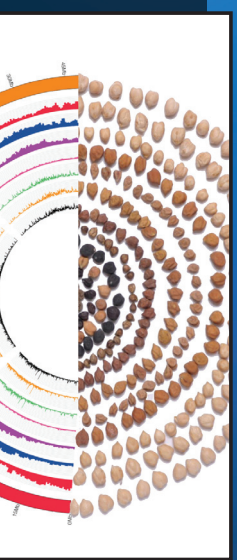
Translational Genomics for Crop Breeding

Volume I: Biotic Stress

Editors: **Rajeev K. Varshney**
Roberto Tuberosa

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Translational Genomics for Crop Breeding, Volume I: Biotic Stress

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Foreword

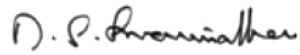
Drs. Rajeev K. Varshney and Roberto Tuberosa have done a great service by bringing out this important book, *Translational Genomics for Crop Breeding, Vol. 1: Biotic Stresses*. This volume deals with the application of genomics in crop breeding for biotic stress tolerance. It will be useful to refer briefly to the transformational role the new genetics based on genomic applications is playing today in improving agriculture, industry, medicine, and environment, following the elucidation of the double-helix structure of the DNA molecule 60 years ago by James Watson, Francis Crick, Maurice Wilkins, and Rosalind Franklin. Their discovery opened up uncommon opportunities for the advancement of science as related to all aspects of life. During recent decades, many Nobel Prizes in Physiology and Medicine have gone to molecular geneticists. At the same time, public concern about the proper measurement of risks and benefits has grown, particularly in the fields of agricultural and food biotechnology. Biotechnology provides an opportunity to convert bioresources into economic wealth. This has to be done in such a manner as to ensure no adverse impact either on the environment or on human and animal health. The bottom line of Indian national agricultural biotechnology policy should be the economic well-being of farm families, food security of the nation, health security of the consumer, protection of the environment, and the security of our national and international trade in farm commodities.

This volume is an epitome of advances in the area of translational genomics application for improving crops with resilience to important components of biotic stress. Integration of high-throughput genotyping with precise phenotyping is the key for dissecting mechanism of complex traits at the molecular level. There are a number of races and biotypes known for a particular disease and insect, and so it is necessary to have a complete knowledge of the causal organism so that race-specific or biotype-specific resistance can be attained. This encourages optimal and target approach to breeding for the trait of interest. Hence, a more holistic approach and, more importantly, a holistic perspective such as that of systems biology is the need of the hour. The chapters in this volume not only provide in-depth review of the problem at hand but also enlighten readers about the advances and possibility of integrating genomics approach in tackling a research problem. In addition, the successful example and success stories discussed are thought provoking to young plant scientists and make them prepare for the challenges ahead.

New approaches for identifying marker-trait association such as genome-wide and candidate gene association studies are gaining fast acceptance due to advantages such as amenability to phenotype at multi-location for multiple traits and genotyping only once, not at each generation. In addition, marker-trait association is validated simultaneously in order to allow the deployment of markers directly in the breeding

program. Another upcoming and promising approach termed as genomic selection is fast gaining importance among the crop specialists. It relies on the genomic-assisted breeding values, rather than phenotypic selection alone, in order to select the lines for crossing and advancing them to next generation. These approaches along with others are covered comprehensively in this book.

I hope this book will be widely read by scientists and scholars, since we must harness the best in the new genetics to overcome the serious threats to human well-being caused by malnutrition, hunger, and disease. The contents of the book show the ways to enhancing productivity in perpetuity without ecological harm. I congratulate and thank Drs. Varshney and Tuberosa for their labor of love in helping harness the best in modern science for enhancing the quality of human life.



Chennai
Date: June 15, 2013

MS Swaminathan
Founder Chairman
M S Swaminathan
Research Foundation

Preface

Recent years have witnessed significant progress in the area of crop genomics mainly due to advances in next-generation sequencing and high-throughput genotyping. Such advances are driving genomics-assisted breeding (GAB), a discipline that has grown tremendously during the past decade, particularly for its applications to improve crop productivity and quality. This quantum leap has been possible through the continuous effort and dedication of those engaged in the translation of the findings of genomics research into improved genotypes and populations. As we anticipate a further reduction in genotyping/sequencing cost, translational genomics is expected to become a more integral part of crop breeding.

Biotic stress is one of the major factors behind crop losses. While a number of reports have been available on genomics approaches such as deciphering marker-trait association either through linkage or association mapping, some success stories have also been reported in recent years on translational aspects of this genomics research in crop breeding. However, the ever-changing and dynamic world of causal organisms of diseases and pests pose serious challenges to crop specialists to identify new resistant alleles and to target disease and pest resistance as well as to accelerate development of superior lines with enhanced resistance to biotic stresses. Therefore, there was an urgent need for a book in which translational genomics activities for resistance to key pests and diseases, success stories completed and in progress, and useful take-home messages from GAB efforts in different crops would be compiled. Along these lines, the 16 chapters of *Translational Genomics for Crop Breeding, Volume 1: Biotic Stresses* include not only details on the aforementioned issues but also address perspectives and challenges in translational genomics for developing superior varieties and lines with enhanced resistance to biotic stresses.

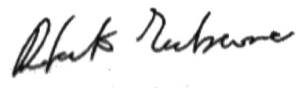
We thank the authors (Appendix I) of different chapters for their commendable effort in summarizing the published and unpublished research and putting all the pieces together in a well-knitted, up-to-date manner, for the benefit of the research challenge in hand. In addition, the cooperation they have extended in terms of timely completion and revision of chapters is greatly appreciated. While editing this book, the strong support received from many other colleagues (Appendix II) willing to review the chapters is equally appreciated. Their constructive comments and suggestions have been instrumental in further improving the contents.

The editors are also grateful to colleagues and staff from their respective laboratories who helped complete the editing of the two volumes in parallel with their demanding responsibilities. In particular, Manish Roorkiwal, B. Manjula, Pawan Khera, and Mahendar Thudi helped RKV with the editorial work. The editors also wish to thank their respective families, as the editorial work for this book took away precious moments they should have spent together with their families. RKV is thankful to his wife Monika for her constant encouragement and

support, and to Prakhar (son) and Preksha (daughter) for their love and cooperation. Similarly, RT is equally thankful to his wife Kay for her support and editorial help. RKV would also like to extend his sincerest thanks to Dr. William D. Dar, Director General, ICRISAT, for his guidance and support in completing this book. The cooperation and help received from Justin Jeffryes, Anna Ehlers, Kelvin Matthews, Erin Topp of Wiley Publishers, and Shikha Sharma of Aptara Corp. during various stages of development and completion of this book are gratefully acknoweldged. RKV would also like to mention that the book was edited during the tenure of RKV as Director, Center of Excellence in Genomics (CEG), ICRISAT, Hyderabad (India), Theme Leader – Comparative and Applied Genomics (CAG), Generation Challenge Programme (GCP) and Adjunct positions at the University of Western Australia, Crops Research Institute of Guangdong Academy of Agricultural Sciences (GAAS), China and BGI-Hongkong Research Institute, China.

We hope that this book will be helpful and useful as a ready guide to students, young researchers, crop specialists, GAB and translational genomics practitioners, and policy makers for developing crops more resilient to biotic stress.

Hyderabad, India (Rajeev K. Varshney)
June 10, 2013



Bologna, Italy (Roberto Tuberosa)
June 11, 2013

Chapter 1

Translational Genomics in Crop Breeding for Biotic Stress Resistance: An Introduction

Rajeev K. Varshney and Roberto Tuberosa

Abstract

Biotic stresses pose a major threat to crop productivity. Crops are challenged by a plethora of biotic stresses, but only a limited number of key pests and diseases cause the vast majority of economic losses in a particular crop. Plant protection measures such as application of pesticides and deployment of resistant gene(s)/quantitative trait loci (QTLs) into cultivars have so far been quite successful in curtailing the losses; however, these measures have also led to the constant evolution of new biotypes/pathotypes/strains/races of pest and disease organisms. Hence, there is a continuous need to identify genomic regions that can impart resistance against these variants. The availability of large-scale genomic resources in many crop species has enhanced our understanding on the path to developing host-plant resistance. As a result, numerous race-specific gene(s) and QTLs have now been identified and cloned with the help of molecular markers. It is quite exciting that these genomic regions are being introgressed into breeding programs of many crops. The objective of this book is to critically review the current availability and utilization of genomic tools for major biotic stresses in important cereals, legumes, vegetables, and tuber and oilseed crop. The book also summarizes the success stories achieved through application of genomics-assisted breeding (GAB), as well as the scope for deployment of modern breeding methods such as marker-assisted backcrossing (MABC) and genomic selection in the era of next-generation sequencing (NGS) technologies, which have the potential to advance the genetic gains for enhancing resilience against biotic stress. This chapter summarizes highlights of different chapters included in the book that is expected to be a resource for young researchers, GAB practitioners, and policy makers for employing better strategies toward achieving food security.

Introduction

Several biotic and abiotic stresses challenge crop productivity. Breeders try to develop superior lines by making crosses and selecting the best lines based on their agronomic performance, but the entire process is expensive and takes several years. During the past two decades, remarkable progress in the area of genomics and molecular genetics has greatly improved our basic

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understanding of resistance to biotic stresses and tolerance of abiotic stresses. Genomics approaches can enhance the precision and efficiency of breeding programs through a better prediction of phenotype from a given genotype – process generally referred to as genomics-assisted breeding (GAB) (Varshney et al. 2005).

Among different GAB approaches, the marker-assisted backcrossing (MABC) approach has been quite successful in transferring the target genomic regions in elite cultivars (Varshney et al. 2012). MABC for gene pyramiding coupled with selection for the genetic background of the recurrent parent and recombination at the target region(s) could lead to faster and better product delivery, thereby increasing productivity and improving livelihoods of the smallholder farmers (Collard et al. 2008).

Biotic stress caused by pests and diseases continues to pose a significant risk to crop productivity in spite of years of investments in research and development aimed at understanding host-plant interaction and finding more effective methods to control it (Lucas 2011). It has been estimated that even after the deployment of pesticides and improved cultivars in the target environment with resistance to biotic stresses, yield losses resulting from pests and diseases can still reach 20-30% (Oerke 2006). This loss may be attributed to the constant and rapid evolution of new virulent pathogens/pests such as Ug99 for wheat stem rust (Levine and D'Antonio 2003), as well as to their spread to new regions in response to climate change and the adoption of different agricultural practices (e.g., minimum tillage).

Abiotic stresses, such as drought, salinity, cold, submergence, mineral toxicity, and others, also hamper growth, yield, and yield quality of crop plants. In fact, these abiotic stresses represent the main cause of crop failure worldwide, reducing average yields for major crops by more than 50%. Overall, as compared to biotic stresses, abiotic stresses pose more serious constraints to crop production, particularly in view of rapidly deteriorating environmental conditions. Quality traits are the other important class of target traits that breeders select for in order to improve crop productivity as well as nutritional quality.

In recent years, large-scale genomic resources have been developed and are being utilized in breeding programs for several crop species (Varshney et al. 2009; Tuberosa et al. 2011). These advances in genomics research have greatly contributed to the conversion of so-called orphan crops to genomic resources-rich crops (Varshney et al. 2009, 2010) and to the enhanced precision and speed of breeding programs. In several cases, GAB has delivered superior lines that have been used for developing new varieties or hybrids (Simpson et al. 2003; Sundaram et al. 2008; Ceballos et al. 2012; Singh et al. 2012). However, introgression of QTLs has not always been successful in crop breeding, and even less so for the improvement of tolerance to abiotic stresses (Collins et al. 2008). Therefore, GAB practices have also offered some lessons to the molecular breeding practitioners.

In view of the above, the two volumes on *Translational Genomics for Crop Breeding* compile a number of manuscripts that report on success stories either completed or still in progress, as well as the lessons learned from GAB work on different crops. Volume I compiles 16 chapters that review the current status and recent advances in the application of GAB approaches for biotic stress resistance. Volume II is a compendium of 13 chapters on GAB for enhancing abiotic stress tolerance and improving crop quality.

This introductory chapter of Volume I provides key highlights of GAB applications to enhance biotic stress tolerance. Since the majority (estimated to be ca. 60-70%) of our major caloric intake is obtained directly or indirectly from cereals, the first five chapters summarize the progress on the improvement of biotic stress tolerance in five major cereals, namely rice, maize, wheat, barley, and sorghum. The contribution of legumes to enhancing nutrition in the daily diet has been largely recognized apart from their well-known ability for nitrogen-fixation. The next five chapters deal with GAB

applications for important biotic stresses in legumes, namely soybean, peanut, common bean, cowpea, and chickpea. Two additional chapters deal with GAB for enhancing the tolerance of potato and tomato to late blight, one of the most devastating diseases of these two important vegetable crops. The three final chapters highlight GAB efforts toward improving disease resistance in lettuce, cassava, and *Brassica* species.

Improving Disease Resistance in Cereals

Bacterial blight (BB), effected by *Xanthomonas oryzae* pv. o*ryzae* (*Xoo*), is a major constraint for rice production, with reported yield losses of up to 50% (Ou 1985). Recently several genes and QTLs have been identified for various virulent strains. Chapter 2 by Kou and Wang provides a comprehensive review of and valuable insights to understanding the interaction between rice and *Xoo* pathogen. This review provides strategies and prior knowledge for effective deployment of resistance genes in target environment against *Xoo* pathogen. Until now, more than 35 BB rice resistance genes have been identified and 7 of these have been isolated. MABC has been quite successful in the case of BB, and various genes such as *Xa4*, *xa5*, *Xa7*, *xa13*, *Xa21*, *Xa23* in single or in pyramided form have been introgressed in popular varieties/parental lines such as, Samba Mahsuri, Pusa Basmati 1, Minghui 63, and have been developed and released in India and China (Gopalakrishnan et al. 2008; Sundaram et al. 2008; Perumalsamy et al. 2010; Huang et al. 2012; Singh et al. 2012).

Chapter 3 by Jamann, Nelson, and Balint-Kurti provides a comprehensive survey of the genetic basis of disease resistance in maize, especially against fungal diseases. In the past, bi-parental linkage mapping was commonly adopted for mapping important genes and QTLs. However, in recent years, modern mapping approaches such as nested association mapping (NAM), which is an effective combination of linkage and linkage-disequilibrium approaches, are becoming increasingly popular (Yu et al. 2008). The chapter reports on the use of the NAM approach to identify genomic regions responsible for three important diseases in maize, namely southern leaf blight, northern leaf blight, and gray leaf spot (Benson et al. 2011; Kump et al. 2011; Poland et al. 2011). In addition, the authors outline the potential of genomic selection to accelerate the breeding efforts for disease resistance, especially in cases where small-effect and environment-sensitive QTLs are involved, as in *Aspergillus* ear rot and aflatoxin accumulation (Warburton et al. 2009). These genetic studies provide an insight into the disease resistance mechanism, thereby helping molecular breeders understand the genes to be used for their deployment in elite cultivars.

In the case of wheat, among several other diseases, Fusarium head blight (FHB) is an age-old and severe one (Leonard and Bushnell 2003). Importantly, contamination caused by fusarium secondary metabolites, known as mycotoxins, poses a major threat to animal and human health (Van Egmond 2004). Extensive QTL studies for FHB resistance have led to the identification of 19 meta-QTLs spread across wheat chromosomes (Buerstmayr et al. 2009; Liu et al. 2009; Löffler et al. 2009). These GAB efforts for FHB have been summarized in Chapter 4 by Hermann Buerstmayr, Maria Buerstmayr, and Schweiger and Steiner. A closely linked codominant marker is always a prerequisite for making any MABC program a success. In particular, *Umn10*, a PCR-based marker linked to a major gene (*Fhb1*) located on the long arm of chromosome 3B and explaining 40-50% of phenotypic variance (Rosyara et al. 2009), is being used routinely in breeding programs of both hexaploid and tetraploid wheat.

In barley, improving virus resistance is one of the top research priorities because it has a serious impact on its production, particularly in Western Europe. Much work has been done in the recent past toward identification of resistance genes for four major viruses affecting barley (Ordon et al.

2009). As a result, molecular markers are now available for fast introgression. In a recent study, improved DH-lines have been developed for Barley Yellow Dwarf Virus through markers (Riedel et al. 2011). Chapter 5 by Ordon and Perovic covers recent advances toward development of genomic tools for transferring virus resistance into elite cultivars via GAB. The authors also highlight the importance and use of allele mining and utilization of high-throughput SNP technologies for carrying out precision breeding activities in barley.

In sorghum, *Striga* is the most damaging obligate parasite pest that leads to yield loss of up to 90% (Ejeta 2007). It is particularly severe in East Africa and some regions in the United States and Asia. Although much progress has been made toward QTL analysis and Marker-assisted selection (MAS) for improving resistance to *Striga*, the molecular mechanisms behind the establishment of parasitism are still not well understood. In Chapter 6, Deshpande, Mohamed, and Hash describe several aspects for elucidating the molecular mechanisms of *Striga* resistance through development of bioassays, exploring the pathway, and identifying the stages as entry points for breeding resistance to *Striga*, as well as GAB approaches to developing sorghum lines with enhanced resistance to *Striga.* The authors also discuss the utility of next-generation sequencing (NGS) technologies for identifying the functional basis of *Striga* resistance.

Improving Disease Resistance in Legumes

Among different legumes, soybean, known for its edible oil and protein content, is an important industry crop. North America and South America are the major production areas, accounting for nearly 86% of total soybean production worldwide (http://www.soystats.com). Cyst, root-knot, and reniform nematode are the major pests of soybean, with annual losses of more than $1 billion (Koenning and Wrather 2010). Chapter 7 by Vuong, Jiao, Shannon, and Nguyen provides a comprehensive review of nematode resistance in soybean. This work highlights the different nematode problems, their biology and candidate genes for host plant response. Notably, the continuous effort toward the identification of genetic markers closely linked to soybean cyst nematode has led to the development and release of three varieties, namely JTN-5503, JTN-5303, and JTN-5109 in the United States, which are essentially gene pyramids of *Rhg1, Rhg4*, and *Rhg5* (Arelli et al. 2006, 2007; Arelli and Young 2009).

Grown in more than 100 countries, peanut is one of the most widespread legume crops in the world (Nwokolo 1996). Chapter 8 by Burow, Leal-Bertioli, Simpson, Ozias-Akins, Chu, Denwar, Chagoya, Starr, Moretzsohn, Pandey, Varshney, Holbrook, and Bertioli describes molecular mapping and MAS for several diseases and pest challenges faced by peanut. As to improving the resistance to root knot nematode, a serious problem in the United States caused by *Meloidogyne* species, the effectiveness of MAS has been demonstrated through the development and release of a nematode-resistant variety 'NemaTAM' in the United States (Simpson et al. 2003). With the availability of more than 6,000 SSR markers, extensive studies have also led to the identification of QTLs with high phenotypic variance for resistance to late leaf spot and rust (Sujay et al. 2012) and tomato spotted wilt virus (Qin et al. 2012). In addition, this chapter presents the prospects and progress of the International Peanut Genome Project toward sequencing the peanut genome, which should help in the identification of candidate genes for stress tolerance and to accelerate GAB in peanut (http://www.peanutbioscience.com/peanutgenomeproject.html).

In common bean, the fungal pathogen *Colletotrichum lindemuthianum* (Sacc. & Magnus) causes a devastating disease known as anthracnose. Several resistance genes against race-specific isolates for anthracnose have been reported in the past. Ferreira, Campa, and Kelly in Chapter 9 report on the inheritance pattern of

the pathogen and the related allelism tests, and discuss GAB approaches for anthracnose resistance. Furthermore, the authors propose a new system of naming anthracnose resistance gene(s) based on the location on the genetic map. Efforts toward marker-assisted introgression in common bean have led to the release of variety 'USPT-ANT-1' with gene *Co-4*^{2} conferring resistance to anthracnose in the United States (Miklas et al. 2003). Recently, line A3308 carrying genes *Co-2* and *Co-3/9* for anthracnose and bean common mosaic (BCM) resistance by genotype *I* + *bc-3* has also been developed (Ferreira et al. 2012).

Cowpea is an important leguminous crop in the tropical and subtropical areas, especially in Latin America, Asia, and Sub-Saharan Africa (Singh et al. 1997). Recent advances in the development of genomic tools in cowpea have enabled the identification of molecular markers for resistance to critical biotic stresses. This notwithstanding, application of modern breeding approaches is still in its infancy. In Chapter 10, Huynh, Ehlers, Close, Cissé, Drabo, Boukar, Lucas, Wanamaker, Pottorf, and Roberts review initial MABC work for various disease resistance and genomic resources available for carrying out GAB in cowpea. The transgenic approach has also been discussed as an option to increase resistance to pod borer and cowpea weevil, as the level of resistance to these pests in the available germplasm is negligible.

Chickpea is another important leguminous crop, mainly grown in Asia and the Mediterranean regions of the world, which is highly nutritious and rich in protein, carbohydrates, and vitamins (Abu-Salem and Abou-Arab 2011). India is the largest producer of chickpea in the world, accounting for more than 65% of global production (FAO 2011). Among important biotic stresses, *Fusarium* wilt and *Ascochyta* blight can cause yield losses of more than 90% (Singh and Reddy 1991, 1996). Efforts to develop genomic resources have led to the identification of molecular markers for agronomic as well as biotic stress, paving the way for GAB activities in this crop (Varshney et al. 2013a). In Chapter 11, Millan, Madrid, Imtiaz, Kharrat, and Chen extensively review disease resistance aspects in chickpea. Furthermore, as genome sequencing of 90 chickpea lines is now available, molecular breeding efforts can now be accelerated to develop tolerant lines for disease resistance (Varshney et al. 2013b).

Improving Disease Resistance in Vegetables

Potato is one of the major staple and vegetable crops, covering more than 100 countries, with an annual production of more than 300 million tons (FAO 2011). *Phytophthora infestans*, which causes late blight, is the main, devastating disease in potato, with an annual yield loss of more than $3 billion (Duncan 1999). Chapter 12 by Śliwka and Zimnoch-Guzowska discusses recent advances in discovering, identifying, mapping, and cloning the resistance genes in potato. This information could be quite useful for the deployment of race-specific resistance in improved lines for target environments.

Tomato is another major vegetable crop for which late blight is a major devastating disease causing vast yield loss. In Chapter 13, Nowicki, Kozik, and Foolad make a special emphasis on late blight resistance in tomato. The chapter provides comprehensive insight into the disease, its chemical control, and GAB aspects. Furthermore, the recently sequenced tomato genome (Tomato Genome Consortium 2012) and *Phytophthora* genome (Haas et al. 2009) provide much-needed understanding of *R-Avr* interaction for late blight. Molecular breeding activities have been quite successful in imparting resilience against late blight, and several varieties such as NC1 CELBR, NC2 CELBR, Mountain Magic, and Mountain Merit have been developed by stacking two genes (*Ph-2* + *Ph-3*) and released in the United States (Gardner and Panthee 2010; Panthee and Gardner 2010).

Lettuce, one of the most commercially important leafy vegetables, has an annual production of more than 23 million tons (FAO 2011).

The crop is grown for a variety of purposes such as salad, stem, and oilseed. The crop is challenged by many biotic stresses leading to huge economic losses. In Chapter 14, Simko reviews recent developments in MAS for resistance to downy mildew, corky root, lettuce mosaic, and lettuce dieback. To achieve these traits, both public and private sectors are routinely utilizing allele-specific assays in their breeding programs. Furthermore, details and current status regarding mapping efforts for other important traits are discussed. Important progress has been made in generating large-scale genomic resources/platforms in lettuce, such as an EST database that includes sequences of more than 700 candidate resistance genes (McHale et al. 2009), microarray chip with more than 6.5 million feature Affymetrix genechip (Stoffel et al. 2012), and complete genome sequencing of cultivated and wild lettuce (https://lgr.genomecenter.ucdavis.edu/; Lavelle et al. 2013), which promises to facilitate faster diagnostics, gene expression analysis, high-throughput genotyping, and cloning of genes.

Improving Disease Resistance in Cassava and *Brassica*

In addition to the aforementioned cereal, legume, and vegetable crops, Volume I includes GAB activities in cassava and *Brassica*, two other important crops for human diet. Cassava, a starchy root crop, is a major food source for more than 800 million people in Sub-Saharan Africa, Asia, and South America. It is cultivated on more than 20 million hectares, with an annual production of more than 240 million tons (FAO 2011). Cassava suffers from several biotic stresses and is highly vulnerable to viral diseases. Cassava mosaic disease (CMD), caused by cassava mosaic Gemini virus, is one of the major viral diseases of cassava, causing reported yield loss of up to 40% (Taylor et al. 2004). Much success has been achieved in identification of molecular markers for CMD, and MAS for this trait is currently being employed in several popular cultivars of Africa and India. The release in 2010 of cassava cultivar CR41-10 in Nigeria, made possible through the activities of the CGIAR Generation Challenge Program (GCP), is the first example of MAS-derived product in cassava (Ceballos et al. 2012). In Chapter 15, Okogbenin, Moreno, Tomkins, Fauquet, Mkamilo, and Fregene present an informative and critical review of GAB activities in cassava.

The agricultural and horticultural uses of the *Brassica* genus contribute an important part to the human diet and to the global economy. Like with all other crops, a plethora of pests and diseases curtail the yield in *Brassica*. In Chapter 16, Li and McVetty review the recent progress on the genetics and gene mapping for disease resistance in *Brassica* species. Tangible progress has been achieved toward GAB for resistance to blackleg and clubroot. However, the development of MAS of sclerotinia stem rot has seen slower progress, mostly because germplasm accessions with high levels of resistance have yet to be identified.

Summary and Outlook

In summary, this volume presents recent advances, useful insights, and comprehensive reviews for GAB approaches to improve biotic stress tolerance in a range of crops. Although the potential for utilization of GAB in crop improvement programs appears almost endless, its application varies greatly among different crop species, reflecting to a certain extent the state-of-the-art genomics of each single species and their economic importance. In crops such as rice, maize, wheat, and barley, MAS and MABC is already well integrated in breeding programs, whereas in many others, the deployment of molecular breeding activities is under way. Notably, GAB for several traits has recently been initiated in orphan crops.

Thanks to the advent of NGS, it has become possible to generate reference genome sequence data of the main crops and also to (re)sequence several varieties/lines. In parallel, modern genetic mapping approaches such as

genome-wide association studies (GWAS; Rafalski 2010; Hamblin et al. 2011) and nested association mapping (NAM; Yu et al. 2008; McMullen et al. 2009) for trait mapping and modern breeding methodologies like marker-assisted recurrent selection (MARS) (Charmet et al. 1999) and GS (Heffner et al. 2009; Jannink et al. 2010) are being increasingly adopted in several crop species. In addition, molecular breeding decision support tools such as an integrated system for marker-assisted breeding (ISMAB) (https://www.integrated breeding.net/ib-tools/breeding-decision/ismab), OptiMAS (http://moulon.inra.fr/optimas/index .html), GS modules (Pérez-Rodríguez et al. 2012; de Los Campos et al. 2013), and platforms like Integrated Breeding Platform (IBP) (https://www.integratedbreeding.net/) are being developed. These advances are expected to accelerate GAB for a range of traits, including biotic stress resistance in crop breeding.

As mentioned earlier, Volume II of this series documents the application of genomics for abiotic stress tolerance and quality traits in several crops. Therefore, together with Volume II, this volume provides an informative and critical update of genomics applications in crop breeding. We hope these chapters will allow young researchers, including graduate students and postdoctoral scholars, to better appreciate GAB and encourage them to devote their career to this exciting area of crop improvement. Additionally, we hope that GAB practitioners as well as policy makers will find these volumes useful for developing the road map toward a more effective improvement of target crops in their respective geographical areas.

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Chapter 2

Bacterial Blight Resistance in Rice

Yanjun Kou and Shiping Wang

Abstract

Rice is one of the most important cultivated food crops. Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the major constraints for sustainable production of rice. Researchers have made tremendous progress in trying to elucidate the interaction between rice and *Xoo*. The genomes of three *Xoo* strains have been sequenced. Some factors affecting pathogenicity of *Xoo*, such as type III secretion system, effectors translocated by type II and III secretion systems, have been identified. In rice, a number of genes contributing to qualitative and quantitative resistance against *Xoo* have been characterized. At least 37 major disease (*MR*) genes have been identified and named, and 7 (*Xa1*, *Xa3/Xa26*, *xa5*, *xa13*, *Xa21*, *xa25*, and *Xa27*) of them have been isolated. Importantly, some key components functioning in *Xa3/Xa26*- and *Xa21*-mediated defense signaling pathways have been characterized, which is helpful to understand molecular mechanisms of qualitative resistance to BB. At least 74 resistance QTLs against *Xoo* have been identified in different rice cultivars interacting with different *Xoo* strains. One major resistance QTL (*WRKY45*) and eight minor resistance QTLs (*NRR*, *WRKY13*, *OsDR8*, *MPK6*, *GH3-1*, *GH3-2*, *GH3-8*, and *C3H12*) have also been identified. The wealth of information about molecular components that function in rice defense response is now accessible for rice improvement in breeding programs.

Rice (*Oryza sativa* L.) is perhaps the most widely cultivated food crop worldwide; it is consumed by approximately 50% of the world's population, and its consumption has been dramatically increased in many parts of the world (White 1994). Various factors affect rice productivity, including diseases. Bacterial blight (BB) is the most devastating bacterial disease of rice. It occurs in epidemic areas of the world and can result in yield loss of up to 50% (Ou 1985). Traditional management methods, including cultivation strategies, chemical control, and biological control, are useful tools to combat BB. However, these methods can be labor intensive, expensive, and may cause environment pollution. The most economical and environmentally friendly way to control BB is to use resistant varieties carrying major disease resistance (*MR*) genes and/or resistance quantitative trait loci (QTLs) in combination with agricultural management practices. Resistance genes and QTLs have been identified and provide valuable resources for developing broad-spectrum and/or durable resistance against BB in rice breeding programs.

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The Disease and Pathogen

BB, also called "kersek" at early growth stage of the plant, is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and is one of the oldest known crop diseases. It was first reported by the farmers of Fukuoka (Japan) in 1884 (Yamanuki et al. 1962). Subsequently, it was found in various parts of Asian countries, Australia, African countries, and the United States. BB occurs in both temperate and tropical regions, but outbreaks are more frequent in irrigated and rainfed lowland areas. Severe epidemics often occur with strong winds and continuous heavy rains (Ou 1985). *Xoo* may be seed-borne and can be spread by irrigation water, but this is disputed (Mizukami 1961; Premalatha and Devadath 1983). The pathogen may survive on infected cultivated rice plants or other hosts (wild rice and gramineous weeds) over winter (Ou 1985). Under favorable conditions, *Xoo* invades rice leaves through hydathodes or wounds, multiplies in the intercellular space of the underlying epithem, and spreads into the plant through the xylem vessels, resulting in yellow lesions with wavy margins along the veins that may systemically extend to the sheath (Figures 2.1A, 2.1B). BB is observed on both seedlings and adult plants and peaks at the flowering stage.

Xoo is a gram-negative bacterium that is rod-shaped, round-ended, motile, and slime-producing with a polar flagellum. The length and width of individual cells are approximately 0.7 to 2.0 μm and 0.4 to 0.7 μm, respectively. Bacterial colonies on nutrient solid media are yellow, round, and convex (Webster and Gunnell 1992) (Figure 2.1C). *Xoo* is aerobic, catalase-positive, able to produce acids from carbohydrates, and unable to use nitrate. The optimal temperature range for *Xoo* growth is 25°C to 30°C (Bradbury 1984). Identification and classification of the bacterial pathotypes of *Xoo* are helpful for resistance breeding and disease control of BB. However, the morphological, physiological, and biochemical characters of different pathotypes are identical (Reddy and Reddy 1990). Based on the infection responses elicited in rice lines, Japanese *Xoo* strains have been classified into 6 virulence groups (I to VI), Philippines *Xoo* strains have been classified into 10 virulence groups (race 1 to 10), Chinese *Xoo* strains include 7 virulence groups (C1 to C7), and Indian *Xoo* strains can be classified into 13 clusters and 5 broad groups (Ezuka and Horino 1974; Vera Cruz 1984; Fang 1990; Nayak et al. 2008).

The genomes of three *Xoo* strains, including Japanese strain MAFF311018, Korean strain

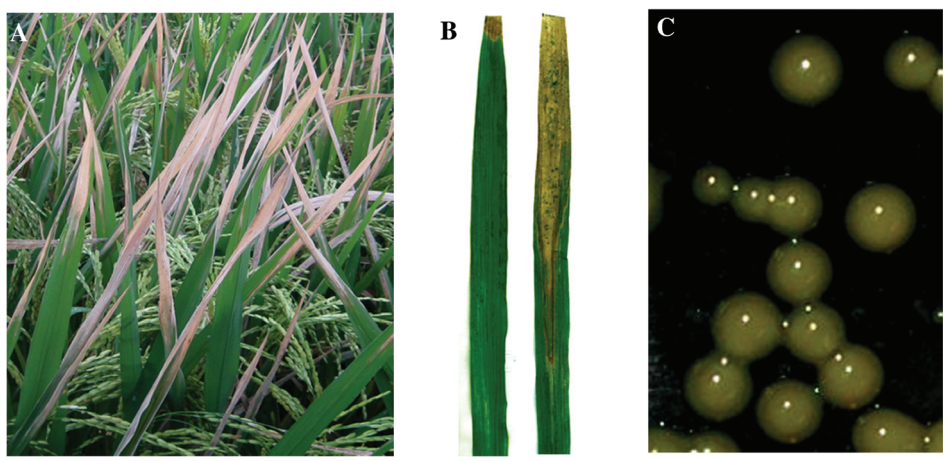

Fig. 2.1. Bacterial blight disease of rice. (A) Rice cultivar infected by *Xoo*. (B) Infected rice leaves after artificial inoculation of *Xoo*. (C) *Xoo* colonies. For a color version of this figure, please refer to the color plate.

KACC10331, and Philippine strain PXO99A, have been sequenced (Lee et al. 2005; Ochiai et al. 2005; Salzberg et al. 2008). The *Xoo* genome is a single circular chromosome of about 50 million bases (Mb), and it contains nearly 5,000 open reading frames (ORFs). It features remarkable plasticity and evolves rapidly. There are large numbers of major rearrangements and indels between the three strains, which contributes to the genomic variation in *Xoo*. This genomic variation explains the diversity of *Xoo* genotypes and pathotypes (Salzberg et al. 2008).

Factors Affecting Pathogenicity of *Xoo*

Key to *Xoo* pathogenicity is the type III secretion system that is encoded by hypersensitive response and pathogenicity (*Hrp*) genes (Boch and Bonas 2010). The *Hrp* gene cluster is necessary for pathogenicity in susceptible hosts and for a hypersensitive response in resistance plants and nonhost plants. In the *Xoo* genome, the *Hrp* gene cluster includes 26 genes that have a high sequence similarity (Ochiai et al. 2005). These genes are regulated by two crucial components, HrpG and HrpX, in the *Xanthomonas* genus. The expression of *HrpX* gene is upregulated by HrpG protein (Koebnik et al. 2006).

The type III secretion system translocates effector proteins into plant cells to support bacterial virulence, proliferation, and dissemination. The largest effector family of *Xoo* is the transcription activator-like (TAL) effector family (also called the avrBs3/pthA family) (Boch and Bonas 2010). A common feature shared by TAL effectors is the central repeat region that consists of 1.5 to 28.5 repeats, with each repeat containing 33–34 amino acids, and contributes to binding the *cis*-elements named UTP (upregulated by TAL effector) boxes of plant gene promoters, the amino-terminal translocation region, the carboxyl-terminal nuclear localization signal, and carboxyl-terminal acidic transcription activator-like domain (Boch et al. 2009; Kay and Bonas 2009; Yuan and Wang 2012). TAL effectors function as specific transcriptional activators in the plant cell nucleus. The specificity of DNA recognition by the TAL effector is determined by the variable amino acids at residues 12 and 13 of each repeat. However, some TAL effectors have been identified as avirulence (Avr) proteins in disease resistance (*R*) gene-mediated *Xoo* resistance (Boch and Bonas 2010).

In addition to the effectors translocated by the type III secretion system, the other important virulence factors of *Xoo* are extracellular enzymes and polysaccharide and a diffusible signal factor (Feng et al. 1996; Büttner and Bonas, 2010; He et al. 2010). The extracellular enzymes, such as endoglucanases, xylanase, cellobiosidase, and esterase, are secreted by the type II secretion system of *Xoo* to degrade the plant cell wall (Büttner and Bonas 2010). The extracellular polysaccharide protects bacteria against environmental stress. Null mutation of *rpfC* in *Xoo* strain T3000 substantially influences the synthesis of extracellular polysaccharide and virulence in rice (Feng et al. 1996). The diffusible signal factor is a cell-cell communication signal, and it can affect the expression of virulence genes (He et al. 2010). Repeats in the structural toxin (RTX toxin), which has functions in biofilm development, cellular adherence, and eukaryotic cell targeting, represent another type of important virulence factors among gram-negative bacteria (Coote 1992; Satchell 2011). Several RTX toxins, including phenylacetic acid, trans-3-methylthioacrylic acid, and 3-methylthio-propionic acid, have been identified in *Xoo* (Noda et al. 1989). Thus, RTX toxins may also be virulence factors of *Xoo*. In addition, the *rax* genes (such as *raxA*, *raxB*, *raxC*, and *raxST*) of *Xoo* are involved in secretion by the type I secretion system and sulfation of peptide Ax21 (activator of *Xa21*-mediated immunity), which elicit rice Xa21 protein-mediated resistance (Lee et al. 2009).

Xoo Resistance in Rice

Overview of Disease Resistance Mechanism in Plants

Physical and biochemical barriers provide a first line of defense against potential pathogen attack. These constitutive defenses include the presence of many preformed barriers such as waxy epidermal cuticles, cell wall, bark, antimicrobial enzymes, and secondary metabolites. However, pathogens have evolved strategies to breach these passive defense barriers. When *Xoo* enters a leaf apoplast through hydathodes or wounds, the plant relies on its innate immune system to detect the invading organisms and activate inducible defenses.

The current view of plant-pathogen interactions has revealed that the innate immune system consists of a two-branched defense response. The first branch is pathogen (microbe)-associated molecular patterns (PAMPs/MAMPs)-triggered immunity (PTI) or basal resistance, which is initiated by the direct recognition pathogen PAMPs through plant pattern-recognition receptors (PRRs) (Jones and Dangl 2006; Boller and Felix 2009). PRRs are plasma membrane proteins. PAMPs, which are essential for microbe fitness or survival, are relatively conserved molecules within a class of microbes during evolution, such as flagellin, peptidoglycan, and lipopolysaccharides. The other branch is effector-triggered immunity (ETI) or race-specific resistance that is activated on direct or indirect detection of pathogen effectors by plant proteins encoded by *R* genes (Jones and Dangl 2006; Thomma et al. 2011). R proteins are either intracellular, plasma membrane, or extracellular, and each of these R proteins recognizes one or a few specific effectors. Pathogen effectors are rapid evolving, which results in loss of function of R proteins.

After the presence of PAMPs or effectors activates PPRs or R protein, the plant receptors transfer the defense signal to downstream components encoded by defense-responsive or defense-related genes, which leads to defense responses. Defense-responsive genes are characterized by their response to a pathogen attack via changed expression levels or posttranslational modifications of their encoding proteins (Kou and Wang 2010). In general, PTI is a relative weak defense response and ETI is a high-level defense response. However, strong PTI and weak ETI have also been reported (Thomma et al. 2011). Furthermore, PAMPs and effectors as well as PRRs and R proteins cannot be strictly maintained, because there is a continuum between PTI and ETI (Thomma et al. 2011). For example, rice *Xa21*-mediated *Xoo* resistance is triggered by a narrowly conserved PAMP, Ax21, and Xa21 protein is considered to be both a PRR and an R protein (Lee et al. 2009). In addition, the defense signaling pathways initiated by PRRs and R proteins are partially overlapping (Kou and Wang 2010).

According to the speed and strength of the plant response to pathogen invasion, plant resistance can be divided into two major categories: qualitative or complete resistance and quantitative or partial resistance. Qualitative resistance is a rapid and high level of defense response mediated by *MR* genes, including *R* and *PRR* genes that confer a high level of resistance. More than 30 *MR* genes that mediate qualitative resistance and have different resistance spectra against *Xoo* have been named. Quantitative resistance is controlled by multiple genes or resistance QTLs and can be broad spectrum and/or durable (Kou and Wang 2010). A large number of resistance QTLs have been identified in the interactions of different rice varieties and *Xoo* strains (Kou and Wang 2012).

In addition to innate immunity, plants have different types of induced resistance, including systemic acquired resistance (SAR) and induced systemic resistance (ISR). Genetic studies in *Arabidopsis* revealed that NPR1 (non-expressor of pathogenesis-related genes 1) is important for SAR, and TGA transcription factors are repressors of SAR (Vlot et al. 2009). Some evidence supports rice having a similar SAR pathway for *Xoo* resistance. Overexpression of rice NH1,

which is a sequence and functional ortholog of *Arabidopsis* NPR1, results in enhanced resistance to *Xoo* (Chern et al. 2005). In rice, NH1 interacts with TGA2.1 transcription factor and negative regulator of resistance (NRR). TGA2.1 negatively regulates basal defense responses to *Xoo* (Fitzerald et al. 2005). Rice NRR negatively regulates SAR in *Arabidopsis* and basal and *Xa21*-mediated *Xoo* resistance in rice (Chern et al. 2005, 2008). It is also known that a rice mitogen-activated protein kinase, MPK6, negatively regulates SAR in rice-*Xoo* interaction (Shen et al. 2010). ISR of plants against pathogens is a widespread phenomenon that activates multiple defense mechanisms including increased activity of pathogenesis-related gene (PR) proteins. Attenuated UV-mutant *Xoo* strains have been documented to induce rice ISR against BB (Thein and Prathuangwong 2010).

Qualitative Resistance to *Xoo*

Asian-cultivated rice (AA genome) consists of two major subspecies, *indica* (*O. sativa* L. ssp. *indica*) and *japonica* (*O. sativa* L. ssp. *japonica)*. At least 37 *MR* genes against *Xoo* have been identified and designated in a series from *Xa1* to *Xa36*, with one symbol having been used for two different genes (Table 2.1). Most of these genes were identified in Asian-cultivated rice while only a few were identified from wild rice species, which were then introgressed into cultivated rice. It is generally accepted that R proteins encoded by dominant *R* genes recognize specific pathogen effectors and initiate defense signal transduction leading to rapid and race-specific disease resistance in most plant-pathogen systems, including rice *R* gene-mediated resistance to fungal pathogen *Magnaporthe oryzae* (Dangl and Jones 2001; Martin et al. 2003; Liu et al. 2010). However, more than one-third of identified *MR* genes against *Xoo* confer recessive resistance, namely *xa5*, *xa8*, *xa9*, *xa13*, *xa15*, *xa19*, *xa20*, *xa24*, *xa25/Xa25(t)*, *xa26(t)*, *xa28(t)*, *xa31(t)*, *xa33(t)*, and *xa34(t)* (Table 2.1). Only 7 (*Xa1*, *Xa3/Xa26*, *xa5*, *xa13*, *Xa21*, *xa25*, and *Xa27*) of the 37 identified *MR* genes against *Xoo* have been isolated. Most of the characterized *MR* genes encode proteins that are different from the most common R protein, such as nucleotide-binding site (NBS)-leucine-rich repeat (LRR) protein (Liu et al. 2010). This feature suggests that the molecular mechanisms of qualitative resistance in rice-*Xoo* system are more complicated than in other plant-pathogen systems.

Xa1

Xa1, localized on the long arm of chromosome 4, was used in Japanese rice breeding for BB resistance from 1967. It confers resistance to Japanese *Xoo* race I, which is the most dominant race in Japan. *Xa1*, which was cloned by a map-based cloning strategy from the *japonica* rice cultivar Kogyoku and *indica* rice line IRBB1, encodes a cytoplasmic NBS-LRR protein (Yoshimura et al. 1998) (Figure 2.2). The expression of *Xa1* can be induced by *Xoo* and wounding. The induction of expression is speculated to be involved in enhanced resistance to *Xoo* (Yoshimura et al. 1998).

Xa3/Xa26

Xa3/Xa26 gene, localized on the long arm of chromosome 11, was isolated as *Xa26* from an *indica* rice cultivar Minghui 63 (AA genome) with a map-based cloning strategy. It encodes a plasma membrane–localized LRR receptor kinase-type protein with an extracellular LRR domain, a transmembrane motif, and a cytoplasmic kinase domain (Sun et al. 2004). Further study revealed that *Xa3*, a previously named *MR* gene, and *Xa26* are actually the same gene, which was then renamed as *Xa3/Xa26* (Xiang et al. 2006) (Figure 2.2). *Xa3/Xa26* gene confers relatively broad-spectrum resistance to different *Xoo* races; rice cultivars carrying *Xa3/Xa26* gene have been widely used in rice production in China for a long period of time (Xu et al. 2004; Gao et al. 2010; Li et al. 2012). The *Xa3/Xa26* alleles, *Xa3/Xa26-2* from wild rice *Oryza officinalis* (CC genome) and *Xa3/Xa26-3* from the CC

Table 2.1. Summary of major disease resistance genes against *Xoo* in rice

Gene	Resistance to *Xoo* race	Donor cultivar[a]	Chromosome	Reference[a]
Xa1	Japanese race I	Kogyoku, IRBB1	4	Yoshimura et al. 1998
Xa2	Japanese races I and II	IRBB2	4	He et al. 2006
Xa3/Xa26	Chinese, Philippine, and Japanese races	Minghui 63, IRBB3	11	Sun et al. 2004, Xiang et al. 2006
Xa4	Philippine races	IRBB4	11	Sun et al. 2003
xa5	Philippine and Japanese races	IRBB5	5	Iyer and McCouch 2004
Xa6	Philippine race 1	Zenith	11	Sidhu and Noori 1978a
Xa7	Philippine races	IRBB7	6	Chen et al. 2008
xa8	Philippine races	PI231128	7	Sidhu and Noori 1978b
xa9	Philippine races	Sateng	11	Singh et al. 1983
Xa10	Philippine and Japanese races	IRBB10	11	Gu et al. 2008
Xa11	Japanese races	IR8		Goto et al. 2009
Xa12	Japanese and Indonesian races	Kogyoku, Java14	4	Ogawa et al. 1978
xa13	Philippine race 6	IRBB13	8	Chu et al. 2006
Xa14	Japanese races and Philippine races 3 and 5	CBB14	4	Tan et al. 2004
xa15	Japanese races	M41 Harebare mutant		Noda 1989
Xa16	Japanese races	Tetep		Noda 1989
Xa17	Japanese races	Asominori		Ogawa et al. 1989
Xa18	Burmese races	IR24, Miyang23, Toyonishiki		Ogawa and Yamamoto 1986
xa19	Japanese races	XM5 (mutant of IR24)		Taura et al. 1991
xa20	Japanese races	XM6 (mutant of IR24)		Taura et al. 1992
Xa21	Philippine and Japanese races	IRBB21	11	Song et al. 1995
Xa22(t)	Chinese races	Zhachanglong	11	Wang et al. 2003
Xa23	Indonesian races	*O. rufipogon* (CBB23)	11	Zhou et al. 2005
xa24(t)	Philippine race 6	DV86	2	Wu X. et al. 2008
	Philippine race 9	Minghui 63	12	Liu et al. 2011
xa25/Xa25(t)				
Xa25	Chinese and Philippine races	HX-3 (somaclonal mutant of Minghui 63)		Gao et al. 2005
xa26(t)	Philippine races	Nep Bha Bong		Lee et al. 2003
Xa27	Chinese strains and Philippine races 2 to 6	IRBB27	6	Gu et al. 2005
xa28(t)	Philippine race 2	Lota sail		Lee et al. 2003
Xa29(t)	Chinese races	*O. officinalis* (B5)	1	Tan et al. 2004
Xa30(t)	Indonesian races	Y238	11	Cheema et al. 2008
xa31(t)	Chinese races	Zhachanglong	4	Wang et al. 2009
Xa32(t)	Philippine races	C406	11	Zheng et al. 2009
xa33(t)	Thai races	Ba7	6	Korinsak et al. 2009
xa34(t)	Chinese race V	BG1222	1	Chen et al. 2011
Xa35(t)	Philippine races	*Oryza minuta* (Acc. No. 101133)	11	Guo et al. 2010
Xa36(t)	Philippine races	C4059	11	Miao et al. 2010

[a]Rice cultivars or rice lines and references are those reporting the characterization of the genes or fine-mapping the genes.

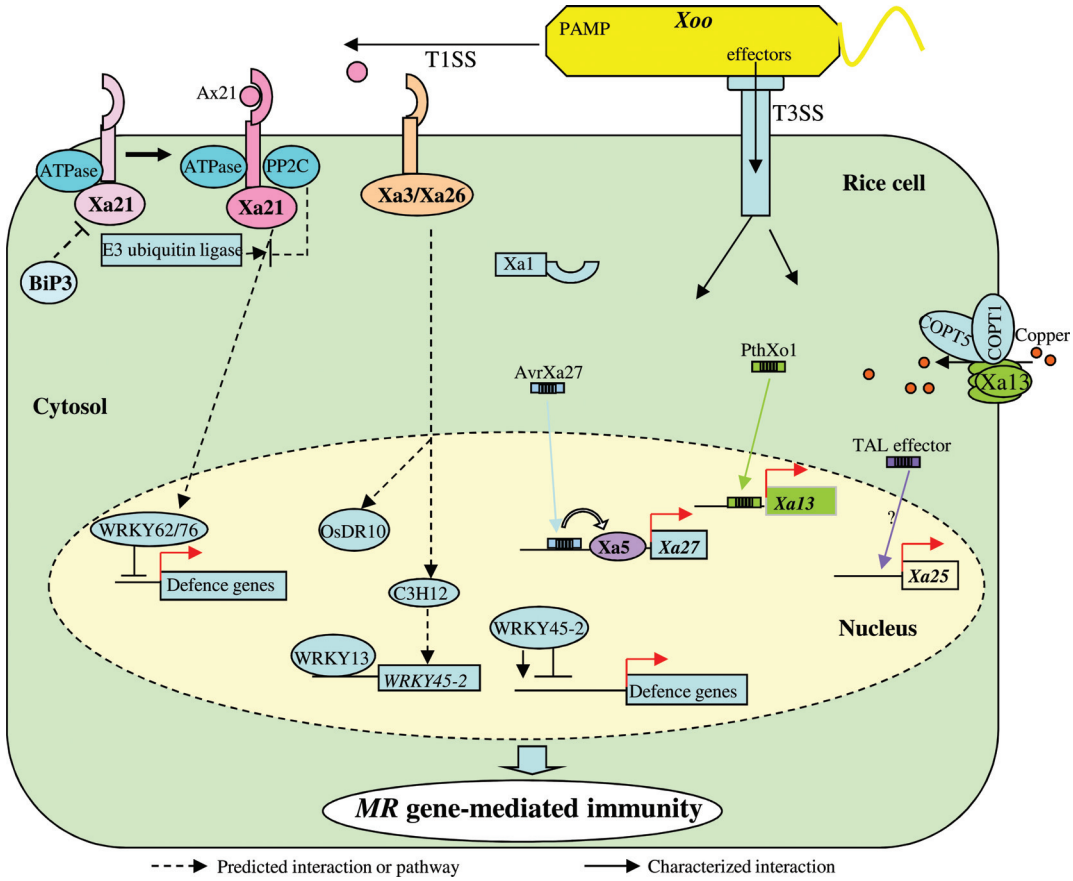

Fig. 2.2. Molecular mechanisms of characterized major disease resistance gene-mediated resistance to *Xoo*. For a color version of this figure, please refer to the color plate.

genome of wild rice *Oryza minuta* (BBCC genome), encode proteins with high sequence similarity to the Xa3/Xa26 protein and can mediate a similar spectrum of resistance against *Xoo* (Li et al. 2012). The speciation of the AA and CC genomes is approximately 7.5 million years ago. These characteristics suggest that the *Xa3/Xa26* locus may confer a durable resistance.

Xa3/Xa26-mediated resistance is influenced by the genetic background and the developmental stage of a plant. This gene confers higher level of resistance in a *japonica* background than in an *indica* background, and rice plants carrying *Xa3/Xa26* gene have full resistance to some *Xoo* strains at both seedling and adult stages, but have full resistance to other *Xoo* strains at adult stage (Yang et al. 2003; Sun et al. 2004; Cao et al. 2007a). Further study has demonstrated that the expression level of *Xa3/Xa26* gene is associated with genetic background- and development-controlled resistance (Cao et al. 2007a; Zhao et al. 2009). *Xa3/Xa26*-mediated resistance is dose dependent: as the expression of *Xa3/Xa26* gene increases, the plant's resistance increases. A *japonica* background facilitates the expression of *Xa3/Xa26* gene compared with an *indica* background. In addition, the expression of *Xa3/Xa26* gene gradually increases with development and reaches the highest level at the maximum tillering to booting (panicle development) stages. Rice plants constitutively overexpressing *Xa3/Xa26* have a high level and broad

spectrum of resistance to *Xoo* at both seedling and adult stages, without any effects on morphology and agronomic performance (Gao et al. 2010). Furthermore, other factors may also contribute to genetic background-controlled resistance conferred by *Xa3/Xa26* gene in addition of the one influencing *Xa3/Xa26* expression (Zhou et al. 2009).

Domain swap analyses suggest that the LRR domain of Xa3/Xa26 protein is an important determinant of race-specific recognition during rice-*Xoo* interaction; in addition, the juxtamembrane region of this protein also appears to contribute to resistance specificity (Zhao et al. 2009). Four components in Xa3/Xa26 protein-initiated defense-signaling pathway have been identified (Figure 2.2). Although they function downstream of Xa3/Xa26 protein in the defense signaling leading to *Xoo* resistance, these components can mediate a broad-spectrum resistance compared with Xa3/Xa26 protein. For example, WRKY45-2, a WRKY-type transcription factor, positively regulates rice resistance to *Xoo*, *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) causing bacterial streak, and *M. oryzae* causing fungal blast (Tao et al. 2009). WRKY13, which is also a transcription factor and functions upstream of WRKY45-2 in the rice-*Xoo* interaction, positively controls rice resistance to *Xoo* and *M. oryzae* (Qiu et al. 2007; Tao et al. 2009). C3H12, a nucleic acid–binding protein upstream of WRKY45-2 in the rice-*Xoo* interaction, promotes rice resistance against *Xoo* and *Xoc* (Deng et al. 2012; Deng H. and Wang S. unpublished data). *OsDR10*, a gene of *de novo* origin and encoding an unknown protein, negatively regulates rice resistance to *Xoo*, and transgenic plants with suppressed expression of *OsDR10* gene have been shown to have broad-spectrum resistance to *Xoo*, including the *Xoo* strain that is compatible with *Xa3/Xa26* gene (Xiao et al. 2009). OsDR10 protein appears to function upstream of WRKY13 in the rice-*Xoo* interaction.

Xa3/Xa26 gene belongs to a tandem clustered multiple gene family, and paralogs of this family have a similar tissue-specific expression pattern (Sun et al. 2006; Xu S et al. 2007; Xu L et al. 2008). One paralog of this family, *MRKa* gene, can mediate partial resistance to *Xoo* when it is overexpressed (Cao et al. 2007b). The kinase domain of MRKa protein can partially replace the function of the kinase domain of Xa3/Xa26 protein in *Xoo* resistance, suggesting that the functions of the paralogs in this family may be partially conserved. This hypothesis is also supported by a recent report that another paralog of this family, *NRKe* gene, regulates rice response to raised temperature (Zhang et al. 2011). The kinase domain of Xa3/Xa26 protein can replace the function of the kinase domain of NRKe protein in response to temperature change.

xa5

The recessive *xa5*, localized on the short arm of chromosome 5, was first identified in varieties of the DZ192 group in 1977 (Iyer and McCouch 2004). It mediates specific resistance to Japanese races and Philippine races 1, 2, 3, and 5 by restriction of bacterial movement, but not multiplication (Iyer and McCouch 2004; Iyer-Pascuzzi et al. 2008). This gene was cloned by a map-based cloning approach combined with allele sequence analysis (Iyer and McCouch 2004), and further complementation testing confirmed this gene (Jiang et al. 2006). The *xa5* encodes a typical gamma subunit of transcription factor IIA (TFIIAγ), which is one of general transcription factors required for transcription by RNA polymerase II (Iyer and McCouch 2004). There are two nucleotide substitutions in the recessive allele, which results in an amino acid substitution of dominant (susceptible) *Xa5* gene. It is speculated that *Xoo* TAL effectors usurp parts of plant basal transcription machinery to regulate rice gene expression; the missense mutation of *xa5* allele does not compromise its general function in transcription, but it may evade TAL virulence functions (Gu et al. 2009; Boch et al. 2010). Thus, *xa5* displays resistance to *Xoo*. *Xoo avrXa5* is an avirulence gene, which encodes a TAL-type protein, corresponding to *xa5* (Zou et al. 2010).