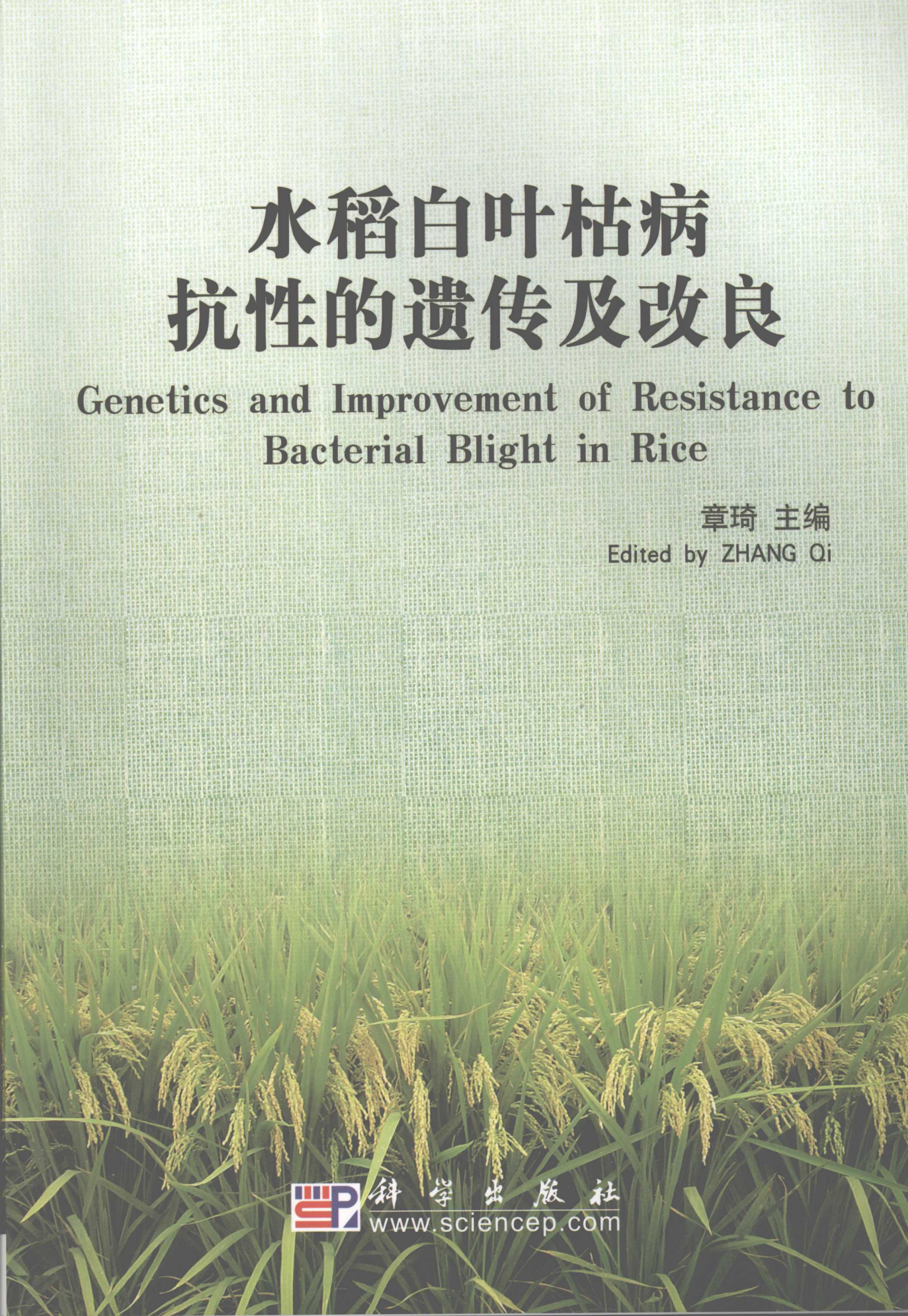
水稻白叶枯病抗性的遗传及改良

Genetics and Improvement of Resistance to Bacterial Blight in Rice

章琦 主编
Edited by ZHANG Qi



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内 容 简 介

本书是综合运用植物病理学、遗传育种学和分子生物学知识，系统论述水稻品种白叶枯病抗性及其遗传改良研究的一部专著。全书共分11章，第1章概述水稻白叶枯病的发生、流行和病原学等一般基础知识。第2、3章探讨病原菌致病力变异及其群体遗传结构与寄主抗性基因的协同演变关系。第4章至第8章分别讨论质量抗性遗传的一般规律与主基因鉴定，数量抗性遗传新进展，国内外稻种资源抗性评价，常规抗性育种和分子标记辅助选择育种技术新进展。第9章至第11章分别论述白叶枯病病原菌无毒基因及相关调节因子，寄主抗性机理和抗性基因的克隆、结构与功能，以及分子进化等专题。每章附中、英文小结。

本书可供从事植物抗病育种、植物病理学科研教学的大专院校师生、研究生以及生产管理、科研开发工作者等参考。

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图 1-2　江苏连云港市东海安峰乡严重发生白叶枯病的稻田（徐大勇摄，2005）

Fig. 1-2　Rice cultivar infected by bacterial blight in Donghai Anfeng Jiangsu Province (Xu , 2005)

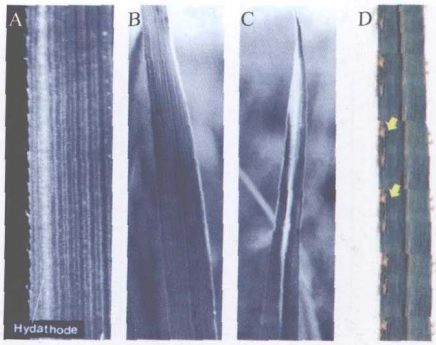

图1-3　白叶枯病症状。A. 叶缘伸长的浅色的水孔；B. 叶缘受 *Xoo* 自然侵染的病斑；C. 扩展的病斑（Mew et al., 1984）；D. 病叶上的*Xoo*菌脓（浙江农业大学,1978）

Fig. 1-3　Symptom of infected leaves. A. The hydathode is near edge of blade. B. Natural infection of *Xoo* at the edge of blade. C. Expanded lesion（Mew et al., 1984）. D. *Xoo* ooze on the infected leaves (Zhejiang Agricultural University, 1978)

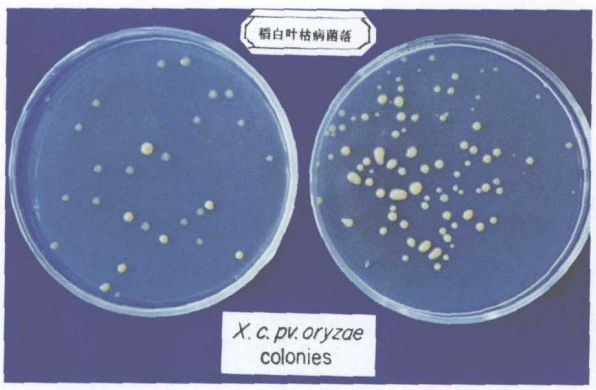

图 1-4　*Xoo* 菌单菌落（Zhang et al., 1985）

Fig. 1-4　*Xoo* colonies（Zhang et al., 1985）

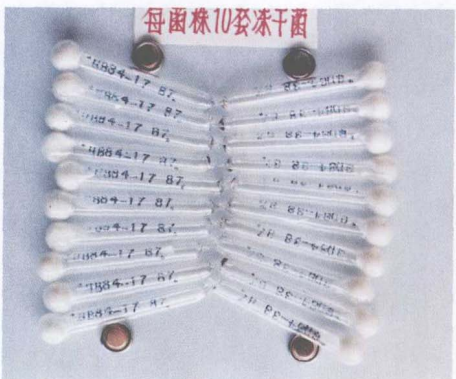

图 1-5　冻干菌种（Zhang et al., 1987）

Fig. 1-5　*Xoo* lyophilized stock（Zhang et al.,1987）

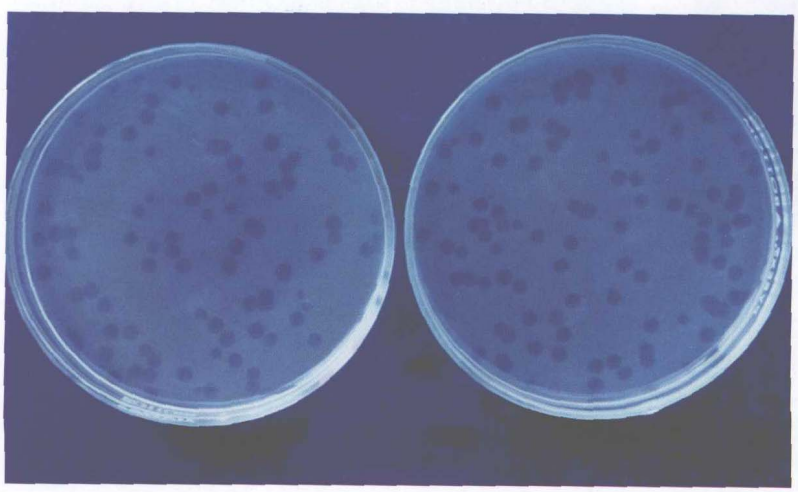

图1-6　水稻白叶枯病菌的噬菌斑(Zhang et al., 1985)

Fig. 1-6　Plaques of *Xoo* (Zhang et al., 1985)

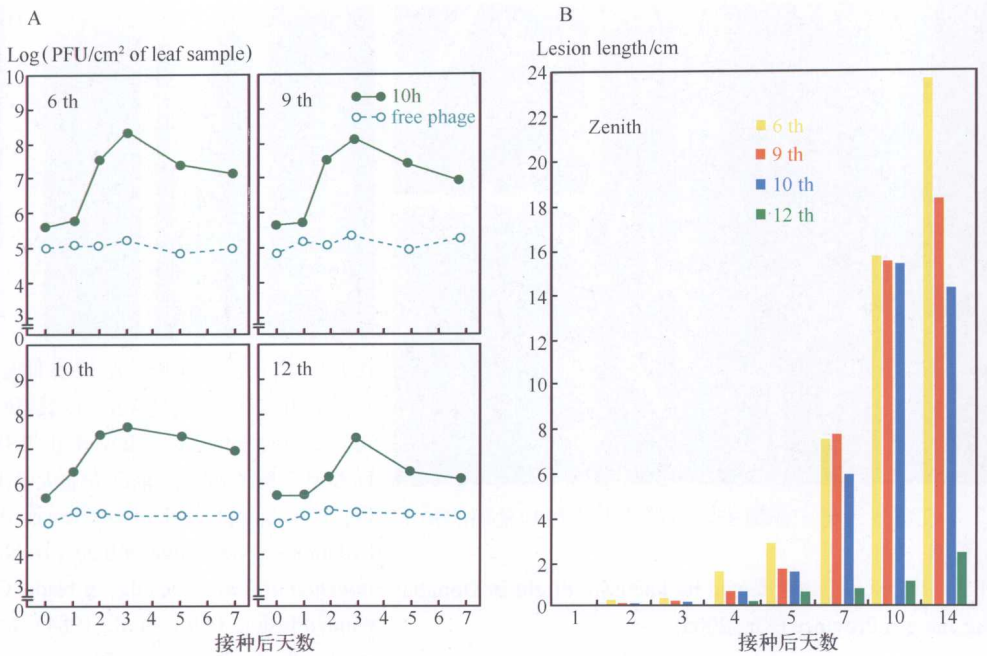

图 1-7　A. 用噬菌体法测定成株抗性品种 Zenith，其第 6、9、10 叶片（感病期）和第 12 叶片（抗病期）接种 *Xoo* 菌株 PXO61 后定期测定细菌增殖趋势；B. Zenith 被接种后的病斑延伸长度（章琦等，1987）

Fig. 1-7　A. Population trends of *Xoo* in the 6th, 9th, 10th and 12th leaves of adult plant resistance variety Zenith at different days after inoculation by using phage test method. B. Development of BB lesion at different leaf positions in Zenith（Zhang et al., 1987）

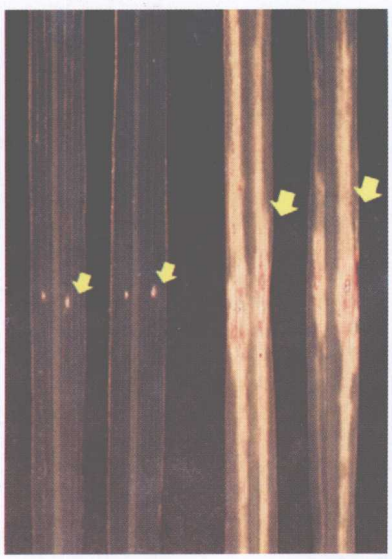

图4-3　双针接种法将日本*Xoo*小种 I 接种在品种黄玉（抗病，左）和金南风（感病，右）的反应型 （Ezuka and Horino, 1974）

Fig. 4-3　Reactions of Kogyoku (left, resistant) Kinmaze (right, susceptible) to Japanese *Xoo* race I by double needle- pricking method (Ezuka and Horino, 1974)

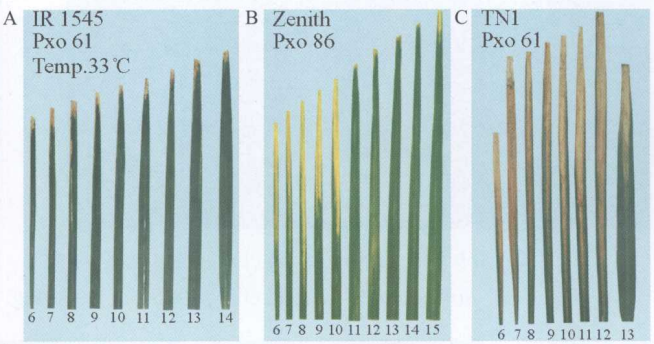

图 5-1　水稻对白叶枯病不同生育期抗性类型品种的病斑反应（章琦等，1985）。A. 全生育期抗性；B. 成株抗性；C. 感病品种

Fig. 5-1　Lesion reactions of cultivars with different BB resistance type. (Zhang et al., 1985)

A. Over-all resistance. B. Adult plant resistance. C. susceptible cultivar

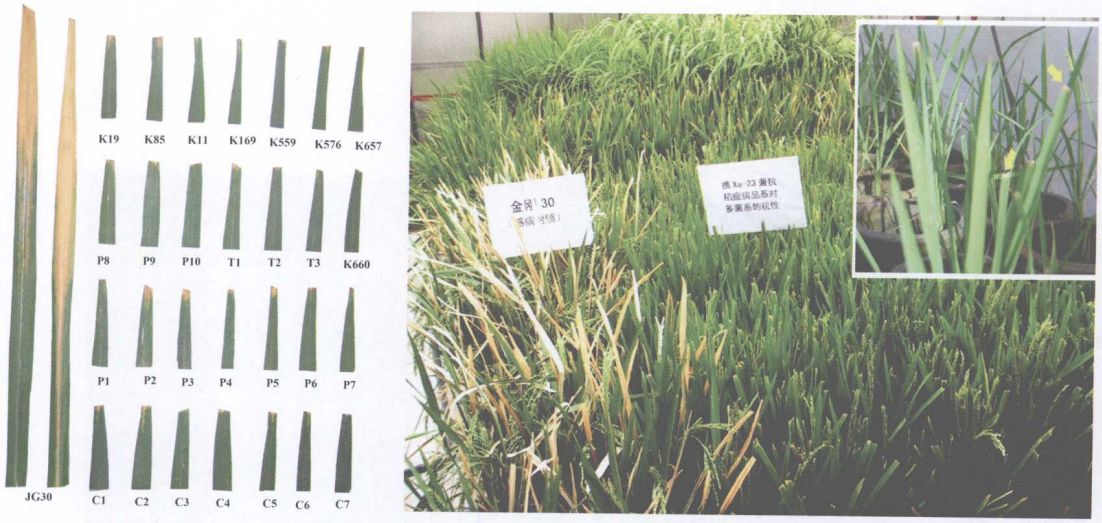

图 5-2　*Xa23* 对 28 个国内外白叶枯病小种代表菌株的抗性反应（章琦等，2004）

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图 5-5　中国粳稻白叶枯近等基因系（Zhang et al.,1996）。A. 6 个 CBB 系统的粳稻白叶枯近等基因系的田间表现。B. 该套近等基因系的轮回亲本（沈农 1033）及其中两个品系 CBB2 和 CBB3

Fig 5-5　The Chinese near - isogenic japonica rice lines for BB resistance (Zhang et al.,1996). Performance of six near - isogenic japonica rice lines for BB resistance in the field. The recurrent parent (SN1033) of the NILs,and 2 out of the six lines, CBB2 and CBB3

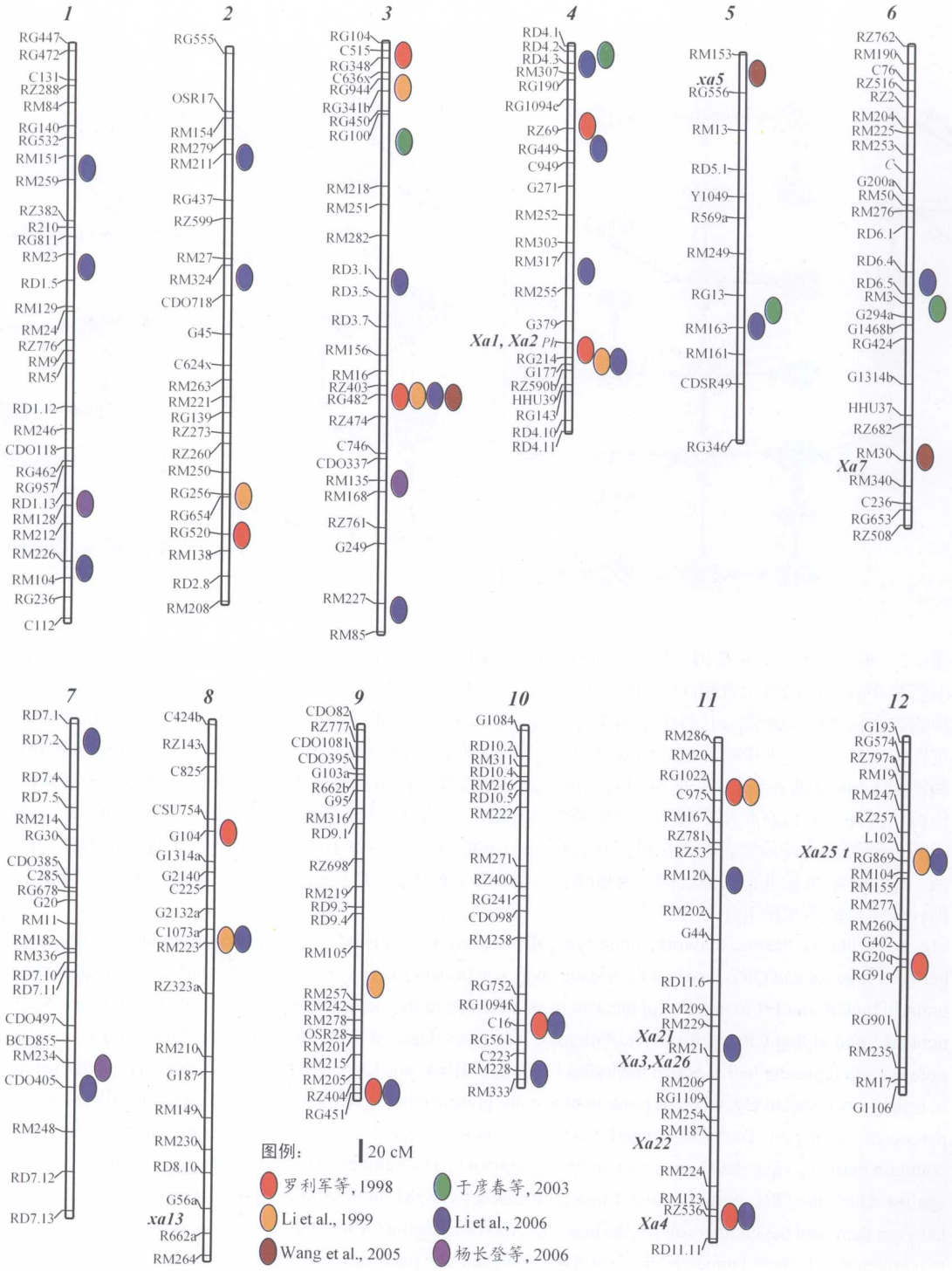

图 6-2　目前已报道的水稻白叶枯病数量抗性位点（QRL）以及部分抗性主基因的染色体位置示意图（连锁图谱参考 GRAMENE 数据库）

Fig. 6-2　Chromosome positions of the quantitative loci and some major genes for resistance to *Xanthomonas oryzae* pv . *oryzae* in rice published papers (The linkage map refers to the GRAMENE database)

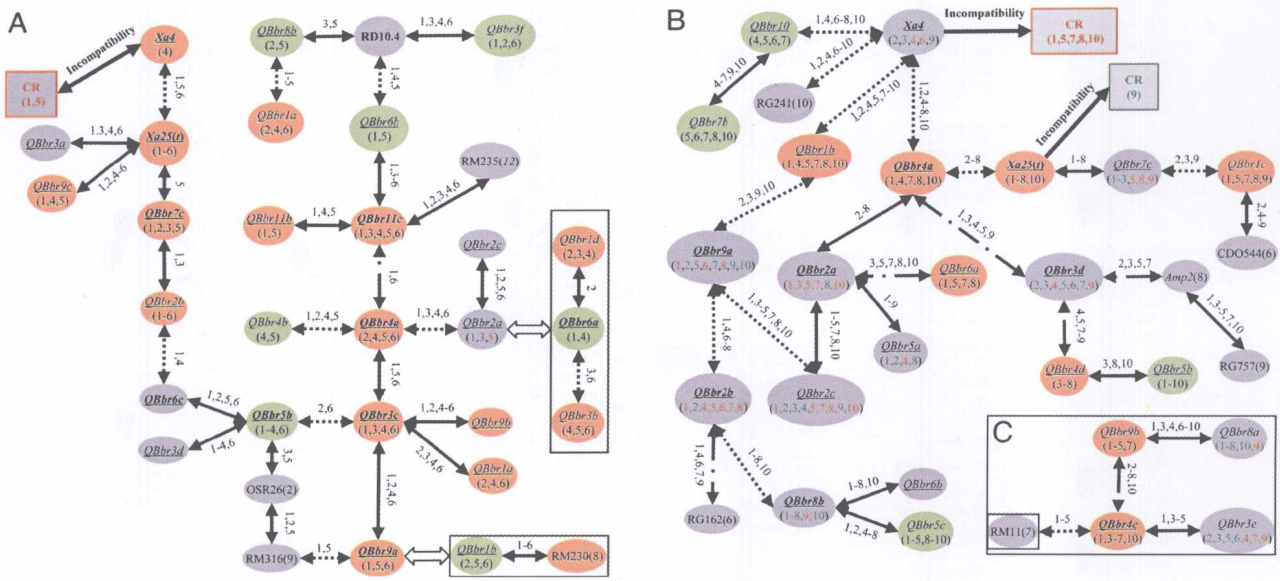

图 6-3　根据主基因（R 基因）与数量抗性位点（QRL）间上位性互作关系推断水稻对白叶枯病防卫系统中所存在的遗传网络示意图(Li et al., 2006)。A. 利用 Lemont/Teqing 重组自交系群体接种对 6 个菲律宾小种的完全抗性(CR)和部分抗性(PR)的遗传网络；B. IR64/Azucena 双单倍体群体对 10 个菲律宾小种的完全抗性(CR)和部分抗性(PR)的遗传网络（红色和绿色分别表示该位点上的抗性基因来自籼稻(Teqing或IR64)和粳稻(Lemont或 Azucena)，蓝色则表示该位点上的抗性等位基因来源因小种而异。下划线表示根据比较作图法所提示的信息在两个群体中均检测到的 QRL。下方括号里的数字表示该位点所控制的小种。QRL 间的双箭头线条表示显著的上位性互作，线条上方的数字表示互作中所针对的小种；实线为亲本型互作抗病，虚线表示为重组型互作抗病，点划线表示亲本型 / 重组型互作抗病因小种而异。A 和 C 的方框表示根据比较作图的结果的几组互作位点

Fig. 6-3 Putative genetic networks underlying the defensive system of rice based on the epistatic relationships between R genes and QRL detected for resistance to *Xoo* in rice(Li et al., 2006). A. The putative genetic networks underlying CR and PR to six Philippine *Xoo* races detected in the Lemont/Teqing RILs; B. The putative genetic networks underlying CR and PR to ten Philippine *Xoo* races detected in the IR64/Azucena DHLs. QRL in red and green colors represent loci at which the indica (Teqing or IR64) and japonica (Lemont or Azucena) alleles resulted in resistance, respectively, whereas those in blue color present cases where resistance resulted from alleles of either parents depending on *Xoo* races. Underlined QRL are those detected in the both populations, inferred based on the common markers using the comparative mapping approach. The numbers under each QRL represent the *Xoo* races against which the QRL was effective. Lines connecting two QRL indicate that significant epistasis was detected between them and the numbers above the lines are *Xoo* races against which the epistasis was detected. Cases where two interacting loci are connected by a solid line represent the parental type of interaction (the parental genotypes were associated with resistance), those connected by a broken line represent the recombinant type of interaction (the recombinant genotypes were associated with resistance), and those connected by a half-broken line represent cases where either parental or recombinant type interaction was associated with resistance depending on *Xoo* races. Rectangular boxes in A and C represent cases where groups of interacting loci could be included into the networks based on the comparative mapping results

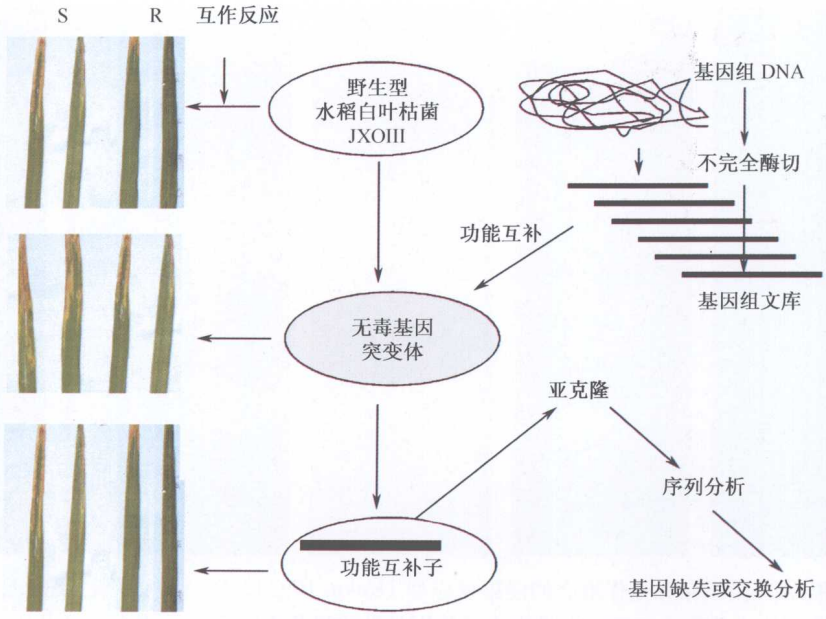

图 9-4　克隆与鉴定水稻白叶枯病菌无毒基因的一般步骤

Fig. 9-4　General procedure of cloning and characterizing avirulence genes from *Xoo*

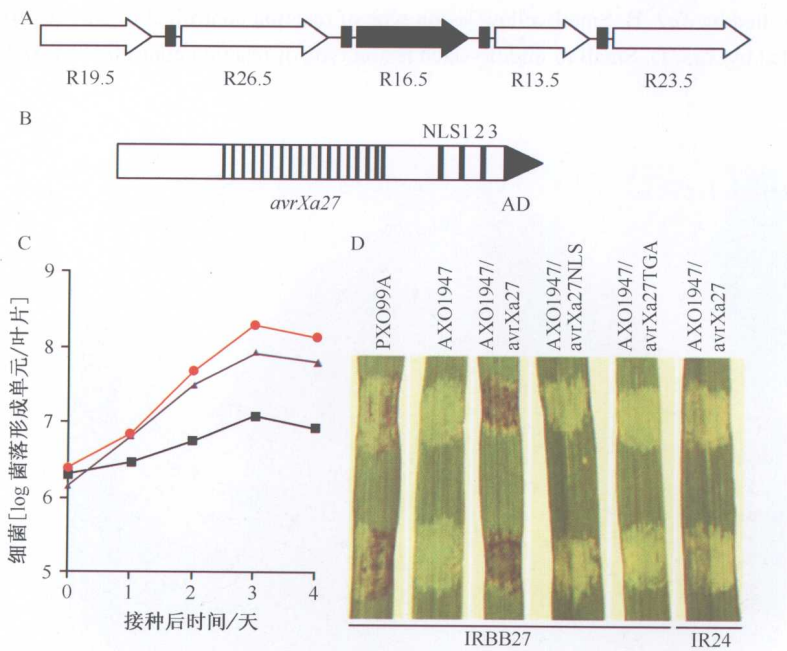

图 9-5　Pxo99 中的 *AvrXa27* 及其他 4 个同源基因(Gu et al., 2005)。A. 黑箭头表示 AvrXa27，R 后数字为重复序列数；B. AvrXa27 的结构与其他 *avrBs3/pthA* 基因家族成员相似；C. 表示亲和性菌株 AXO1947 含 AvrXa27（下）和不含 AvrXa27 重组菌（上）在 IRBB27（*Xa27*）水稻上的生长曲线，中间为含 AvrXa27 的 AXO1947 在 IRBB24 上的生长曲线；D. AvrXa27 需要 NLS 基序和激活结构域才产生依赖 *Xa27* 的抗性

Fig. 9-5　AvrXa27 is one of 5 homologous genes of the AvrBs3/PthA family. (Gu et al., 2005) . A. Black arrow expresses AvrXa27, and R for repeat number. B. AvrXa27 has similar structure to that of AvrBs3/PthA family members. C. Growth curves of AXO1947 containing AvrXa27(down), or not containing AvrXa27 (upper). D. Indicating NLS and AAD are required for eliciting *Xa27*-dependent resistance

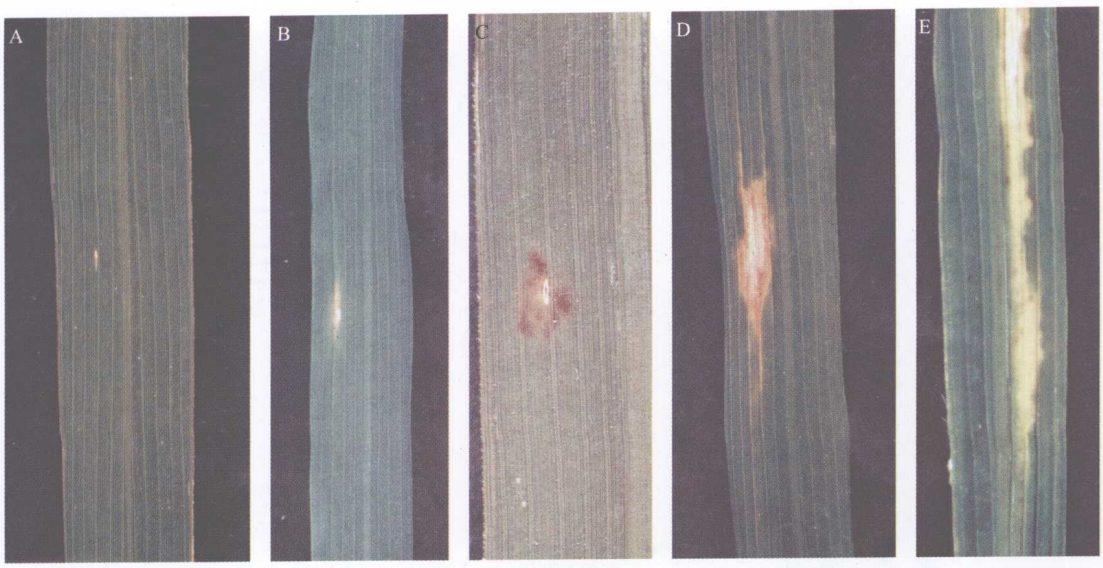

图 10-4　水稻 -*Xoo* 菌不亲和互作组合的侵染反应型（Kaku, 1993）。A. *Xa1* 基因控制的无症状反应型；B. *Xa2* 基因控制的小黄斑反应型；C. 携有 *Xa3* 基因品种的褐色病斑型；D. 携有 *Xa4* 基因品种的中小型病斑型；E. 感病反应型

Fig. 10-4　Infection types in rice - *Xanthomonas oryzae* pv. *oryzae* interaction(Kaku, 1993). A. Symptomless type of reaction controlled by *Xa1*. B. Small yellow lesion type of reaction controlled by *Xa2*. C. Browning type of reaction controlled by *Xa3*. D. Small to middle-sized lesion type of reaction controlled by *Xa4*. E. Susceptible reaction

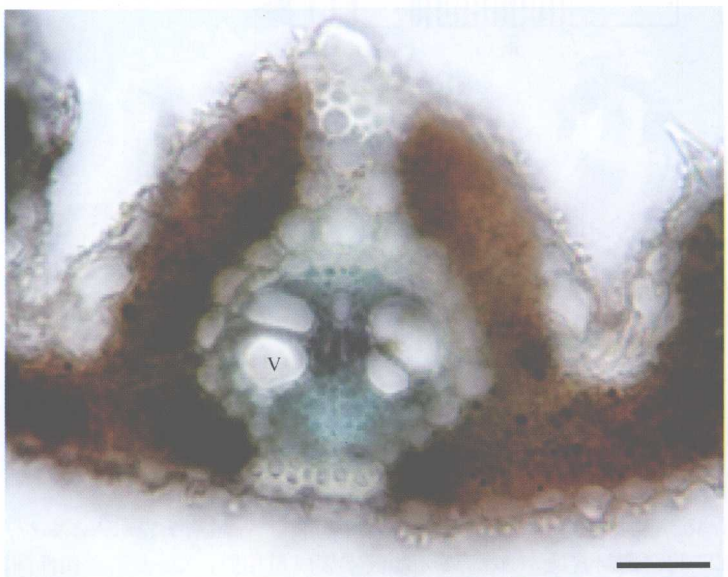

图 11-9　*Xa13* 基因在水稻叶片组织中的表达模式。转基因水稻叶片组织中 *Xa13* 启动子调控葡糖醛酸酶（GUS）的表达。蓝色表示 GUS 在维管束（V）周围的细胞中表达。标尺 = 30 μm

Fig. 11-9　The expression pattern of *Xa13* in leaves. *Xa13* promoter-β-glucuronidase (GUS) expression in transgenic rice plant. The blue color indicates that GUS was preferentially expressed in the cells surrounding the vascular element (V). Scale bar, 30 μm

Preface by T. W. Mew

To draw a general picture of bacterial blight (BB) caused by *Xanthomonas oryxae* pv. *oryzae* (*Xoo*) on rice production in the fields today is not an easy job. Although the disease has been seen "quiet" for some time, yet in recent years it has gradually emerged prevalently in fields planted with newly released rice varieties, particularly in China. This situation poses a major concern for scientists and inevitably raised several questions: what causes the resurgence and what should we do next? A more specific question is: do we need to conduct research to prevent the disease intensity reaching the levels as damaging as in the 1960s to 1980s? To answer these questions we need to examine the road map leading to what has been achieved from the past three decades on research in BB, and also on issues of crop improvement and disease management:

(1) a description of the problem, and

(2) an account of its function on crop production.

Research often takes the first step to understand the biology of the problem, an analysis of the actual and potential damage the problem may impose on the crop production, and then to find solution to correct the problem. As in most agricultural systems, the rice production system is dynamic and continually evolving. The change is caused by new varieties being developed and planted, new cultural methods being formulated, introduced and practiced by farmers. But plant pathogens that cause the diseases are shifty enemies. They have the intrinsic mechanism to adjust to new crop cultivars and to new crop production environment. The rate or frequency by which old diseases reemerge and new diseases and virulent pathogen populations emerge depend on the inherent ability of the pathogen to adapt and on the efficiency by which farmers use their inputs and resources. There are numerous examples of reemergence of old diseases, sudden outbreaks of new ones, and emergency of virulent populations of a pathogen in rice production systems in modern times. Ragged stunt, grassy stunt emerged in the 1970s, and red stripe in the 1990s due to changes in new varieties and new crop management practices. Another example is blast which continues to be a devastating disease despite decades of research devoted to its management because of the ability of the pathogen's high ability to evolve to new forms of virulence. In modern times blast epidemics not only devastated the rice production in the fields causing heartfelt sufferings to millions of rice farmers but also had serious political and social consequences in many countries. Would this situation be different for rice bacterial blight? It is unlikely. However, due to the fact that its epidemic potential, and thus damage to rice crop and production may not be at par in magnitude and frequency with that of the rice blast situation. Nonetheless, it

remains a threat to rice production because of its increase in prevalence in rice fields where newly release varieties are introduced to rice farmers. Its resurgence after a decade of "quietness" seems to suggest three possible reasons:

(1) more virulent populations of the bacterial pathogen have emerged,

(2) newly released varieties do not carry resistance genes, and/or

(3) varieties were not adequately screened prior to their release.

Clearly something has gone wrong during this period of BB "quietness". Are we too relaxed on the success achieved? It often happens that when the problem is not seen, it is either not important or non-existent. No matter what the reason is, the situation does not conform well for the stability of rice production in rice growing countries. The pressure of human population is not lessened and the rice production has to meet the demand of the hungry majority in developing world. In the essence of rice production, rice improvement has to be taken as a continuous effort in agricultural production, and there is no room for complacence. We cannot underestimate the intrinsic potential of plant pathogens to flourish in a new crop environment. Resistance breeding has to be in the agenda of crop improvement, or we will pay the price.

To look forward on bacterial blight in rice production, let us pause for a while to look back and to assess where we came from. Active research on bacterial blight of rice did not probably take place until the launching of modern rice improvement program in the 1950s or 1960s. Research intensified during the post-green revolution era. The overall goal at the time was to improve the performance of modern varieties in rice-growing areas in the tropics. To manage bacterial blight, the specific objective was to develop resistant varieties. Scientists initially succeeded in achieving this objective as proven by the low incidence of bacterial blight throughout Asia for some time. For about two decades, varieties grown by farmers possessed various degrees of resistance to bacterial blight. Nowadays, there are several questions we need to ask when bacterial blight is frequently observed in farmers' fields.

(1) Is the resistance still effective?

(2) More strategically, is resistance breeding still one of the main objectives of rice improvement?

(3) Can we continue to use the same resistance gene to prolong or achieve such a scenario for another decade or two? and,

(4) With the numerous new tools, what approach do we take on resistance breeding so that the resistance will be more durable, and the R-gene will be used more efficiently?

The remarkable success making BB "quiet" in the forefront of rice production has to be attributed to an exerted effort of over 40 years of resistance breeding beginning in the 1950s in China and 1960s in tropical Asia. In this effort and process thousands of accessions of rice germplasm were evaluated to identify new sources of resistance. Conse-

quently research on BB and its bacterial pathogen was mushrooming in the 1970s and 1980s in most of the rice growing countries, and it was especially obvious in China judging the number of scientists working on bacterial blight and also the number of publications that appeared in technical and semi-technical journals. Prior to the introduction of the modern semi-dwarf high yielding varieties, BB was not known to occur in epidemic proportion whether it was in China or the rest of Asia except in Japan. However, after the release of the first generation of the high-yielding and nitrogen responsive rice varieties, such as TN1 and IR8, the disease had become a serious constraint. Recognizing it as a major threat, resistance breeding began. Resistance genes were identified and were incorporated into the breeding programs. Among the R-genes, *Xa4* was the first to be used in modern rice varieties and it had become the most widely deployed resistance gene in rice throughout Asia except in Japan. In resistance breeding, however, finding and utilizing diverse sources of resistance is only part of the ingredients. The other vital component is a good understanding of the pathogen variability. Analysis of pathogenic variation is essential for the detection of pathogen effectors that matches the resistance. The research results coming from different rice growing countries has provided a clear picture of race or pathotype diversity and distribution of the bacterial blight pathogen.

Change is the norm of the biological world, so are the pathogen populations. When a single source of resistance is being extensively utilized in the breeding programs, and varieties carrying this resistance gene have been widely grown, new virulence of the pathogen would evolve to overcome its resistance. This begins the "boom and bust" cycle. In rice and resistance to bacterial blight, new strains of *Xoo* virulent to rice varieties carrying the *Xa4* resistance gene were detected after its release. The good news is, although the resistance conferred by *Xa4* has been matched by the virulence, its useful life has never been totally displaced. In a screening process designed to accumulate minor effects of the resistance, it has continued to demonstrate that *Xa4* is a good combiner in the resistance breeding program. Although some of the varieties still carry *Xa4* alone, their resistance to BB has never been totally "broken" down. The information, nevertheless, suggests that the battle against rice diseases is never ending. We need not only develop resistant varieties with different R-genes, but also continue monitoring the pathogen population in response to the newly released varieties in order to be "a step ahead" of the disease problem. We need to identify more resistance genes that are effective and durable, and also know how these resistance genes should be utilized in breeding. The guiding principle in doing so is the information of pathogen population dynamics in response to new resistance. Such information would also guide the deployment once the resistant varieties are developed and introduced to farmers for commercial planting. A smart use of the R-genes should be based on information of host-pathogen interactions. The past 20 years of research on bacterial blight of rice has prepared us to do just that. We believe that a good knowledge of pathogenic variability is essential to

formulate strategy for disease management based on resistance deployment. We have used conventional tools like differential varieties as well as molecular markers to analyze pathogenic variation and to use the information to design and develop durable resistance. Genetic resource is a limited natural resource, and resource of R-genes is no exception to this. The future of rice production using host plant resistance has to address resource use efficiency. Only then can we move forward to design a more sustainable rice production system.

As scientists, we all can do research on our own; but we may not be able to use our research results to solve a problem on our own. Sooner or later we would have to realize that to solve a problem like bacterial blight across the region and across the Asian rice growing countries we need collaboration among scientists of different disciplines. Even on research to evaluate how stable is the sources of resistance from the germplasm that we have identified, there is a need to collaborate testing them on multi-locations. The objective is to expose the resistance to the pathogen populations in different rice production environments. We need to work with scientists from different countries to evaluate the resistance. After we have confirmed that there was physiologic specialization among isolates of the pathogen in the tropics, a collaborative research was organized among scientists to test the differential varieties against strains from various localities. To design more stable (and durable) resistance we also need to have a bigger picture in mind on virulence pattern that may be present in rice growing environments, i. e. tropics, subtropics and temperate; or East to West Asia. Would the set of differentials we just formulated provide a more useful tool to allow us to more accurately assess the virulence pattern to compare the amount of variation in different populations of bacterial blight pathogen? Our target was clear and focused. The next step was to bring scientists working on rice bacterial blight together to formulate work plans and to share and execute different research activities. At the end we could compare and analyze the data to draw the right picture. After extensive data gathering, the initial information available at that time, appeared to suggest that the virulence spectrum was continuous geographically as the strains were more virulent in South Asia than in East Asia. This means that if a resistance were functional in South Asia, it would likely be resistant in East Asia. When more information was available and new tool was used to assess coevolution of rice and *Xoo*, the hypothesis did not seem to hold true. This was further confirmed when *Xa21*, originally thought to be the most resistant gene against the South Asian strains of *Xoo*, was found to be susceptible to some strains in Korea and in China. This information suggests that we need to look into the pathogenic variability not only nationwide but also on a geographical scale in consideration of resistance breeding and R-gene deployment. The virulence of the bacterial pathogen is not continuous. The rice-*Xoo* coevolution is determined obviously by the R-genes-virulence interactions in specific host environments.