi.org/10.1038/nmeth.1253

Rasmann S, De Vos M, Casteel CL, Tian D, Halitschke R, Sun JY, Agrawal AA, Felton GW, Jander G (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. Plant Physiol 158(2):854–863. https://doi.org/10.1104/pp.111.187831

Rathinam M, Mishra P, Vasudevan M, Budhwar R, Mahato A, Prabha AL, Singh NK, Rao U, Sreevathsa R (2019) Comparative transcriptome analysis of pigeonpea, *Cajanus cajan* (L.) and one of its wild relatives *Cajanus platycarpus* (Benth.) Maesen. PLoS One 14(7):e0218731. https://doi.org/10.1371/journal.pone.0218731

Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell 12(5):707–719. https://doi.org/10.1105/tpc.12.5.707

Rich-Griffin C, Stechemesser A, Finch J, Lucas E, Ott S, Schäfer P (2020) Single-cell transcriptomics: a high-resolution avenue for plant functional genomics. Trends Plant Sci 25(2):186–197. https://doi.org/10.1016/j.tplants.2019.10.008

Robinson GE, Hackett KJ, Purcell-Miramontes M, Brown SJ, Evans JD, Goldsmith MR, Lawson D, Okamuro J, Robertson HM, Schneider DJ (2010) Creating a buzz about insect genomes. Science 331(6023):1386. https://doi.org/10.1126/science.331.6023.1386

Rodriques SG, Stickels RR, Goeva A, Martin CA, Murray E, Vanderburg CR, Welch J, Chen LM, Chen F, Macosko EZ (2019) Slide-seq: a scalable technology for measuring genome-wide expression at high spatial resolution. Science 363(6434):1463–1467. https://doi.org/10.1126/science.aaw1219

Saadati M, Toorchi M (2017) The study of plant protein accumulation in gut of insect using proteomics technique: wheat–sunn pest interaction. J Saudi Soc Agric Sci 16(3):205–209. https://doi.org/10.1016/j.jssas.2015.06.005

Sanchez-Arcos C, Kai M, Svatoš A, Gershenzon J, Kunert G (2019) Untargeted metabolomics approach reveals differences in host plant chemistry before and after infestation with different pea aphid host races. Front Plant Sci 10:188. https://doi.org/10.3389/fpls.2019.00188

Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270(5235):467–470. https://doi.org/10.1126/science.270.5235.467

Schranz ME, Manzaneda AJ, Windsor AJ, Clauss MJ, Mitchell-Olds T (2009) Ecological genomics of *Boechera stricta*: identification of a QTL controlling the allocation of methionine-vs branched-chain amino acid-derived glucosinolates and levels of insect herbivory. Heredity 102(5):465–474. https://doi.org/10.1038/hdy.2009.12

Schreinemachers P, Tipraqsa P (2012) Agricultural pesticides and land use intensification in high, middle and low income countries. Food Policy 37(6):616–626. https://doi.org/10.1016/j.foodpol.2012.06.003

Sharma HC, Ortiz R (2002) Host plant resistance to insects: an eco-friendly approach for pest management and environment conservation. J Environ Biol 23(2):111–135

Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GP, Handa N, Kohli SK, Yadav P, Bali AS, Parihar RD, Dar OI (2019) Worldwide pesticide usage and its impacts on ecosystem. SN Appl Sci 1(11):1446. https://doi.org/10.1007/s42452-019-1485-1

Shashko AY, Bandarenka UY, Charnysh MA, Przhevalskaya DA, Usnich SL, Pshybytko NL, Smolich II, Demidchik VV (2020) Modern phenotyping platforms and their application in plant biology and agriculture. J Belarusian State Univ Biol 2:15–25. https://doi.org/10.33581/2521-1722-2020-2-15-25

Smith CM (2005) Plant resistance to arthropods: molecular and conventional approaches. Springer, Netherlands, p 426. https://doi.org/10.1007/1-4020-3702-3

Stam JM, Kroes A, Li Y, Gols R, van Loon JJ, Poelman EH, Dicke M (2014) Plant interactions with multiple insect herbivores: from community to genes. Annu Rev Plant Biol 65:689–713. https://doi.org/10.1146/annurev-arplant-050213-035937

Stanley J, Preetha G (2016) Pesticide toxicity to non-target organisms. Springer, Berlin, pp 99–152. https://doi.org/10.1007/978-94-017-7752-0

Stiling P, Cornelissen T (2007) How does elevated carbon dioxide (CO_2) affect plant–herbivore interactions? A field experiment and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance. Glob Change Biol 13(9):1823–1842. https://doi.org/10.1111/j.1365-2486.2007.01392.x

Subramanian I, Verma S, Kumar S, Jere A, Anamika K (2020) Multi-omics data integration, interpretation, and its application. Bioinform Biol Insights 14:1177932219899051.

Thompson GA, Goggin FL (2006) Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. J Exp Bot 57(4):755–766. https://doi.org/10.1093/jxb/erj135

Tollefsbol TO (2011) Advances in epigenetic technology. Methods Mol Biol 791:1–10. https://doi.org/10.1007/978-1-61779-316-5_1

Tonhasca A Jr, Byrne DN (1994) The effects of crop diversification on herbivorous insects: a meta-analysis approach. Ecol Entomol 19(3):239–244. https://doi.org/10.1111/j.1365-2311.1994.tb00415.x

Tu X, Liu Z, Zhang Z (2018) Comparative transcriptomic analysis of resistant and susceptible alfalfa cultivars (*Medicago sativa* L.) after thrips infestation. BMC Genomics 19(1):1–8. https://doi.org/10.1186/s12864-018-4495-2

Wang Y, Carolan JC, Hao F, Nicholson JK, Wilkinson TL, Douglas AE (2010) Integrated metabonomic–proteomic analysis of an insect–bacterial symbiotic system. J Proteome Res 9(3):1257–1267. https://doi.org/10.1021/pr9007392

Wang H, Shi Y, Wang L, Liu S, Wu S, Yang Y, Feyereisen R, Wu Y (2018) CYP6AE gene cluster knockout in *Helicoverpa armigera* reveals role in detoxification of phytochemicals and insecticides. Nat Commun 9(1):1–8. https://doi.org/10.1038/s41467-018-07226-6

Wang D, Shi X, Liu D, Yang Y, Shang Z (2020a) Transcriptome profiling revealed potentially critical roles for digestion and defense-related genes in insects use of resistant host plants: a case study with *Sitobion avenae*. Insects 11(2):90. https://doi.org/10.3390/insects11020090

Wang P, Wu H, Zhao G, He Y, Kong W, Zhang J, Liu S, Liu M, Hu K, Liu L, Xu Y (2020b) Transcriptome analysis clarified genes involved in resistance to *Phytophthora capsici* in melon. PLoS One 15(2):e0227284. https://doi.org/10.1371/journal.pone.0227284

Wang YT, Shen RX, Xing D, Zhao CP, Gao HT, Wu JH, Zhang N, Zhang HD, Chen Y, Zhao TY, Li CX (2021) Metagenome sequencing reveals the midgut microbiota makeup of *Culex pipiens quinquefasciatus* and its possible relationship with insecticide resistance. Front Microbiol 12:228. https://doi.org/10.3389/fmicb.2021.625539

Whitehill JG, Popova-Butler A, Green-Church KB, Koch JL, Herms DA, Bonello P (2011) Interspecific proteomic comparisons reveal ash phloem genes potentially involved in constitutive resistance to the emerald ash borer. PLoS One 6(9):e24863. https://doi.org/10.1371/journal.pone.0024863

Windram O, Madhou P, McHattie S, Hill C, Hickman R, Cooke E, Jenkins DJ, Penold CA, Baxter L, Breeze E, Kiddle SJ, Rhodes J, Atwell S, Kliebenstein DJ, Kim YS, Stegle O, Borgwardt K, Zhang C, Tabrett A, Legaie R, Moore J, Finkenstadt B, Wild DL, Mead A, Rand D, Beynon J, Ott S, Buchanan-Wollaston V, Denby KJ (2012) Arabidopsis defense against Botrytis cinerea: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. Plant Cell 24(9):3530–3557. https://doi.org/10.1105/tpc.112.102046

Wu X, Yan J, Wu Y, Zhang H, Mo S, Xu X, Zhou F, Ding H (2019) Proteomic analysis by iTRAQ-PRM provides integrated insight into mechanisms of resistance in pepper to *Bemisia tabaci* (Gennadius). BMC Plant Biol 19(1):1–19. https://doi.org/10.1186/s12870-019-1849-0

Xia X, Gurr GM, Vasseur L, Zheng D, Zhong H, Qin B, Lin J, Wang Y, Song F, Li Y, Lin H (2017) Metagenomic sequencing of diamondback moth gut microbiome unveils key holobiont adaptations for herbivory. Front Microbiol 8:663. https://doi.org/10.3389/fmicb.2017.00663

Yang N, Xie W, Yang X, Wang S, Wu Q, Li R, Pan H, Liu B, Shi X, Fang Y, Xu B (2013) Transcriptomic and proteomic responses of sweetpotato whitefly, *Bemisia tabaci*, to thiamethoxam. PLoS One 8(5):e61820. https://doi.org/10.1371/journal.pone.0061820

Yang M, Wang Z, Wang R, Zhang X, Li M, Xin J, Qin Y, Zhang C, Meng F (2020) Transcriptomic and proteomic analyses of the mechanisms of overwintering diapause in soybean pod borer (*Leguminivora glycinivorella*). Pest Manag Sci 76(12):4248–4257. https://doi.org/10.1002/ps.5989

Yu QY, Fang SM, Zhang Z, Jiggins CD (2016) The transcriptome response of *Heliconius melpomene* larvae to a novel host plant. Mol Ecol 25(19):4850–4865. https://doi.org/10.1111/mec.13826

Zebelo SA, Maffei ME (2012) Signal transduction in plant–insect interactions: from membrane potential variations to metabolomics. In: Plant electrophysiology. Springer, Berlin, pp 143–172. https://doi.org/10.1007/978-3-642-29110-4_6

Zhang W (2018) Global pesticide use: profile, trend, cost/benefit and more. Proc Int Acad Ecol Environ Sci 8(1):1

Zhang Q, Lu YX, Xu WH (2013a) Proteomic and metabolomic profiles of larval hemolymph associated with diapause in the cotton bollworm, *Helicoverpa armigera*. BMC Genomics 14(1):1–13. https://doi.org/10.1186/1471-2164-14-751

Zhang Y, Zhang F, Li X, Baller JA, Qi Y, Starker CG, Bogdanove AJ, Voytas DF (2013b) Transcription activator-like effector nucleases enable eficient plant genome engineering. Plant Physiol 161(1):20–27. https://doi.org/10.1104/pp.112.205179

Zhang S, Shen S, Yang Z, Kong X, Liu F, Zhen Z (2020) Coding and non-coding RNAs: molecular basis of forest-insect outbreaks. Front Cell Dev Biol 8:369. https://doi.org/10.3389/fcell.2020.00369

Zheng SJ, Dicke M (2008) Ecological genomics of plant-insect interactions: from gene to community. Plant Physiol 146(3):812–817. https://doi.org/10.1104/pp.107.111542

Zhu F, Poelman EH, Dicke M (2014) Insect herbivore-associated organisms affect plant responses to herbivory. New Phytol 204(2):315–321. https://doi.org/10.1111/nph.12886

Chapter 14
MicroRNA-Mediated Insect Resistance in Field Crops

Bijayalaxmi Mahanty, Suhas Sutar Bharat, Jatindra Nath Mohanty, Rukmini Mishra, and Raj Kumar Joshi

14.1 Introduction

The ever-increasing global population together with changing climatic conditions significantly affects agricultural productivity worldwide. Modern agriculture is tremendously affected by several biotic and abiotic stresses, insect pest being one of the major challenges accounting for up to 25% annual crop yield losses worldwide (Deutsch et al. 2018). Several pest management measures have been utilized by farmers across the world without any tangible success, and important crop plants continue to suffer from yield losses. Moreover, the application of chemical pesticides for controlling insect pests is highly detrimental to both man and its environment. Traditional breeding has significantly contributed to generate high-yielding crop varieties, but the process is time-consuming and cumbersome. Therefore, it is imperative on the part of the plant biologist to formulate new strategies towards development of high-yielding, stress-tolerant crop varieties with existing land and resources to satisfy the current food demand and nutritional security. Genetic

Bijayalaxmi Mahanty and Suhas Sutar Bharat contributed equally with all other contributors.

B. Mahanty · R. K. Joshi (✉)
Department of Biotechnology, Rama Devi Women's University, Bhubaneswar, Odisha, India
e-mail: rkjoshi@rdwu.ac.in

S. S. Bharat · R. Mishra
School of Applied Sciences, Centurion University of Technology and Management, Bhubaneswar, Odisha, India
e-mail: rukmini.mishra@cutm.ac.in

J. N. Mohanty
Centre for Biomedical Informatics, Siksha O Anusandhan University, Bhubaneswar, Odisha, India

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_14

engineering and biotechnology is one such strategy which is currently being practiced for pest management in crop plants through genetic modification of several insect resistance genes (especially *Bacillus thuringiensis* Bt-ICPs) that significantly contribute to the productivity and agricultural sustainability. However, techniques have become highly apprehensive due to the emergence of Bt-toxin-resistant insect-pest population. Moreover, as a single trait might be controlled by many genes or vice versa, genetic introgression of a trait may result in pleotropic effect with aberration in plant phenotypes. Therefore, efficient genetic modulators are required that could facilitate genetic manipulation of agronomic traits' high precision and specificity.

Over the last decade, small interfering RNAs (siRNAs) and microRNAs (miRNAs) have been identified as prominent regulators of developmental and physiological responses in plants (Khraiwesh et al. 2012). The first evidence came from the identification of unique miRNAs and their target genes in stressed *Arabidopsis* plants (Jones-Rhoades and Bartel 2004). Since then, miRNAs which are 20- to 24-nucleotides long have been reported to regulate the expression of target genes in different plants through post-transcriptional silencing or translational inhibition of the mRNA sequences (Jones-Rhoades and Bartel 2006). In plants, these small RNA molecules are processed through a Dicer-like (DCL) enzyme and bind with endonuclease Argonaute (AGO) proteins to form RNA-induced silencing complex that migrate to the target region and facilitate mRNA cleavage or inhibition of translation. Emerging evidences from the recent past have shown the potential role of miRNA towards stress response in a host of economically important crop plants including rice, wheat, maize, and cotton (reviewed in Chaudhary et al. 2021). The uniquely conserved miRNA biogenesis pathway in plants has also contributed to the development of novel strategies to manipulate miRNA sequences, such as the artificial miRNA (amiRNA) technology and CRISPR/Cas9 technology towards improvement of field crops. This chapter primarily reviews the role of miRNAs following herbivore attack on plants and how they could be exploited for insect-pest management. Additionally, we also highlight the basics of amiRNA and CRISPR/Cas9 technology and their application for protection of field crops against insect pests.

14.2 Plant miRNAs: Biogenesis and Mechanism of Action

MicroRNAs are a class of approximately 20- to 24-nucleotides (nt) endogenous small RNAs that negatively regulate gene expression and play vital roles in multiple biological processes, including plant growth, development, and responses to environmental stresses (Sunkar et al. 2012; Khraiwesh et al. 2012). In 2002, the first group of plant miRNAs (miR156 to miR173) was identified from *Arabidopsis thaliana* (Reinhart et al. 2002). Since then, 38,589 precursor miRNAs (pre-miRNAs) expressing 48,885 mature miRNAs have been identified in 271 species (miRBase, ver. 22). Increasing evidence indicates that miRNAs play critical roles in plant disease-resistance responses (Katiyar-Agarwal et al. 2006; Jin 2008; Padmanabhan et al. 2009; Yang and Huang 2014).

miRNAs recognize mRNA targets through sequence complementarity and downregulate the expression of the target genes by cleavage or repression of translation (Jones-Rhoades et al. 2006; Shukla et al. 2008).

14.2.1 Biogenesis of Plant miRNAs

MicroRNAs are generally transcribed from independent units of endogenous miRNA (*MIR*) genes often located in the intergenic regions of the genomes (Coruh et al. 2014). These genes could be intronic, exonic, or located within the transposable elements as has been reported from *Arabidopsis*, rice, and wheat (Lucas and Budak 2012). During biogenesis, RNA polymerase II (RNA Pol II) mediates the synthesis of primary miRNA transcripts (pri-miRNA) from nuclear-encoded MIR genes (Joshi et al. 2017).Subsequently, the pri-miRNAs are stabilized by the addition of 7-methylguanosine cap at the 5′-end and a polyadenylated tail at the 3′ region (Bartel 2004; Xie et al. 2005; Zhang et al. 2005) (Fig. 14.1). Further, the RNA-binding protein DAWDLE (DDL) interacts with the ribonuclease Dicer-like 1 (DCL1) and stabilizes the pri-miRNAs in the dicing bodies (Dbodies) of the nucleus (Ha and Kim 2014). DCL1 together with the dsRNA-binding protein Hyponastic Leaves 1 (HYL1) (Vazquez et al. 2004), zinc-finger protein SERRATE (SE) (Yang et al. 2006), and the G-patch domain protein (TGH) (Ren et al. 2012) forms a nuclear cap-binding complex and processes the pri-miRNAs into a hairpin precursor called pre-miRNAs (Joshi et al. 2017). SE optimizes the DCL1 activity (Iwata et al. 2013), HYL1 enables accurate pri-miRNA processing (Yang et al. 2010), while TGH interacts with all the components suggesting a crucial but still unclear role in the DCL1 machinery (Ren et al. 2012; Ren and Yu 2012). The pri-miRNAs ranges from 60 to 300 nt in length and is processed from the free end opposite to the loop to one or several mature miRNA duplex consisting of miRNA-miRNA* sequences, each of 20–24 nt in length. miRNA* refers to the strand complementary to miRNA, with a 2 nt overhang at 3′-end of this duplex. The miRNA-miRNA* duplex is methylated at the 3′-end by the nuclear protein HUA1 enhancer (HEN1), thereby blocking the uridylation and 3′ exonuclease degradation of miRNAs (Yu et al. 2005; Zhai et al. 2013). The methylated duplex is then transported into the cytoplasm by a nuclear exportin called HASTY1 (HST1) (Kim 2004; Park et al. 2005). In the cytoplasm, an unknown helicase separates the two strands of the duplex, and one of the strand (mature miRNA) binds to the cytoplasmic Argonaute (AGO) protein from the RNA-induced silencing complex (RISC). The activated RISC helps in binding the mature miRNA through sequence complementarity leading to cleavage and/or repression (Mi et al. 2008; Montgomery et al. 2008). Argonaute protein consists of two conserved RNA-binding domains, namely, a PAZ domain and a PIWI domain. The PAZ domain bind with the 3′-end of the mature miRNA molecule, whereas the PIWI domain with endonucleolytic activity associates with the 5′-end of the guide strand during the process of mature miRNA formation and mRNA degradation (Pratt et al. 2009). Readers may refer to many important available reviews for a detailed description of miRNA biogenesis in plants (Bologna and Voinnet 2014).

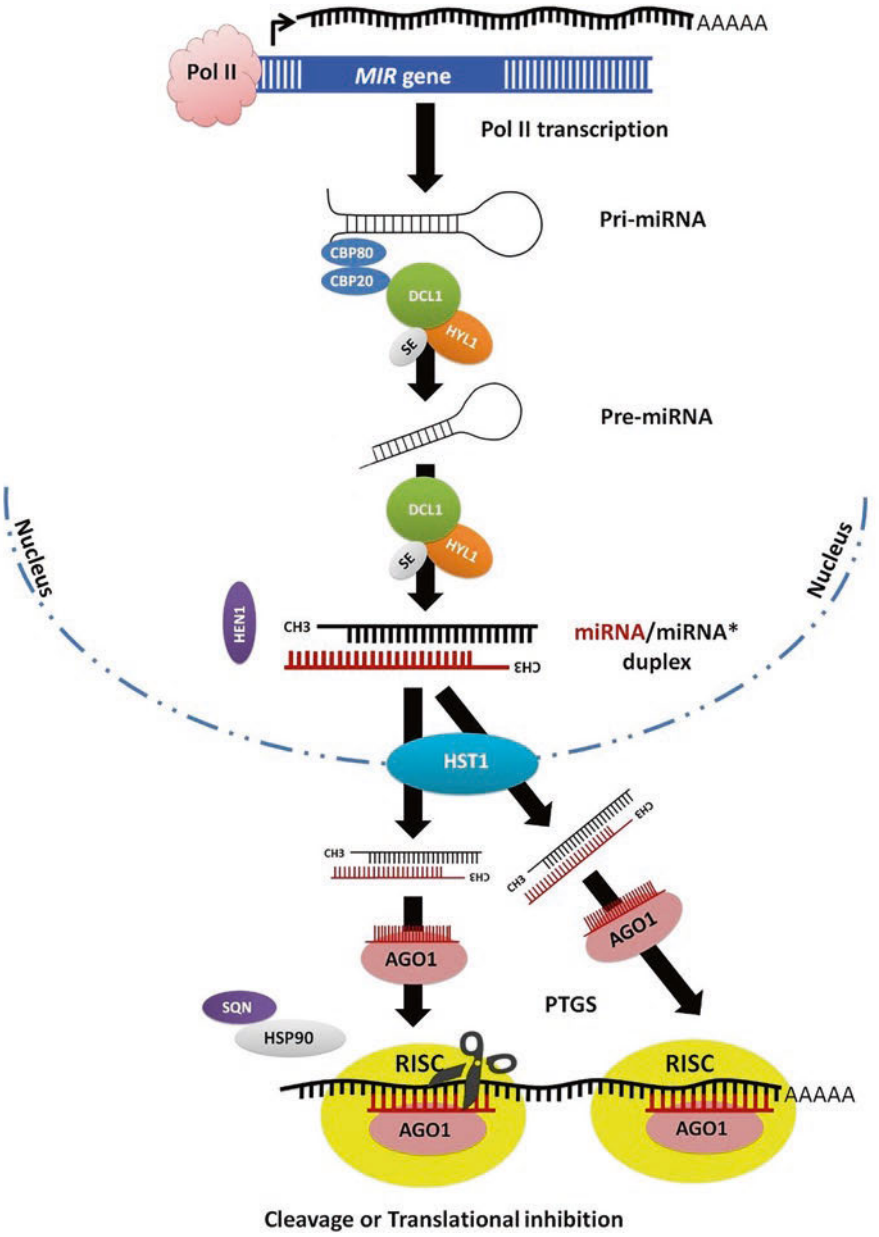

Fig. 14.1 miRNA biogenesis and silencing pathway. Primary miRNA (pri-miRNA) transcribed from MIR genes is processed into pre-miRNA by DCL1, HYL1, and SERRATE (SE) proteins. Pre-miRNA is further processed into 20–24-nucleotides long miRNA-miRNA* duplex. The duplex is methylated by Hua Enhancer 1 (HEN1) and transported into the cytoplasm through HASTY (HST1) transporter. The guide strand of mature miRNA binds with argonaute 1 (AGO1) protein to form the RNA-induced silencing complex (RISC). The RISC complex binds to the target mRNA through complementary interaction between mature miRNA and mRNA region leading to target cleavage or translational repression

14.2.2 Mechanistic Action of Plant miRNA

miRNAs are prominent determinants of post-transcriptional gene regulation through the execution of two main mechanisms, mRNA cleavage and translational inhibition (Sunkar et al. 2012). The mode of action largely depends upon the degree of complementarity between the miRNA and its binding site (Mallory et al. 2004; Liu et al. 2014). If the complementarity of miRNA with its target is weak, then gene expression is suppressed by blocking the translation of mRNA (Doench and Sharp 2004). However, a perfect complementarity between miRNA and mRNA results in targeted degradation (Kidner and Martienssen 2004). The target sites of plant miRNAs are mostly found in the open reading frames (ORFs) and occasionally in the 5′ untranslated regions (UTRs), 3′ UTRs, or in non-coding RNA (Addo-Quaye et al. 2008; German et al. 2008). Majority of the plant miRNAs demonstrate extensive complementarity with the targets with less than five mismatches. AGO-mediated mRNA splicing critically occurs between 9 and 11 positions from the 5′-end of the miRNAs which the target repression depends upon binding affinity from 2 to 13 positions (Mallory et al. 2004; Schwab et al. 2005). mRNA degradome and ligation-mediated rapid amplification of cDNA ends (RLM-RACE) analysis in many plants have shown that majority of target sites are subjected to AGO1 endonucleolytic cleavage (Gregory et al. 2008). However, the recent identification of *Arabidopsis* mutants exhibiting miRNA-mediated gene repression without cleavage of functional transcripts suggest that miRNA-directed translational repression also has distinctive occurrence in plants (Iwakawa and Tomari 2013). A study suggests that plant miRNA-mediated translation repression occurs on the endoplasmic reticulum (Li et al. 2013). The study demonstrated that ER surface protein ALTERED MERISTEM PROGRAM1 (AMP1) reconcile the disproportionate effect of miRNA towards repression of the target gene. AMP1 together with AGO-bound miRNAs thwarts the binding of target mRNA to the ER bound polysomes, thus inhibiting the translation of the target gene. Although many other methods of miRNA-mediated translational repression has been reported in animals (Fabian et al. 2010), they are yet to be verified in plants. Additionally, a few silencing effectors including the decapping proteins and Glycine Tryptophan repeat proteins have been proposed to be associated with translational repression of miRNA targets (Motomura et al. 2012; Zekri et al. 2013).

14.3 miRNAs in Plant Stress Responses

miRNAs are either over- or under-expressed or sometimes novel sequences of miRNAs are produced to overcome the environmental stresses impeding plant growth and development (Covarrubias and Reyes 2010). Multiple stress-regulated miRNAs have been identified and characterized from model plants as well as cultivated crops under various biotic and abiotic conditions such as drought (Zhao et al. 2007a, b; Liu et al. 2008; Zhou et al. 2010), low temperature (Zhou et al.

2008), salinity (Liu et al. 2008; Sunkar et al. 2008), heat stress, nutrient deficiency (Fujii et al. 2005), UV radiation (Zhou et al. 2007), oxidative and mechanical stress (Lu et al. 2005), and bacterial, viral, and fungal infections (Navarro et al. 2006; TenOever 2013; Campo et al. 2013).

14.3.1 Plant miRNAs Responsive to Abiotic Stresses

Abiotic stresses adversely affect plant growth and development by decelerating seed germination, root development, and chlorophyll synthesis. miRNA-mediated regulation of gene expression is a key strategy involved in mitigating these stresses. Deficiency of water and excess evaporation lead to drought stress in plants (Shukla et al. 2008; Bartels and Sunkar 2005). Sequencing of small RNA libraries and miRNA expression profiling performed in several plants including *Arabidopsis*, *Triticum dicoccoides*, cowpea, soybean, *Phaseolus vulgaris*, and tobacco has revealed the role of multiple miRNAs responsive to drought (Sunkar and Zhu 2004; Kantar et al. 2011; Barrera-Figueroa et al. 2011; Kulcheski et al. 2011; Arenas-Huertero et al. 2009; Frazier et al. 2011). In *Arabidopsis*, miR396, miR168, miR167, miR165, miR319, miR159, miR393, miR394,miR156, miR393, miR397, miR171, miR158, and miR169 were revealed to be drought responsive (Liu et al. 2008; Sunkar and Zhu 2004). In rice, miR169g, miR171a, andmiR393 were found to be regulated in response to drought stress (Zhao et al. 2007a, b; Zhou et al. 2010; Jian et al. 2010). A microarray platform-based genome-wide profiling and analysis of miRNAs from drought-challenged rice revealed that 30 miRNAs are critically implicated in regulating the dehydration stress (Zhou et al. 2010). In *Populus*, miR171l-n, miR1445, miR1446a-e, miR1444a, miR1450, miR482.2, miR530a, miR827, miR1448, and miR1447 were found to be drought responsive (Lu et al. 2008). In *Phaseolus vulgaris*, miR159.2, miR393, miR2118, miRS1, miR1514a, and miR2119 showed high transcript accumulation upon drought treatment (Arenas-Huertero et al. 2009). In *Medicago truncatula*, miR169, miR398a, miR398b, and miR408 demonstrated differential spatial expression under drought stress (Trindade et al. 2010). The expression of miR169 was reduced inroots, while miR398a, miR398b, and miR408 were highly accumulated in the shoots and roots under water-scarce conditions. Likewise, miRNA sequencing in emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) characterized 13 miRNAs, namely, miR1867, miR896, miR398, miR528, miR474, miR1450, miR396, miR1881, miR894, miR156, miR1432, miR166, and miR171, that were found to be differentially regulated in response to drought stress (Kantar et al. 2011). More than 6% of cultivable lands across the world are affected with high salt concentration (Munns 2005). A wide number of genes and associated pathway components are affected by salinity (Zhu 2002). Several studies have shown that the expression levels of many miRNAs were regulated under salt-stress conditions. In *Arabidopsis*, 13 conserved miRNAs were upregulated, while miR398 was downregulated under salt shock conditions (Liu et al. 2008). In rice, four miRNAs, namely, miR169g, miR169n, miR169o, and

miR393, regulated the plant growth under salt stress by cleaving the NF-YA transcription factors (Zhao et al. 2009; Gao et al. 2011). Microarray study on salt-tolerant and salt-sensitive *Zea mays* have reported that 98 miRNAs from 27 families were differentially expressed, of which miR156, miR164, miR167, and miR396 family members were found to be downregulated, while miR162, miR168, miR395, and miR474 families were found to be upregulated in salt-shocked maize roots (Ding et al. 2009). Members of the miRNA families, such as miR171, miR393, miR855, and miR408, were significantly induced under salinity stress in wheat (Wang et al. 2014). A recent study have demonstrated that the endonuclease AGO1 interacts with the chromatin at MIR161 and MIR173 leading to disassembly of the transcriptional complex leading to a stabilized expression of miR161 and miR173 under salt stress conditions (Dolata et al. 2016). Heat and cold stresses are chief abiotic elements that can induce severe plant damage leading to decrease in crop productivity. miRNA-mediated regulation of cold stress was first demonstrated by Sunkar and Zhu (2004). They found that miR393 was strongly induced while miR319c and miR398a were repressed when *Arabidopsis* seedlings were exposed to 0 °C for 24 h. Since then, the role of miRNAs in response to low-temperature stress has been confirmed in several plant species including *Arabidopsis* (Liu et al. 2008), rice (Lv et al. 2010), *Brachypodium distachyon* (Zhang et al. 2009), *Prunus* spp. (Barakat et al. 2012; Karimi et al. 2016), wheat (Tang et al. 2012), *Citrullus lanatus*(Li et al. 2016), grape vine (Sun et al. 2015), and *Glycine max* (Xu et al. 2016). Sudden increase in temperature leads to denaturation of proteins (enzymes) causing cellular damages, and accumulated evidences indicate that miR398, miR156, and miR172 play essential roles in plant response to heat stress (Zhao et al. 2016). Alternatively, DNA methylation and alternative splicing could also affect miRNA expression under heat stress (Ci et al. 2015). miRNAs are key factors in the regulation of nutrient homeostasis (Khraiwesh et al. 2012). Multiple studies have shown that miR399, miR395, and miR398 are significantly induced in response to phosphate, sulfate, and copper deficiency in *Arabidopsis*, respectively (Sunkar et al. 2006; Aung et al. 2006; Bari et al. 2006; Yamasaki et al. 2007; Khraiwesh et al. 2012). miR399 binds with the *cis* element GNATATNC in the promoter regions of the MYB transcription factor gene encoding phosphate starvation response (PHR1) and positively regulate their expression during phosphate-deprived condition (Rubio et al. 2001; Franco-Zorrilla et al. 2004; Chiou 2007). Similarly, miR395 targets the ATP sulfurylase genes APS1, APS3, and APS4 which function in the sulfur assimilation pathway (Jones-Rhoades et al. 2006; Allen et al. 2005; Sunkar et al. 2007; Liang and Yu 2010). miR398 facilitate the degradation of copper-zinc super oxide dismutases (CSD1 and CSD2) and make copper available for different essential processes including photosynthesis and oxidative responses (Yamasaki et al. 2007; Beauclair et al. 2010).

Other abiotic stresses including oxidative stress and mechanical stress also regulate the differential expression of miRNAs in plants. Multiple abiotic and biotic stresses also lead to the accumulation of reactive oxygen species (ROS) and superoxide radicals (O_2^-), and hydroxyl radicals (OH^-) are the main cause of oxidative damage to the cells (Mittler 2002; Bartels and Sunkar 2005). Superoxide is

converted into free oxygen and hydrogen peroxide by Cu-Zn SODs. Sunkar et al. (2006) reported that miR398 targeting CSD1 and CSD2 are significantly downregulated under oxidative stress. Further, the transgenic overexpression of amiR398-resistant form of CSD2 resulted in increased accumulation of CSD2 due to relaxation of miR398-directed cleavage (Sunkar et al. 2006). A genome-wide study on rice seedlings has identified seven H2O2-responsive miRNAs of which miR169, miR397, miR827, and miR1425 were found to be upregulated while miR528, miR319a.2 and miR408-5p was downregulated by H2O2 treatments (Li et al. 2010). Similarly, dynamic expression pattern of miR156, miR162, miR164, miR475, miR480, and miR481 has been reported in response to mechanical stress and is critical to the maintenance of structural and mechanical fitness in plants (Lu et al. 2005; Khraiwesh et al. 2012). All these reports clearly suggest that miRNAs are crucial components in gene regulatory networks modulating the response of plants to various external cues required for sustainable growth and development.

14.3.2 Plant miRNAs Responsive to Biotic Stresses

Along with abiotic stresses, the yield and quality of crops are severely affected by the opportunistic infection by biotic components including viruses, bacteria, fungi, insects, and nematodes. Recent studies have shown that miRNAs and the mediated RNA interference (RNAi) pathway components are crucial to plant immunity against multiple phytopathogens (Navarro et al. 2006; Jagadeeswaran et al. 2009; TenOever 2013; Campo et al. 2013; Li et al. 2014) (Table 14.1). The earliest of the report is of miR393 which promoted pathogen-associated molecular pattern-triggered immunity (PTI) in *A. thaliana* (Navarro et al. 2006). *flg22*, a pathogen-associated molecular pattern (PAMP), induced the expression of miR393 which in turn mediated the suppression of auxin signaling pathway through cleavage of the auxin receptor TIR1. In addition to this, several miRNAs including miR160, mi168, miR398, and miR773 regulates *flg22*-induced callose deposition as part of the PTI response (Li et al. 2010). Negative regulation of an F-boxgene by miR393 in *Zea mays* plays an important role in defense against *Rhizoctonia solani* infection (Luo et al. 2014). Plant miRNAs responsive to fungal infection have also been identified in many plant species. Small RNA profiling of *Magnaporthe oryza*-challenged resistant and susceptible cultivars of rice revealed that miR156, miR160, miR169, and miR164 are induced whereas miR394 and miR396 were downregulated upon infection in resistant cultivars but not in susceptible one (Li et al. 2014). In addition, miR169, miR172, and miR398 were induced in both resistant and susceptible cultivars suggesting their dogmatic role in basal responses (Li et al. 2014). Moreover, the overexpression of miR160 in a susceptible rice cultivar led to enhanced disease resistance toward *M. oryzae* suggesting their critical involvement in defense response against fungal pathogen (Li et al. 2014). Similarly, Osa-miR7695 reported positive regulation of defense against *M. oryzae* by downregulating the expression of elicitor-responsive *OsNramp6* (Natural resistance-associated macrophage

Table 14.1 Plant miRNAs targeting herbivore insects

Plants	miRNAs	Insect pest	References
Oryza sativa	Multiple miRNAs	*Nilaparvata lugens*	Cheng et al. (2013), Wu et al. (2017)
	miR156	*Nilaparvata lugens*	Ge et al. (2018)
	miR396	*Nilaparvata lugens*	Dai et al. (2019)
	miR2871a-3p, miR172a, miR166a-5p, miR2120, miR1859	*Nilaparvata lugens*	Nanda et al. (2020)
Zea mays	Multiple miRNAs	*Spodoptera frugiperda*	Moné et al. (2018)
Solanum lycopersicon	Multiple miRNAs	*Bemisia tabaci*	Ketao et al. (2018)
Gossypium arboreum	Multiple miRNAs	*Bemisia tabaci*	Li et al. (2019)
Nicotiana attenuata	Multiple miRNAs	*Manduca sexta*	Bozorov et al. (2012)
Cucumis melo	miR164, miR167, miR390, miR393	*Aphis gossypii*	Sattar et al. (2012)
Chrysanthemum morifolium	miR159a, miR160a, miR393a	*Macrosiphoniella sanborni*	Xia et al. (2015)
Camellia sinensis	Multiple miRNAs	*Ectropis obliqua*	Jeyaraj et al. (2017)
Medicago truncatula	Multiple miRNAs	*Acyrthosiphon kondoi*	Gao et al. (2010)

protein 6) gene (Campo et al. 2013). Also, a genome-wide sRNA sequencing and transient overexpression analysis revealed that miR319, miR394, and slymiRn1 are involved in the regulation of tomato immunity against *Botrytis cinerea* (Jin and Wu 2015). Moreover, several studies on wheat, oil seeds, and other crops have reported the possible role of miRNA-mediated gene silencing in plant defense against multiple fungal phytopathogens (Xin et al. 2010; Campo et al. 2013; Shen et al. 2014). Among the miRNAs mediating viral responses, miR156 and miR164 were induced by viral silencing suppressor P1/HC-Pro encoded by Turnip mosaic virus (TuMV) in *Arabidopsis thaliana* (Zhou and Luo 2013). In *Brassica rapa*, two novel miRNAs, miR1885 and miR158, were found to be induced by TuMV infection (He et al. 2008). miR159 was found upregulated while miR164 and miR171 were downregulated in response to Tomato leaf curl virus (ToLCV) infection (Naqvi Afsar et al. 2008). In another study related to interaction between tomato and Tomato leaf curl New Delhi virus (ToLCNDV), the miR159/319 and miR172 were detected as biomarkers for infection (Naqvi Afsar et al. 2010). Of late, miRNAs have been reported as principal regulators of nucleotide-binding site leucine-rich repeat (NBS-LRR) defense gene family through production of trans-acting small interfering RNAs (tasiRNAs) (Zhai et al. 2011). Genome-wide sequencing has led to the identification of microRNA-targeted NBS-LRR resistance (R) genes in a number of plant species including grapevine, sugarcane, loblolly pine, and eggplant (Yang and Huang 2014). miRNA families such as nta-miR6019 and nta-miR6020 from tobacco; stumiR1507,

stu-miR2109, and stumiR2118 from potato; as well as tomato-specific slymiR482f and sly5300 have been identified as resistance regulators by directing the cleavage NBS-LRR class R genes (Zhai et al. 2011; Li et al. 2012; Ouyang et al. 2014). On the other hand, ata-miR398 and ata-miR733 were described as negative regulators of the PAMP response by preventing callose deposition and cleaving the Cu/Zn superoxide dismutase genes leading to elevated reactive oxygen species (ROS) (Jagadeeswaran et al. 2009; Li et al. 2010). miR400-mediated dysfunction of the mRNA encoding pentatricopeptide repeat (PPR) protein renders *Arabidopsis thaliana* more susceptible to *B. cinerea* (Park et al. 2014). In another study, transgenic *Arabidopsis* plants overexpressing miR844 degraded the putative target mRNA Cytidine phosphate diacylglycerol synthase 3 (CDS3) leading to enhanced susceptibility to the bacterium *Pseudomonas syringae* pv. tomato DC3000 and fungus *Botrytis cinerea* (Lee et al. 2002). Also, the overexpression of potato miR482e demonstrated enhanced plant sensitivity to *Verticillium dahliae* due to cleavage of nucleotide-binding site leucine-rich repeat (NBSLRR)-resistant target transcripts (Yang et al. 2015). A recent study have shown that tomato miR1918 silences a RING finger gene by cleavage of its target mRNA, thereby enhancing the susceptibility of tomato to *Phytophthora infestans* (Luan et al. 2016). Most recently, the overexpression of miR169a in rice made them hyper-susceptible to different *M. oryzae* strains associated with decreased expression of defense-related genes and poor accumulation of hydrogen peroxide at the infection site (Li et al. 2017). In another study, reduced expression of miR396 enables the upregulation of GRF (growth regulating factor) target genes to trigger host reprogramming leading to broad resistance against necrotrophic and hemibiotrophic fungal pathogens (Soto-Suárez et al. 2017). Emerging evidences also indicate that immune-regulated miRNAs that are differentially regulated upon pathogen attack are translocated into interactive organisms and induce cross-kingdom RNA interference (RNAi) (Knip et al. 2014). While the host-induced RNAi triggers the silencing of pathogen genes in a process referred to as HIGS, pathogen-secreted small RNAs also mimics host miRNAs and suppresses plant immunity (Weiberg et al. 2015). For example, three *Botrytis cinerea* small RNAs (BcsiRNAs) impersonate plant miRNAs and suppress *Arabidopsis* and tomato defense responsive genes in vivo (Weiberg et al. 2013). Similarly, a wide range of miRNAs regulate the expression genes that are implicated in the development, biological processes, and cellular homeostasis in insects (reviewed in Chauhan et al. 2017). All these reports clearly suggest that miRNAs play critical role in fine-tuning the interaction between plant and the biotic components around it toward reprogramming of immune responses.

14.4 Plant Insect Interactions: A Molecular Perspective

At one end, plants attract a wide range of insect pollinators and beneficial insects while at the same time defend themselves against insect herbivory. As such, plants have developed constitutive strategies to prevent herbivore yet attract other

beneficial insects. A complex and diverse mechanism governs the interaction between the plant and the invading insects (Erb and Reymond 2019). The molecular components of the jasmonic acid (JA) signaling pathway are believed to the core elements that regulate plant defense response against herbivores (Howe et al. 2018). Plants perceive insect herbivory by recognizing the herbivore- and damage-associated molecular patterns (HAMPs and DAMPs) using specialized pattern recognition receptors (PRRs). Cellular damages allow DAMPs consisting of cellular fragments and small metabolites into the apoplast. Herbivory also stimulates the production of secondary danger signals (SDS) such as peptides (pep) and systemin (sys) that can activate the defense response by binding to the PRRs. The interaction between HAMPs/DAMPs with PRRs results in the activation of one or more cellular responses including membrane depolarization, Ca^{2+} signaling, reactive oxygen species (ROS) signaling, or downstream mitogen-activated protein kinase (MAPK) signaling leading to the biosynthesis of the canonical jasmonates—Jasmonoyl-L-isoleucine (JA-Ile). JA-Ile binds with the nuclear receptor complex consisting of CORONATINE INSENSITIVE 1 (COI1) and JASMONATE ZIM-DOMAIN (JAZ) protein. Binding of JA-Ile to JAZ causes the degradation of JAZ repressor which in turn releases the suppression of transcription factors. The activation of transcription factors regulates the production of defense metabolites including phenylpropanoids, sesquiterpenes, and glucosinolates that exhibit resistance response against a wide variety of herbivores. While the JA-mediated herbivore defense response in plant has been extensively studied, the involvement of miRNA or small RNA repertoire in the reciprocal interaction between plant and herbivore is still at its infancy. In the subsequent sections, we have discussed recent evidences indicating the role of miRNAs in regulating defense process in plants against insect pest and their application in improvement of field crops.

14.5 Plant miRNAs Targeting Herbivore Insects

Interaction between the plants and insects is a very complex process, and miRNAs play a significant role in the regulation of genes involved in plant's defense response to insect herbivory (Table 14.1). A distinct profile of miRNA expression was reported in resistant and susceptible *Cucumis melo* under aphid herbivory (Sattar et al. 2012). Fifty-nine miRNAs and two trans-acting small interfering RNAs (tasiRNAs) were expressive in tobacco plants following infestation with tobacco hornworm (*Manduca sexta*) (Bozorov et al. 2012). Further, these miRNAs were regulated by both JA-dependent and JA-independent signaling pathways (Bozorov et al. 2012). Likewise, miR159a, miR160a, and miR393a were predicted to be involved in *Chrysanthemum morifolium* and aphid interaction (Xia et al. 2015). Genome-wide profiling also revealed multiple miRNAs responsive to *Ectropis oblique* feeding in *Camellia sinensis* (Jeyaraj et al. 2017). In yet another study, 33 known and 13 novel miRNAs were associated with resistance to whitefly (*Bemisia tabaci*) attack in *Solanum lycopersicon* and *Solanum habrochaites* (Ketao et al. 2018).

Genome-wide analysis and subsequent validation have revealed that several miRNAs and their corresponding target genes reported dynamic spatio-temporal expression pattern in cotton post infestation of *Bemisia tabaci* (Li et al. 2019). Significant progress has been made in the identification of analysis of miRNAs. Brown plant hopper (BPH) is a rice-specific herbivore responsible for devastating yield losses throughout the Asian subcontinent (Cheng et al. 2013). Multiple miRNAs involved in the regulation of pathways that contribute to the basal as well as specific resistance response to BPH has been reported (Wu et al. 2017). In another study, the silencing of miR156 exhibited enhanced resistance to BPH as demonstrated by low rate of nymph survival and high fecundity of BPH. Additionally, three genes, namely, mitogen-activated protein kinase 3 (MPK3), MPK6, and WRKY70 that are previously reported to be involved in the process of JA signaling-mediated BPH resistance reported reduced expression in the miR156-silenced rice lines (Ge et al. 2018). In yet another study, the OsmiR396-OsGRF8-OsF3H-flavanoid pathway has been revealed as a potent mechanism for BPH resistance in rice (Dai et al. 2019). While the silencing of OsmiR396 negatively regulated BPH resistance, overexpression of its target OsGRF8 (*Oryza sativa* growth regulating factor 8) showed resistance to BPH. OsGRF8 directly regulate a BPH-responsive flavanone 3-hydroxylase (OsF3H) leading to increased flavonoid contents and BPH resistance (Dai et al. 2019). More recently, dynamic small RNA profiling in the BPH-resistant rice genotype IR56 identified five know miRNAs that might be involved in rice defense response against BPH (Nanda et al. 2020).

Alternatively, plant miRNAs also influence the gene expression in insect pests through cross-kingdom RNA interference (Gualtieri et al. 2020). Deep sequencing has revealed 13 sorghum miRNAs and 3 barley miRNAs that target aphid (cereal aphid *Schizaphis graminum*) genes implicated in detoxification and sucrose metabolism leading to resistance response (Wang et al. 2017). Many cross-kingdom transfer of miRNAs also contributes to the communication between plants and the feeding insects (Zhang et al. 2019). For instance, miR159a, miR166a-3p, and the novel miRNA7703-5p regulate the cellular and metabolic processes in *Plutella xylostella* through binding and suppression of basic juvenile hormone-repressible protein 1 and 2 (BJHSP1 and BJHSP2) and polyphenol oxidase subunit 2 (PPO2) genes (Zhang et al. 2019). All these studies clearly suggest that endogenous plant miRNAs function extensively in plant-insect interactions and associated defense responses.

14.6 Strategies for miRNA-Based Insect Resistance in Crops

The functional validation of plant miRNAs towards insect resistance are being carried out using various genetic tools including high-throughput sequencing, quantitative real-time PCR, and microarray. However, these approaches are primarily meant for identification and revalidation and do not provide direct evidence about gene functionality. Overexpression or repression of miRNA genes may alter the

expression of genes related to growth, development, and insect-resistant plants. However, some miRNAs are implicated in the regulation of several genes which may result in undesirable phenotypic changes. Therefore, in the recent times, various target-specific miRNA-based strategies have been developed for precise alteration or introduction of desired traits to develop insect resistance in crop plants.

14.6.1 Artificial miRNA (amiRNA) Technology

Host delivered RNAi technology utilizes long hair-pin (hp)-RNA (dsRNA) constructs to produce multiple siRNAs for silencing of target genes. However, they suffer from off-target effects and environmental safety concerns owing to their effect in beneficial insects (Auer and Frederick 2009). In contrast, amiRNA technology exploits the silencing ability of miRNAs and acts as a precise gene silencing tool with no or limited off-target effects (Schwab et al. 2005). amiRNAs are designed from the precursor of an endogenous miRNAs in which the region of the mature miRNA is replaced with a specific amiRNA sequence complementary to the target sequence (Fig. 14.2a) (Alvarez et al. 2006). The Web MicroRNA Designer 3 tool is often used to generate potential amiRNA from the gene sequences which have the conserved stem loop structure like the original pre-miRNA and the complementary sequence to target the specific mRNA. The amiRNA/miRNA* duplex is directly introduced into the transgenic plants to target the mRNA with high specificity. The replacement of endogenous miRNA sequence of the native miRNA precursor with the amiRNA sequence is carried out through overlapping PCR (Van vu et al. 2018). Like the natural miRNAs, amiRNAs also possesses varying numbers of target mismatches and could silence single as well multiple genes with high precision (Tiwari et al. 2014). miRNA family members including miR159a, miR164b, miR167b, miR169d, miR171a, miR172a, miR319a, and miR395a are often utilized in the amiRNA-mediated gene silencing owing to the effectiveness and consistency of their precursors (Tiwari et al. 2014). What more, amiRNA technology is considered as the second-generation RNAi technology with significant contribution towards development of biotic stress resistance in crops (Kamthan et al. 2015). Guo et al. (2014) showed that genetic transformation of tobacco with amiRNA vectors targeting MpAChE2 gene reported improved resistance to aphid (*Myzus persicae*) infestation as demonstrated by evident reduction in target gene transcript level. Tobacco plants transformed with amiRNA cassette of endogenous insect gene has shown significant resistance against polyphagous insect pest, *Helicoverpa armigera* (Saini et al. 2018). Arabidopsis pre-miRNA164b was modified by replacing it with amiRNA/amiRNA* targeting the *H. armigera* acetylcholine esterase 1 (*HaAce1*) gene, and the vector cassette was used for tobacco transformation. Transgenic tobacco lines expressing HaAce1-amiR1 reported 70–80% defective adults and 25% larval mortality. Plant expressed insect pre-amiRs (plin-amiRs) strategy also demonstrated significant efficacy in controlling *H. armigera Nicotiana benthamiana* (Bally et al. 2020). Transgenic *N. benthamiana* expressing the plin-amiRs

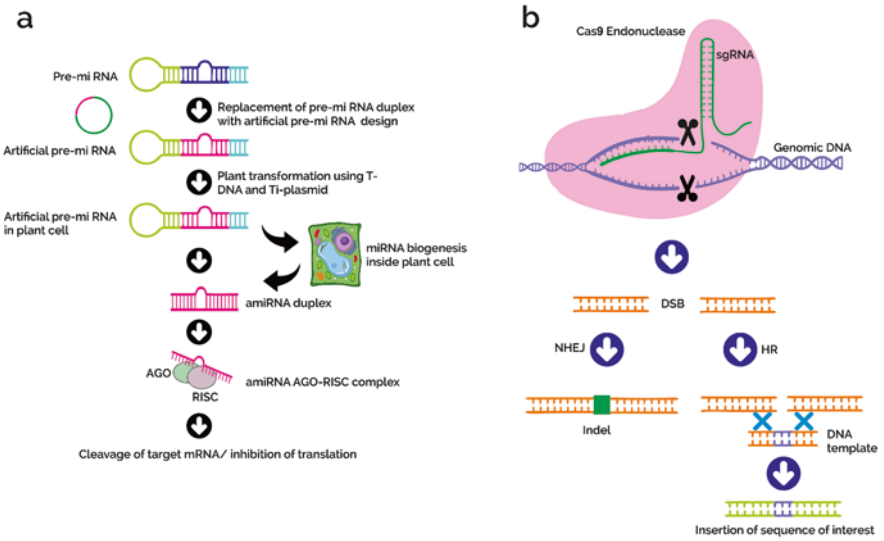

Fig. 14.2 miRNA-based strategies for developing pest resistance in field crops. (**a**) amiRNA technology. Designed amiRNA is inserted into the endogenous miRNA precursor by replacing the miRNA/miRNA* sequence with amiRNA/amiRNA* sequence. The pre-miRNA is processed by DCL1 to generate amiRNA-amiRNA* duplex. Matured amiRNA strand binds with the RISC containing the AGO1 leading to target mRNA cleavage or translational inhibition. (**b**) CRISPR/Cas9 technology. The genomic sequence of the targeted miRNA gene is used to design 20 nt crispr RNA (crRNA) which binds with trans-activating crispr RNA (tracrRNA) to form the single-guide RNA (sgRNA). The sgRNA together with sequence-specific nuclease Cas9 construct is introduced into plant cell using a transformation technique. In the transformed plant, the CRISPR/Cas9 complex cleaves the target DNA and causes knockout of the targeted miRNA gene

resulted in increased mortality, developmental abnormalities, and delayed growth rates in *H. armigera.* In another study, *A. thaliana* miR159 precursor was modified and engineered to express amiRNAs targeting three developmental specific genes in whitefly (*Bemisia tabaci*) (Zubair et al. 2020). The transgenic tobacco plant showed significant resistance against whitefly and the number of whitefly were substantially reduced in the next generation. In yet another study, amiRNA-mediated gene silencing was used to generate insect-resistant tomato plants (Yogindran and Rajam 2021). In this approach, the developmental specific gene ecdysone receptor (HaEcR) from *H. armigera* was targeted using the amiRNA-319a-*HaEcR* cassette and the insect feeding of the resultant transgenic lines affected the overall growth and survival of the insect pest. Most recently, an RNAi plasmid vector containing amiRNA sequence targeting the *Myzus persicae* acetylcholine esterase 1 gene (*Ace 1*) gene was engineered and successfully transformed into tomato varieties that exhibited resistance to aphids (Faisal et al. 2021). A significant drop in aphid colonies was reported when fed with T1 transgenic tomato plants. All these studies suggest that host expression of insect-specific amiRNAs is a promising strategy towards development of insect-resistant crop varieties.

14.6.2 CRISPR/Cas9 Technology

The advent of genome-editing technologies has revolutionized the field of modern biology through precise and targeted modification at specific genomic loci (Zhang et al. 2018). Genome editing makes use of sequence-specific nucleases (SSNs) that cut specific genome target DNA sequences and introduce double-stranded breaks (DSBs). The DSBs are subsequently repaired through endogenous DNA repair systems-non homologous end joining (NHEJ) leading to insertion or deletion (InDel)-based gene knockouts and homologous recombination in the presence of donor DNA template resulting in precise base modification or gene replacement (Chen and Gao 2014). The genome-edited plants produce only a few nucleotide modifications and are often indistinguishable from the similar to naturally occurring populations. As such, genome-editing technologies have become major players in the breeding programs towards crop improvement against biotic and abiotic stresses. Among others, the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR associated protein (Cas) system has wider scientific acceptability for its simplicity in development, high specificity in target cleavage, and universal applicability (Zaidi et al. 2018). It is a two component editing system consisting of a target-specific single-guide RNA (sgRNA) molecule and a *Cas9* endonuclease (Hsu et al. 2013) (Fig. 14.2b). The binding of the Cas9-sgRNA complex and cleavage of the target DNA depend on the presence of a protospacer adjacent motif (PAM) in the downstream of the target site. Hence, the need of only different spacer sequences makes CRISPR/Cas9 a very simple and highly effective editing tool. Moreover, CRISPR/Cas9 editing causes complete loss-of-function in the target genes while the RNAi generally results in hypomorphic effect leading to partial loss of gene function. What more, CRISPR/Cas9 and its variants have been greatly exploited in recent years for improvement of model plants and major crops against myriads of stresses (Tyagi et al. 2021). The application of genome-editing tools for insect management envisage modification of both insects and plants. Genome editing in insects for pest management has been largely achieved by modification of Cry protein-binding receptors and inhibition of detoxification enzymes. CRISPR/Cas9 mediated modification of cadherin receptors that are genetically linked to Cry1Ac toxin resistance was used to substantially reduce *H. armigera* colonies (Wang et al. 2016). In another study, CRISPR/Cas9 mediated knockout of gossypol-inducing cytochrome P450 (CYP6AE) gene cluster resulted in significant reduction of *H. armigera* population (Wang et al. 2018a, b). CRISPR/Cas-mediated knocking out of developmental genes of insects could also be effective for pest management. For instance, CRISPR/Cas9-induced loss of function mutation of Abdominal-A (abd-A) gene reported adult deformity, embryonic lethality, and reduced growth rates in multiple agricultural pests (Sun et al. 2017; Wu et al. 2018). Alternatively, exploitation of genome editing of plant genes for insect pest management is still at its infancy. While the change in the plant volatile blends prevents insect from host plants, alteration in the anthocyanin pigmentation content also acts as deterrent to herbivores (Beale et al. 2006; Zhang et al. 2020). Currently, CRISPR/Cas9-mediated

genome editing focuses towards modification of genes from anthocyanin pathway or those encoding plant volatiles to develop insect resistance in crop plants.

14.7 Conclusions and Future Prospects

Over the past decade, several studies have shown the involvement of miRNAs in almost all kinds of biochemical and molecular networks in plants. Therefore, they have become suitable candidates towards development of stress-tolerant crop varieties including for insect resistance. Many miRNA families are now known to play an important role in inhibiting insect growth and control of insect population in crop fields. In-depth elucidation of the molecular mechanism regulated by miRNAs in the plant molecular networking systems could largely enable molecular breeders and agronomists to modify unique agronomical traits in crops. However, it is always challenging to select specific miRNA for targeting a candidate agronomic trait given that multiple genes and networks are regulated by single miRNA in plants. Moreover, the alteration of a specific miRNAs may also result in modification at the non-target loci and thereby undesirable phenotypes. Therefore, more efficient tools are required for deciphering the characteristics of pri-miRNA-mediated regulatory mechanisms for unique trait expression. Currently, amiRNA-mediated gene silencing has developed into a potential technique for genetic engineering of crop plants. amiRNA also make it feasible to explore genes with deleterious effects which are not possible through traditional mutation analysis. Several reports have shown the successful utilization of amiRNAs for generating transgenic crop plants resistant to various insect pests. More recently, genome editing has become the tool of choice by the scientific community for elucidating gene functions and modification of specific agronomic traits. The editing strategies especially the CRISPR/Cas9 system have developed into a beneficial tool for their specificity, efficiency, and universal acceptance. However, its use in the management of insect pest is yet to be fully exploited. A few recent reports have shown the utilization of CRISPR/Cas9 system towards insect management through manipulation of insect genes. However, editing plant genes for insect resistance is still slow, owing to the lack of availability of target genes as compared to other stresses. Therefore, it is essential to identify novel resistance sources which could be used for manipulation towards insect management. Given that a large group of miRNAs are known to be involved in defense response against insect pests, strategies are required to be developed for potential miRNA-based genome editing towards development of pest resistance in field crops.

Acknowledgement BM is supported in the form of a research fellowship (Fellowship no. 544/83/OSHEC-Part-II) under the OURIP fellowship scheme of Odisha State Higher Education Council, Dept. of Higher Education, Govt. of Odisha, India. Research work in the laboratory of RKJ is supported by grants from Dept. of Biotechnology (Grant no. BT/PR23412/BPA/118/284/2017), Govt. of India. RM is supported by grant from Science and Engineering Research Board (SERB) (Grant no. EMR/2016/005234). RM is also thankful to the President, Centurion University of Technology and Management for his encouragement and support.

References

Addo-Quaye C, Eshoo TW, Bartel DP, Axtell MJ (2008) Endogenous siRNA and miRNA targets identified by sequencing of the Arabidopsis degradome. Curr Biol 18:758–762. https://doi.org/10.1016/j.cub.2008.04.042

Allen E, Xie Z, Gustafson AM, Carrington JC (2005) MicroRNA-directed phasing during trans-acting siRNA biogenesis in plants. Cell 121:207–221. https://doi.org/10.1016/j.cell.2005.04.004

Alvarez JP, Pekker I, Goldshmidt A, Blum E, Amsellem Z, Eshed Y (2006) Endogenous and synthetic microRNAs stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. The Plant Cell 18:1134–1151

Arenas-Huertero C, Pérez B, Rabanal F, Blanco-Melo D, De la Rosa C, Estrada-Navarrete G, Reyes JL (2009) Conserved and novel miRNAs in the legume Phaseolus vulgaris in response to stress. Plant Mol Biol 70:385–401. https://doi.org/10.1007/s11103-009-9480-3

Auer C, Frederick R (2009) Crop improvement using small RNAs: applications and predictive ecological risk assessments. Trends Biotechnol 27:644–651

Aung K, Lin SI, Wu CC, Huang YT, Su CL, Chiou TJ (2006) pho2, a phosphate over accumulator, is caused by a nonsense mutation in a microRNA399 target gene. Plant Physiol 141:1000–1011. https://doi.org/10.1104/pp.106.078063

Bally J, Fishilevich E, Doran RL, Lee K, de Campos SB, German MA, Narva KE, Waterhouse PM (2020) Plin-amiR, a pre-microRNA-based technology for controlling herbivorous insect pests. Plant Biotechnol J 18:1925–1932. https://doi.org/10.1111/pbi.13352

Barakat A, Sriram A, Park J, Zhebentyayeva T, Main D, Abbott A (2012) Genome wide identification of chilling responsive microRNAs in Prunus persica. BMC Genomics 13:481. https://doi.org/10.1186/1471-2164-13-481

Bari R, Datt Pant B, Stitt M, Scheible WR (2006) PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. Plant Physiol 141:988–999. https://doi.org/10.1104/pp.106.079707

Barrera-Figueroa BE, Gao L, Diop NN, Wu Z, Ehlers JD, Roberts PA, Liu R (2011) Identification and comparative analysis of drought-associated microRNAs in two cowpea genotypes. BMC Plant Biol 11:127. https://doi.org/10.1186/1471-2229-11-127

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297. https://doi.org/10.1016/S0092-8674(04)00045-5

Bartels DP, Sunkar R (2005) Drought and salt tolerance in plants. Crit Rev Plant Sci 24:23–58. https://doi.org/10.1080/07352680590910410

Beale MH, Birkett MA, Bruce TJA, Chamberlain K, Field LM, Huttly AK, Martin JL, Parker R, Phillips AL, Pickett JA, Prosser IM, Shewry PR, Smart LE, Wadhams LJ, Woodcock CM, Zhang Y (2006) Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. Proc Natl Acad Sci U S A 103:10509–10513. https://doi.org/10.1073/pnas.0603998103

Beauclair L, Yu A, Bouché N (2010) microRNA-directed cleavage and translational repression of the copper chaperone for superoxide dismutase mRNA in Arabidopsis: post-transcriptional control of superoxide dismutases. Plant J 62:454–462. https://doi.org/10.1111/j.1365-313X.2010.04162.x

Bologna NG, Voinnet O (2014) The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis. Annu Rev Plant Biol 65:473–503

Bozorov TA, Pandey SP, Dinh ST, Kim SG, Heinrich M, Gase K, Baldwin IT (2012) DICER-like proteins and their role in plant-herbivore interactions in Nicotiana attenuata: DICER-like proteins in plant-insect interactions. J Int Plant Biol 54:189–206. https://doi.org/10.1111/j.1744-7909.2012.01104.x

Campo S, Peris-Peris C, Siré C, Moreno AB, Donaire L, Zytnicki M, San Segundo B (2013) Identification of a novel microRNA (miRNA) from rice that targets an alternatively spliced transcript of the Nramp6 (Natural resistance-associated macrophage protein 6) gene involved in pathogen resistance. New Phytol 199:212–227. https://doi.org/10.1111/nph.12292

Chaudhary S, Grover A, Sharma PC (2021) MicroRNAs: potential targets for developing stress-tolerant crops. Life 11:289. https://doi.org/10.3390/life11040289
Chauhan S, Yogindran S, Rajam MV (2017) Role of miRNAs in biotic stress reactions in plants. Indian J Plant Physiol 22:514–529. https://doi.org/10.1007/s40502-017-0347-3
Chen K, Gao C (2014) Targeted genome modification technologies and their applications in crop improvements. Plant Cell Rep 33:575–583. https://doi.org/10.1007/s00299-013-1539-6
Cheng X, Zhu L, He G (2013) Towards understanding of molecular interactions between rice and the brown planthopper. Mol Plant 6:621–634. https://doi.org/10.1093/mp/sst030
Chiou TJ (2007) The role of microRNAs in sensing nutrient stress. Plant Cell Environ 30:323–332. https://doi.org/10.1111/j.1365-3040.2007.01643.x
Ci D, Song Y, Tian M, Zhang D (2015) Methylation of miRNA genes in the response to temperature stress in Populus simonii. Front Plant Sci 6:921. https://doi.org/10.3389/fpls.2015.00921
Coruh C, Shahid S, Axtell MJ (2014) Seeing the forest for the trees: annotating small RNA producing genes in plants. Curr Opin Plant Biol 18:87–95. https://doi.org/10.1016/j.pbi.2014.02.008
Covarrubias AA, Reyes JL (2010) Post-transcriptional gene regulation of salinity and drought responses by plant microRNAs. Plant Cell Environ 33:481–489. https://doi.org/10.1111/j.1365-3040.2009.02048.x
Dai Z, Tan J, Zhou C, Yang X, Yang F, Zhang S, Shi Z (2019) The OsmiR396-OsGRF8-OsF3H-flavonoid pathway mediates resistance to the brown plant hopper in rice (Oryza sativa). Plant Biotech J 17:1657–1669. https://doi.org/10.1111/pbi.13091
Deutsch CA, Tewksbury JJ, Tigchelaar M, Battisti DS, Merrill SC, Huey RB, Naylor RL (2018) Increase in crop losses to insect pests in a warming climate. Science 361:916–919. https://doi.org/10.1126/science.aat3466
Ding D, Zhang L, Wang H, Liu Z, Zhang Z, Zheng Y (2009) Differential expression of miRNAs in response to salt stress in maize roots. Ann Bot 103:29–38
Doench JG, Sharp PA (2004) Specificity of microRNA target selection in translational repression. Genes Dev 18:504–511. https://doi.org/10.1093/aob/mcn205
Dolata J, Bajczyk M, Bielewicz D, Niedojadlo K, Niedojadlo J, Pietrykowska H, Jarmolowski A (2016) Salt stress reveals a new role for ARGONAUTE1 in miRNA biogenesis at the transcriptional and posttranscriptional levels. Plant Physiol 172:297–312
Erb M, Reymond P (2019) Molecular interactions between plants and insect herbivores. Annu Rev Plant Biol 70:527–557. https://doi.org/10.1104/pp.16.00830
Fabian MR, Sonenberg N, Filipowicz W (2010) Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem 79:351–379. https://doi.org/10.1146/annurev-biochem-060308-103103
Faisal M, Abdel-Salam EM, Alatar AA (2021) Artificial microRNA-based RNA interference and specific gene silencing for developing insect resistance in *Solanum lycopersicum*. Agronomy 11:136. https://doi.org/10.3390/agronomy11010136
Franco-Zorrilla JM, González E, Bustos R, Linhares F, Leyva A, Paz-Ares J (2004) The transcriptional control of plant responses to phosphate limitation. J Exp Bot 55:285–293. https://doi.org/10.1093/jxb/erh009
Frazier TP, Sun G, Burklew CE, Zhang B (2011) Salt and drought stresses induce the aberrant expression of microRNA genes in tobacco. Mol Biotechnol 49:159–165. https://doi.org/10.1007/s12033-011-9387-5
Fujii H, Chiou TJ, Lin SI, Aung K, Zhu JK (2005) A miRNA involved in phosphate-starvation response in Arabidopsis. Curr Biol 15:2038–2043. https://doi.org/10.1016/j.cub.2005.10.016
Gao P, Bai X, Yang L, Lv D, Pan X, Li Y, Zhu Y (2011) osa-MIR393: a salinity- and alkaline stress-related microRNA gene. Mol Biol Rep 38:237–242. https://doi.org/10.1007/s11033-010-0100-8
Gao LL, Kamphuis LG, Kakar K, Edwards OR, Udvardi MK, Singh KB (2010) Identification of potential early regulators of aphid resistance in Medicago truncatula via transcription factor expression profiling. New Phytol 186:980–994
Ge Y, Han J, Zhou G, Xu Y, Ding Y, Shi M, Wu G (2018) Silencing of miR156 confers enhanced resistance to brown plant hopper in rice. Planta 248:813–826. https://doi.org/10.1007/s00425-018-2942-6
German MA, Pillay M, Jeong DH, Hetawal A, Luo S, Janardhanan P, Green PJ (2008) Global identification of microRNA-target RNA pairs by parallel analysis of RNA ends. Nat Biotechnol 26:941–946. https://doi.org/10.1038/nbt1417

Gregory BD, O'Malley RC, Lister R, Urich MA, Tonti-Filippini J, Chen H, Ecker JR (2008) A link between RNA metabolism and silencing affecting Arabidopsis development. Dev Cell 14:854–866. https://doi.org/10.1016/j.devcel.2008.04.005

Gualtieri C, Leonetti P, Macovei A (2020) Plant miRNA cross-kingdom transfer targeting parasitic and mutualistic organisms as a tool to advance modern agriculture. Front Plant Sci 11:930. https://doi.org/10.3389/fpls.2020.00930

Guo L, Zhao Y, Yang S, Zhang H, Chen F (2014) An integrated analysis of miRNA, lncRNA, and mRNA expression profiles. Biomed Res Int 2014:345605. https://doi.org/10.1155/2014/345605

Ha M, Kim VN (2014) Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 15:509–524. https://doi.org/10.1038/nrm3838

He XF, Fang YY, Feng L, Guo HS (2008) Characterization of conserved and novel microRNAs and their targets, including a TuMV-induced TIR-NBS-LRR class R gene-derived novel miRNA in Brassica. FEBS Lett 582:2445–2452. https://doi.org/10.1016/j.febslet.2008.06.011

Howe GA, Major I, Koo AJ (2018) Modularity in jasmonate signaling for multi stress resilience. Annu Rev Plant Biol 69:387–415. https://doi.org/10.1146/annurev-arplant-042817-040047

Hsu P, Scott D, Weinstein J et al (2013) DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol 31:827–832

Iwakawa HO, Tomari Y (2013) Molecular insights into microRNA-mediated translational repression in plants. Mol Cell 52:591–601. https://doi.org/10.1016/j.molcel.2013.10.033

Iwata Y, Takahashi M, Fedoroff NV, Hamdan SM (2013) Dissecting the interactions of SERRATE with RNA and DICER-LIKE 1 in Arabidopsis microRNA precursor processing. Nucleic Acids Res 41:9129–9140. https://doi.org/10.1093/nar/gkt667

Jagadeeswaran G, Saini A, Sunkar R (2009) Biotic and abiotic stress down-regulate miR398 expression in Arabidopsis. Planta 229:1009–1014. https://doi.org/10.1007/s00425-009-0889-3

Jeyaraj A, Liu S, Zhang X, Zhang R, Shangguan M, Wei C (2017) Genome-wide identification of microRNAs responsive to Ectropis oblique feeding in tea plant (*Camellia sinensis* L.). Sci Rep 7:13634. https://doi.org/10.1038/s41598-017-13692-7

Jian X, Zhang L, Li G, Zhang L, Wang X, Cao X, Chen F (2010) Identification of novel stress-regulated microRNAs from Oryza sativa L. Genomics 95:47–55. https://doi.org/10.1016/j.ygeno.2009.08.017

Jin H (2008) Endogenous small RNAs and antibacterial immunity in plants. FEBS Lett 582:2679–2684. https://doi.org/10.1016/j.febslet.2008.06.053

Jin W, Wu F (2015) Characterization of miRNAs associated with *Botrytis cinerea* infection of tomato leaves. BMC Plant Biol 15:1. https://doi.org/10.1186/s12870-014-0410-4

Jones-Rhoades MW, Bartel DP (2004) Computational identification of plant microRNAs and their targets, including a stressinduced miRNA. Mol Cell 14:787–799. https://doi.org/10.1016/j.molcel.2004.05.027

Jones-Rhoades MW, Bartel DP, Bartel B (2006) MicroRNAS and their regulatory roles in plants. Annu Rev Plant Biol 57:19–53. https://doi.org/10.1146/annurev.arplant.57.032905.105218

Joshi R, Gupta P, Singla-Pareek SL, Pareek A (2017) Biomass production and salinity response in plants: role of MicroRNAs. Ind J Plant Physiol 22:448–457. https://doi.org/10.1007/s40502-017-0327-7

Kamthan A, Chaudhuri A, Kamthan M, Datta A (2015) Small RNAs in plants: recent development and application for crop improvement. Front Plant Sci 6:208. https://doi.org/10.3389/fpls.2015.00208

Kantar M, Lucas SJ, Budak H (2011) miRNA expression patterns of Triticum dicoccoides in response to shock drought stress. Planta 233:471–484. https://doi.org/10.1007/s00425-010-1309-4

Karimi M, Ghazanfari F, Fadaei A, Ahmadi L, Shiran B, Rabei M, Fallahi H (2016) The small-RNA profiles of almond (*Prunus dulcis mill*.) reproductive tissues in response to cold stress. PLoS One 11:e0156519. https://doi.org/10.1371/journal.pone.0156519

Katiyar-Agarwal S, Morgan R, Dahlbeck D, Borsani O, Villegas A, Zhu JK, Staskawicz BJ, Jin H (2006) A pathogen-inducible endogenous siRNA in plant immunity. Proc Natl Acad Sci U S A 103:18002–18007. https://doi.org/10.1073/pnas.0608258103

Ketao W, Xiaomei SU, Xia CUI, Yongchen DU, Shuaibin ZHANG, Jianchang GAO (2018) Identification and Characterization of microRNA during Bemisia tabaci Infestations in Solanum lycopersicum and Solanum habrochaites. Horticultural Plant Journal 4(2):62–72

Khraiwesh B, Zhu JK, Zhu J (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta 1819:137–148. https://doi.org/10.1016/j.bbagrm.2011.05.001

Kidner CA, Martienssen RA (2004) Spatially restricted microRNA directs leaf polarity through ARGONAUTE1. Nature 428:81–84. https://doi.org/10.1038/nature02366

Kim VN (2004) MicroRNA precursors in motion: exportin-5 mediates their nuclear export. Trends Cell Biol 14:156–159. https://doi.org/10.1016/j.tcb.2004.02.006

Knip M, Constantin ME, Thordal-Christensen H (2014) Trans-kingdom cross-talk: small RNAs on the move. PLoS Genet 10:e1004602. https://doi.org/10.1371/journal.pgen.1004602

Kulcheski FR, de Oliveira LF, Molina LG, Almerão MP, Rodrigues FA, Marcolino J, Margis R (2011) Identification of novel soybean microRNAs involved in abiotic and biotic stresses. BMC Genomics 12:307. https://doi.org/10.1186/1471-2164-12-307

Lee Y, Jeon K, Lee JT, Kim S, Kim VN (2002) MicroRNA maturation: stepwise processing and subcellular localization. EMBO J 21:4663–4670

Li Y, Zhang Q, Zhang J, Wu L, Qi Y, Zhou JM (2010) Identification of microRNAs involved in pathogen-associated molecular pattern-triggered plant innate immunity. Plant Physiol 152:2222–2231. https://doi.org/10.1104/pp.109.151803

Li F, Pignatta D, Bendix C, Brunkard JO, Cohn MM, Tung J, Baker B (2012) MicroRNA regulation of plant innate immune receptors. Proc Natl Acad Sci U S A 109:1790–1795. https://doi.org/10.1073/pnas.1118282109

Li S, Liu L, Zhuang X, Yu Y, Liu X, Cui X, Chen X (2013) MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in Arabidopsis. Cell 153:562–574. https://doi.org/10.1016/j.cell.2013.04.005

Li Y, Lu YG, Shi Y, Wu L, Xu YJ, Huang F, Wang WM (2014) Multiple rice microRNAs are involved in immunity against the blast fungus Magnaporthe oryzae. Plant Physiol 164:1077–1092. https://doi.org/10.1104/pp.113.230052

Li H, Dong Y, Chang J, He J, Chen H, Liu Q, Zhang X (2016) High-throughput MicroRNA and mRNA sequencing reveals that MicroRNAs may be involved in melatonin-mediated cold tolerance in Citrullus lanatus L. Front Plant Sci 7:1231. https://doi.org/10.3389/fpls.2016.01231

Li Y, Zhao S, Li JL, Hu XH, Wang H, Cao XL, Wang WM (2017) Osa-miR169 negatively regulates rice immunity against the blast fungus Magnaporthe oryzae. Front Plant Sci 8:2. https://doi.org/10.3389/fpls.2017.00002

Li J, Hull JJ, Liang S, Wang Q, Chen L, Zhang Q, Jin S (2019) Genome-wide analysis of cotton miRNAs during Whitefly infestation offers new insights into plant-herbivore interaction. Int J Mol Sci 20:5357. https://doi.org/10.3390/ijms20215357

Liang Y, Yu L (2010) A new class of semiconducting polymers for bulk heterojunction solar cells with exceptionally high performance. Accounts Chem Res 43:1227–1236. https://doi.org/10.1021/ar1000296

Liu HH, Tian X, Li YJ, Wu CA, Zheng CC (2008) Microarray-based analysis of stress-regulated microRNAs in Arabidopsis thaliana. RNA 14:836–843. https://doi.org/10.1261/rna.895308

Liu H, Guo S, Xu Y, Li C, Zhang Z, Zhang D, Chong K (2014) OsmiR396d-regulated OsGRFs function in floral organogenesis in rice through binding to their targets OsJMJ706 and OsCR4. Plant Physiol 165:160–174. https://doi.org/10.1104/pp.114.235564

Lu S, Sun YH, Shi R, Clark C, Li L, Chiang VL (2005) Novel and mechanical stress-responsive MicroRNAs in Populus trichocarpa that are absent from Arabidopsis. Plant Cell 17:2186–2203. https://doi.org/10.1105/tpc.105.033456

Lu S, Sun YH, Chiang VL (2008) Stress-responsive microRNAs in Populus. Plant J 55:131–151. https://doi.org/10.1111/j.1365-313X.2008.03497.x

Luan Y, Cui J, Wang W (2016) MiR1918 enhances tomato sensitivity to *Phytophthora infestans* infection. Sci Rep 6:35858. https://doi.org/10.1038/srep35858

Lucas SJ, Budak H (2012) Sorting the wheat from the chaff: identifying miRNAs in genomic survey sequences of Triticum aestivum chromosome 1AL. PloS One 7:e40859. https://doi.org/10.1371/journal.pone.0040859

Luo M, Gao J, Peng H, Pan G, Zhang Z (2014) MiR393-targeted TIR1-like (F-box) gene in response to inoculation to R. solani in Zea mays. Acta Physiol Plant 36:1283–1291. https://doi.org/10.1007/s11738-014-1509-9

Mallory AC, Reinhart BJ, Jones-Rhoades MW, Tang G, Zamore PD, Barton MK, Bartel DP (2004) MicroRNA control of PHABULOSA in leaf development: importance of pairing to the microRNA 5′ region. EMBO J 23:3356–3364. https://doi.org/10.1038/sj.emboj.7600340

Mi S, Cai T, Hu Y, Chen Y, Hodges E, Ni F, Qi Y (2008) Sorting of small RNAs into Arabidopsis argonaute complexes is directed by the 5′ terminal nucleotide. Cell 133:116–127. https://doi.org/10.1016/j.cell.2008.02.034

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410. https://doi.org/10.1016/S1360-1385(02)02312-9

Moné Y, Nhim S, Gimenez S et al. (2018) Characterization and expression profiling of microRNAs in response to plant feeding in two host-plant strains of the lepidopteran pest Spodoptera frugiperda. BMC Genomics 19:804

Montgomery TA, Howell MD, Cuperus JT, Li D, Hansen JE, Alexander AL, Carrington JC (2008) Specificity of ARGONAUTE7-miR390 interaction and dual functionality in TAS3 trans-acting siRNA formation. Cell 133:128–141. https://doi.org/10.1016/j.cell.2008.02.033

Motomura K, Le QT, Kumakura N, Fukaya T, Takeda A, Watanabe Y (2012) The role of decapping proteins in the miRNA accumulation in Arabidopsis thaliana. RNA Biol 9:644–652. https://doi.org/10.4161/rna.19877

Munns R (2005) Genes and salt tolerance: bringing them together: Tansley review. New Phytol 167:645–663. https://doi.org/10.1111/j.1469-8137.2005.01487.x

Nanda S, Yuan SY, Lai FX, Wang WX, Fu Q, Wan PJ (2020) Identification and analysis of miRNAs in IR56 rice in response to BPH infestations of different virulence levels. Sci Rep 10:19093. https://doi.org/10.1038/s41598-020-76198-9

Naqvi Afsar R, Choudhury NR, Haq QMR, Mukherjee SK (2008) MicroRNAs as biomarkers in tomato leaf curl virus (ToLCV) disease. Nucleic Acids Symp Ser 52:507–508. https://doi.org/10.1093/nass/nrn257

Naqvi Afsar R, Haq QMR, Mukherjee SK (2010) MicroRNA profiling of tomato leaf curl New Delhi virus (tolcndv) infected tomato leaves indicates that deregulation of mir159/319 and mir172 might be linked with leaf curl disease. Virol J 7:281. https://doi.org/10.1186/1743-422X-7-281

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JDG (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science 312:436–439. https://doi.org/10.1126/science.1126088

Ouyang S, Park G, Atamian HS, Han CS, Stajich JE, Kaloshian I, Borkovich KA (2014) MicroRNAs suppress NB domain genes in tomato that confer resistance to Fusarium oxysporum. PLoS Pathog 10:e1004464. https://doi.org/10.1371/journal.ppat.1004464

Padmanabhan C, Zhang X, Jin H (2009) Host small RNAs are big contributors to plant innate immunity. Curr Opin Plant Biol 12:465–472. https://doi.org/10.1016/j.pbi.2009.06.005

Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS (2005) Nuclear processing and export of microRNAs in Arabidopsis. Proc Natl Acad Sci U S A 102:3691–3696. https://doi.org/10.1073/pnas.0405570102

Park YJ, Lee HJ, Kwak KJ, Lee K, Hong SW, Kang H (2014) MicroRNA400-guided cleavage of pentatricopeptide repeat protein mRNAs renders Arabidopsis thaliana more susceptible to pathogenic bacteria and fungi. Plant Cell Physiol 55:1660–1668. https://doi.org/10.1093/pcp/pcu096

Pratt ZL, Kuzembayeva M, Sengupta S, Sugden B (2009) The microRNAs of Epstein-Barr virus are expressed at dramatically differing levels among cell lines. Virology 386:387–397. https://doi.org/10.1016/j.virol.2009.01.006

Ren G, Yu B (2012) Critical roles of RNA-binding proteins in miRNA biogenesis in Arabidopsis. RNA Biol 9:1424–1428. https://doi.org/10.4161/rna.22740

Ren G, Xie M, Dou Y, Zhang S, Zhang C, Yu B (2012) Regulation of miRNA abundance by RNA binding protein TOUGH in Arabidopsis. Proc Natl Acad Sci U S A 109:12817–12821. https://doi.org/10.1073/pnas.1204915109

Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP (2002) MicroRNAs in plants. Genes Dev 16:1616–1626

Rubio V, Linhares F, Solano R, Martín AC, Iglesias J, Leyva A, Paz-Ares J (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes Dev 15:2122–2133. https://doi.org/10.1101/gad.204401

Saini RP, Raman V, Dhandapani G, Malhotra EV, Sreevathsa R, Kumar PA, Sharma TR, Pattanayak D (2018) Silencing of HaAce1 gene by host-delivered artificial microRNA disrupts growth and development of Helicoverpa armigera. PLoS One 13:e0194150. https://doi.org/10.1371/journal.pone.0194150

Sattar S, Song Y, Anstead JA, Sunkar R, Thompson GA (2012) Cucumis melo microRNA expression profile during aphid herbivory in a resistant and susceptible interaction. Mol Plant-Microbe Interact 25:839–848. https://doi.org/10.1094/MPMI-09-11-0252

Schwab R, Palatnik JF, Riester M, Schommer C, Schmid M, Weigel D (2005) Specific effects of microRNAs on the plant transcriptome. Dev Cell 8:517–527. https://doi.org/10.1016/j.devcel.2005.01.018

Shen D, Suhrkamp I, Wang Y, Liu S, Menkhaus J, Verreet JA, Cai D (2014) Identification and characterization of microRNAs in oilseed rape (Brassica napus) responsive to infection with the pathogenic fungus Verticillium longisporum using Brassica AA (Brassica rapa) and CC (Brassica oleracea) as reference genomes. New Phytol 204:577–594. https://doi.org/10.1111/nph.12934

Shukla LI, Chinnusamy V, Sunkar R (2008) The role of microRNAs and other endogenous small RNAs in plant stress responses. Biochim Biophys Acta 1779:743–748. https://doi.org/10.1016/j.bbagrm.2008.04.004

Soto-Suárez M, Baldrich P, Weigel D, Rubio-Somoza I, San Segundo B (2017) The Arabidopsis miR396 mediates pathogen-associated molecular pattern-triggered immune responses against fungal pathogens. Sci Rep 7:44898. https://doi.org/10.1038/srep44898

Sun X, Fan G, Su L, Wang W, Liang Z, Li S, Xin H (2015) Identification of cold-inducible microRNAs in grapevine. Front Plant Sci 6:595. https://doi.org/10.3389/fpls.2015.00595

Sun B, Yang P, Kattawar GW, Mishchenko MI (2017) On Babinet's principle and diffraction associated with an arbitrary particle. Opt Lett 42:5026. https://doi.org/10.1364/OL.42.005026

Sunkar R, Zhu JK (2004) Novel and stress-regulated microRNAs and other small RNAs from Arabidopsis. Plant Cell 16:2001–2019. https://doi.org/10.1105/tpc.104.022830

Sunkar R, Kapoor A, Zhu JK (2006) Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in Arabidopsis is mediated by downregulation of miR398 and important for oxidative stress tolerance. Plant Cell 18:2051–2065. https://doi.org/10.1105/tpc.106.041673

Sunkar R, Chinnusamy V, Zhu J, Zhu JK (2007) Small RNAs as big players in plant abiotic stress responses and nutrient deprivation. Trends Plant Sci 12:301–309. https://doi.org/10.1016/j.tplants.2007.05.001

Sunkar R, Zhou X, Zheng Y, Zhang W, Zhu JK (2008) Identification of novel and candidate miRNAs in rice by high throughput sequencing. BMC Plant Biol 8:25. https://doi.org/10.1186/1471-2229-8-25

Sunkar R, Li YF, Jagadeeswaran G (2012) Functions of microRNAs in plant stress responses. Trends Plant Sci 17:196–203. https://doi.org/10.1016/j.tplants.2012.01.010

Tang Z, Zhang L, Xu C, Yuan S, Zhang F, Zheng Y, Zhao C (2012) Uncovering small RNA-mediated responses to cold stress in a wheat thermosensitive genic male-sterile line by deep sequencing. Plant Physiol 159:721–738. https://doi.org/10.1104/pp.112.196048

TenOever BR (2013) RNA viruses and the host microRNA machinery. Nat Rev Microbiol 11(3):169–180

Tiwari M, Sharma D, Trivedi PK (2014) Artificial microRNA mediated gene silencing in plants: progress and perspectives. Plant Mol Biol 86:1–18. https://doi.org/10.1007/s11103-014-0224-7

Trindade I, Capitão C, Dalmay T, Fevereiro MP, Santos DMD (2010) miR398 and miR408 are up-regulated in response to water deficit in Medicago truncatula. Planta 231:705–716. https://doi.org/10.1007/s00425-009-1078-0

Tyagi S, Siddarth M, Mishra BK, Banerjee BD, Urfi AJ, Madhu SV (2021) High levels of organo chlorine pesticides in drinking water as a risk factor for type 2 diabetes: a study in North India. Environ Pollut 271:116287. https://doi.org/10.1016/j.envpol.2020.116287

Vazquez F, Gasciolli V, Crété P, Vaucheret H (2004) The nuclear dsRNA binding protein HYL1 is required for microRNA accumulation and plant development, but not posttranscriptional transgene silencing. Curr Biol 14:346–351

Wang B, Sun YF, Song N, Wei JP, Wang XJ, Feng H, Kang ZS (2014) MicroRNAs involving in cold, wounding and salt stresses in Triticum aestivum L. Plant Physiol Biochem 80:90–96. https://doi.org/10.1016/j.plaphy.2014.03.020

Wang J, Chen J, Sen S (2016) MicroRNA as biomarkers and diagnostics. J Cell Physiol 231:25–30. https://doi.org/10.1002/jcp.25056

Wang H, Zhang C, Dou Y, Yu B, Liu Y, Heng-Moss TM, Sarath G (2017) Insect and plant-derived miRNAs in greenbug (Schizaphis graminum) and yellow sugarcane aphid (Sipha flava) revealed by deep sequencing. Gene 599:68–77. https://doi.org/10.1016/j.gene.2016.11.014

Wang H, Shi Y, Wang L, Liu S, Wu S, Yang Y, Feyereisen R, Wu Y (2018a) CYP6AE gene cluster knockout in Helicoverpa armigera reveals role in detoxification of phytochemicals and insecticides. Nat Commun 9:4820. https://doi.org/10.1038/s41467-018-07226-6

Wang K, Su X, Cui X, Du Y, Zhang S, Gao J (2018b) Identification and characterization of microRNA during Bemisia tabaci infestations in *Solanum lycopersicum* and *Solanum habrochaites*. Hortic Plant J 4:62–72. https://doi.org/10.1016/j.hpj.2018.03.002

Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, Jin H (2013) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science 342:118–123. https://doi.org/10.1126/science.1239705

Weiberg A, Bellinger M, Jin H (2015) Conversations between kingdoms: small RNAs. Curr Opin Biotechnol 32:207–215. https://doi.org/10.1016/j.copbio.2014.12.025

Wu Y, Lv W, Hu L, Rao W, Zeng Y, Zhu L, He G (2017) Identification and analysis of brown plant hopper-responsive microRNAs in resistant and susceptible rice plants. Sci Rep 7:8712. https://doi.org/10.1038/s41598-017-09143-y

Wu K, Shirk PD, Taylor CE, Furlong RB, Shirk BD, Pinheiro DH, Siegfried BD (2018) CRISPR/Cas9 mediated knockout of the abdominal-A homeotic gene in fall armyworm moth (Spodoptera frugiperda). PLoS One 13:e0208647. https://doi.org/10.1371/journal.pone.0208647

Lv DK, Bai X, Li Y, Ding XD, Ge Y, Cai H, Ji W, Wu N, Zhu YM (2010) Profiling of cold-stress-responsive miRNAs in rice by microarrays. Gene 459:39–47

Xia X, Shao Y, Jiang J, Du X, Sheng L, Chen F, Chen S (2015) MicroRNA expression profile during aphid feeding in chrysanthemum (Chrysanthemum morifolium). PLoS One 10:e0143720. https://doi.org/10.1371/journal.pone.0143720

Xie Z, Allen E, Fahlgren N, Calamar A, Givan SA, Carrington JC (2005) Expression of Arabidopsis MIRNA genes. Plant Physiol 138:2145–2154. https://doi.org/10.1104/pp.105.062943

Xin M, Wang Y, Yao Y, Xie C, Peng H, Ni Z, Sun Q (2010) Diverse set of microRNAs are responsive to powdery mildew infection and heat stress in wheat (Triticum aestivum L.). BMC Plant Biol 10:123. https://doi.org/10.1186/1471-2229-10-123

Xu S, Liu N, Mao W, Hu Q, Wang G, Gong Y (2016) Identification of chilling-responsive microRNAs and their targets in vegetable soybean (Glycine max L.). Sci Rep 6:26619. https://doi.org/10.1038/srep26619

Yamasaki H, Abdel-Ghany SE, Cohu CM, Kobayashi Y, Shikanai T, Pilon M (2007) Regulation of copper homeostasis by micro-RNA in Arabidopsis. J Biol Chem 282:16369–16378. https://doi.org/10.1074/jbc.M700138200

Yang L, Huang H (2014) Roles of small RNAs in plant disease resistance: small RNAs and plant disease resistance. J Int Plant Biol 56:962–970. https://doi.org/10.1111/jipb.12200

Yang L, Liu Z, Lu F, Dong A, Huang H (2006) SERRATE is a novel nuclear regulator in primary microRNA processing in Arabidopsis. Plant J 47:841–850. https://doi.org/10.1111/j.1365-313X.2006.02835.x

Yang SW, Chen HY, Yang J, Machida S, Chua NH, Yuan YA (2010) Structure of Arabidopsis HYPONASTIC LEAVES1 and its molecular implications for miRNA processing. Structure 18:594–605. https://doi.org/10.1016/j.str.2010.02.006

Yang L, Mu X, Liu C, Cai J, Shi K, Zhu W, Yang Q (2015) Overexpression of potato miR482e enhanced plant sensitivity to *Verticillium dahliae* infection: MiR482e is involved in potato Verticillium wilt. J Int Plant Biol 57:1078–1088. https://doi.org/10.1111/jipb.12348

Yogindran S, Rajam MV (2021) Host-derived artificial miRNA-mediated silencing of ecdysone receptor gene provides enhanced resistance to Helicoverpa armigera in tomato. Genomics 113:736–747. https://doi.org/10.1016/j.ygeno.2020.10.004

Yu B, Yang Z, Li J, Minakhina S, Yang M, Padgett RW, Chen X (2005) Methylation as a crucial step in plant microRNA biogenesis. Science 307:932–935. https://doi.org/10.1126/science.1107130

Zaidi SSEA, Mukhtar MS, Mansoor S (2018) Genome editing: targeting susceptibility genes for plant disease resistance. Trends Biotechnol 36:898–906. https://doi.org/10.1016/j.tibtech.2018.04.005

Zekri L, Kuzuoğlu-Öztürk D, Izaurralde E (2013) GW182 proteins cause PABP dissociation from silenced miRNA targets in the absence of deadenylation. EMBO J 32:1052–1065. https://doi.org/10.1038/emboj.2013.44

Zhai J, Jeong DH, De Paoli E, Park S, Rosen BD, Li Y, Meyers BC (2011) MicroRNAs as master regulators of the plant NB-LRR defense gene family via the production of phased, trans-acting siRNAs. Genes Dev 25:2540–2553. https://doi.org/10.1101/gad.177527.111

Zhai J, Zhao Y, Simon SA, Huang S, Petsch K, Arikit S, Meyers BC (2013) Plant microRNAs display differential 3′ truncation and tailing modifications that are ARGONAUTE1 dependent and conserved across species. Plant Cell 25:2417–2428. https://doi.org/10.1105/tpc.113.114603

Zhang BH, Pan XP, Wang QL, Cobb GP, Anderson TA (2005) Identification and characterization of new plant microRNAs using EST analysis. Cell Res 15:336–360. https://doi.org/10.1038/sj.cr.7290302

Zhang J, Xu Y, Huan Q, Chong K (2009) Deep sequencing of Brachypodium small RNAs at the global genome level identifies microRNAs involved in cold stress response. BMC Genomics 10:449. https://doi.org/10.1186/1471-2164-10-449

Zhang LL, Jing XD, Chen W, Wang Y, Lin JH, Zheng L, You MS (2019) Host plant-derived miRNAs potentially modulate the development of a cosmopolitan insect pest, Plutella xylostella. Biomolecules 9:602. https://doi.org/10.3390/biom9100602

Zhang Y, Massel K, Godwin ID et al (2018) Applications and potential of genome editing in crop improvement. Genome Biol 19:210

Zhang P, van Leeuwen CHA, Bogers D, Poelman M, Xu J, Bakker ES (2020) Ectothermic omnivores increase herbivory in response to rising temperature. Oikos 129:1028–1039. https://doi.org/10.1111/oik.07082

Zhao B, Liang R, Ge L, Li W, Xiao H, Lin H, Jin Y (2007a) Identification of drought-induced microRNAs in rice. Biochem Biophys Res Commun 354:585–590. https://doi.org/10.1016/j.bbrc.2007.01.022

Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Srivastava D (2007b) Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. Cell 129:303–317. https://doi.org/10.1016/j.cell.2007.03.030

Zhao B, Ge L, Liang R, Li W, Ruan K, Lin H, Jin Y (2009) Members of miR-169 family are induced by high salinity and transiently inhibit the NF-YA transcription factor. BMC Mol Biol 10:29. https://doi.org/10.1186/1471-2199-10-29

Zhao F, Zhang D, Zhao Y, Wang W, Yang H, Tai F, Hu X (2016) The difference of physiological and proteomic changes in maize leaves adaptation to drought, heat, and combined both stresses. Front Plant Sci 7:1471. https://doi.org/10.3389/fpls.2016.01471

Zhou M, Luo H (2013) MicroRNA-mediated gene regulation: potential applications for plant genetic engineering. Plant Mol Biol 83:59–75. https://doi.org/10.1007/s11103-013-0089-1

Zhou X, Wang G, Zhang W (2007) UV-B responsive microRNA genes in Arabidopsis thaliana. Mol Syst Biol 3:103. https://doi.org/10.1038/msb4100143

Zhou X, Wang G, Sutoh K, Zhu JK, Zhang W (2008) Identification of cold-inducible microRNAs in plants by transcriptome analysis. Biochim Biophys Acta 1779:780–788. https://doi.org/10.1016/j.bbagrm.2008.04.005

Zhou L, Liu Y, Liu Z, Kong D, Duan M, Luo L (2010) Genome-wide identification and analysis of drought-responsive microRNAs in Oryza sativa. J Exp Bot 61:4157–4168. https://doi.org/10.1093/jxb/erq237

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273. https://doi.org/10.1146/annurev.arplant.53.091401.143329

Zubair M, Khan MZ, Rauf I, Raza A, Shah AH, Hassan I, Amin I, Mansoor S (2020) Artificial micro RNA (amiRNA)-mediated resistance against whitefly (*Bemisia tabaci*) targeting three genes. Crop Prot 137:105308. https://doi.org/10.1016/j.cropro.2020.105308

Chapter 15
Challenges in Molecular Insect Resistance Studies for Crop Improvement

Amarjit S. Tanda and Ravneet Kaur

15.1 Introduction

Crop plants are always exposed to different biotic stresses including insect pests and diseases which suppress their growth and development. Insect herbivory mostly compromises host plant balance and growth, ultimately killing the plant. A number of plants with great economic value are attacked by many insect pests globally, causing big reduction in yields of billions of dollars (Cheng et al. 2013a; Satyabrata et al. 2021). Hypersensitive responses (HR), organized cell killing, tissue augmentation at the site of injury, and expression of defense-responsive genes are related to defensive mechanisms against insect pests (Cheng et al. 2013b). Insect attack results in oxygen burst inside the tissues discharging intermediate signal molecules, for instance, reactive oxygen species (ROS), superoxides (O_2^-), nitric oxide (NO), and hydrogen peroxide (H_2O_2) which in turn produces the defense reaction through activation. Many plant hormones like abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and gibberellins (GA) (Fig. 15.1) govern defense responses and regulate expression of several downstream target genes (Berens et al. 2017). In addition, calcium-reliant protein kinase (CDPK), cyclin-dependent protein kinase (CDK), and mitogen-stimulated protein kinase (MAPK) act as a significant factor of the defense signaling forces (Berens et al. 2017). Furthermore, plant defense reactions against insect herbivory can be more varied and may be engaged constitutively or transiently. Additionally, host plant defense responses against insects show temporal dynamics with some defense reactions being obtained within

A. S. Tanda (✉)
School of Horticulture, University of Western Sydney, Richmond, NSW, Australia

R. Kaur
Department of Zoology, Post Graduate Govt. College for Girls, Chandigarh, India

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_15

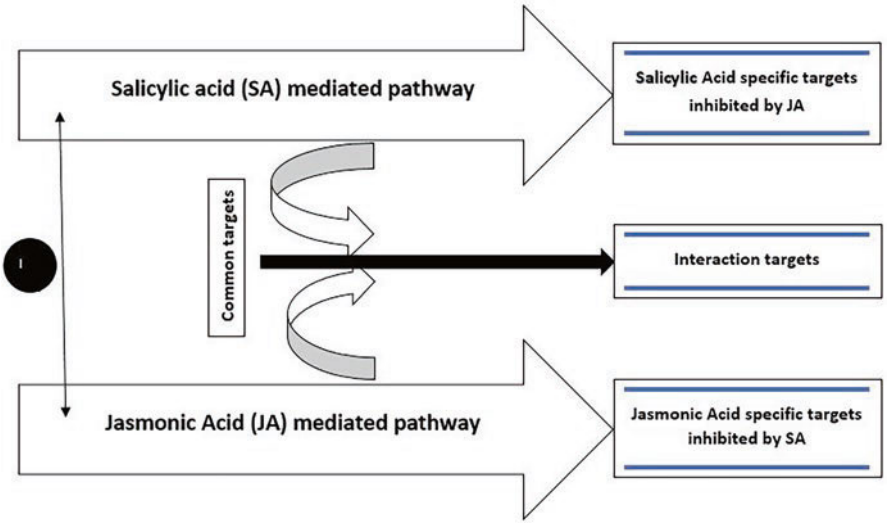

Fig. 15.1 Impact of phytohormones like jasmonic acid and salicylic acid on targets like herbivores

minutes of insect attack while others being exhibited later on (Fürstenberg-Hägg et al. 2013). Few years back, important progress has been made in comprehending insect pest defense methods, insect-resistant gene identification, and solving the molecular procedure of host-insect interaction in crops. All these defense plans used by plants are yet to be distinguished and classified depending on the elicitation nature. In this chapter, we have highlighted several plant defense reactions against insect attack and grouped them as per their defensive mechanism. Moreover, we offer a genetic and molecular mechanism description of insect resistance in detail and the implementation of multiple genomic bio-techniques for further improvement in insect resistance in the newly designed cultivars.

15.2 Host Plant Reactions to Insect Attack

The counter-defense reactions against insect herbivory can be of different nature such as integral, induced, direct, or indirect. Plants have developed multi-layered defense mechanisms to prevent insect damage. This diverse defense structure shows preventive procedures beginning with physical barriers to phyto-metabolites including inducible/adaptive defense structures. Sometimes, when the direct defense via secondary metabolites against the insects fails, plants hide substances that allure the scavengers of the insect herbivory (Erb and Reymond 2019). During this indirect defense, plants harbor the scavengers of pests to decrease the insect attack. A plant can be regarded as resistant, relying on modifying strategies for self-defense. When the physical and chemical responses of a host plant can alarm insects and subside

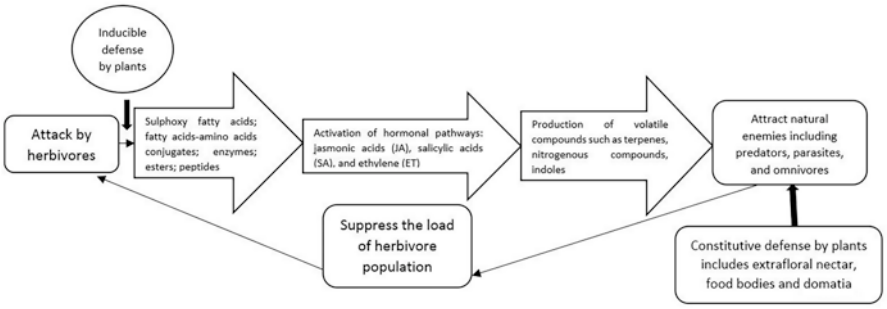

Fig. 15.2 Induced and constitutive defense by plants in response to herbivores attack and their neutralization by natural enemies

the damage caused by herbivory, it is considered to be resistant or tolerant (Fig. 15.2). The resistant crops try to discourage insect development and mostly foist strong selection pressure on the pest. Contrastingly, tolerant crops are helpless to deter the insects but can decrease the harmful effects of insect attack. Tolerant plants apply less selection pressure, and the growth and multiplication of the insects are undisputed. Insect attack, egg laying, and settlement can bring about many plant defense reactions, for example, building up or adapting physical barriers, release of antagonistic secondary substances, discharge of herbivore-induced plant volatiles (HIPVs), HR/ROS generation, defense gesturing, and exhibition of defense-associated genes. In addition, the briefing of plant defense against insects eases fast activation of defense responses (Blenn et al. 2012). Consequently, plants react to insect herbivory by starting any one kind or a cocktail of the procedures to reduce insect attack.

15.3 Insect Attack and Physical Plant Defenses

To prevent the insects of various nourishing guilds, crops have evolved modified structural characteristics, for example, trichomes, waxes, cuticle depositions, and spines, that act as physical barriers against insect attack and egg laying. The epicuticular wax layer inhibits insects to stay, feed, or oviposit on the leaves (Blenn et al. 2012). The wax production in the plant system differs from its natural traits under various insect-induced stresses. For instance, the egg laying of a cabbage white butterfly on *A. thaliana* activates the change in wax make-up by raising the concentration of tetratriacontanoic acid (C34) and decreasing tetracosanoic acid (C24) (Bricchi et al. 2012). This alteration in the wax composition allures the egg parasitoid wasps *Trichogramma brassicae*. Likewise, plants discourage insects by increasing the leaf and root rigidity. The strengthening of plant epidermis prohibits the feeding by herbivores. The roots' rigidity is built up mostly by the lignin polymer accumulation to discourage insect feeding. Additionally, accumulation of silica, suberin, callose, and cellulose culminates in cell wall bracing which limits

herbivore attack. Crops also encourage extensive root regrowth under insect attack which encourages root density and number of roots. Adaptation of leaves to thorns and spines and trichomes also limit the crop from herbivory. Although thorns and spines prevent the bigger insects from attacking the plants, the trichomes stop the insect mobility and connection1. Glandular and non-glandular trichomes help in pest management, by reducing the plant taste and insect movement. The glandular trichomes in *N. attenuate* manufacture sufficient amounts of *O*-acyl sugars that indirectly encourage the larvae of *M. sexta* to liberate volatile metabolites, which results in alluring its predators (Cheng et al. 2013b). Likewise, in raspberries, the more leaf trichome density repulses and decreases egg laying by the mite *Tetranychus urticae* (Karley et al. 2016).

15.4 Host Plant Metabolites and Insect Damage

Numerous bioactive substances and secondary metabolites are manufactured by the plants which are antagonistic to herbivory. These metabolites not only minimize the insect attacks but also reduce the extent of insect damage and regulate subsequent plant defense systems. These secondary chemicals mainly help in direct defenses and, however, can also contribute in indirect defenses like dwelling the predators of the specific herbivory (Erb and Reymond 2019). Many plant-synthesized bioactive compounds work as toxins to insect pests and influence their digestive and nervous system, affecting development or death. Moreover, they control the taste, odor, and color of a host plant or its parts (Kessler et al. 2006). Almost in all plants, alkaloids are present and contribute to the defense mechanism against herbivory. Aphid attacks were limited in *Festuca arundinacea* due to the presence of pyrrolizidine alkaloids (PA) by the endophytic fungi *Acremonium coenophialum* (Johnson 2011). The PAs get converted to a toxic nature, as they access the alkaline digestive system of insects, empowering PAs as potent anti-feeders which generally debar aphids and other insects (Johnson 2011). Deglycosylation of 2-β-Dglucopyranosyloxy-4,7-dimethoxy-1, and 4-benzoxazin-3-one glucoside (HDMBOA-Glc) procreates HDMBOA, which limits damages by the moths *S. frugiperda* and *S. littoralis* (Glauser et al. 2011). Moreover, the glucosinolate derivatives, for example, the indole glucosinolate in *Arabidopsis*, give out more resistance to *M. persicae* (Erb and Reymond 2019). Plant terpenoids serve as toxins, insect repellents, and anti-feeders as a plant defense system. When these terpenoids are found as volatile compounds, resins and vital components of plant essential oils also influence the herbivory adversely. Several plant peptides and other chemicals assist in the plant defense system by damaging the insect digestion. Lectins, chitinases, and α-amylase inhibitors in plants work as anti-digestive proteins by impeding the digestion of the consumed plant or as anti-nutritive by interfering with the consumption of plant parts by the insects. Proteinase inhibitors (PIs) produced by the plants assist to impair the digestive process in the herbivory. Cysteine proteases and metalloproteinase are major enzymes available in *Hemiptera*, and suppression of these enzymes

in the guts of insects can lead to mortality (Erb and Reymond 2019). Similarly, the plant α-amylase inhibitors (α-AI) chunk the αamylases of attacking insects, limiting their starch catabolism. The α-AIs were observed to hinder the mealworms in wheat, beetles, and wheat weevils from damaging the crop and cereals (Fürstenberg-Hägg et al. 2013). The heterologous expression of bean αamylase inhibitor1 in *Pisum sativum* established resistance against the weevil *Bruchus pisorum* (Morton et al. 2000). In addition, plants synthesize chitinase to neutralize insect attacks as chitin is a main compound in the exoskeleton of insects. The transgenic tomato lines having poplar chitinase found resistance to the beetle, *Leptinotarsa decemlineata*, by restricting their growth (Lawrence and Novak 2006). Likewise, polyphenol oxidase (PPO) enzymes also help in plant defense, regularly on mechanical damage or injury. During insect attack, the plant cell disruption produces PPOs which consecutively release ROS (Mahanil et al. 2008). In tomato, overexpression of PPO resulted in increased resistance to the moth *S. litura* by reducing its growth and enhancing its mortality rate (Mahanil et al. 2008). Numerous plants acquire laticifers and resin ducts which stock latex and resins. These ducts get punctured and the latex is discharged at the site of injury to hinder or to trap the herbivory during an attack. In addition, being sticky to entrap insects, the latex of few plants can also be lethal. The latex of *A. cannabinum* possesses phenolics, alkaloids, terpenoids, and PIs, which work as toxins or anti-feedants when ingested by herbivory (Erb and Reymond 2019).

15.5 Insect Pest Attack and Chemical Defense Mechanisms

Plants manufacture several metabolites, for example, the VOCs, food bodies, and nectars which allure, nurture, and accommodate the insect scavengers, thus encouraging insect's defense. VOCs are synthesized mainly in flowers and roots to entice crop pollinators (Tanda, 1983, 1984, 1985, 2019a, b, c, 2020, 2021a, b, c, d, e, f, g) and insect scavengers. About 30 volatile compounds, for example, sesquiterpenes, (*E*)-α-bergamotene, and other aromatic chemicals, were released by the attack of leaf-worm *S. littoralis* in maize (Erb and Reymond 2019). Oddly, the VOCs or HIPVs also help in intra- and inter-communication in crops and briefing the defense reactions against herbivory. The plants *Artemisia tridentata* and *N. attenuate* were found to share this behavior, where attacked *A. tridentata* plants produced VOCs provided chemical defense in *N. attenuate* (Kessler et al. 2006). Likewise, exposure of volatiles in the not-harmed leaves produced from injured leaves showed elevated defensive reactions against the moth *Lymantria dispar* (Maffei et al. 2012). Plants release food bodies (FBs) rich in nutrients to begin a mutualistic relation with other organisms which result in prevention against herbivory. The connections between *Piper fimbriatum* and *Pheidole bicornis* ants are mutualistic as the ants prevent the plant from many insects while nourishing on its FBs (Fischer et al. 2002). Several plants release nectars to allure pollinators, predators of insects and pests, and parasitoids which help greatly in indirect defense (Tanda, 2019a, b, c, 2020, 2021a, b, c,

d, e, f, g; Erb and Reymond 2019). Nevertheless, nectars are manufactured by flowers; EFNs are released and put down on shoots and leaves of plants. The production and secretion of these EFNs enhance when the plant is attacked by herbivory. Gall formation was inhibited on excised roots of okra by co-culturing with sesame. Sesame callus reduced penetration, discouraged nematode build-up in okra, and caused an increase in numbers of males showing antagonism of sesame to root-knot nematode on okra (Tanda and Atwal 1988; Tanda et al. 1988, 1989).

15.6 Intrinsic Physiological Processes and Response to Herbivory

Apace with structural and chemical defense mechanisms, crop plants depend on several intrinsic physiological procedures like identification of insect effectors, ion flux gradients across the plasma membrane, Ca^{2+} burst, ROS generation, or oxidative burst and gesturing cascades for acumen and reaction to insects. This signaling afterwards influences the deposition of plant hormones, defense genes expression, biosynthesis of phytohormone genes, and plant hormone-controlled genes (Blenn et al. 2012). The acumen of the herbivory attack and stimuli is mostly at the site of the injury but can disperse to adjoining cells and start systemic defense reactions. The insects produce oral secretions (OS) or elicitors frequently into the plant system. To discern these secretions, many specific receptors are found on the cell membrane (Maffei et al. 2012). The signals produced by insects include change of the plasma membrane, ionic influxes or effluxes, and oxidative or Ca^{2+} ruptures. Many insect OS consists of fatty acid-amino acid conjugates (FACs) which work as potential elicitors for defense mechanisms. Feeding on leaves and OS by cotton leaf worm in lima beans quickly produced the depolarization of plasma membrane potential, thus starting defense reactions against the herbivory (Bricchi et al. 2012). Mousavi et al. (2013) described the role of change of membrane potentials and specific membrane proteins like glutamate receptor-like (GLRs) proteins in regulating the JA-induced gene expressions and signaling of wounds. The OS and FACs successively stimulate the kinase signaling cascades that act in regulating defense against herbivory. For example, in case of *N. attenuata*, the exogenous action of *Manduca sexta* obtained FACs to the injured cells induced MAPKs, wound-activated protein kinase (WIPK), salicylic acid-induced protein kinase (SIPK) and led in the deposition of plant hormones such as JA, SA and ET (Wu et al. 2007). Likewise, the attack of brown plant hoppers in rice activated the expression of multiple OsMPKs (Nanda et al. 2018). Oxidative production or burst of ROS is another quick response of plants against herbivory. ROS contribute in modulating anti-insect plant defense via redox potential-based signaling (Erb and Reymond 2019). The part of ROS in insect defense has been well established in *N. attenuata* (Wu et al. 2013). Injury of the *N. attenuata* plants caused production of NaRBOHD, a member of the respiratory rupture oxidase homolog (RBOH) family. Additionally, the treatment of OS from *M. sexta* led to elevated transcription of NaRBOHD. The ROS induction even after the OS treatment

was significantly reduced in the NaRBOHD-silenced plants, and they were more susceptible to insect pests. The plant oxidases such as RBOHs have the capacity to combine with Ca^{2+} and MAPK signaling alongside ROS generation, showing its nexus in insect-induced defense reactions. Amalgamation of ROS release and Ca^{2+} signaling has been established already in *Arabidopsis* where the combining of Ca^{2+} synergistically stimulates RBOH12. ROS-mediated defense mechanism against phloem and sap-sucking herbivory is often registered through the collection of H_2O_2 and increased task of peroxidases (POD), superoxide dismutases (SOD), and catalases (CAT) in a number of crops (Kerchev et al. 2012). In many ion species, Ca^{2+} ion contribute to the change of cell membrane potential and signal alteration during insect attack. The concentration of Ca^{2+} ions in the cytoplasm and in the apoplast remains in the nanomolar and micromolar range, respectively, under normal conditions (Dodd et al. 2010). The Ca^{2+} homeostasis gets disrupted, and a gush of Ca^{2+} ions runs into the cytosols during insect injury. This change of the ionic concentrations by the Ca^{2+} burst starts downstream signaling cascades for defense reactions (Drerup et al. 2013). The Ca^{2+} signals are mostly observed by calcium sensor proteins, for example, calmodulin (CaM) and CDPKs, which further connect with downstream targets to spread the acquired signal to the nucleus (Du et al. 2011). The Signal responsive1 (AtSR1) transcription factor protein to CaM activates insect resistance in *Arabidopsis thaliana*, while the atSR1 mutants are susceptible to insects (Laluk et al. 2012). The defense feedbacks against aphid attack in *Arabidopsis* (vs. *Myzus persicae*) and wheat (vs. *Diuraphis noxia*) were observed to be controlled by the expression of CaM-binding proteins (Smith and Boyko 2007). Similarly, AtCPK3 and AtCPK13 regulated the CPK-mediated Ca^{2+} signaling, modulating the defense responses against *S. littoralis* (Kanchiswamy et al. 2010). Phytohormones contribute to the fine-tuning of plant defense mechanisms. JA works as a main participant in regulating defenses against herbivory by playing as direct and indirect defenses (Yang et al. 2019). Insect injury of leaves causes the rise of intracellular concentration and collection of JA in the plant tissues. Plant defense reactions activated by JA accumulation may be like the formation of trichomes to the liberation of volatile organic compounds (VOCs), production of extra foliar nectars (EFNs), secretion of secondary substances, and expression of JA-responsive genes (Wasternack and Hause 2013). In addition, the SA pathway is omnipresent in vascular plants and plays an important part in quick adaptation to insect infestation. SA regulates the defense reaction against the bollworm *Helicoverpa armigera* in tomatoes by producing ROS (Peng et al. 2004). SA-activated H_2O_2 collection also stops insect feeding as higher concentration of H_2O_2 adversely influences the insect digestion and growth (Maffei et al. 2007). Likewise, ET signaling works with JA and/or SA to induce or suppress defense responses to herbivory. For example, ethylene biosynthesis helped the development of fall armyworm *Spodoptera frugiperda* in maize (Harfouche et al. 2006). On the other hand, the deterioration of ethylene signaling aiding poor aphid development in tomato and more resistance to *S. littoralis* in *Arabidopsis* (Mantelin et al. 2009). In addition, ET is answerable for the activated emission of several VOCs in plant-insect interplay as observed in the European alder plants, lima beans and maize (Erb and Reymond 2019).

15.7 Insect Resistance and Molecular Strategies in Field Crops

Insect resistance in crops mostly includes two main defense mechanisms. On the other hand, constitutive defenses save crops from insect herbivory by setting up various physical barriers and chemical metabolites, activated defense ease perception of insect elicitors or effector molecules inducing the secretion of specific chemicals, induction of downstream signaling modules, and genetic rearranging of transcriptional methods (Du et al. 2020). Numerous insect resistance genes in crops convert plasma membrane-localized/intracellular-localized receptors implying that activated defense is important to crop resistance against herbivory (Du et al. 2020). Namely, plant defense response against insect injury shows a high similarity to that against the disease. Many important investigations have made it possible to comprehend the perceptions of the molecular mechanism of resistance to insect pests in crops in the last decade. Plant resistance to many insects and diseases is mostly elucidated by a zig-zag model (Jones and Dangl 2006). Nevertheless, such a system is not completely accepted for the host plant-insect interplay and the degree up to which it is applicable for the same, is yet unrevealed.

15.8 Molecular Patterns and Herbivory

Insect attack mostly induces the secretion of conserved molecules called as herbivore-associated molecular patterns (HAMPs) or damage-associated molecular patterns (DAMPs), which are resembling the pathogen-associated molecular patterns (PAMPs). Such molecules can be identified by the pattern recognition receptor (PRRs) in crops and trigger the PTI (PRR-triggered immunity), which is identical to that of the PAMP-activated resistance (Cheng et al. 2013b). HAMPs comprise secretory proteins from insect pest saliva, oral secretions (OS), and egg laying fluid containing fatty acid-amino acid conjugates (FACs), volicitin, alkaline phosphatase carliferins, bruchins, and glucose oxidase that activate defense reaction via JA signaling pathway (Erb and Reymond 2019). In addition, the DAMPs produced from the injured cells on insect attack contain oligogalacturonides, cutin monomers, and endogenous peptides comprising systemin, VOC, HypSys, and RALF. Alongside HAMPs and DAMPs, insect-released effectors, for example, endo-β-1,4-glucanase N1EG1 from brown plant hoppers (BPH) or HARP1 from cotton ballworm, can subdue or surpass the PTI to activate the effector-triggered susceptibility (ETS) (Malik et al. 2020).

Opposite to this, plant induces specific receptors or resistance (R) proteins that can identify these insect effector molecules, thus stimulating the effector-triggered immunity (ETI). In rice, Bph (Du et al. 2011) converts a NB–LRR protein that serves as a specific receptor for the effectors from BPH in rice-BPH interplay

(Du et al. 2020). Especially, when the PTI found the response to herbivory in a plant comprises cell wall callose deposition (structural), induction of ROS signaling (chemical) and causing signaling cascades (MAPK), ETI includes a more like gene-for-gene interaction for defense reaction via the stimulation of specific genes or transcription elements (Du et al. 2020). Depending upon the research on transcriptome and proteome dynamics, many genes found in insect tolerance have been cloned and distinguished in myriads of crop species. Although these few genes show a clear gene-for-gene association with the insect effectors, others do not advance by this theory. For example, NB-LRR class R-gene Mi-*1.2* from tomato and *vat* from melon convert protein that directly bestows immunity to *Macrosiphum euphorbiae* and *Aphis gossypii*, respectively (Rossi et al. 1998; Villada et al. 2009).

On the other hand, a rice long-chain-based gene *OsLCB1*a aided defense against insect feeding not by directly interplaying with the elicitor but by enhancing the concentration of the defense protein across the cell membranes (Begum et al. 2016). Nonetheless, plants do acquire many genes that contribute in the plant-insect interactions and modulate plant defenses disregarding their connection with the insect effectors. Three lectin receptor kinases (*OsLecRK1*, *OsLecRK2*, and *OsLecRK3*) and multiple *OsMPKs* were found to be engaged in rice resistance against BPH attack (Liu et al. 2015). Likewise, *LecRK1* in *N. attenuata* works as an important player in defense against *M. sexta* by stopping the collection of SA and raised concentration of nicotine, diterpeneglucosides, and trypsin protease inhibitors (Gilardoni et al. 2011).

A leucine-rich repeat receptor-like kinase, *OsLRR-RLK1*, in rice was observed to start defense responses against the *Chilo suppressalis* (Hu et al. 2018). While the transcription of *OsLRR-RLK1* was highly down-regulated by the insect injury, gene silencing revealed lessened resistance to *C. suppressalis*. Additionally, the MAPK cascade works downstream to *OsLRR-RLK1* and is positively controlled by *OsLRR-RLK1* regulating the expressions of MAPK and WRKY transcription factors (Hu et al. 2018). Similarly, the recognition of specific effectors in herbivory and their utilization through various functional genomic methods demonstrated new intuitions in plant-insect interplays. In earlier reports, the transcriptome analysis of the salivary glands of the pea aphid *Acyrthosiphon pisum* has led to the identification of C002, a key effector of insect attack (Mutti et al. 2008). The silencing of C002 resulted in enhanced aphid mortality as the aphids were unable to access the plant sieve tube components. Intriguingly, when the C002 ortholog MpC002 from green peach aphid was upregulated in transgenic *Arabidopsis* plants, it encouraged aphid severity establishing its role in plant defenses. Also, overexpression of candidate aphid effectors Me10 and Me23 also helped in the increased aphid virulence in *N. benthamiana* (Atamian et al. 2013). Insect attack produces jasmonic acid resulting in important transcriptional reprogramming, proposing the participation of multiple transcription factors (TFs) in activating herbivory resistance (Du et al. 2020).

15.8.1 Transcription Factors (TF'S)

It is demonstrated that combining jasmonate-isoleucine (JA-Ile) to coronatine-insensitive 1 (COI1) leads to the degradation of JAZ proteins and induction of the basic helix loop helix (bHLH) TF MYC2 during jasmonate signaling (Pauwels and Goossens 2011). The latest research has shown new insights into the role of various TFs in transcriptional reprogramming during JA signaling. MYC2 along with MYC3 and MYC4 has an extra defense against insect attack (Fernández-Calvo et al. 2011). Schweizer et al. (2013) established a systemic transcriptome profiling to illustrate the resistant result of nine TFs counting WRKYs, NACs, and ERFs in resistance to *S. littoralis*. Nevertheless, in comparison to myc234 triple mutant, the knockout lines of these TFs were moderately sensitive to *S. littoralis*, *showing* that MYC2, MYC3, and MYC4 are the main controllers of resistance to insects in *Arabidopsis*. On the contrary, in rice, the WRKY TFs were reported to be mainly responsible for generalist insect resistance. *Os*WRKY89 established increased WBPH resistance via more accumulation of leaf waxes, culm lignification, and SA deposition (Wang et al. 2007). *Os*WRKY70 observed increased resistance to striped stem borer with control of JA synthesis and susceptibility to BPH via negative management of gibberellic acid (GA) (Li et al. 2015a). Likewise, *Os*WRKY45 eased BPH resistance through enhanced accumulation of H_2O_2 and in rice ET29 and *Os*WRKY53 established SSB resistance through negative control of OsMPK3/6 signaling (Hu et al. 2016). In rice, *Os*bHLH61 and *Os*bLHL96 found great defense-responsive genes resulting in resistance to BPH (Wang et al. 2018). These investigations demonstrated the participation of multiple novel TFs in host plant defense mechanisms against insect infestations. RNA interference or the antisense arbitrated homologous gene silencing using double-stranded RNA (dsRNA) intermediates is an important reverse genetic technology that has been manipulated to comprehend the working of genes and bio-control of major crop insect pests (Zhang et al. 2017). dsRNA addressing important genes in herbivory have been instituted into crops which when consumed by the insects cause poor development or kill of the insect (Zhang et al. 2017). Since the first proof of concept study toward the usage of RNAi towards growth retardation and death of the Western corn rootworm (WCR) *Diabrotica virgifera virgifera*, the technology has been effectively employed towards development of resistance against multiple Coleopteran and Lepidopteran insects. Li et al. (2015b) have observed that BPH or Asian corn borers fed with rice or maize treated with a solution having dsCes (carboxylesterase gene) or dsKTI (Kunitz-type trypsin inhibitors gene) established significant decrease in their survival. Likewise, in rice, the expression of dsNlMLP (mucin-like protein gene) prevented it from BPH attack due to impairment of salivary sheath and decreased survival rate of insects when nourished on its plant parts (Shangguan et al. 2018).

An RNAi-based insecticide named SmartStax Pro has been manufactured by Monsanto and Dow Agrosciences in a new development. In maize, possessing a protectant utilizing a pyramided process engaging multiple Bt proteins and dsRNA targeting the WCR *Snf7* gene provided a good management of *Diabrotica virgifera*

virgifera (Head et al. 2017). In maize, possessing a protectant utilizing a pyramided process engaging multiple Bt proteins and dsRNA targeting the WCR *Snf7* gene provided a good management of *Diabrotica virgifera virgifera* (Head et al. 2017).

15.8.2 Interference RNA (RNAi)

Though the conveyance of RNAi in transgenic plants is sure, it is also anticipated that the RNA-built products are involved in a non-transformative way to prevent the controlling matter connected with GM goods. A report described the exogenous application of siRNA molecules against the diamondback moth *Plutella xylostella*.

15.8.3 siRNAs

Brassica spp. leaves treated with siRNAs targeting the acetylcholine esterase genes AchE2 of *Plutella xylostella* resulted in higher than 60% of the larval feeding (Gong et al. 2013). Likewise, the foliar spray of naked dsRNA aiming at the actin gene led to notable management of the Colorado potato beetle, *Leptinotarsa decemlineata* (San Miguel and Scott 2016). These investigations reveal that RNAi-based gene silencing is a practicable and an effective method to switch off important genes in plant protection against herbivory. The microRNAs (miRNAs), the endogenous small RNAs that negatively control gene expression, are incriminated in multiple biological procedures such as plant growth, development, and defense reactions to environmental pressures (Khraiwesh et al. 2012).

15.8.4 miRNAs

Similarly, siRNAs and miRNAs have also been related in insect-associated responses in crops. The fecundity of aphids was extremely oppressed in *Arabidopsis thaliana* lines mutated with DCL1 and ARGONAUTE1 (AGO1), the two key enzymes engaged in miRNA conversion (Kettles et al. 2013). More studies also show that miRNAs operate as the controlling modulators of herbivory tolerance in major field crops. About 32 resistant line-specific miRNAs were established via high-throughput sequencing of *Solanum lycopersicon* post attack with whitefly, *Bemisia tabaci* (Wang et al. 2018). Resistance-specific miRNAs have been described in response to *Aphis gossypii* aphid infestation in *Cucumis melo* (Sattar et al. 2012). Similarly, more than 150 miRNAs were distinctively identified in response to insect attack in the tea plant, *Camellia sinensis* by the moth *Ectropis oblique* (Jeyaraj et al. 2017). About 104 resistance-specific and 80 basal defense-responsive miRNAs were found post infection with brown plant hopper (BPH) under compatible and noncompatible interplay in rice (Weinhold

and Baldwin 2011). In miRNAs, *Os*miR156 and *Os*miR396 have been identified as basic regulators of BPH resistance in rice crop. *Os*miR156 negatively controls BPH resistance by controlling the JA biosynthetic process (Fürstenberg-Hägg et al. 2013). OsmiR396 enhances rice response to BPH by managing the expression of the *Os*F3H (flavanone 3-hydroxylase), the rate limiting enzyme in the flavonoid biosynthetic routes (Dai et al. 2019). Recently, in rice small RNA profiling line integrated with the BPH-resistant gene, BPH6 found 29 opposite identified and 9 specifically identified miRNAs in early or late infesting stages showing their engagement in BPH6-mediated tolerance to BPH (Tan et al. 2020). These studies establish that plant miRNAs are significant in the resistance response against herbivory and function as a beneficial resource in comprehending the contribution of post-transcriptional silencing elements in host plant-insect defense responses. The modern tool of genome editing technologies (GETs) has revealed new avenues for insect resistance studies in key field crops. GETs are constituted by a number of advanced molecular bio-techniques that empower targeted alteration of genomic loci in a precise and effective way (Zhang et al. 2018).

15.8.5 CRISPR/Cas9

CRISPR/Cas9 is the most facile and revolutionary technology with broad application in crop improvement programs (Zhang et al. 2018). Though many plant species have been improved by this methodology for multiple trait improvements such as resistance to bacterial, viral, and fungal plant diseases, its utilization for insect resistance is being manipulated in recent studies. Important research has been recently carried out inducing BPH and SSB resistance in rice using CRISPR/Cas9-mediated suppression of serotonin (Lu et al. 2018). In rice crop, the cytochrome P450 monooxygenase gene *CYP71A1* incites the act of tryptamine 5-hydroxylase enzyme and catalyzes the transformation of tryptamine to serotonin. A CRISPR/Cas9 mutation of *CYP71A1* gene in rice led to greater SA levels, no serotonin production, and increased resistance to SSB and BPH (Lu et al. 2018). Genome editing biotechnology basically targets editing of susceptible genes that help the herbivory. The gene editing tool has the potential to change susceptible alleles into resistant types averting the requirement of traditional backcross breeding systems for resistance introgression. Recently, GETs are increasingly being studied to design gene drives in herbivores to avoid them from insect attack. However, more investigations are needed to completely use this biotechnology in insect resistance in field crops.

15.9 Challenges and Conclusions

The interactions between host plant-insects are greatly intricate and multi-faceted. The co-evolution of crops and herbivory and their challenging arm races for survival are very interesting. The multi-level defense plans as described are used by plants to

manage herbivory. Important advances have been achieved recently to comprehend the molecular technology of insect resistance in field crops and its use in the resistance breeding projects. The transgenic cultivars with raised callose depositions have been observed to show enhanced resistance against the sucking insects, especially plant hoppers. Overexpression of specific metabolite genes has also caused better insect resistance. An important biotechnological development has been demonstrated in comprehending crop and insect genomes, proteomes, and transcriptomes. The useful genomic procedures and genetic engineering methods have aided the cloning and description of resistance genes, identification of supposed insect effectors, and exploration of signaling routes in plant-insect responses. The genetic program of plant-insect interaction is still insufficient in several crops. For example, as in few plants insect resistant R genes have been cloned, however, their putative effector is unrevealed. In many plant herbivory structures, the effector molecules have been established while the R-genes have not been distinguished. In future more comprehensive and exhaustive research is needed to be carried out in identification of host plant genes and insect-produced effectors to evolve a distinctive regulatory network related with effector-activated signaling-mediated resistance against herbivory. Modern arising biotechnologies, for example, RNAi and CRISPR/Cas9 genome editing are encouraging methods for crop insect pest management. However, many limitations make them impossible to utilize beyond the research laboratories. For instance, greater genetic variations in the natural pest abundance could lead to more varying results for RNAi under field environments. Additionally, high concentration of dsRNA may not be feasibly dispensed into herbivory in spite of its needs for gene silencing as it may result in imbalanced dietary options (Satyabrata et al. 2021). RNAi demands more in-depth research dealing with dsRNA stability and field-applicable efficient experiments to be regarded as effective against insect pest management programs. Similarly, GETs need precise understanding about plant susceptibility elements which would be efficiently mutated for use in insect pest control strategies. Nonetheless, all such bio-tools will be crucial for unravelling the significance of plant R-genes and insect effectors in the transformation of crop resistance to herbivory.

In the field, as several pests are present at the same time, the indiscriminate application of chemicals for pest control is more applicable, economical, and efficient than developing insect-resistant crop cultivars. Thus, insect resistance breeding programs must require the incorporation of broad-spectrum resistance genes to reduce the investment in crop management programs, designing a new technique more suitable for the future crop improvement strategies. Newly developed biotechnologies, for example, CRISPR/Cas9 gene editing to alter insect susceptible alleles to insect resistance alleles, as well as changing the levels of specific secondary metabolites in vivo, encourage the potential to develop field crops that can be repaired. Moreover, these emerging molecular tools will be invaluable for uncovering the contributions of insect effectors and plant target proteins in the regulation of crop immune systems.

References

Atamian HS, Chaudhary R, Cin VD, Bao E, Girke T, Kaloshian I (2013) In planta expression or delivery of potato aphid *Macrosiphum euphorbiae* effectors Me10 and Me23 enhances aphid fecundity. Mol Plant-Microbe Interact 26(1):67–74

Begum MA, Shi XX, Tan Y, Zhou WW, Hannun Y, Obeid L, Mao C, Zhu ZR (2016) Molecular characterization of rice OsLCB2a1 gene and functional analysis of its role in insect resistance. Front Plant Sci 7:1789

Berens ML, Berry HM, Mine A, Argueso CT, Tsuda K (2017) Evolution of hormone signaling networks in plant defense. Annu Rev Phytopathol 55:401–425

Blenn B, Bandoly M, Küffner A, Otte T, Geiselhardt S, Fatouros NE, Hilker M (2012) Insect egg deposition induces indirect defense and epicuticular wax changes in *Arabidopsis thaliana*. J Chem Ecol 38(7):882–892

Bricchi I, Bertea CM, Occhipinti A, Paponov IA, Maffei ME (2012) Dynamics of membrane potential variation and gene expression induced by *Spodoptera littoralis*, *Myzus persicae* and *Pseudomonas syringae* in Arabidopsis. PLoS One 7(10):e46673

Cheng C, Gao X, Feng B, Sheen J, Shan L, He P (2013a) Plant immune response to pathogens differs with changing temperatures. Nat Commun 4:2530

Cheng X, Zhu L, He G (2013b) Towards understanding of molecular interactions between rice and the brown planthopper. Mol Plant 6(3):621–634

Dai Z, Tan J, Zhou C, Yang X, Yang F, Zhang S, Sun S, Miao X, Shi Z (2019) The OsmiR396-OsGRF8-OsF3H-flavonoid pathway mediates resistance to the brown planthopper in rice (*Oryza sativa*). Plant Biotechnol J 17(8):1657–1669

Dodd AN, Kudla J, Sanders D (2010) The language of calcium signaling. Annu Rev Plant Biol 61:593–620

Drerup MM, Schlücking K, Hashimoto K, Manishankar P, Steinhorst L, Kuchitsu K, Kudla J (2013) The Calcineurin B-like calcium sensors CBL1 and CBL9 together with their interacting protein kinase CIPK26 regulate the Arabidopsis NADPH oxidase RBOHF. Mol Plant 6(2):559–569

Du L, Yang T, Puthanveettil SV, Poovaiah BW (2011) Decoding of calcium signal through calmodulin: calmodulin binding proteins in plants. In: Luan S (ed) Coding and decoding of calcium signals in plants, signaling and communication in plants. Springer, Berlin, pp 177–233

Du B, Chen R, Guo J, He G (2020) Current understanding of the genomic, genetic and molecular control of insect resistance in rice. Mol Breed 40:1–24

Erb M, Reymond P (2019) Molecular interactions between plants and insect herbivores. Annu Rev Plant Biol 70:527–557

Fernández-Calvo P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-Ibanez S, Geerinck J, Eeckhout D, Schweizer F, Godoy M, Franco-Zorrilla JM, Pauwels L, Witters E, Puga MI, Paz-Ares J, Goossens A, Reymond P, De Jaeger G, Solano R (2011) The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. Plant Cell 23(2):701–715

Fischer RC, Richter A, Wanek W, Mayer V (2002) Plants feed ants: food bodies of myrmecophytic Piper and their significance for the interaction with *Pheidole bicornis* ants. Oecologia 133(2):186–192

Fürstenberg-Hägg J, Zagrobelny M, Bak S (2013) Plant defense against insect herbivores. Int J Mol Sci 14(5):10242–10297

Gilardoni PA, Hettenhausen C, Baldwin IT, Bonaventure G (2011) *Nicotiana attenuata* LECTIN RECEPTOR KINASE1 suppresses the insect-mediated inhibition of induced defense responses during Manduca sexta herbivory. Plant Cell 23(9):3512–3532

Glauser G, Marti G, Villard N, Doyen GA, Wolfender JL, Turlings TC, Erb M (2011) Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. Plant J 68(5):901–911

Gong L, Chen Y, Hu Z, Hu M (2013) Testing insecticidal activity of novel chemically synthesized siRNA against *Plutella xylostella* under laboratory and field conditions. PLoS One 8(5):1–8

Harfouche AL, Shivaji R, Stocker R, Williams PY, Luthe DS (2006) Ethylene signaling mediates a maize defense response to insect herbivory. Mol Plant-Microbe Interact 19(2):189–199

Head GP, Carroll MW, Evans SP, Rule DM, Willse AR, Clark TL, Storer NP, Flannagan RD, Samuel LW, Meinke LJ (2017) Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. Pest Manag Sci 73(9):1883–1899

Hu L, Ye M, Li R, Lou Y (2016) OsWRKY53, a versatile switch in regulating herbivore-induced defense responses in rice. Plant Signal Behav 11(4):e1169357

Hu L, Ye M, Kuai P, Ye M, Erb M, Lou Y (2018) OsLRRRLK1, an early responsive leucine-rich repeat receptor-like kinase, initiates rice defense responses against a chewing herbivore. New Phytol 219(3):1097–1111

Jeyaraj A, Liu S, Zhang X, Zhang R, Shangguan M, Wei C (2017) Genome-wide identification of microRNAs responsive to Ectropis oblique feeding in tea plant (*Camellia sinensis* L.). Sci Rep 7(1):13634

Johnson MTJ (2011) Evolutionary ecology of plant defences against herbivores. Funct Ecol 25(2):305–311

Jones JD, Dangl JL (2006) The plant immune system. Nature 444(7117):323–329

Kanchiswamy CN, Takahashi H, Quadro S, Maffei ME, Bossi S, Bertea C, Zebelo SA, Muroi A, Ishihama N, Yoshioka H, Boland W, Takabayashi J, Endo Y, Sawasaki T, Arimura G (2010) Regulation of Arabidopsis defense responses against *Spodoptera littoralis* by CPK-mediated calcium signaling. BMC Plant Biol 10:97

Karley AJ, Mitchell C, Brookes C, McNicol J, O'Neill T, Roberts H, Graham J, Johnson S (2016) Exploiting physical defence traits for crop protection: leaf trichomes of *Rubus idaeus* have deterrent effects on spider mites but not aphids. Ann Appl Biol 168:159–172

Kerchev PI, Fenton B, Foyer CH, Hancock RD (2012) Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signaling pathways. Plant Cell Environ 35(2):441–453

Kessler A, Halitschke R, Diezel C, Baldwin IT (2006) Priming of plant defense responses in nature by airborne signaling between *Artemisia tridentata* and *Nicotiana attenuate*. Oecologia 148(2):280–292

Kettles GJ, Drurey C, Schoonbeek HJ, Maule AJ, Hogenhout SA (2013) Resistance of Arabidopsis thaliana to the green peach aphid, *Myzus persicae*, involves camlexin and is regulated by microRNAs. New Phytol 198(4):1178–1190

Khraiwesh B, Zhu JK, Zhu J (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta 1819(2):137–148

Laluk K, Prasad KV, Savchenko T, Celesnik H, Dehesh K, Levy M, Mitchell-Olds T, Reddy AS (2012) The calmodulinbinding transcription factor SIGNAL RESPONSIVE1 is a novel regulator of glucosinolate metabolism and herbivory tolerance in Arabidopsis. Plant Cell Physiol 53(12):2008–2015

Lawrence SD, Novak NG (2006) Expression of poplar chitinase in tomato leads to inhibition of development in Colorado potato beetle. Biotechnol Lett 28(8):593–599

Li R, Zhang J, Li J, Zhou G, Wang Q, Bian W, Erb M, Lou Y (2015a) Prioritizing plant defence over growth through WRKY regulation facilitates infestation by non-target herbivores. elife 4:e04805

Li H, Guan R, Guo H, Miao X (2015b) New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests. Plant Cell Environ 38(11):2277–2285

Liu Y, Wu H, Chen H, Liu Y, He J, Kang H, Sun Z, Pan G, Wang Q, Hu J, Zhou F, Zhou K, Zheng X, Ren Y, Chen L, Wang Y, Zhao Z, Lin Q, Wu F, Zhang X, Guo X, Cheng X, Jiang L, Wu C, Wang H, Wan J (2015) A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. Nat Biotechnol 33(3):301–305

Lu HP, Luo T, Fu HW, Wang L, Tan YY, Huang JZ, Wang Q, Ye GY, Gatehouse AMR, Lou YG, Shu QY (2018) Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. Nat Plants 4(6):338–344

Maffei ME, Mithöfer A, Boland W (2007) Insects feeding on plants: rapid signals and responses preceding the induction of phytochemical release. Phytochemistry 68(22–24):2946–2959

Maffei ME, Arimura GI, Mithöfer A (2012) Natural elicitors, effectors and modulators of plant responses. Nat Prod Rep 29(11):1288

Mahanil S, Attajarusit J, Stout MJ, Thipyapong P (2008) Overexpression of tomato polyphenol oxidase increases resistance to common cutworm. Plant Sci 174(4):456–466

Malik NA, Kumar IS, Nadarajah K (2020) Elicitor and receptor molecules: orchestrators of plant defense and immunity. Int J Mol Sci 21(3):963

Mantelin S, Bhattarai KK, Kaloshian I (2009) Ethylene contributes to potato aphid susceptibility in a compatible tomato host. New Phytol 183(2):444–456

Morton RL, Schroeder HE, Bateman KS, Chrispeels MJ, Armstrong E, Higgins TJ (2000) Bean alpha-amylase inhibitor 1 in transgenic peas (Pisum sativum) provides complete protection from pea weevil (Bruchus pisorum) under field conditions. Proc Natl Acad Sci U S A 97:3820–3825

Mousavi SA, Chauvin A, Pascaud F, Kellenberger S, Farmer EE (2013) GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signaling. Nature 500(7463):422–426

Mutti NS, Louis J, Pappan LK, Pappan K, Begum K, Chen MS, Park Y, Dittmer N, Marshall J, Reese JC, Reeck GR (2008) A protein from the salivary glands of the pea aphid, Acyrthosiphon pisum, is essential in feeding on a host plant. Proc Natl Acad Sci U S A 105:9965–9969

Nanda S, Wan PJ, Yuan SY, Lai FX, Wang WX, Fu Q (2018) Differential responses of OsMPKs in IR56 rice to two BPH populations of different virulence levels. Int J Mol Sci 19(12):4030

Pauwels L, Goossens A (2011) The JAZ proteins: a crucial interface in the jasmonate signaling cascade. Plant Cell 23(9):3089–3100

Peng J, Deng X, Huang J, Jia S, Miao X, Huang Y (2004) Role of salicylic acid in tomato defense against cotton bollworm, Helicoverpa armigera Hubner. Z Naturforsch C J Biosci 59(12):856–862

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene Mi of tomato confers resistance against the potato aphid. Proc Natl Acad Sci U S A 95:9750–9754

San Miguel K, Scott JG (2016) The next generation of insecticides: dsRNA is stable as a foliar-applied insecticide. Pest Manag Sci 72(4):801–809

Sattar S, Addo-Quaye C, Song Y, Anstead JA, Sunkar R, Thompson GA (2012) Expression of small RNA in Aphis gossypii and its potential role in the resistance interaction with melon. PLoS One 7(11):e48579

Satyabrata N, Mishra R, Joshi RK (2021) Molecular basis of insect resistance in plants: current updates and future prospects. Res J Biotechnol 16(3):194–205

Schweizer F, Bodenhausen N, Lassueur S, Masclaux FG, Reymond P (2013) Differential contribution of transcription factors to Arabidopsis thaliana defense against Spodoptera littoralis. Front Plant Sci 4:13

Shangguan X, Zhang J, Liu B, Zhao Y, Wang H, Wang Z, Guo J, Rao W, Jing S, Guan W, Ma Y, Wu Y, Hu L, Chen R, Du B, Zhu L, Yu D, He G (2018) A mucin-like protein of planthopper is required for feeding and induces immunity response in plants. Plant Physiol 176(1):552–565

Smith CM, Boyko EV (2007) The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomol Exp Appl 122:1–16

Tan J, Wu Y, Guo J, Li H, Zhu L, Chen R, He G, Du B (2020) A combined microRNA and transcriptome analyses illuminates the resistance response of rice against brown planthopper. BMC Genomics 21(1):144

Tanda AS (1983) Assessing the role of honey bees in a field of Asiatic cotton (Gossypium arboreum L.). Am Bee J 123:593–594
Tanda AS (1984) Bee pollination increases yield of 2 interplanted varieties of Asiatic cotton (Gossypium arboretum L.). Am Bee J 124(7):539–540
Tanda AS (1985) Floral biology, pollen dispersal, and foraging behaviour of honeybees in okra (*Abelmoschus esculentum*). J Apic Res 24(4):225–227
Tanda AS (2019a) Entomophilous crops get better fruit quality and yield: an appraisal. Indian J Entomol 81(2):227–234
Tanda AS (2019b) Floral biology, foraging behavior and efficiency of European honey bee (Apis mellifera) in bitter gourd (*Momordica charantia* L.) pollination at Sydney Australia. Bee World. Submitted
Tanda AS (2019c) Entomofaunal effect enhances the quality and quantity in okra (*Abelmoschus esculenlum* (L.)) plantation. Indian J Entomol 81(1):16–17
Tanda AS (2020) Entpollinatology-a strong relationship between plants and insects for crop improvement. 6th Edition of Global conference on plant sciences and molecular biology (GPMB 2020) to be held on 10–12 Sept 2020, at Paris, France. Accepted 26 May 2020
Tanda AS (2021a) Why insect pollinators important in crop improvement? Indian J Entomol. https://doi.org/10.5958/IJE.2021.4
Tanda AS (2021b) Insect pollinators matter in sustainable world food production. Indian J Entomol. Accepted
Tanda AS (2021c) Urbanization and its impact on native pollinators. The 1st international electronic conference on entomology will be held on 1st–15th July 2021 virtually
Tanda AS (2021d) Native bees are important and need immediate conservation measures: a review. The 1st international electronic conference on entomology will be held on 1st–15th July 2021 published in the Proceedings 1 July 2021, 68, x. https://doi.org/10.3390/xxxxx
Tanda AS (2021e) Wild bees and their conservation. Indian J Entomol. Accepted
Tanda AS (2021f) Biofloralphenology, foraging behaviour and entpollinatological effect of honey bees in pomegranate (*Punica granatum*) fruit quality and yield. J Hortic 08:2
Tanda AS (2021g) Insect resistance and host plant relations: a milestone in sustainable crop production. Indian J Entomol. Accepted
Tanda AS, Atwal AS (1988) Effect of sesame intercropping against the root-knot nematode (*Meloidogyne Incognita*) in okra. Nematologica 34(4):484–492
Tanda AS, Atwal AS, Bajaj YPS (1988) Antagonism of sesame to the root-knot nematode (*Meloidogyne Incognita*) on okra in tissue culture. Nematologica 34(1):78–87
Tanda AS, Atwal AS, Bajaj YPS (1989) In vitro inhibition of root-knot nematode *Meloidogyne incognita* by sesame root exudate and its amino acids. Nematologica 35:115–124
Villada ES, González EG, López-Sesé AI, Castiel AF, Gómez-Guillamón ML (2009) Hypersensitive response to Aphis gossypii Glover in melon genotypes carrying the Vat gene. J Exp Bot 60(11):3269–3277
Wang H, Hao J, Chen X, Hao Z, Wang X, Lou Y, Peng Y, Guo Z (2007) Overexpression of rice WRKY89 enhances ultraviolet B tolerance and disease resistance in rice plants. Plant Mol Biol 65(6):799–815
Wang K, Su X, Cui X, Du Y, Zhang S, Gao J (2018) Identification and characterization of microRNA during Bemsia tabaci infestations in Solanum lycopersicum and Solanum habrochaites. Hort Plant J 4(2):62–72
Wasternack C, Hause B (2013) Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. Ann Bot 111(6):1021–1058
Weinhold A, Baldwin IT (2011) Trichome-derived O-acyl sugars are a first meal for caterpillars that tags them for predation. Proc Natl Acad Sci U S A 108:7855–7859
Wu J, Hettenhausen C, Meldau S, Baldwin IT (2007) Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of Nicotiana attenuate. Plant Cell 19(3):1096–1122

Wu Y, Zhang X, Kang X, Li N, Wang R, Hu T, Xiang M, Wang X, Yuan W, Chen A, Meng D, Chen S (2013) Oxidative stress inhibits adhesion and transendothelial migration and induces apoptosis and senescence of induced pluripotent stem cells. Clin Exp Pharmacol Physiol 40(9):626–634

Yang J, Duan G, Li C, Liu L, Han G, Zhang Y, Wang C (2019) The crosstalks between jasmonic acid and other plant hormone signaling highlight the involvement of jasmonic acid as a core component in plant response to biotic and abiotic stresses. Front Plant Sci 10:1349

Zhang J, Khan SA, Heckel DG, Bock R (2017) NextGeneration insect-resistant plants: RNAi-mediated crop protection. Trends Biotechnol 35(9):871–882

Zhang Y, Massel K, Godwin ID, Gao C (2018) Applications and potential of genome editing in crop improvement. Genome Biol 19(1):210

Index

A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1