Progress in Biological Control

Biological Control of Rice Diseases

by

Samuel S. Gnanamanickam

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Preface

There is sufficient need to document all the available data on biological control of rice diseases in a small volume. Part of this need rests on the global importance of rice to human life. In the first chapter, I have tried to show that rice is indeed life for most people in Asia and shortages in production and availability can lead to a food crisis.

While rice is cultivated in most continents, biological disease management attains special relevance to rice farmers of Africa, Asia, and also perhaps, Latin America. These farmers are resource-poor and might not be able to afford the cost of expensive chemical treatments to control devastating rice pathogens such as *Magnaporthe oryzae* (blast), *Xanthomonas oryzae* pv. *oryzae* (bacterial leaf blight), *Rhizoctonia solani* (sheath blight) and the virus, rice tungro disease.

In an earlier volume that I developed under the title, Biological Control of Crop Diseases (Dekker/CRC Publishers, 2002), I included transgenic crops generated for the management of plant pathogens as biological control under the umbrella of a broad definition. Dr Jim Cook who wrote the Foreword for the volume lauded the inclusion of transgenic crops and induced systemic resistance (ISR) as a positive trend toward acceptance of host plant resistance as part of biocontrol. I continue to subscribe to this view.

This volume is small but presents adequate and important information on major rice diseases and research on biological control of rice diseases. If I presented the information on biological control alone, I feared that the reader will not get the whole picture. I do hope that this volume will be useful as a reference volume for all students and scientists in crop sciences and plant pathology.

More than two third of the work that is covered in this volume comes from research that was carried out in my laboratory at the University of Madras in southern India during 1980–2006 and the research group that was headed by Dr. T. W. Mew at the International Rice Research Institute (IRRI) in the Philippines. A number of Ph.D dissertations were prepared from the research that was carried out in my laboratory and the reader has a chance to come across these in literature cited under each chapter of the volume. As I prepared the volume I realized how fortunate I was

to have all these graduate students do doctoral research on biocontrol of different rice diseases and also felt thankful for the opportunities I have had to associate with Drs. Tom Mew and Swapan Datta at IRRI.

Dallas, TX Sam Gnanamanickam

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I would like to acknowledge the international collaborations I had with T. W. Mew, S. K. Datta, S. McCouch, S. Leong, M. Levy, A. Alvarez, J. E. Leach, S. Muthukrishnan, A. K. Chatterjee, B. A. McDonald, G. Defago, R. J. Cook, L. S. Thomashow, and M. Hofte and national collaborations with the Kerala Agricultural University at Pattambi, National Chemical Laboratory at Pune, and the Central Leather Research Institute at Chennai, India.

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My wife, Beulah Samuel, stood by me and encouraged me to prepare this volume.

I would like to thank the University of Madras, India who appointed me to a faculty position and afforded all the freedom to carry out rice research during 1978–2006 and Novozymes Biologicals in the United States who appointed me to an industry position in their R&D during 2006–2008.

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Dallas, TX Sam Gnanamanickam

Contents

Chapter 1
Rice and Its Importance to Human Life

RICE is life, for most people living in Asia. Rice has shaped the cultures, diets and economies of thousands of millions of people. For more than half of humanity rice is life (Fig. 1.1). Considering its important position, the United Nations designated year 2004 as the International Year of Rice. Devoting a year to a commodity was unprecedented in United Nations history. However, the 57th session of the United Nations General Assembly noted that rice is the staple food of more than half the world's population, affirmed the need to heighten the awareness of the role of rice in alleviating poverty and malnutrition and reaffirmed the need to focus world attention on the role rice can play in providing food security and eradicating poverty and declared the year 2004 as the International Year of Rice (adopted on December16, 2002; www.fao.org/ag/irc).

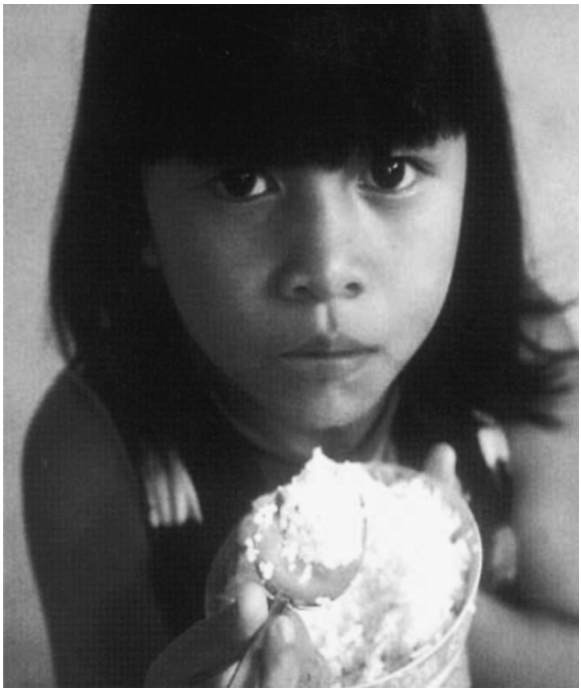

Fig. 1.1 A bowl of rice is life

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Rice, *Oryza sativa*, is a cereal food crop that belongs to the grass family (Family: Poaceae) of the plant kingdom (Fig. 1.2; USDA-NRCS PLANTS Database/ Hitchcock, A.S. [rev. A. Chase]. 1950). Domesticated rice comprises two species of food crops, *Oryza sativa* and *Oryza glaberrima*. These plants are native to tropical and subtropical southern Asia and southeastern Africa (Crawford & Shen, 1998). Rice is a grass "autogame", a crop that is grown more easily in the tropics. Originally rice was probably cultivated without submersion, but it is believed that mutations led it to become a semi aquatic plant. Although it can grow in diverse environments, it grows faster and more vigorously in wet and warm conditions. This plant develops a main stem and many tillers and may range from 0.6 to 6 m (floating rice) in height. The tiller bears a ramified panicle that measures between 20 and 30 cm wide. Each panicle has 50–300 flowers (floret or spikelet), which form the grains. The fruit obtained is a caryopsis (UNCTAD.org).

Fig. 1.2 Rice, *Oryza sativa*, L.

Origin, History and Spread

It is believed that rice cultivation began simultaneously in many countries over 6500 years ago. The first crops were observed in China (Hemu Du region) around

5000 B.C. as well as in Thailand around 4500 B.C. They later appeared in Cambodia, Vietnam and southern India. From there, derived species *Japonica* and *Indica* expanded to other Asian countries, such as Korea, Japan, Myanmar, Pakistan, Sri Lanka, Philippines and Indonesia. *Japonica* is an irrigated rice of the temperate zone, with medium or short grains, also called round grain, and is a rainfed lowland rice of warm tropical zones. *Indica* is an irrigated rice of warm tropical zones, with long, thin and flat grains. The Asian rice (*Oryza sativa*) was adapted to farming in the Middle East and Mediterranean Europe around 800 B.C. After the middle of the 15th century, rice spread throughout Italy and then France, later propagating to all the continents during the great age of European exploration. In 1694 rice arrived in South Carolina, probably originating from Madagascar. The Spanish took it to South America at the beginning of the 18th century (Source: UNCTAD. org).

The origins of rice have been debated for some time, but the plant is of such antiquity that the precise time and place of its first development will perhaps never be known. It is certain, however, that the domestication of rice ranks as one of the most important developments in history, for rice is the longest, continuously grown cereal crop in the world.

Nutritional Value of Rice

Oryza sativa (rice) is recognized as one of the most important crops in the world and it provides the main source of energy (Table 1.1) for more than half of the world

Table 1.1 Nutritional value of white, long grain, un-enriched raw rice per 100 g/3.5 oz) (USDA Nutrient Database)

Nutrient	Amount/Percent (Percentages are relative to U.S. recommendations for adults)
Carbohydrates	79.95 g
Sugars	0.12 g
Dietary fiber	1.3 g
Fat	0.66 g
Protein	7.13 g
Thiamin	0.07 mg (5%)
Riboflavin	0.05 mg (3%)
Niacin	1.6 mg (11%)
Pantothenic acid	1.01 mg (20%)
Vitamin B6	0.16 mg (13%)
Calcium	28 mg (3%)
Iron	0.8 mg (6%)
Magnesium	25 mg (7%)
Phosphorus	115 mg (16%)
Potassium	115 mg (2%)
Zinc	1.09 mg (11%)
Manganese	1.09 mg

population. It is the major food crop in India, China and the rest of Asia where 92% of the world's rice is grown.

Cultivation Methods and Rice Farming Systems

The traditional method of cultivating rice is flooding the direct-seeded fields with or after transplanting the young seedlings. This is also known the irrigated rice commonly in most of Asia. Seeds are machine drilled in puddled soils in the United States and Australia. The other methods of rice farming systems are: the rainfed lowland rice (mainly in Africa and Madagascar), upland or dryland rice (in mountains or plateaus), and the deep water or flood-prone rice (in Bangladesh and in the Mekong, Chao Phraya and Niger deltas). The world average yield is 3.9 tons/ha. Higher yields of 9.5 tons have been harvested in Australia and lower yields of 0.70 tons/ha are harvested from traditional upland areas of Africa.

Rice Production

Rice production represents 30% of the world cereal production today. It has doubled in the last 30 years, in part due to the introduction of new varieties, but its present growth barely follows consumption. In 2025 there will be 4.6 billion people that depend on rice for their daily nourishment, compared with three billion today. A new leap in production is therefore expected. At the same time, small producers will have to use land which is less favorable for cultivation, such as brackish or briny soils, and the availability of water resources will become more and more problematic (en.wikipedia.org/wiki/Rice).

In agriculture, the term "Green Revolution" refers to the transformation of agriculture that occurred from the 1940s through the 1960s, when farmers used the discoveries of science, planting higher-yielding rice varieties to great success. In 1968, rice scientists at the International Rice Research Institute (IRRI) in the Philippines released a variety of rice that yielded 5 tons of rice per hectare with almost no fertilizer and 9.4 tons/ha with fertilizer. This was nearly 10 times the yield of traditional rice and came to be known as Miracle Rice. The introduction of IR8 and new management practices changed a hungry landscape to one of food self-sufficiency in Asia. It is difficult to overstate this achievement; rice sustains about 3.5 billion people either partially or fully for caloric intake around the world, mostly in Asia.

Production and Export

Rice is the second largest produced cereal in the world. At the beginning of the 1990s, annual production was around 350 million tons and by the end of the century

it had reached 410 million tons. World production totaled 395 million tons of milled rice in 2003, compared with 387 million tons in 2002. This reduction since the end of the previous millennium is explained by the strong pressure put on land and water resources, which led to a decrease of seeded areas in some Western and Eastern Asian countries.

Production is geographically concentrated in Western and Eastern Asia with more than 90% of world output. China and India account for more than one-third of global population (52.3% over the 1999–2003 period) and supply over half of the world's rice. Brazil is the most important non-Asian producer, followed by the United States. Italy ranks first in Europe. World production has shown a significant and very steady growth, almost exclusively due to increasing production in Western and Eastern Asia (Fig. 1.3).

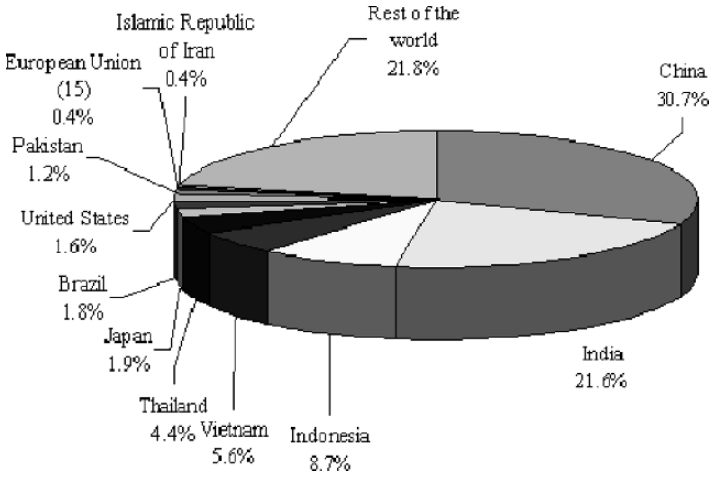

Fig. 1.3 Distribution of the world paddy rice production (average 1999–2003) (UNCTAD.org)
Source: Secretariat from the Food and Agriculture Organization of the United Nations (FAO) data

World trade figures are very different, as only about 5–6% of rice produced is traded internationally. The largest three exporting countries are Thailand (26% of world exports), Vietnam (15%), and the United States (11%), while the largest three importers are Indonesia (14%), Bangladesh (4%), and Brazil (3%). Although China and India are the top two largest producers of rice in the world, both countries consume the majority of the rice produced domestically leaving little to be traded internationally (Rice Wikipedia).

Worldwide Consumption

Between 1961 and 2002, per capita consumption of rice increased by 40%. Rice consumption is highest in Asia, where average per capita consumption is higher than 80 kg/person per year. In the subtropics such as South America, Africa, and

the Middle East, per capita consumption averages between 30 and 60 kg/person per year. People in the developed West, including Europe and the United States, consume less than 10 kg/person per year (United Nations Conference on Trade and Development, UNCTAD).

Rice is the most important crop in Asia. In Cambodia, for example, 90% of the total agricultural area is used for rice production. US rice consumption has risen sharply over the past 25 years, fueled in part by commercial applications such as beer production (UNCTAD briefing, 24 April 2008; Rice Wikipedia). Almost one in five adult Americans now report eating at least half a serving of white or brown rice per day.

Place of Rice in the Global Economy

On December 16, 2002, the UN General Assembly declared the year 2004 the International Year of Rice. The declaration was sponsored by more than 40 countries. It is one of the three major cereal grains (maize, wheat and rice) that feeds the growing population. As the population nearly doubled between the years 1961 and 1999 from 3.07 to 6.05 billion, the production of these three major cereals increased 2.5–3.0 times. IR8 was created through a cross between an Indonesian variety named "Peta" and a Chinese variety named "Dee Geo Woo Gen" (IRRI, 2006). As it was mentioned in the preceding paragraphs, the introduction of IR8 rice and new management practices changed a hungry landscape to one of food self-sufficiency in Asia and it is difficult to overstate this achievement. Rice sustains about 3.5 billion people either partially or fully for caloric intake around the world, mostly in Asia. These achievements in increasing rice production have greatly helped in hunger alleviation in the world.

The importance of rice to human life across the globe can be emphasized strongly by looking at the current global food crisis of 2007–2008. Beginning in 2007 and going forward in 2008, a global food crisis loomed as Asia's rice bowl emptied and world price soared. The crisis over rice showed no signs of easing as the price of the world's benchmark jumped 10% in just one week during April 2008, fanning fears that millions across Asia will struggle to afford their staple food. Increased food demand from rapidly developing countries, such as China and India, the use of biofuels, high oil prices, global stocks at 25-year lows and market speculation are all blamed for pushing prices of staples such as rice to record highs around the globe (Rice Wikipedia).

Rice Germplasm and Cultivars

The largest collection of rice cultivars is at the International Rice Research Institute (IRRI), with over 100,000 rice accessions held in the International Rice Genebank (IRRI, 2006). Rice cultivars are often classified by their grain shapes and texture.

For example, Thai Jasmine rice is long-grain and relatively less sticky, as long-grain rice contains less amylopectin than short-grain cultivars. Chinese restaurants usually serve long-grain as plain unseasoned steamed rice. Japanese mochi rice and Chinese sticky rice are short-grain. Chinese people use sticky rice which is properly known as "glutinous rice" (note: glutinous refer to the glue-like characteristic of rice; does not refer to "gluten") to make zongzi. The Japanese table rice is sticky, short-grain rice. Japanese sake rice is another kind as well.

Indian rice cultivars include long-grained and aromatic Basmati (grown in the North), long and medium-grained Patna rice and short-grained Sona Masoori (also spelled Sona Masuri). In South India the most prized cultivar is "Ponni" which is primarily grown in the delta regions of Kaveri River. Kaveri is also referred to as ponni in the South and the name reflects the geographic region where it is grown. In the Western Indian state of Maharashtra, a short grain variety called Ambemohar is very popular. This rice has a characteristic fragrance of mango blossom.

Aromatic rices have definite aromas and flavors; the most noted cultivars are Thai fragrant rice, Basmati, Patna rice, and a hybrid cultivar from America sold under the trade name, Texmati. Both Basmati and Texmati have a mild popcorn-like aroma and flavor. In Indonesia there are also *red* and *black* cultivars.

High-yielding cultivars of rice suitable for cultivation in Africa and other dry ecosystems called the new rice for Africa (NERICA) have been developed. It is hoped that their cultivation will improve food security in West Africa.

In a major advancement to rice science, the draft genome sequences for the two most common rice cultivars, *indica* and *japonica*, were published in 2002 (Goff et al., 2002; Sasaki, 2002; Yu et al., 2002). Rice was chosen as a model organism for the biology of grasses because of its relatively small genome (∼430 megabase pairs). Rice became the first crop whose genome sequence was fully mapped (Gillis, 2005).

Potentials for the Future

As the UN Millennium Development project seeks to spread global economic development to Africa, the "Green Revolution" is cited as the model for economic development. With the intent of replicating the successful Asian boom in agronomic productivity, groups like the Earth Institute are doing research on African agricultural systems, hoping to increase productivity. An important way this can happen is the production of "New Rices for Africa" (NERICA). These rices, selected to tolerate the low input and harsh growing conditions of African agriculture, are produced by the African Rice Center, and billed as technology from Africa, for Africa. The NERICA have appeared in *The New York Times* (October 10, 2007) and *International Herald Tribune* (October 9, 2007), trumpeted as miracle crops that will dramatically increase rice yield in Africa and enable an economic resurgence (en.wikipedia.org/wiki/Rice).

Rice Improvement Towards Nutrition Security

Improving Vitamin A Deficiency (VAD): Golden Rice

Ingo Potrykus (ZTH-Zentrum) of Switzerland and Peter Beyer of the University of Freiburg, Germany teamed up to engineer rice that will produce beta-carotene, with the intent that it might someday be used to treat vitamin A deficiency (Ye et al., 2000). In the first prototype of Golden Rice developed in 1999, two genes were inserted into the rice genome by genetic engineering, to account for the turned-off genes. This intervention leads in turn to the production and accumulation of β-carotene in the grains. The intensity of the golden color is an indicator of the concentration of β-carotene in the endosperm. According to the World Health Organization, dietary vitamin A deficiency (VAD) causes some 250,000–500,000 children to go blind each year (www.goldenrice.org).

Additional efforts are being made to improve the quantity and quality of other nutrients in golden rice (Beyer et al., 2002; Potrykus, 2001, 2003). The addition of the carotene turns the rice gold. The figures (Fig. 1.4a, b) below show the biosynthetic pathway used by researchers to engineer provitamin-A-rich golden rice (on the left) and kernels of golden rice in Indica rice background.

Improving Iron Deficiency-Ferretin Rice

Iron deficiency is widespread problem in developing countries. In particular, children appear to be much more deficient in iron. Rice varieties are low in iron. Therefore to remedy this, ferretin rices have been created by scientists at the International Rice Research Institute (IRRI) (Datta et al., 2003, 2007).

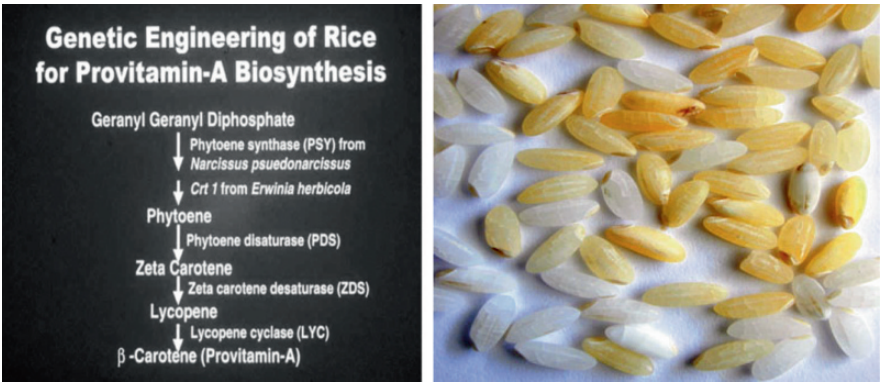

Fig. 1.4 **a**. Biosynthetic pathway for provitamin-A; **b**. Golden rice created in an indica rice background by researchers at the International Rice Research Institute (Datta et al., 2003)

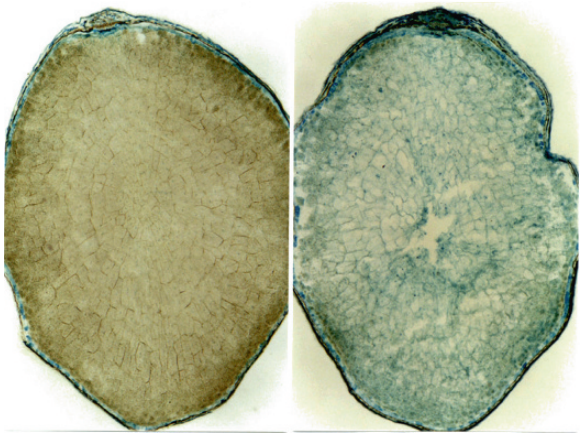

Fig. 1.5 Localization of iron in the endosperm of transgenic rice (ferretin rice) (Datta et al., 2007)

Ferretin rices are transgenic rices that have increased iron in their endosperm (Fig. 1.5).

Improving Protein Content in Rice: Expression of Human Proteins

Bethell and Huang (2004) of Ventria Bioscience genetically modified rice to express lactoferrin and human lysozyme which are proteins usually found in breast milk and have antiviral, antibacterial, and antifungal properties. Using Ventria's ExpressTec™ system, Ventria researchers expressed these two human proteins (lactoferrin and human lysozyme) in rice (Bethell & Huang, 2004). They observed that these proteins have the potential to provide not only the benefits of reduced stool volume and improved weight gain, but also shorten the course of diarrheal episodes via antimicrobial activity against the causative agent.

Rice Pests and Diseases

Entomologists at the International Rice Research Institute (IRRI) have carried out volumes of work on ecology-conscious management of different rice pests. Rice pests are any organisms or microbes with the potential to reduce the yield or value of the rice crop (or of rice seeds) (Jahn, Litsinger, Chen, & Barrion, 2007). Rice pests include weeds, pathogens, insects, rodents, and birds. A variety of factors can contribute to pest outbreaks, including the overuse of pesticides and high rates of nitrogen fertilizer application (e.g. Jahn, Almazan, & Pacia, 2005). Weather conditions also contribute to pest outbreaks. For example, rice gall midge and army worm outbreaks tend to follow high rainfall early in the wet season, while thrips outbreaks are associated with drought (Douangboupha, Khamphouko, Inthavong, Schiller, & John, 2006).

One of the challenges facing crop protection specialists is to develop rice pest management techniques which are sustainable. In other words, to manage crop pests in such a manner that future crop production is not threatened (Jahn et al., 2001, 2007). Rice pests are managed by cultural techniques, pest-resistant rice varieties, and pesticides.

Major rice pests include the brown planthoppers (Preap, Zalucki, & Zahn, 2006), armyworms, the green leafhopper, the rice gall midge (Jahn & Khiev, 2004), the rice bug (Jahn, Domingo, Almazan, & Pacia, 2004), hispa (Murphy et al., 2006), the rice leaffolder, stemborer, rats (Leung Peter, Cox, Jahn, & Nugent, 2002), and the weed *Echinochloa crusgali* (Pheng, Khieve, Pil, & John, 2001). Rice weevils are also known to be a threat to rice crops in the US, China and Taiwan.

Major rice diseases include, sheath blight (ShB), blast (bl), bacterial leaf blight (BB) and tungro (RTD) virus (Ou, 1985). Rice diseases, rice pathogen populations and biological disease control are the central themes of this book. These are dealt with in greater detail in separate, later chapters of this volume.

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Chapter 2
Major Diseases of Rice

The purpose of this chapter is to introduce the reader to the major or devastating diseases of rice. In the Fig. 2.1, three fungal diseases, blast, sheath blight and sheath-rot, the bacterial disease, bacterial blight (BB) of rice and the viral disease, rice tungro disease (RTD), are listed as major diseases of rice. The list may not be entirely correct for certain rice ecologies of the world. In Asia where more than half of the world's rice is produced and consumed, these five diseases are major production constraints. These are also diseases for which lots of scientific information is

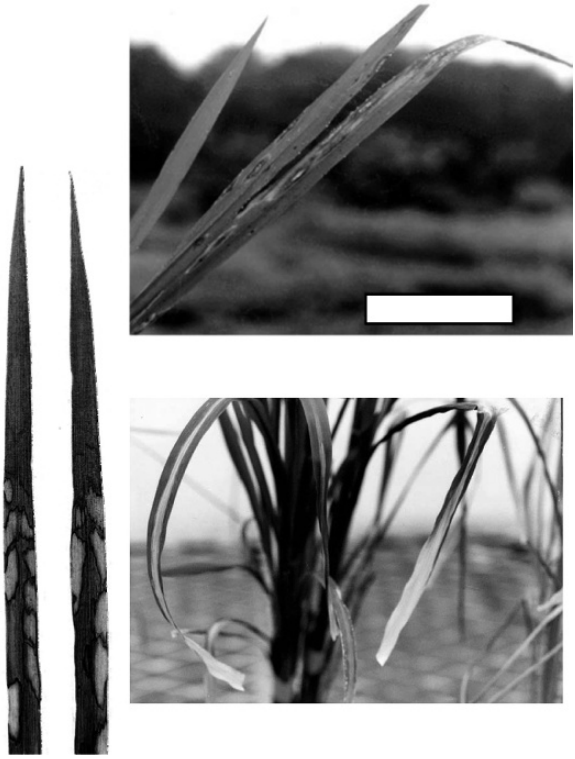

Fig. 2.1 Major diseases of rice. Fungal: *Blast* (Bl), *Sheath bligh* (ShB), & *Sheath-rot* (Sh-R). Bacterial: *Bacterial blight* (BB). Viral: *Rice tungro disease* (RTD)

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available because they have been studied in detail due to the devastations they cause to rice production.

In the following pages the importance of each of these diseases is described. The description covers the pathogen, prevailing pathogen population, symptoms, crop losses, and methods of disease management. Disease management or control practices do not include biological control which is the theme of this book. Biological control of these diseases and their pathogens are dealt with in separate Chapters 3–8 of the book.

Blast (Bl)

Pathogen: Magnaporthe oryzae

Importance of the Disease and the Causal Agent

Blast is considered the principal disease of rice because of its wide distribution and high incidence under favorable conditions. Valent (2004) considered the disease as the world's chief disease of rice about which a lot has to be learned yet. The disease is distributed in about 85 countries in all continents where rice is cultivated. It is a potentially damaging disease in upland environment where drought and soil stress predispose the rice crop to severe attacks by the pathogen. Yield loss due to blast can be as high as 50% when the disease occurs in epidemic proportions. The damage to the rice crop is often influenced by environmental factors. Rice blast disease finds its place in biological terrorism because of the potential devastation it can cause to rice production.

Causal Organism

On the basis of a multilocus gene geneology analysis, the correct name of the rice blast fungus, known earlier (until 2002) as *Magnaporthe grisea* (Hebert) Barr (syn: *Pyricularia grisea* Sacc.), was described as *Magnaporthe oryzae*. It is a new species (*Magnaporthe oryzae* B. Couch sp. Nov.), distinct from *M. grisea*, now considered a genus complex consisting of *M. oryzae* (rice and closely related grass/weed isolates) *and M. grisea* (*Digitaria* type grass isolates) (Couch & Kohn, 2002). This filamentous heterothallic ascomycetous fungus is the causal organism of blast. The genus *Magnaporthe* collectively paratisizes more than 50 hosts, while individual isolates have limited host range and cross-infectivity is relatively rare.

In another significant recent advancement, the genome sequence of the rice blast fungus was produced by Dean et al. (2005). Their reported draft sequence of the *M. grisea* genome and analysis of the gene set provides an insight into the adaptations required by a fungus to cause disease. The genome encodes a large and diverse set of secreted proteins, including those defined by unusual carbohydrate-binding domains. This fungus also possesses an expanded family of G-protein-coupled

receptors, several new virulence-associated genes and large suites of enzymes involved in secondary metabolism. Consistent with a role in fungal pathogenesis, the expression of several of these genes is upregulated during the early stages of infection-related development. The *M. grisea* genome has been subject to invasion and proliferation of active transposable elements, reflecting the clonal nature of this fungus imposed by widespread rice cultivation.

The ability of this fungus to quickly overcome resistance within a short time after the release of a new cultivar has made breeding for resistance a constant challenge. An understanding of the structure and dynamics of pathogen population is essential for prudent implementation of strategies for management of the disease. Such molecular data have been used to develop concepts for breeding for blast-resistance. In a comprehensive overview Babujee and Gnanamanickam (2000) documented the information on molecular methods that have been used to characterize the genetic variation of *M. oryzae*, including the DNA fingerprinting tools for the pathogen. The extent of genetic variation and instability in *M. oryzae* has been a topic of long-standing debate among blast researchers; only few believed the organism was stable. Theories centered on mitotic recombination parasexual recombination, hyphal fusion, etc. were advanced to explain the high levels of variation encountered in the blast pathogen. Considerable effort has indeed gone into designing new strategies to understand and document genetic variation in *M. oryzae* (reviewed by Babujee & Gnanamanickam, 2000).

Parts of Rice Plant Infected and Symptoms

The fungus, *M. oryzae*, infects all parts of the rice plant, including the roots (Sesma & Osbourn, 2004). The common symptoms that are observed frequently in an infected rice field are the leaf blast, neck blast (Figs. 2.1 and 2.2) and the panicle blast. Of these, neck blast (Fig. 2.2) causes most damage to rice production. Detailed descriptions of these symptoms have been recorded (Ou, 1985; IRRI,

Fig. 2.2 Neck blast symptoms

2003). The blast fungus also causes the collar rot and nodal blast symptoms which are less common than the leaf and neck blast symptoms. Sesma and Osbourn (2004) made a landmark discovery and reported that the leaf blast fungus infects rice roots and through root invasion becomes systemic in the plant to be able to cause characteristic symptoms on the aerial parts. These findings have significant implications for fungal development, epidemiology, plant breeding and disease control.

Disease Cycle

The traditional picture of blast disease cycle has dramatically changed with the new knowledge that *M. oryzae* invades the rice roots, can become systemic and causes aerial symptoms (Sesma & Osbourn, 2004). The disease cycle can begin either with root infection or with a conidium that lands on young rice foliage of a susceptible genotype to begin a new leaf blast lesion development. Under optimal conditions of prolonged leaf surface wetness and cooler night temperatures (12–32 °C), the infection cycle continues. It is known that a single leaf blast lesion can generate 20,000 conidia and an infected rice spikelet can produce up to 60,000 conidia in a night (Kato, 2001) to keep the leaf blast infection cycle going. It is also common knowledge that that an incidence of about 5% or more of leaf blast infection can lead to neck blast incidence in the same crop (Fig. 2.2 shows neck blast incidence in a rice field in Kerala, southern India). Neck blast is the most serious phase of devastation as the broken necks lead to dead panicles and chaffy grains. Between cropping seasons, the pathogen survives or over-winters (in temperate zones) on residues of diseased rice plants, ratoons of stubbles, infected seeds and on alternate weed hosts (Kato, 2001; Ou, 1985).

Management of Blast

In developing countries, poor farmers cannot afford to control blast disease by the application of chemicals and pesticides. Chemical control of plant pathogens is most effective and yet the use of chemicals is not generally desired due to the serious environmental threat it poses. Besides, their continuous use leads to the resurgence of resistant races of the pathogen under selection pressure. However, chemical method of rice blast control has been effective and therefore, it is believed that they will have a role in fighting blast, particularly in countries where farmers can afford the cost of treatments.

Many fungicides are routinely used to control rice blast. These include benomyl, fthalide, edifenphos, iprofenphos, tricyclazole, isoprothiolane, probenazole, pyroquilon, meferimzone, diclocymet, carpropamid, fenoxanil, and metominostrobin along with antibiotics such as blasticidin and kasugamycin (Kato, 2001). These chemicals belong to the following classes: plant activator (probenazole), choline

biosynthesis inhibitors and melanin biosynthesis inhibitors (tricyclazole). Their continued use for a number of years illustrates the value of retaining different classes of chemicals to interfere with the resurgence of resistance in *M. oryzae* (Kato, 2001).

Resistant Cultivars

Use of resistant cultivars is the best alternative to overcome yield losses. The variability of the pathogen and the history of resistance breakdown have led to the development of a number of different plant breeding approaches to achieve durable blast resistance.

Resistance (R) Genes and Cloned R Genes

Resistance to blast may be conditioned by major genes or by quantitative trait loci (QTLs). Over 25 blast *R* genes have been mapped on the rice genome, many of which are allelic or closely linked (Kiyosawa, 1989; Inukai, Nelson, Zeigler, Sarkarung, & Mackill, 1994; Wang, Mackill, Bonman, McCouch, & Nelson, 1994; Pan, Wang, Ikehashi, & Tanisak, 1998; Chao, Moldenhauer, & Ellingboe, 1999) For example, 5 blast *R* genes have been identified at the *Pi-k* locus of chromosome 11 (Kiyosawa, 1989; Inukai et al., 1994). *Pi-ta* and $Pi\text{-}ta^2$ are allelic or at least very close to each other in the centromere region of chromosome 12 (Rybka, Miyamoto, Ando, Saito, & Kawasaki, 1997), while *Pi5(t)* and *Pi3(t)* map at the same location on rice chromosome 5 (Inukai et al., 1996; Jeon, Chen, Yi, Wang, & Ronald, 2003). In recent years, 5 blast *R* genes, *Pib* (Wang et al., 1999), *Pi-ta* (Bryan et al., 2000), *Pi36* (Liu, Lin, Wang, & Pan, 2007), *Pi37* (Lin et al., 2007) and more importantly, the broad-spectrum R gene, *Pi9* (Qu et al., 2006), have been cloned. *Pib, Pi9* and *Pi37* are members of the nucleotide-binding site-leucine rich repeat (NBS-LRR) class of genes, a multi-gene family of rice (Wang et al., 1999; Qu et al., 2006). *Pi-ta* differs from *Pib* and *Pi9*, the NBS–LRR genes in that the protein predicted to be encoded by *Pi-ta* lacks a classic LRR in its C-terminal region, containing instead a highly imperfect repeating structure with 10 repeats of various lengths (from 16 to 75 amino acids), referred to as a leucine-rich domain (LRD) (Bryan et al., 2000). Rice transformation with individual candidate genes determined that *Pi9* transgenic line had the exact resistance spectrum with the *Pi9* donor line (Qu et al., 2006).

Gene Pyramiding

Durable resistance is that which remains effective while a cultivar possessing it is widely cultivated. Gene pyramiding is one of the strategies recommended to increase durability of resistance. This term refers to the combining of two or more major genes for resistance in a single plant genotype. While the use of single major genes limits the useful life span of resistant cultivars to few years, gene pyramiding could delay resistance breakdown by conferring “horizontal resistance” effective against all prevalent pathotypes of a pathogen.

Lineage-Exclusion Hypothesis

The organization of the blast fungus population into well defined lineages and their distribution in specific geographic locations have led researchers to employ resistance genes targeted against pathogen populations prevalent in that region. This concept was proposed by Zeigler, Leong, and Teng (1994), and has been called the "lineage-exclusion" hypothesis. In many regions, it might be useful to combine or pyramid two or more genes in a cultivar since resistance genes effective against members of a lineage might not be so against members of another lineage. On the other hand, the combination of resistance genes can confer resistance to the entire population by effective complementation. This strategy thus allows judicious use of host resistance, which is essential for resistance to be durable. Lineage-exclusion presumes that lineage-specific avirulences represent an evolutionary genetic barrier to pathotype diversification within the lineage. The pyramided resistance (for instance, with *Pi*-1 and *Pi*-2 blast resistance genes) will be durable in places where compatibility to the component resistance genes is distributed among the prevalent lineages (Fig. 2.3).

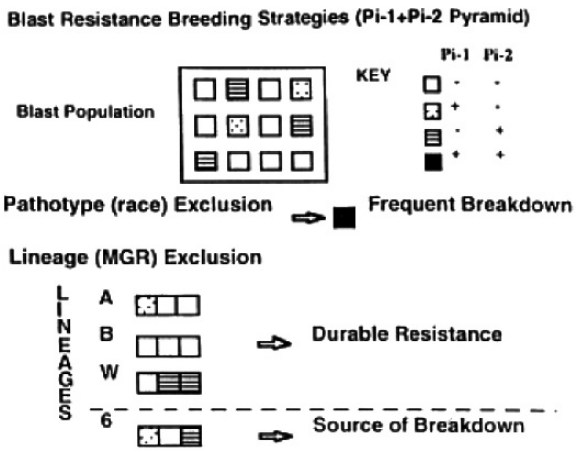

Fig. 2.3 Blast resistance breeding strategies using a two-resistance gene *Pi*-1 + *Pi*-2, pyramid against a rice blast population with isolates that are differentially compatible with each resistance gene. Conventional pathotype (race) exclusion directed against pathotypes not yet detected, leads to frequent breakdown. Lineage-exclusion directed against the distribution of virulence and avirulence among genetic lineages predicts durable resistance when each lineage is avirulent with at least one component resistance (Sivaraj et al., 1998)

Breeding for Resistance and Marker-Assisted Selection

A breeding program with blast resistance as its principal objective should be structured such that major genes are combined to exclude the known lineages in a target region, and supported by a high level of general resistance conferred by QTLs. In breeding for disease and pest resistance at present, the segregating populations derived from crosses between the resistant sources and otherwise desirable and productive genotypes are selected either under natural disease or pest hotspots or under

artificially created disease and pest nurseries or by infecting individual plants under controlled environments. These procedures are time consuming and expensive and are prone to be ambiguous. Besides, there are always susceptible plants that escape attack. Screening of plants with different pathogens and their pathotypes simultaneously or even sequentially is difficult if not impossible.

Molecular markers offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait. Molecular markers are especially advantageous for agronomic traits that are otherwise difficult to tag such as resistance to pathogens. Durability of resistance has been increased in several crops by incorporating genetically diverse major resistance genes. Marker-assisted selection (MAS) is of enormous use in gene pyramiding where the presence of more than one gene has to be confirmed. Jena and Mackill (2008) have provided a complete review of the information on molecular markers and their use in marker-assisted selection in rice.

With the use of molecular techniques, it would now be possible to hasten the transfer of desirable genes among varieties. Techniques which are particularly promising in assisting selection for desirable characters involve the use of molecular markers such as RAPD, RFLP, microsatellites, AFLP, and PCR-based DNA markers such as SCAR, Sequence Tagged Sites (STS), Cleaved Amplicon Polymorphisms (CAPs) Etc. Detailed reviews on the application of these techniques in plant improvement are available (see Jena & Mackill, 2008). The potential benefits of MAS strategy have been widely discussed but actual examples of the application of this approach are few at present.

Crop Diversification as an Ecological Method of Blast Control (Zhu et al., 2000)

By establishing a unique cooperation among farmers, researchers and extension personnel in Yunnan Province of China, genetically diversified rice crops were planted in all the rice fields in five townships during 1998 and ten townships during 1999. Control plots had monocultured crops. Disease-susceptible rice varieties planted in mixtures with resistant varieties had 89% greater yield and 94% less blast severity than in monocultured control (Zhu et al., 2000). Biological control is described in Chapter 4 of this volume.

Concluding remarks. Rice blast is a much dreaded plant disease of very serious nature and character. Perhaps, there is so much more to be learned about the rice blast fungus, *M. oryzae*. For disease management, it can be said that the lineage-exclusion approach which in some ways is similar to the crop diversification method can be an effective strategy to manage rice blast, the success of which hinges on the extent of knowledge about the structure and dynamics of pathogen population. In order to facilitate greater understanding of the genetics of pathogenesis and host–plant resistance and to serve as a guide to breeders for resistance gene deployment, a rice blast database has been created (Yap et al., 1998). Blast disease

can be controlled by the deployment of resistant cultivars or by using a variety of methods listed in previous paragraphs in an integrated management.

Sheath Blight (ShB)

Pathogen: Rhizoctonia solani Kuhn [Thanatephorus cucumeris (Frank) Donk].

Rhizoctonia solani, the widespread destructive and versatile plant pathogen was first observed by Julies Kuhn in the year 1958. Earlier French mycologist De Condolle (1815) had described the genus *Rhizoctonia* for the violet root rot organism. It is a filamentous Basidiomycete fungus. This group of fungi are found in all parts of the world and they have the capability to attack a wide range of hosts causing seed decay, damping-off, stem canker, root rots, fruit decay and a number of foliage diseases. The competitive saprophytic ability with lethal pathogenic potential to attack wide range of host makes this fungus *R. solani*, a highly destructive pathogen. A close correlation occurred between the severities of sheath blight (ShB) development and yield loss in rice (Hori, 1969; Hashiba, 1984). The yield loss may be more than 50% in susceptible cultivars when all the leaf sheaths and leaf blades are infected (Lee & Rush, 1983). On an average, 20–50% annual yield losses caused by ShB were reported in both tropical and temperate conditions (Miruta, 1956; Boyatee & Lee, 1979; Rajan, 1987).

The possible involvement of toxin(s) has been discussed and debated for many years. Production of a host-specific toxin by isolates of the sheath blight pathogen, *R. solani* was announced by Vidhyasekaran et al. (1997). By this we understand that this toxin is the primary determinant of ShB symptom development in rice.

Symptoms

Seedlings rarely show the symptoms of sheath blight disease. But under very high humidity and at favorable temperatures even the seedlings may get wilted. The characteristic symptoms of the disease are water-soaked, circular to oblong, ellipsoid to ovoid or even irregularly elongated discolored lesions on the leaf sheath at or above the water level in lowland and at ground level in upland fields (Fig. 2.4). The natural infection usually starts during the tillering stage (Premlatha Dath, 1990).

Under favorable conditions of microenvironments, the disease progression is of three types:

(i) Inward spread – from outer to inner sheaths with bleached center and irregular purple brown border (Ou, 1985).
(ii) Vertical spread – a rapid upward spread that invades the lamina, loosens the sheath from the culms and causes blight of boot, flag leaf and panicle with

Fig. 2.4 Symptoms of rice sheath blight in rice fields of Kerala, India (Immanuel, 2006)

ultimate lodging of plants. Grains become chaffy or partially filled; particularly in the lower part of the panicle and entire panicle may even be matted together (Singh, Devi, Singh, 1988; 1989; Ou, 1985).

(iii) Horizontal lateral spread – the disease spreads from the tiller to hill apparently by physical contact in a densely crowded planting. Under moist conditions cobweb like mycelia spread externally and sclerotia are formed superficially on these spots. Sclerotia are initially white but turn brown at maturity. Mature sclerotia, loosely attached to the developed lesions fall down to the ground and function as a source of inoculum for the next season. Depending upon the source of inoculum and growth stage of the crop, different types of symptoms may be produced, of which sheath blight is the most prominent one (Acharya, Basu, & Sengupta, 1997).

Host Range

R. solani isolated from rice had been shown to infect many other plants too and similar fungi isolated from other crops were able to infect rice (Yokogi, 1927; Tervet, 1937; Ryker, 1939; Sato & Shoji, 1957). Other host plants include sugarcane, bean, soybean, tomato, egg plant, tobacco, water hyacinth (Matsumoto & Hirane, 1933), hyacinth bean and green gram (Padwick, 1950). Kozaka (1965) stated that plants of 188 species in 32 families were infected by the rice isolates of *R. solani*. Water hyacinth, a common aquatic weed was reported to be the possible host of *R.*

solani by Talukdar (1968). Tsai (1970) reported that the rice fungus also infected 20 species of weeds that belonged to 11 families.

Distribution of the Disease

Reinking (1918) and Palo (1926) found the disease in the Philippines and defined that the fungus belongs to the *Rhizoctonia solani* group. Park and Berks (1932) reported the disease in Sri Lanka and called the organism as *Rhizoctonia solani* Kuhn. Its occurrence was also reported from Burma, Indonesia, Iran, Korea, Liberia, Thailand, Malaysia, China (Chin, 1976), Africa, Brazil, Surinam, Venezuela, Madagascar, USA and many countries in Asia. Paracer and Chahal (1963) first reported the occurrence of ShB from Gurdeespur in Punjab. Singh and Pavji (1969) reported the widespread occurrence of the disease in Varanasi (Uttar Pradesh). Now the disease is prevalent in most of the rice growing states in India, and in Kerala it was found to be one of the major constraints in rice production in the region of Kuttanad (Rajan, 1981).

Disease Cycle

Disease development is most rapid during early heading and grain filling stages. The pathogen survives between crops as soil-borne sclerotia and as mycelium in plant debris and this constitutes the primary inoculum for the disease (Ou, 1985).

The fungus is attracted to the plant by chemical stimulants released by actively growing plant cells and by decomposing plant residues. Mature sclerotia germinate and initiate infection when they come in contact with the rice plant. The germinating hyphae produce an infection cushion (Matsumoto & Hirane, 1933) on the leaf sheath, from which haustoria grow and penetrate the host tissue (Dodman & Flentje, 1970; Marshall & Rush, 1980). The infection of the host tissue is either through cuticle or through stomata. The mycelium first ramifies from the outer surface of the sheath to the inner surface resulting in formation of primary lesions. Later the mycelium grows rapidly on the surface of the plant and inside its tissue proceeding upward as well as laterally and initiates secondary lesions. Kobayashi, Mew, and Hashiba (1997) confirmed the existence of mycelium in the plant debris by isolating the pathogen from debris containing ShB infected rice remains.

Characteristics of the Pathogen

The distinct characteristics of *R. solani* are, (i) pale to brown, fast growing mycelium with branching near the distal septum of hyphal cells; (ii) constriction of branch hyphae at the point of origin and formation of a septum in the branch near it; (iii) formation of monilioid cells, often called chlamydospores or sporodochia; (iv) production of sclerotia of nearly uniform texture and varying size and shape; (v)

possession of prominent septal pore apparatus, and (vi) possession of basiodiomycetous perfect stage (Ou, 1985).

The basic color of *R. solani* colony is brown. In the substrate the mycelium is generally hyaline and irregular in shape. The mycelium of *R.solani* is colorless in young cultures and yellow-brown in old cultures. The hyphae have a prominent dolipore septum. The hyphae are multinucleated and are generally 8–12 μm in diameter. The young hyphae are typically branched at 45° or 90° angles and hyphal branches are constricted at the point of origin. The pathogen produces three types of specialized mycelium. They are runner hyphae, lobate hyphae and monilioid cells.

The runner hyphae have thick, parallel walls and spread rapidly over the sheath and leaf surface of the rice plant. On the host the runner hyphae give rise at intervals to swollen, lobate hyphae (appressoria) or masses of broad cells produced in short chains that continue to form sclerotia.

Hymenia of *T. cucumeris* are effuse cream to grayish-white in color and composed of a spidery network of hyphae from which loose patches of basidia arise in clusters. Basidia are barrel shaped, slightly wider than the supporting hyphae and have an average size of 14 μm. Sterigmata are long, stout, tapered, usually four (rarely two or three) per basidium and have an average size of 13.5 μm. Basidiospores are hyaline, oblong to ellipsoid, often with flattened side and a prominent truncate apiculus. These measure usually 9 μm in size and are capable of repetitive germination.

In general growth rates of the isolates of *R. solani* may vary a few hundredth of a millimeter per hour to at least 1 mm per hour. The minimum, optimum and maximum pH for the growth of ShB fungus are 2.5, 5.5 and 7.8 respectively (Endo, 1935). Misawa (1965) reported that pH affects the utilization of carbon source in the medium. Optimum growth rate occurs between 20–30 °C. Hemmi and Endo (1931) found that sclerotia were formed most abundantly in light. Inoue and Uchino (1963) reported that no sclerotia were formed on media containing ammonium sulfate and peptone as nitrogen sources. The size and the number of sclerotia formed on agar plates were affected by the carbon and nitrogen sources present in the medium (Santos, 1970).

Pathogen Populations

R. solani, a ubiquitous pathogen, incites one of the most serious diseases of rice, sheath blight. Its pathogen population world-wide has been described as 14 anastomosis groups (AGs) to date. Among these, isolates of AG1-IA have been associated with rice sheath blight pathogen. *R. oryzae* and *R. oryzae-sativae*, causal agents of sheath spot and aggregate sheath-spot respectively are known to occur in California, Argentina and East Asia and they both produce lesions on the rice leaf sheath similar to those of sheath blight. In addition to the similarity of disease symptoms, distinguishing the species is difficult due to the lack of stable morphological characters on which to base a definitive classification of the genus *Rhizoctonia* and

species assigned to it. Also, identification of three intraspecific groups (ISGs) of AG1 based on anastomosis grouping on slide is not accurate because of AG1-IA isolates fuse very well with not only with other isolates of IA, but also with isolates of IB and IC. This difficulty observed in *R. solani* pathogen populations demanded the need for the use of molecular tools to analyze their population structure more precisely.

The population structure of *R.solani* in India has been analyzed by the use of different tools in order to understand and identify the virulence patterns. Lack of this information makes breeding for ShB resistance difficult. Neeraja, Vijayabhanu, Shenoy, Reddy, and Sarma (2002) assessed the variability in 18 pathogen isolates collected from different rice growing regions of India by characterizing their electrophoretic profiles for 13 isoenzymes and virulence to rice (cv. IR50, susceptile and Swarnadhan, less susceptible/tolerant). Sixteen of the virulent pathogen isolates formed a major cluster while two of the avirulent isolates formed a second group. These researchers concluded that isozymes, esterases (both α and β) and 6-phosphogluconic dehydrogenase could be used to fingerprint the individual isolates.

Two recent studies carried out by Gnanamanickam and his collaborators to determine the genetic structure of *R. solani* populations in southern India by sing molecular tools. Linde, Zala, Paulraj, McDonald, and Gnanamanickam (2005); David Paulraj (2003) evaluated the population structure of 96 isolates of *R. solani* AG-1 IA that caused sheath blight symptoms on rice using RFLP loci. Nineteen of the isolates did not hybridize to AG-1 IA-specific RFLP probes and these were either characterized as isolates of *Ceratobasidium oryzae-sativae* or another *Rhizoctonia* sp by *rDNA* analyses. The remaining 77 isolates of AG1 IA from southern India conformed to the previously characterized Texas population (Linde et al., 2005).

Clonal dispersal of *R. solani* isolates within rice fields in southern India was moderate and no clones were shared among field populations. These observations on low levels of population subdivision and small genetic distances were consistent with high levels of gene flow. The southern Indian population also had frequent sexual reproduction indicated by their Hardy-Weinberg equilibrium (HWE). Although the Indian and Texas populations were geographically very distant, they both exhibited only moderate population subdivision, with an F_{ST} value of 0.193.

The above *R. solani* populations from southern India were analyzed differently by Taheri et al. (2007). When conventional and polymerase chain reactions were used 99 of 110 isolates were identified as *R. solani* (96 were AG1-IA, 1 was AG1A-IB and 2 were AG1-IC) and 11 of 110 isolates were *R. oryzae-sativae*. Amplified fragment-length polymorphism (AFLP) was used to delineate sheath blight-inducing isolates of AG1-IA, IB and IC and also to differentiate these from aggregate sheath-spot inducing *R. oryzae-sativae* isolates (Fig. 2.6). The distinguishing symptoms were observed after rice plants (cv. Zenith) were artificially inoculated with different isolates of *R. solani* or *R. oryzae-sativae* (Fig. 2.5) (Taheri et al., 2007).

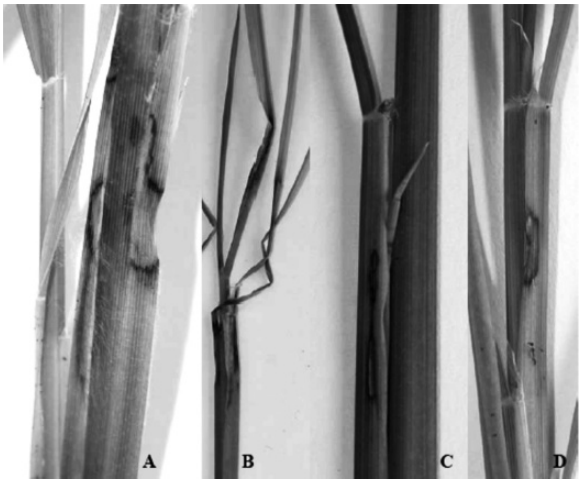

Fig. 2.5 Sheath blight symptoms induced on rice (cv. Zenith) by Indian isolates of (A) *R. solani* AG1-IA, *R. solani* AG1-IB and (C) *R. solani* AG1-IC. D represents aggregate sheath-spot symptoms initiated by an isolate of *R. oryzae-sativae* (Taheri, Gnanamanickam, & Hofte, 2007)

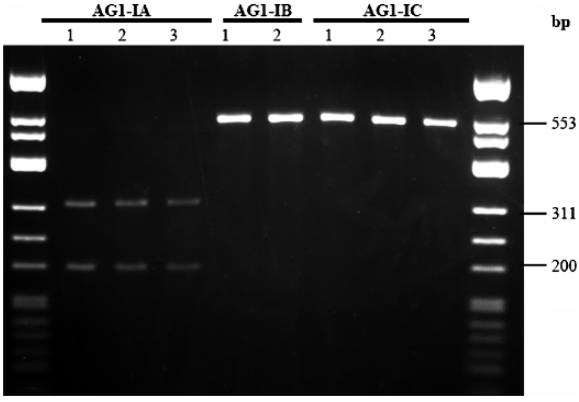

Fig. 2.6 Restriction patterns obtained by RFLP analysis of ITS regions of southern India *R.solani* isolates of AG1-IA, IB and IC amplified with primers RS1 and RS4 digested with *MunI*. (Taheri et al., 2007)

These studies demonstrate the complex genetic structure of *R. solani* isolates that form the rice sheath blight-sheath spot-aggregate sheath spot disease complex and point to the usefulness of the some of the molecular tools that can be deployed to identify isolates within this population (Fig. 2.6).

Disease Management

Lack of adequate levels of ShB resistance in rice is a major constraint in sheath blight management. The other factor which influences disease management is the host range and variability of the pathogen. Cultural practices, chemical control and biological control are the strategies used in disease management. Several studies have shown the potential of rice ShB control with plant-associated strains of *Pseudomonas*. These and cultural practices are discussed in chapter 6 on biological control of sheath blight.

Chemical Control

Chemical control offers a significant level of disease reduction. Organic mercury compounds were used at first, but later organic arsine compounds and organic tin compounds were found to be more effective. Fungicides like bavistin, hinosan, daconil and thiabendazole were also effective in controlling sheath blight (Roy, 1981). Validamycin and polymyxin produced by *Streptomyces* sp. have also been commercially used in Japan to control the disease. In addition, fungicides like triazole and flutolanil were also effective (Suryadi & Kadir, 1989). Recently a systemic fungicide kitazin has been demonstrated to offer reduction of ShB disease (Bera & Purkayastha, 1999). Bavistin and hinosan are effective but induced abnormalities in the hyphal growth of *R. solani* (Roy, 1981). Behera, Dash, and Mishra (1982) reported that bavistin and benlate were most effective in inhibiting growth and mycelial dry weight of *R. solani*. Agallol and bavistin totally killed the sclerotia at 1000 ppm as reported by Upadhyay and Singh (1985).

Bacterial Blight (BB)

Pathogen: Xanthomonas oryzae pv. oryzae

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) Swings et al. (1990), is one of the most important and very serious diseases of rice. BB is also one of the oldest known diseases and was first noticed by the farmers of the Fukuoko area, Kyushu, Japan as early as 1884 (Tagami & Mizukami, 1962). Subsequently, its incidence has been reported from different parts of Asia, Northern Australia, Africa and United States of America. BB, therefore, occurs globally and its distribution ranges from 20 °S in Queensland, Australia to 58 °N in Heilang Jiang, China (Mew, 1989). Considerable research on the disease and its causal agent has been carried out and the information has been reviewed (Mizukami & Wakimoto, 1969; Mew, 1987; Ou, 1985; Mew, 1989; Mew, Alvarez, Leach, & Swings, 1993; Subramoni, Jha, & Sonti, 2006; Gnanamanickam, Priyadarisini, Narayanan, Vasudevan, & Kavitha, 1999; Sridhar, 2002).

BB Pathogen: Morphology and Taxonomy

The causal bacterium of rice bacterial leaf blight *Xanthomonas oryzae* pv. *oryzae*, has cells that are short rods with round ends, 1–2 × 0.8–1 μm, with monotrichous flagellum of 6–8 μm × 30 nm. The organism is gram negative and non-spore forming (Ishiyama, 1922). Bacterial cells are surrounded by mucous capsules. Colonies are circular, convex, whitish to straw yellow with a smooth surface, entire margin and opaque against transmitted light. The flagellum is 8.75 μm × 30 nm.

The bacterial nature of leaf blight was established by Japanese scientists in as early as 1920s. It was initially named as *Bacillus oryzae* by Hori and Bokura in 1911 and has been reclassified numerous times thereafter (Mew et al., 1993). With the establishment of the pathovar system, it was named as *Xanthomonas campestris* pv. *oryzae* and was distinguished from the leaf streak pathogen, *X.c* pv. *oryzicola* (Vera Cruz et al., 1984). Later, Swings et al. (1990) found that the leaf blight and leaf streak pathogens were distinct from other *X. campestris* pathovars and proposed that they can be reclassified as a separate species, *X. oryzae*, consisting of pathovars *oryzae* and *oryzicola*. The taxonomic position of *Xanthomonas oryzae* has been confirmed by analyzing the DNA homology, protein and fatty acid profiles of *Xanthomonas* strains belonging to different pathovars (Vauterin, Hoste, Kesters, & Swings, 1995; Vauterin, Rademaker, & Swings, 2000) and stands acceptable till date.

Xanthomonas oryzae pv. *oryzae* is a yellow, gram-negative bacterium producing copious amounts of extracellular polysaccharides (EPS). EPS deficient mutants are characterized by their inability to produce BB symptoms on rice plants (Subramoni, Jha, & Sonti, 2006). Apart from the conventional pathotyping, several other novel genetic and serological tools like monoclonal antibodies (Gnanamanickam, Shigaki, Medalla, Mew, & Alvarez, 1994) and PCR-based markers have aided the detection and analysis of serological and genetic diversity of the pathogen population (Gnanamanickam, Priyadarisini, Narayanan, Vasudevan, & Kavitha, 1999; Rajebhosale et al., 1997; Shanti et al., 2001).

Symptoms

Bacterial blight is a vascular disease resulting in a systemic infection of rice (Mew, 1987) and it produces tannish-grey to white lesions along the veins. Symptoms are observed at the tillering stage, disease incidence increases with plant growth, peaking at the flowering stage (Mew et al., 1993). There are two different phases of BB disease, the leaf blight phase and the kresek phase. Kresek is the most destructive manifestation of the disease, wherein the leaves of the entire plant turn pale yellow and wilt during the seedling to the early tillering stage, resulting in a partial or total crop failure. Young plants of less than 21 days old are the most susceptible to kresek that is favored by temperatures between 28 °C and 34 °C (Mew, Vera Cruz, Reyes, & Zaragosa, 1979; Mizukami & Wakimoto, 1969). Leaf blight phase of BB has characteristic yellow lesions with wavy margins on leaf blades (Fig. 2.1).

The occurrence of bacterial ooze from infected leaves has been observed in warm and humid climates, which contributes to the spread of this disease. Though leaf blight does occur at all growth stages, it is very common from maximum tillering to maturity. While damage is extensive when kresek precedes bacterial blight, post flowering infections have very little effect on grain yield. However, when infection occurs during panicle initiation or subsequently during stages that precede flowering, a severe impairment of grain development and a consequent increase in sterility has been observed.

Yield Losses

Bacterial blight occurs worldwide and is particularly destructive in Asia during the heavy rains of the monsoon season. In many Asian countries, the disease has become endemic on rice following repeated cultivation. The disease can reduce grain yields to varying levels, depending on the stage of the crop at the time of infection, the degree of cultivar susceptibility and to a great extent, the conduciveness of the environment it occurs. Severe crop losses of 10–20% in moderate conditions (Ou, 1985), or up to 50% in highly conducive conditions (Mew et al., 1993) have been recorded in several parts of Asia and South East Asia.

In Japan the yield losses in severely infected fields were estimated to be 20–30% or as high as 50% on several occasions. In the tropics, the disease has been very destructive where millions of hectares of rice are severely affected. In India, losses in yield varied from 6 to 60% in most of the rice growing states (Srivastava, 1967). The yield loss was maximum in cv. Bala (74.20%) followed by TN1 (57.75%) and least in CR44-45 (6.12%). The reduction in yield was mainly due to a reduction in panicle number and grain weight and increase in chaffy grains. Rao and Kauffman (1977) reported insignificant yield loss in IR20 and greater losses in two other rice cultivars, in IR8 (10%) and Karuna (56%).

Disease Cycle

The soil is not considered as an important source of inoculum (Tagami et al., 1963; Srivastava, 1967). The bacterium can survive in soil only for one to two months (Wakimoto, 1956). It can survive in a dry form on seeds from infected plants, stored rice straw and rice stubble. This dry form of bacteria becomes activated by moisture. Growth form bacteria are normally found in stubble and in some susceptible grasses, especially *Leersia* sp., *Leptocloa chinensis*, *Cyperus rotundus* etc., which serve as alternate hosts. While seed-borne nature of the pathogen is certain (Unnamalai, 1987), the hypothesis that the disease is seed-transmitted consistently from infected seed has not been positively proved. Sensitive PCR-based assays for the detection of the pathogen using primers based on the repetitive element probe *IS113* could not detect the pathogen DNA from seeds collected from infected plants (Gnanamanickam, Sakthivel, Alvarez, Benedict, & Leach, 1996).

The bacterial blight pathogen enters through natural openings like hydathodes and stomata as well as through wounds (Mew, Mew, & Huang, 1984). Upon entry into the host, the pathogen reaches the vascular tissue, particularly the xylem, from where it multiplies and spreads throughout the plant, resulting in a systemic infection. Bacteria in the ooze spread the pathogen across fields via irrigation water, rain and wind. Irrigation water is considered to contribute to the spread of this disease over large areas of cultivated land, as it carries the bacterial ooze that drop into rice field water. However, the role of water as a primary mode of transmission has been disputed as the pathogen survives only for 15 days in field water (Tagami et al., 1963).

Bacterial Blight Management

The severity of losses incurred due to the disease necessitates the development of strategies that are ecology-conscious and cost effective. BB disease management centers around methods that reduce the initial inoculum and subsequent development of the pathogen on host plants and this can be accomplished through the use of chemicals, disease resistant cultivars, and biological agents.

Chemical Control

An ideal agent for chemical control is one that functions at low concentration by either killing or inhibiting the multiplication of the pathogen by blocking an important metabolic pathway. BB has been controlled by chemicals like Bordeaux mixture with or without sugar, copper-soap mixture, and copper-mercury fungicides. Spraying copper oxychloride was recommended to control of rice bacterial blight disease (Sulaiman & Ahmed, 1965). Chlorination of the field water with stable bleaching powder also effectively reduced disease severity in India (Chand, Singh, Singh, & Thind, 1979; Sivaswamy & Mahadevan, 1986). Synthetic organic bactericides such as nickel dimethyl dithiocarbamate, dithianone, phenazine and phenazine N-oxide were also recommended (Fukanaga, 1966).

A foliar spray of cowdung extract (20 g/l) was reported to suppress BB development in the state of Kerala in southern India (Mary, Dev, Karunakaran, & Nair, 1986). Also, dithiocarbamate fungicides were reported to inhibit the growth of *Xoo* by arresting fatty acid (Yoneyama, Sekido, & Misato, 1978) and lipid (Yoneyama & Misato, 1978) biosynthesis. A few antibiotics like steptocycline and fungicides like zineb, carbendazim inhibited the pathogen *in vitro* (Mahto, Singh, & Singh, 1988).

However, an effective and economical chemical control is yet to be developed for BB disease. This may be because the pathogen population is highly variable in its sensitivity to the chemicals used for disease control. The existence and development of drug-resistant strains also poses serious problems in formulating fool-proof control agents.

Host Resistance and R Genes

Planting resistant cultivars has been the major method of BB management. Wild species of crop plants represent natural source of resistance to their pathogens. Till now, about twenty-three major BB resistance genes have been identified (Mew, Vera Cruz, & Medalla, 1992; Zhang et al., 1998), two of which, *Xa1* and *Xa21*, have been cloned from rice (Song et al., 1995; Yoshimura et al., 1998). The R-gene *Xa21* was first identified in a wild rice, *Oryza longistaminata*. A locus for resistance to BB was transferred from the wild species *Oryza longistaminata* to the cultivated rice IR24 generating the introgression line IRBB21 (Khush, Bacalango, & Ogawa, 1990). This locus, *Xa21*, was found to confer resistance to all known *Xoo* races in India and Philippines (Khush et al., 1990; Ikeda, Khush, & Tabien, 1990). However,

Fig. 2.7 Characteristic bacterial blight lesions on leaves of supposedly blight-resistant IRBB21 *(Xa-21)* caused by a subset of Indian *X. oryzae* pv. *oryzae* strains (Venkatesan and Gnanamanickam, 1999)

there were *Xoo* strains in some parts of Asia that overcame this resistance in IRBB21 (Fig. 2.7).

Quantitative resistance, also known as horizontal resistance is a low-level of resistance that generally shows no pathogen race specificity. This type of resistance, governed by quantitative trait loci (QTL), can prevent the breakdown of varietal resistance in a breeding program (Ogawa & Sekizawa, 1980). This type of resistance is complicated for genetic analysis because of their continuous variation with no distinct classes in a segregating population. Washi, Kariya, and Toriyama (1966) were the first to report the slow lesion-developing type of resistance in Japan which was controlled by polygenes.

Pyramiding of R-Genes

In rice, single-gene resistance has been the primary means of control of BB, but unfortunately, due to continuous and large-scale use of single-gene resistance, there has been a shift in the virulence pattern of the strains, leading to breakdown of resistance (Mew et al., 1992). For example, the highly resistant BB locus, *Xa21*, was found to confer resistance to all known *Xoo* races in India and the Philippines (Ikeda et al., 1990; Khush et al., 1990). However, recent studies have shown that Nepalese strains were virulent on R gene *Xa21* present in rice line IRBB21 (Adhikari, Basnyat, & Mew, 1999). In India also, a sub-population of *Xoo* virulent to rice line IRBB21 was isolated during a BB epidemic that occurred in 1998 in Kerala (Venkatesan & Gnanamanickam, 1999) (Fig. 2.7). Hence, pyramiding of R-genes is thought to delay the virulence shifts. According to Kinoshita (1995),

the pyramiding of multiple resistance genes into rice varieties is one way to develop durable resistance to BB. Several workers have started to pyramid lines with different R-gene combinations and these lines have also been included in screening effective gene/gene combinations. Different resistance genes often confer resistance to different isolates, races or biotypes. Combining these resistances broadens the number of races or biotypes that a variety can resist (Mackill & Ni, 2001). Furthermore, combining major and minor gene resistances may lead to increased durability of resistance (Wang et al., 1994; Bharathkumar, Paulraj, Brindha, Kavitha, & Gnanamanickam, 2008).

Marker-assisted selection allows the identification of plants with multiple resistance genes in a population (Jena & Mackill, 2008). Markers have been used to pyramid several BB resistance genes. Yoshimura et al. (1995) used markers to pyramid R-genes *Xa10+Xa4* and *Xa4+xa5*. Huang et al. (1997) pyramided four resistance genes, *Xa4, xa5, xa13* and *Xa21*, using PCR-based markers. This four-gene pyramid was found to be effective against much of the pathogen population. To facilitate selection of progenies containing more than one R-gene and with the advantage of the availability of near-isogenic lines, several genes for BB resistance have been tagged with RFLP and PCR-based markers (McCouch, Khush, & Tanksley, 1991; Ronald & Tanksley, 1991; Blair & McCouch, 1997). In rice breeding programs at the International Rice Research Institute (IRRI) and national rice improvement programs in the Philippines, Indonesia and India, resistance genes *Xa4, xa5, Xa7* and *Xa21* were targeted for transfer to commercially important rice varieties (Nelson et al., 1996). Genes from rice cv. PR106, widely grown in Punjab, India, were introgressed with pyramided resistance genes *xa5, xa13* and *Xa21* from pyramid line IRBB62 using MAS (Singh et al., 2001). Marker-assisted selection has been successfully used in selecting for resistance in the absence of pathogens to pyramid multiple genes for durable resistance against rice bacterial blight (Huang et al., 1997) and for development of multiple disease-resistant germplasm (Kelly, 1995; Narayanan et al., 2002; Narayanan, Baisakh, Vera Cruz, Gnanamanickam, & Datta, (2004); Bharathkumar et al., 2008).

Transgenic rice and BB management. This aspect is described in Chapter 5 on biological control of bacterial blight of rice.

Sheath-Rot

Pathogen: Sarocladium oryzae (Sawada) W. Gams and D. Hawksw

Symptoms

The following symptoms develop following sheath-rot infection: (i) development of irregular spots or lesions, with dark reddish brown margins and gray center on leaf sheath; (ii) discoloration of the sheath; (iii) enlargement and coalescence of lesions often covering the entire leaf sheath; (iv) lack of panicle exertion in severe infection leading to entire or parts of young panicles to remain within the sheath; (v) rotting

of infected panicles that remain non-emerged and change of color of florets to dark brown, (vi) appearance of white powdery growth (consisting of mass of conidiophores) inside infected sheaths and panicles, and (vii) sterile panicles with shriveled and partially filled grains (IRRI, 2003; Sakthivel, 2002; Webster & Gunnell, 1992)

Sheath-rot infection occurs due to predisposing factors such as insect injury, presence of entry points, fertilization of rice with high amount of nitrogen, high relative humidity, dense crop growth and leaf canopy and prevalence of temperature from 20 to 28 °C at heading to maturity of the rice crop.

The fungus produces two phytotoxins, helvolic acid and cerulenin, in culture fluids which are known to reproduce part or all of the sheath-rot symptoms when they are applied to the rice sheath (Gnanamanickam & Mew, 1991; Sakthivel & Gnanamanickam, 1986).

Yield Loss and Disease Control

The disease appears late during the growing season of the rice crop. It caused yield losses from 20 to 85% in Taiwan and 30 to 80% in Vietnam, the Philippines, and India (Nair, 1976; Nyvall, 1999; Ou, 1985; Webster & Gunnell, 1992). In Japan, areas infected ranged from 51,000 to 122,000 ha and annual losses were estimated to be 16,000–35,000 tons. At booting stage, seed treatment and foliar spraying with carbendazim, edifenphos, or mancozeb has been found to reduce sheath rot. Foliar spraying with benomyl and copper oxychloride were also found effective (IRRI, 2003).

Rice Tungro Disease (RTD)

Pathogen: Rice Tungro Bacilliform Virus (RTBV) and Rice Tungro Spherical Virus (RTSV)

Pathogen

Tungro virus complex consists of rice tungro baciliform virus (RTBV) and rice tungro spherical virus (RTSV). RTBV is the more important of the two and its particles are rod-shaped and are 100–300 nm in length and 30–35 nm in width. It contains DNA of 8.3 kb. RTSV particles are isometric and are 30 nm in diameter. It has a polyadenylated single-stranded RNA of about 12 kb (Azzam & Chancellor, 2002; Tiango, Chancellor, Villareal, Magbanua, & Teng, 1996). The viruses are transmitted by leafhoppers, and the most efficient vector is the green leafhopper, *Nephotettix virescens* (Distant). RTBV cannot be transmitted by leafhoppers unless RTSV is present. Insects could acquire the virus from any part of the infected plant. After acquiring the virus, the vector can immediately transmit to the plants (IRRI, 2003). Host transcription factors (proteins) RF2a and RF2b were recently discovered and are known to reduce the spread of RTBV in rice (Dai et al., 2008). Azzam and

Chancellor (2002) reviewed and updated the literature on the biology, epidemiology and management of rice tungro disease in Asia.

Symptoms

Characteristic leaf discoloration (as observed in Fig. 2.8) begins from leaf tip and extends down to the blade or the lower leaf portion. Infected leaves may also show mottled or striped appearance and stunting. Infected rice plants have reduced tillering, show delayed flowering, which may delay maturity. Panicles are small and are not completely exerted. Most panicles are sterile or have partially filled grains and are covered with dark brown blotches. Yield losses result from damage caused to the infected plants by severe leaf discoloration and emergence of sterile or partially filled panicles (Ling, 1972).

Fig. 2.8 Symptoms of rice tungro disease (RTD) Source: Rice Doctor, 2003. The International Rice Research Institute, Los Banos, Philippines

Although tungro symptoms can be confused with nitrogen or zinc deficiencies and other disorders, presence of tungro can be confirmed by some serological tools to detect tungro viruses. These are latex agglutination test, enzyme-linked immunosorbent assay or ELISA, and rapid immunofilter paper assay or RIPA. The presence of the vector *Nephotettix* spp. is indicative of the disease (IRRI, 2003; Ling, 1972; Tiango et al., 1996).

Yield Loss

Tungro is one of the most damaging and destructive diseases of rice in countries of Southeast Asia. Outbreaks of the disease can affect thousands of hectares in

many countries. When plants are infected with the virus at the early crop growth stage, losses can be as high as 100%. The damage caused by the disease depends on the variety used, the plant stage when infection occurs, the virus particles, and the environmental conditions (Azzam & Chancellor, 2002; IRRI, 2003; Link, 1972; Ou, 1985).

Disease Management

There are three limitations for effective tungro management: (1) the absence of symptoms at early growth stage of the disease development, (2) lack of resistant varieties to the tungro viruses, and (3) vector adaptation on GLH-resistant variety. Planting of resistant varieties against tungro virus disease is the most economical means of managing the disease. There are resistant varieties released from the Philippines, Malaysia, Indonesia, India, and Bangladesh. Among the cultural management practices, adjusting the date of planting is recommended. Likewise, farmers are strongly advised to observe a fallow period of at least a month in-between crops to eliminate hosts, viruses and vectors of the disease and to plow and harrow the field to destroy stubbles right after harvest to eradicate other tungro hosts. (Azzam & Chancellor, 2002; IRRI, 2003).

Transgenic Rice for RTD Management

There are recent efforts based on the new knowledge about the RTD. In Beachy's laboratory in the United States, pathogen derived resistance (PDR) and coat-protein (CP) genes were used to construct rice plants resistant to the RTSV (Sivamani et al., 1999). More recently, these researchers have discovered that elevating the expression levels of host transcription factors (proteins), RF2a and RF2b reduces the spread of RTBV (Dai et al., 2008). RNA interference (RNAi) is another strategy being followed by Tyagi, Rajasubramaniam, Venkatrajam, and Dasgupta (2008) of India. These aspects are covered in detail in the Chapter on Biological control of RTD.

Other Diseases

Brown Leaf Spot (Pathogen: Bipolaris oryzae; Cochliobolus miyabeanus)

This disease, previously called *Helminthosporium* leaf spot, is common in rice growing countries of the world. Its incidence is common in Texas in the United States. Most conspicuous symptoms of the disease occur on leaves and glumes of maturing plants. Symptoms also appear on young seedlings and the panicle branches in older plants. Brown leaf spot is a seed-borne disease. Leaf spots may be evident

shortly after seedling emergence and continue to develop until maturity. Leaf spots vary in size, are typically 1/8 inch in diameter, and are circular to oval in shape. The smaller spots are dark brown to reddish brown, and the larger spots have a dark brown margin and reddish brown to gray centers. Damage from brown spot is particularly noticeable when the crop is produced in nutritionally deficient or otherwise unfavorable soil conditions. Significant development of brown spot is often indicative of a soil fertility problem (Texas Agric. Exp. Stn, 1996).

Although this is a disease that occurs invariably in every rice field in Asia that has been planted with indica rices for the last several decades, it has not been responsible for any devastating yield losses even under the most conducive conditions. The earlier observations made by Indian plant pathologists that brown spot was responsible for the 1942 Bengal famine in India has been questioned (Buddenhagen, 1983). It is possible that the Bengal famine was caused by *Xanthomonas oryzae* pv. *oryzae*. On the basis of these, there is no evidence to consider this pathogen as dreaded as the other *Helminthosporium* spp that attack maize and oats.

Researchers at the International Rice research Institute (IRRI) (Vidhyasekaran, Borromeo, & Mew, 1986) reported the production of a host-specific toxin. In spite of everything we have known about the pathogen, brown spot remains as important but not a major production constraint to rice production.

Brown spot may be reduced by balanced fertilization, crop rotation, and the use of high quality planting seed. Foliar fungicides are not economical for controlling brown leaf spot on most commercial long grain varieties. Rice seed with infected glumes can result in diseased seedlings. Seed treatment fungicides reduce the incidence and severity of seedling blight caused by this fungus.

Stem Rot (Pathogen: Sclerotium oryzae)

Stem rot becomes most noticeable in rice fields during the latter stages of maturity. The disease occurs in circular to irregular areas in fields and causes premature death and lodging of the plants. The fungus attacks the rice plant near the water line usually during late tillering or early reproductive stages of growth. It first causes black, rectangular lesions with distinct angular borders on the leaf sheath. Later the lesions become larger, more diffuse, irregular in shape, and penetrate deep into the culm. As rice approaches maturity, injury to the stems increases and reaches its peak at harvest. Weakened stalks break during this stage and plants lodge making harvest difficult. Plants infected early yield poorly. Ratoon cropping in many areas is impractical because of the high percentage of plants killed by the disease. Diagnosis is confirmed by obtaining an infected plant, splitting the base of the stem, and observing the presence of tiny, black sclerotia in internal stem tissues. Control measures include the following: crop rotation, use of early maturing varieties, varying the flood water level, avoiding excessive rates of nitrogen, and rice stubble destruction. Some fungicides help to suppress this disease but are not highly effective. (Texas Agric. Expt. Station, 1996; Webster & Gunnell, 1992).

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Chapter 3
An Overview of Progress in Biological Control

Beginnings of Rice Biological Control Research and Recent Progress in Pathogens and Biocontrol Agents

We prepared a chapter on the biological control of rice diseases for an earlier book on Biological Control of Crop Diseases (Gnanamanickam, 2002). In the first chapter, we described the definitions and principles of biological control (Gnanamanickam, Vasudevan, Reddy, Kloepper, & Defago,2002). In Chapter 2, we documented the work on biological control of rice diseases (Vasudevan, Kavitha, Priyadarisini, Babujee, & Gnanamanickam, 2002). The descriptions provided then in those two chapters form the central theme for this book. In this overview, we look back and see how this method of rice disease management started and describe the advances that have been made. While the contributions made in biocontrol for some of the major diseases of rice may be significant, there has been very little progress made in applying biocontrol agents for minor diseases. The objective for this overview is to document how this work developed in the author's laboratory in India and Asia, to highlight some of the most significant research advances that have been made in advanced laboratories elsewhere and to show how they compliment the overall research on biological control of rice diseases.

Areas and advances that are highlighted in this chapter are,

1. *New knowledge about rice blast fungus*. In 2004 it was shown that in addition to infecting plants through the leaf, *M. grisea* can also infect the plant roots (Sesma & Osbourne, 2004). In another significant event, the genome sequence of the rice blast fungus was produced by Dean et al. (2005). They produced the complete sequence of *Magnaporthe grisea*. Their reported draft sequence of the *M. grisea* genome and analysis of the gene set provides an insight into the adaptations required by a fungus to cause disease and reflects on the clonal nature of this fungus imposed by widespread rice cultivation.
2. *Biocontrol agents*. Plant-associated bacteria, in particular *Pseudomonas* and *Bacillus* strains, still occupy the forefront in research that uses them as inoculants for rice for the control of rice pathogens and plant growth-promotion. The later group of beneficial bacteria are the plant growth-promoting rhizobacteria

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(PGPR). We will also look at diverse groups of other bacteria and fungi that have been used to suppress different rice diseases in future pages of the book.

3. *Mechanisms of biological control*. It is understood that PGPR strains use direct mechanisms for plant growth-promotion and indirect mechanisms for pathogen suppression. In this genomic era, significant achievements have been made in sequencing bacterial genomes of well-known *Pseudomonas* (Paulsen et al., 2005; Loper & Gross, 2007) and *Bacillus* (Chen et al., 2007, 2009; Chen, Koumoutsi, Scholz, & Borriss, 2009) strains. These are very important contributions to our understanding of how genes or gene clusters of these strains are used for plant growth-promotion and biological disease suppression. The genome of another environmental strain used in bioremediation, *P. fluoresccens* PfO-1, has also been sequenced by Silby and Levy of Tufts University School of Medicine (*http://genome.jgi-psf.org/finished_microbes/psefl.info.html*). Even though these sequenced strains have not been applied for rice disease management, the information on genomic analysis of antifungal or antibacterial metabolites such the biosynthetic gene clusters of 2,4-diacetylphloroglucinol, pyrrolnitrin and pyoluteorin must be common for strains which produce these metabolites and are being used in rice (Velusamy, Immanuel, Gnanamanickam, & Thomashow, 2006).
4. *Transformation of rice*. Generation of transgenic rices with different desired genes of rice or non-rice origin, is an area where significant progress has been made in the last few years. The progress in this area of rice disease management will be included in this book as the genes are responsible for products/mechanisms of biocontrol or pathogen suppression. Transformation technology simply becomes a tool for gene delivery and activation of pathogen suppression.

Studies on the Development of Bacterial Biocontrol Agents for Rice Diseases

This work started in the author's laboratory at University of Madras in southern India back in 1982 and continued on over a 24-year period (1982–2006). In the earlier years biological disease control was not a subject that was familiar to many in Asia. In this overview on biological control of rice diseases, I summarize the work that was carried out by this author in India and in the Philippines (during a tenure at the International Rice Research Institute). This includes,

1. Introduction of *Pseudomonas fluorescens* to the nation of India (Unnamalai & Gnanamanickam, 1983)
2. Biological control of rice blast by seed treatment of rice with *Pseudomonas fluorescens* (Gnanamanickam & Mew, 1989)
3. Genetic analysis of antifungal antibiotic production by *Pseudomonas fluorescens* Pf7-14 (Chatterjee et al., 1996; Valasubramanian, 1994)
4. Identification of 2,4-diacetylphloroglucinol produced by tropical strains of *P. fluorescens* as a major mechanism in the suppression of bacterial leaf blight

caused by *Xanthomonass oryzae* pv. *oryzae* (Velusamy & Gnanamanickam, 2003; Velusamy et al., 2006)
5. Role of 2,4-diacetylphloroglucinol in sheath blight (*Rhizoctonia solani*) suppression (Immanuel, 2006)
6. Development of a number of *Pseudomonas fluorescens/ P. putida* and *Bacillus* strains for the suppression of rice diseases (Table 3.1)

Table 3.1 Bacterial biocontrol agents developed for major diseases of rice. Center for Advanced Studies in Botany, University of Madras, India

Disease	Pathogen (causal agent)	Biocontrol agent developed	Reference
Blast (Bl)	*Pyricularia grisea* (Teliomorph: *Magnaporthe oryzae*)	*Pseudomonas fluorescens, Bacillus* spp: *B. polymyxa, B. pumulus, B. coagulans, Enterobacter agglomerans*	Gnanamanickam and Mew (1992), Valasubramanian (2004), Kavitha (2002)
Sheath blight (ShB)	*Rhizoctonia solani* (Teliomorph: *Thanetophorus cucumeris)*	*P. fluorescens, P. putida, Bacillus megaterium, B. polymyxa, B. pumulus, B. coagulans, Enterobacter agglomerans*	Vasantha Devi, Malar Vizhi, Sakthivel, and Gnanamanickam (1989), Thara (1994), Krishnamurthy & Gnanamanickam (1998), Kavitha (2002)
Sheath-rot (Sh-R)	*Sarocladium oryzae*	*P. fluorescens*	Sakthivel (1987), Sakthivel and Gnanamanickam (1987, 1989)
Bacterial Blight (BB)	*Xanthomonas oryzae* pv. *oryzae*	*Bacillus* spp: *B. lentus B. cereus B. circulans*, and *P. fluorescens*	Vasudevan (2002), Velusamy and Gnanamanickam (2003), Velusamy et al. (2006)

Source: Immanuel (2006).

Starting about 1980 or earlier, perhaps, there was a strong research focus on biocontrol of rice diseases at the International Rice Research Institute (IRRI). T.W. Mew and his team of researchers were involved in using not only bacterial biocontrol agents (by bacterization) but also *Trichoderma* spp (Mew & Rosales, 1986; Rosales & Mew, 1997; Gnanamanickam & Mew, 1989, 1992; Gnanamaickam, Candole, & Mew, 1992). They made very important contributions to the biological control of sheath blight, bakane, blast and other problems. Because of their co-ordinated effort in teaching and training rice researchers (starting from 1987 onwards) from rice growing countries of Asia, the message about the benefits derived from the use of environment-friendly and ecology-conscious biocontrol agents over chemicals/fungicides soon spread to other rice-growing countries of Asia. Countries like China, India, Malaysia, Thailand, Vietnam, and Indonesia started applying bacterial and fungal biocontrol agents.

Tamil Nadu Agricultural University (TNAU) which participated in IRRI training started building a very strong research group that will devote its efforts to biological control of rice diseases for years to come. The contributions from this group continue to enrich our understanding of the potential of biocontrol agents for rice disease management.

Mechanisms of Biological Disease Suppression: Recent Advances with PGPR Strains

PGPR: An important group of the rhizosphere microbial community that exerts beneficial effects on plant growth upon root colonization. These root colonizing microorganisms were first defined by J. Kloepper and M. Schroth and termed as Plant Growth-Promoting Rhizobacteria (PGPR) (Kloepper & Schroth, 1978).

The prospect of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria to increase plant growth has shown considerable promise in laboratory and greenhouse studies, but responses have been variable in the field. (Bowen & Rovira, 1999). However, there are potential environmental benefits of the PGPR approach. These are,

1. Reduction in the usage of agricultural chemicals, and
2. Deployment in sustainable and organic agricultural management practices.

According to the mode of action, PGPRs are divided into two groups: 1. biocontrol-PGPRs (indirect growth-promoters), and 2. growth-promoting PGPRs (Bashan & Holguin, 1997; Glick, Patten, Holguin, & Penrose, 1999; Podile & Kishore, 2006). Protection of bacteria-inoculated seedlings against soil-borne pathogens was observed inseparable from the plant growth-promoting activity of several of the reported PGPR (Guo et al., 2004; Manjula & Podile, 2001; Raupach & Kloepper, 2000).

The known mechanisms of growth-promotion and disease suppression by PGPR are both direct and indirect. The direct mechanisms (demonstrated in the absence of plant pathogens) are 1. phytohormones, 2. increased uptake of iron, 3. volatiles, 4. solubilization of phosphates and minerals, 5. fixation of atmospheric nitrogen, 6. production of siderophores, and 7. lowering of ethylene levels. The indirect mechanisms are 1. antibiosis, 2. competition with deleterious microbes, 3. lysis/parasitism, and 4. induced resistance. Significant research advances have been made to elicit each of the above mechanisms and these inform how each of these mechanisms bring about either plant growth-promotion or disease suppression or both.

Role of Antibiotics and Secondary Metabolites in Disease Suppression

While antibiosis is the primary or most important mechanism known to be involved in disease suppression, their role has been analyzed through genome analyses for important and well known PGPR strains with broad-spectrum of activity towards

plant pathogens and thus in biological disease suppression. These advances were made in J. Loper's USDA laboratory at Corvallis, OR in the United States for *Pseudomonas fluorescens* Pf-5 (originally isolated and characterized by Howell & Stipanovic, 1979, 1980) (Paulsen et al., 2005; Loper & Gross, 2007; Loper, Kobayashi, & Paulsen, 2007) and R. Boriss's laboratory at the Humboldt University, Berlin for *Bacillus amyloliquefaciens* FZB42 (Chen et al., 2007, 2008, 2009).

Genome sequencing and genome analyses reveal interesting aspects of PGPR strains that are of particular significance to the ecology of the strain and its interactions with the plant and other plant-associated microorganisms. The circular representation of the 7.07 Mb genome of *P. fluorescens* in ten circles (Paulsen et al., 2005) is presented below in Fig. 3.1.

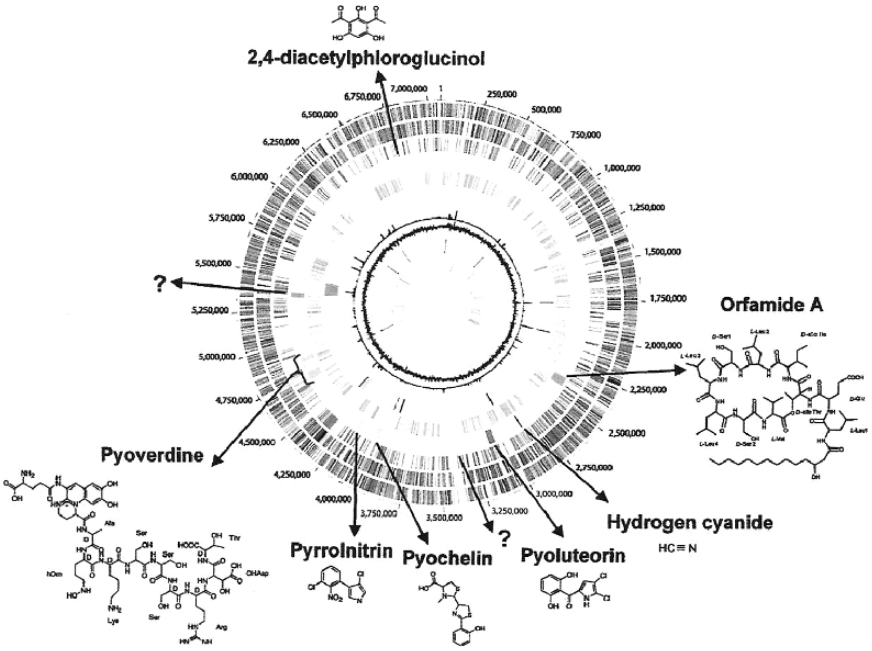

Fig. 3.1 The circular representation of the genome of *Pseudomonas fluorescens* Pf-5 (Paulsen et al., 2005)

The genome reveals the position of gene clusters that code for important antibiotics such as, pyoluteorin, 2,4-diacetylphloroglucinol, pyrrolnitrin and hydrogen cyanide (Raaijmakers, Vlami, & de Souza, 2002; Mavrodi, Blankenfeldt, & Tomashow, 2002) involved in biological disease suppression. The genome also shows the position of gene clusters that code for orfamide A, a newly discovered cyclic lipopeptide (LCP) (Loper & Gross, 2007).

P. fluorescens Pf-5 has not been tested against rice pathogens. However, other promising strains of *P. fluorescens* isolated from rice fields in southern India have been observed to produce 2,4-diacetylphloroglucinol, pyrrolnitrin and pyoluteorin and have been successfully used to suppress rice diseases, bacterial leaf blight

(Velusamy & Gnanamanickam, 2003; Velusamy et al., 2006) and sheath blight (Immanuel, 2006).

The genome sequence analysis of *B. amyloliquefaciens* FZB42 (originally characterized by Bayer Laboratory, Germany) carried out by Chen et al. (2007) showed that 8.5% of the total 3,918 Kb genome was devoted to genes that coded for different antibiotics and siderophores. In further anlysis, Chen et al. (2008) observed that the genome of plant-associated *B. amyloliquefaciens* FZB42 harbored an array of giant gene clusters involved in synthesis of lipopeptides and polyketides with antifungal, antibacterial and nematocidal activity. Five gene clusters, srf, bmy, fen, nrs, dhb, covering altogether 137 kb, were shown to direct synthesis of the cyclic lipopeptides surfactin, bacillomycin, fengycin, an unknown peptide, and the iron-siderophore bacillibactin and thus, the potential of the organism for biological disease suppression.

This author had recently profiled *B. amyloliquefaciens* FZB24 (a relative of FZB42 and from the same Bayer origin) in laboratory, greenhouse and field experiments and showed that it had a very broad-spectrum of activity towards a number of devastating fungal and bacterial plant pathogens. Fungal pathogens suppressed by strain FZB24 were, *Rhizoctonia solani, Fusarium graminearum, Sclerotinia sclerotiorum, Gaeumannomyces graminis* var. *tritici, Phytophthora erythrospetica* among others (Gnanamanickam, Inman, West, & Semones, 2008). Also, strain FZB24 afforded significant levels of suppression of bacterial wilt of tomato (*Ralstonia solanacearum*) and shoot infection of fire blight of apples (*Erwinia amylovora*) in field experiments (Gnanamanickam et al., 2008; Aldwinckle et al., 2008).

Transgenic Plants in Rice Disease Management

This author considers that transformation of rice with genes that code for known products/mechanisms (such as chitinase, thaumatin-like proteins) is part of the broad umbrella of biocontrol. In the earlier volume edited by Gnanamanickam (2002), a whole chapter was devoted to the description of transgenic plants for management of rice diseases (Datta, 2002). The chapter elegantly described the rice transformation protocols developed at the International Rice Research Institute (IRRI) in the Philippines for incorporating a number of pathogenesis-related rice proteins and major genes (such as *Xa21*,a cloned rice gene for bacterial blight resistance) and a pyramid of such genes for sheath blight, bacterial blight and blast suppression. Since then there have been many reports on transgenic rices generated and used for rice disease suppression. These contributions will be listed in later pages of this book under management of different rice diseases.

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Chapter 4
Biological Control of Rice Blast

Antagonistic Bacteria as Biocontrol Agents

Pseudomonas *and* Bacillus *spp.*

Rice blast continues to be a severe production constraint particularly in dryland and unbunded upland rice in tropical Asia. In early research, plant-associated bacterial strains assembled from the rice rhizosphere were used as biocontrol agents for blast suppression. In such efforts it was observed that fluorescent strains of *Pseudomonas* were the dominant group in the rice rhizosphere. In South Indian flooded rice rhizosphere, an analysis showed that biovar 3 was quite dominant among the *P. fluorescens* (Sakthivel & Gnanamanickam, 1989). Representative strains of a dominant biovar were usually chosen as biocontrol agents. An important first criterion that determined whether or not a particular strain will be useful for further work in the greenhouse/filed plots is its consistent antagonism towards the target pathogen, *Magnaporthe grisea*.

Gnanamanickam and Mew (1989, 1992) assembled more than 400 strains of bacterial strains from rice fields at the International Rice Research Institute (IRRI). These strains were characterized for antagonism to *M. grisea* in the laboratory. Inhibition of *M. grisea* in a dual plate laboratory assay by an efficient strain of *P. fluorescens* Pf7-14 is seen in Fig. 4.1. In their first report on biological suppression of leaf and neck blast, they used mutant strains that were resistant to rifampicin ® or rifampicin and nalidixic acid (RN) of two fluorescent strains of *Pseudomonas* and two strains of *Bacillus* in a field trial. Batches of seeds of upland rice cultivar UPLRi-5 were coated with a strain of the bacterium at 10^9 cfu/seed (final concentration) or with the chemical pyroquilon (fungorin) @8.0 g/kg of seed before they were sown in upland field plots. The rice crop received three additional foliar sprays with respective bacterial strains or pyroquilon at 20, 30 and 40 days after seeding.

It was reported that the *Pseudomonas* strains 4–15 and 7–14 afforded 59 and 47% leaf blast reduction in the upland rice cultivar, UPLRi-5 (Table 4.1). In the same experiment, the Bacillus strains 4-03 and 33 reduced leaf blast by 46 and 44%. Of all four strains Pf7-14 was most effective in reducing neck blast while the neck blast reduction was not significant (Table 4.1).

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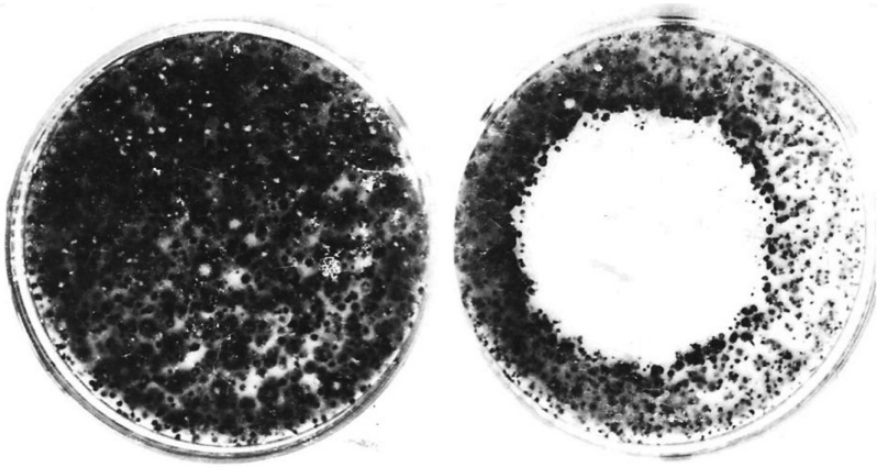

Fig. 4.1 Inhibition of mycelial growth of *Magnaporthe oryzae* by *Pseudomonas fluorescens* Pf7-14 (seen in plate on the *right*) in dual plate laboratory assay. On the *left* is an untreated control (Gnanamanickam & Mew, 1992)

Table 4.1 Effect of seed bacterization and foliar application of antagonistic bacteria on leaf and neck blast incidence and grain yield of UPLRi-5 rice in the Philippines (Gnanamanickam & Mew, 1992)

Treatment	Severity of leaf blast[a]	Severity of neck blast[b]	Grain yield (g)/100 panicles
Bacterial strain			
Bacillus 4-03R	3.3.	3.0	100.5
Bacillus 33R	3.5	3.7	95.5
P.fluorescens 4-15R	2.6	3.0	92.5
P. fluorescens 7-14RN	3.3	2.8	102.7
Pyroquilon	2.0	3.7	106.7
Check	6.3	3.8	96.3
LSD (0.05)	2.2	1.8	43.6

[a]Number of blast lesions/cm^2 leaf area.
[b]Neck blast severity index $= \frac{n(1)+n(2)+n(3)\ldots n(9)}{\text{Total number}} \times 100$, where n is the number of tiller with a disease score of 1 (resistant) to 9 (susceptible).

Blast (leaf and neck blast) suppression was mediated by the production of an antifungal antibiotic (tentatively identified as phenazine-1-carboxylic acid (PCA) by the biocontrol agent, *P. fluorescens* Pf7-14 (Valasubramanian, 1994).

A detailed genetic analysis of antifungal antibiotic production by *P. fluorescens* Pf7-14 was carried out. This strain did not harbor a plasmid indicating that the gene(s) specifying its antibiotic production were located within the chromosome. By using the plasmid vehicle, pUT/km, random mini-*Tn5* km mutagenesis was performed and mutants which produced little or no antifungal antibiotic (afa) were isolated.

Southern hybridization with TRIAL RESTRICTION the mini-Tn5 probe revealed that insertions within 3.3 Kb, 3.9 Kb, 7.2 Kb or 10.6 Kb Sst1 chromosomal fragments affected afa production. Complementary analysis suggested that the afa genes were clustered in the cosmid pAKC901. In a field test for biological suppression of rice blast, strain 7–14 afforded 79 and 82% reduction of leaf and neck blasts while its afa mutants afforded 34 and 12% control of leaf and neck blasts suggesting that afa production by *P. fluorescens* mediated the biological control of rice blast (Table 4.2; Fig. 4.2) and sheath blight suppression. These field experiments conducted in Chennai, southern India (Table 4.2) (Chatterjee et al., 1996; Gnanamanickam, Valasubramanian, Chatterjee, Chatterjee, & Mew, 1994; Valasubramanian, 1994).

Table 4.2 Evaluation of Pf7-14 and its Mini-Tn5 mutants in field plots for suppression of leaf blast in IR50 rice (Chatterjee et al., 1996; Vasudevan, Kavitha, Venkatesan, Babujee, & Gnanamanickam, 2002)

Bacterial strain	Leaf blast incidence (%)	Leaf blast control (%)
Pf7-14	21.30	78.70
Afa^{-} mutant AC2003	75.30	24.70
Afa^{leaky} mutant AC2007	52.90	47.10
Fungicide, tricyclazole	31.00	69.00

Fig. 4.2 Rice panicles (plants on the *right*) from a field experiment show suppression of neck blast due to treatment with *Pf*7-14 and lack of protection against neck blast in plants treated with its afa^{-} mutant (plants on the *left*)

Yoshihiriro, Mitsuo, Hayato, and Futoshi (2003) of Japan conducted biocontrol studies with *Bacillus subtilis* IK1080. In laboratory tests, the bacterium inhibited both mycelial growth and appressorial formation in *M. grisea*. In a greenhouse study, the antagonist applied to rice plants 14 d before inoculation with *M.grisea* reduced the length of leaf blast lesions.

In another detailed study, Kavitha (2002) used *Bacillus* strains in combination with genes for blast resistance (R-genes). In rice cultivars that had the R-genes,

reduction of leaf blast incidence was observed in comparison to those cultivars that did not have the R-genes. An efficient *B. polymyxa* strain VLB16 used in this study produced an antifungal protein that inhibited the growth of *M. oryzae*. In the field, VLB16 suppressed leaf blast by 50% in rice cultivar CO39 (Kavitha, Senthilkumar, Gnanamanickam, Inayathulla, & Jayakumar, 2005). Other *Bacillus* strains, *B. pumilus, B. polymyxa, B. coagulans* and *Enterobacter* also reduced blast severities in this field experiment (Table 4.3).

Table 4.3 Suppression of rice blast in IR50 and CO39 rice by bacterial treatments. Field experiment, RARS, Pattambi, Kerala, southern India

Bacterial strain	Diameter of zone of inhibition of *Pyriculari a grisea* (cm)	Percent blast incidence	centering Percent blast suppression	Production of antifungal metabolite
Bacillus pumilus (IM3)	3.0	51.03	46.22	–
Bacillus polymyxa (KRU22)	4.4	44.31	53.30	–
Bacillus polymyxa (VLB16)	5.0	47.36	50.06	37 kDa antifungal, heat-resistant protein (Kavitha et al., 2005)
Bacillus coagulans (PD7)	3.4	51.22	46.02	–
Enterobacter agglomerans (UPM18)	2.4	41.67	56.09	Antifungal glucanase (9.3 units)

Source: Kavitha (2002) and Kavitha et al. (2005).

Erwinia ananas Transformed with Chitinase Gene of Serratia

Someya et al. (2003) established a novel biocontrol strategy for the control of rice blast by using a transformed bacterium, *E. ananas*, a common epiphyte that colonizes the rice phylloplane efficiently. In their earlier studies, they had evaluated *Serratia marcescens* strain B2 (isolated originally from tomato) as a producer of a set of chitinase genes that were able to inhibit the growth of several pathogenic fungi, including *P. oryzae*, the rice blast fungus (Someya et al., 1997, 2000; Someya, Nakajima, Hirayae, Hibi, & Akutsu, 2001, Someya, Nakajima, Hibi, Yamaguchi, & Akutsu, 2002). However, *S. marcescens* B2 did not colonize the rice phylloplane satisfactorily well, not as much as it colonized the rhizosphere of rice (2002). Therefore, they cloned the endochitinase gene, ChiA of *S. marcescens* B2 (Someya et al., 2003). The cloned gene, *chiA* was introduced into a rice phylloplane strain of *E. ananas*, strain NR-1. The construction vectors were designated pchiA-V1pcf9 and pchiA-V1pcf53 with two types of promoter activity. *E. ananas* NR-1 transformed with either of these vectors produced and secreted ChiA (Fig. 4.3).

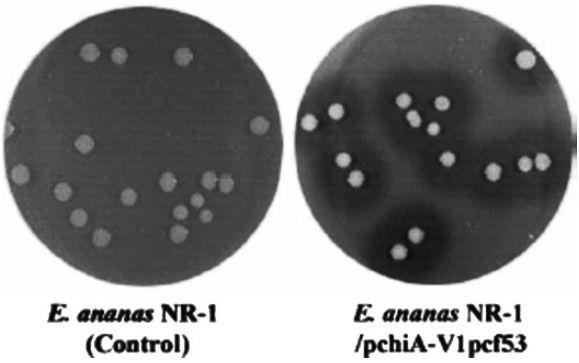

Fig. 4.3 Laboratory assays for chitinase production by a transformed strain of *Erwinia ananas* NR-1 and its non-tranformed control (Someya et al., 2003)

In similar laboratory assays on LB agar plates, the non-transformed wildtype/parent strain NR-1 showed no chitinase activity (Someya et al., 2003). In laboratory tests the transformed bacterium inhibited the mycelial growth and conidial germination of *P. oryzae* (rice blast fungus), and also caused bursts in the mycelial tips.

In a greenhouse experiment, Someya et al. (2003) recorded significant reductions in leaf blast incidence with two of their transformed *E.ananas* NR-1 strains when used as foliar spray treatments over the untreated check. Efficiency of blast suppression depended on the promoter used.

Other Biocontrol Agents

In Iran, *Streptomyces* strains isolated from rice field soils were screened in the laboratory against *M. oryzae* (rice blast fungus) by Zarandi, Ebrahimi, Shahidi, Dehkaei, and Padashst (2009). These researchers observed that 10 of 100 Streptomyces strains showed fungal inhibition. In subsequent characterization, *Streptomyces sindeneusis* 263 was considered effective against blast in a greenhouse study.

Method of Application of Bacterial Biocontrol Agent

In studies performed by this author that have been described in previous sections of this chapter, bacteria were applied to the rice seeds before sowing and or as foliar sprays to the rice foliage. Surface sterilized rice seeds were submerged overnight in aqueous bacterial cell suspensions (OD = 0.1 at 600 nm) prepared in 0.5–1.0% carboxymethylcellulose for seed coating and were then air dried before sowing. Such cell suspensions were also used in foliar spray treatments (Vasudevan et al., 2002). Seed treatments have been very effective in greenhouse and field experiments for biological suppression also of other diseases, sheath-rot (caused by

Sarocladium oryzae) and bakane (caused by *Fusarium moniliforme*) (Sakthivel & Gnanamanickam, 1987; Rosales Roasales & Mew, 1997).

Fungi as Biocontrol Agents

Non-Pathogens: Phylloplane Fungi and Freeze-Killed Mycelium

Ohtaka, Kawamata, and Narisawa (2008) successfully tested an antagonistic fungus that induced systemic resistance in rice to the blast fungus. Several applications of the suppressive fungus MKP5111B, isolated from the phylloplane of rice plants, were tested in an effort to control rice blast disease. Three treatments with MKP5111B [living (Std), killed with liquid nitrogen (FR), and autoclaved (AC)] were either sprayed onto rice seedlings or mixed into seed-sown soil. Three weeks after spraying and 4 weeks after the soil application, *Magnaporthe oryzae*, the causal agent of rice blast, was introduced into the systems. The Std and FR treatments suppressed rice blast, but the AC treatment proved ineffective. Although a suppressive effect was seen on new leaves, no mycelium of MKP5111B was seen. The fungus thus may have induced a systemic resistance in the rice plants. The possibility of a substance from MKP5111B, such as elicitor molecule(s) was suggested to be responsible for the induced resistance. Likewise, Kawamata, Narisawa, and Hashiba (2004) used isolates of rice phylloplane fungi for suppression of leaf blast in rice. Also, an isolate of *Exserohilum monoceras*, a fungal pathogen of *Echinochloa* sp.was observed to reduce rice leaf blast development (Tsukamoto, Tsutsumi, Onodera, Yamada, & Fujimori, 1999).

Avirulent/Weakly Virulent Isolates of *M. Oryzae*

Several researchers in Japan have used weakly virulent or avirulent isolates of *Pyricularia oryzae* (*M. oryzae*) strains which caused incompatible lesion types as biocontrol agents for leaf blast (Ohata and Kozaka, 1967; Fujita, Sonoda, & Yaegashi, 1990; Iwano, 1987). In these studies, pre-inoculation of rice leaves with the avirulent isolate of *P. oryzae* (Ashizawa, Zenbayashi, & Sonoda, 2005) achieved some level of reduction of rice leaf blast induced by a virulent isolate of the blast fungus. Pre-inoculation of rice sheath with an incompatible *P. grisea* (grass isolate) was also observed to reduce leaf blast lesion development (Arase & Fujita, 1992).

The detailed study of Manandhar, Lyngs Jorgensen, Mathur, and Smedegaard-Petersen (1998) provided another example for the suppression of rice blast with an avirulent isolate of *Pyricularia oryzae* and the nonrice pathogen *Bipolaris sorokiniana*, when they were used either in pre-inoculation or foliar spray applications both in greenhouse and field experiments. When used as foliar spray applications in a field experiment in Nepal, *B. sorokiniana* afforded a higher reduction of neck blast incidence (42.2%) than the avirulent isolate of *P. oryzae* which afforded 26.1%

reduction in rice cv. Masuli. These researchers suggested that induced resistance was involved in blast suppression.

In all these studies which used avirulent or incompatible isolates of *P. oryzae* or *P. grisea* or rice phylloplane fungi or their freeze-killed mycelium as biocontrol agents for leaf or neck blast, the underlying principle is the induction of induced systemic resistance (ISR). Induction of ISR as a mechanism of blast suppression was elegantly illustrated by Smith and Métraux (1991) when they used *Pseudomonas syringae* pv. *syringae* as the inducer of local and systemic resistance (ISR) in rice. This phenomenon was further explained by the isolation and characterization of a rice gene which had the bacterial *lemA* function from the rice-*P. syringae* pv. *syringae* interaction that led to ISR in rice (Reimmann, Hofmann, Mauch, & Dudler, 1995). Today we understand that ISR is a mechanism that operates in every host-pathogen interaction and maximizing the efficacy of resistance elicitors can enhance such levels of such resistance and protection of crop plants (Walters, Walsh, Newton, & Lyon, 2005).

Transformation of Rice for Blast Control

In the previous section we discussed how a transformed epiphytic bacterium was developed for blast control in Japan (Someya et al., 2003). This author considers transformed rice plants which carry either rice genes or others that are transiently expressed in rice leaves and are able to suppress the development of blast can be an effective biological control as others we have discussed so far (Gnanamanickam, 2002). Datta (2002) described in detail how disease resistance ® genes and pathogenesis-(PR)-related protein genes can be expressed in transgenic rice plants for strategic management of rice diseases. Perhaps, there are more examples of transgenic rices that have been constructed for control of sheath blight, bacterial blight and virus diseases of rice.

There has been some effort by researchers to engineer rice for resistance to blast. Sometimes, the transgenic plants that were resistant to attack by one of the fungal pathogens, often also had limited resistance to another fungal pathogen such as *P. oryzae*. Indica rice variety IR72 expressing the transformed *bar* gene was resistant to attack by sheath blight fungus, *R. solani* and showed also decreased development of blast symptoms (Tada et al., 2000).

Expression of phytolaexin genes in transgenic rice plants has afforded blast-resistance. Tang et al. (2000) observed that transformation of rice with a stilbene synthase gene from grapevine allowed the expression of the rice phytoalexin momilactone and showed higher levels of resistance to *P. oryzae*. Nishigawa et al. (2000) characterized blast-resistant transgenic rice constitutively expressing chitinase or the β-glucanase gene in an elite japonica cultivar.

Transformation protocols that used either biolistic-, protoplast-, and *Agrobacterium*- mediated transformation of the more difficult indica rices were successfully designed by Datta (2002) at the International Rice Research Institute (IRRI) in the Philippines. Earlier efforts to use chitinase transgenes afforded mixed results.

Transgenic Rices of Indica Rice Cultivars, IR50 and CO39 for Blast Control

In the author's laboratory, research towards the improvement of elite indica rice cultivars, IR50 and CO39 for blast resistance and cv. Jyothi and IR50 for bacterial blight resistance was carried out through pyramiding of R-genes and marker-assisted selection of resistant plants. In order to create multiple resistance in these rices, blast-resistant pyramids of IR50 and CO39 available were subsequently transformed with *Xa21* gene. The bacterial blight resistance gene *Xa21* is known to confer resistance to all known races of *Xanthomonas oryzae* pv. *oryzae* in India and the Philippines. This transformation work was carried out in collaboration with Dr Swapan Datta of IRRI and was supported by the Rockefeller Foundation (Narayanan, 2001; Narayanan et al., 2002, 2004) and I describe the work in some detail.

a. Starting materials for transformation. Target rice pyramids (carrying blast resistant gene/genes) for the transformation were, IR50 (*Piz-5*) and CO39 (*Pi-1* + *Piz-5*). They were used as the starting material for the rice tissue culture and transformation with *Xa21* gene using particle bombardment. Immature embryos and mature seeds were used as explants in both the varieties for the tissue culture process.
b. Construct used for bombardment. The plasmid pC822 that contained *Xa21* coding sequence was supplied by Dr. P.C Ronald, University of California, Davis, USA. The primers U1 and I1 developed to amplify a 1.4-Kb DNA fragment of *Xa21* that was polymorphic to fragments amplified from other *Xa* genes were used for quick genetic analysis of the transgenic progeny. Plasmid pROB5 contained the selectable marker, the hph-coding region, flanked by the cauliflower mosaic virus (CaMv) 35S promoter and polyadenylation signals (Poly (A). This plasmid provided a selectable marker that confers resistance to hygromycin for cotransformation with the pC822 plasmid.
c. Particle bombardment and selection. Immature embryos (IEs), immature embryo derived callus (IECs) and mature seed derived callus (MCs) were arranged and bombarded with the *Xa21* + pROB5 plasmid (purified by CsCl/EtBr method) by the particle gun PDC-1000/He system (BIORAD, Hercules, CA) following manufacturer's instructions. After bombardment, the explants were left in the dark overnight in the same medium. In the morning, the cultures were transferred to MS + 30 g/l maltose + 7.5 g/l agar medium supplemented with 50 mg/l hygromycin B for selection of transformed calli and incubated in dark at 27 °C. The newly developing hygromycin-resistant calli were subcultured in fresh media under continued selection pressure at every fortnight interval for 5 cycles.
d. Plant regeneration. The embryogenic calli were carefully selected and transferred to 50 ml flasks containing plant regeneration medium (for IR50, MS medium with 2 mg kinetin/L and 1 mg NAA/L and for CO39, MS medium with 5 mg kinetin/L and 1 mg NAA/L). The cultures were incubated for 2–3 week in 16 h

photoperiod of 3000-lux intensity at 26 °C. Three to four week old plantlets were transferred to MS basal medium (MS_0) for rooting. These plantlets with vigorous roots were transferred to styrofoam boards with holes in a plastic tray containing Yoshida's culture solution. The regenerants in the culture solution were allowed to grow for 2 weeks for hardening and then were transferred to soil directly in the transgenic greenhouse with a day/night temperature regime of 29/23 °C. The transgenic plants thus generated were subjected to both molecular assays and pathogen-infection assays.

e. Bioassay for blast-resistance. Twenty lines from each of the transgenic test cultivar, CO39, IR50 (susceptible), and C101A51 (*Piz-5*), were sown in plastic trays. Inoculum of *M. grisea* containing 50,000 conidia/ml of inoculum was spray-inoculated onto the rice seedlings at 4th leaf stage. Inoculated seedlings were incubated in a moist chamber for 24 h and then transferred to the dew misty chamber at 22–25 °C for 7 days till scoring. Blast disease severity was scored 10 day after inoculation using a 0-to-9 scoring method.

Leaves of IR50 and CO39 (transgenic T_1) plants showed high levels of resistance to blast. This observation is also supported by data on percent diseased leaf area (DLA) presented in Tables 4.4, 4.5 and Fig. 4.4.

Table 4.4 Screening of IR50 transgenic plants (T_1) against the rice blast fungus *Magnaporthe grisea*

Genotype	Mean size of lesion (mm)	DLA%[a]	Score[b]
CO39	12 ± 0.8	65	9
IR50	8 ± 0.7	60	9
C101A51	1 ± 0.2	0.5	0–1
13-T_1	2 ± 0.4	0.3	0–1
14-T_1	2 ± 0.7	0.6	0–1
15-T_1	1 ± 0.8	0.8	0–1

Source: Narayanan (2001) and Narayanan et al. (2002).
[a]DLA %, Percentage diseased leaf area
[b]Score based on a scale of 0–9 (SES system, IRRI).

Table 4.5 Screening of CO39 transgenic plants (T_1) against the rice blast fungus *Magnaporthe grisea* (Narayanan, 2001; Narayanan et al., 2004)

Genotype	Mean size of lesion (mm)	DLA%[a]	Score[b]
CO39	11 ± 0.6	65	9
IR50	7 ± 0.6	60	9
C101A51	1 ± 0.3	0.5	0–1
C101LAC	1 ± 0.4	0.5	0–1
18-T_1	2 ± 0.4	0.3	0–1
19-T_1	2 ± 0.7	0.6	0–1

[a]DLA %, Percentage diseased leaf area
[b]Score based on a scale of 0–9 (SES system, IRRI).

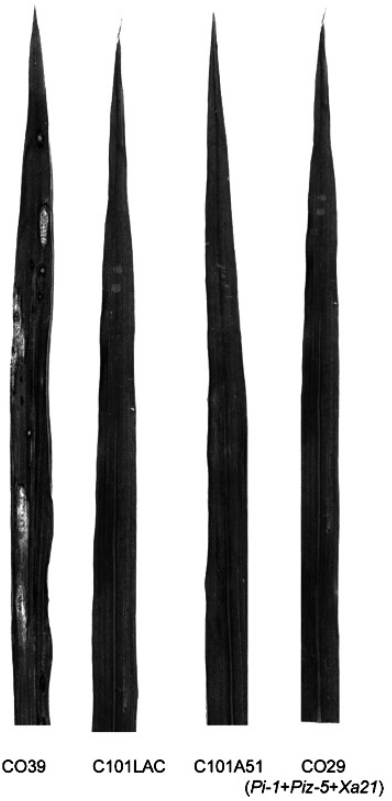

Fig. 4.4 Reactions of transgenic T_1 CO39 plants introgressed with *Piz-5* for blast resistance when inoculated with *Magnaporthe oryzae* isolate IK81-3. Leaves 1 to 4 (*left to right*) represent CO39, C101LAC, C101A51 (*Piz-5*), and the transgenic CO39 (*Pi-1*+*Piz-5*+*Xa21*), respectively (Narayanan et al., 2002)

The bacterial blight-resistant transgenic lines will be described briefly in the next chapter under biocontrol of bacterial blight. These transgenic plants will have to be evaluated in the rice fields in southern India under strict biosafety precautions and their field performances have to be monitored carefully.

Other Transgenic Rices for Chimeric and Non-rice Genes

Uchimiya et al. (2002) developed transgenic rice incorporated with a chimeric gene made up of rice gene, *YK-1* and maize ubiquitin gene, *HM-1*. The transgenic plants showed tolerance to *Magnaprothe oryzae* (blast fungus) and additional tolerance to abiotic stresses such as salt, submergence, uv-c and hydrogen peroxide.

Recently, Chattoo and his co-workers observed high levels of broad-spectrum of resistance to *M. oryzae* and *Rhizoctonia solani* (ShB pathogen) in transgenic rice expressing a plant defensin (usually small peptides of 45–54 amino acids) gene, *Dm-AMP1* from *Dalia merckii* (Jha, Tank, Prasad, & Chattoo, 2008). Because of the strict biosafety guidelines that exist at national levels, these transgenics may or not be field-evaluated to evaluate their importance for blast management.

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Chapter 5
Biological Control of Bacterial Blight of Rice

Pathogen: *Xanthomonas oryzae* pv. *oryzae*

Need for Biological Control

Bacterial blight (BB) of rice is among the most devastating rice diseases that occur globally, ranging in distribution from 20 °S in Queensland, Australia to 58 °N in Heilang Jiang, China (Mew, 1989). The disease is particularly destructive in the tropics, especially in Asia, where rice is grown throughout the year, during heavy rains of the monsoon season, and peaking at the flowering stage. Several disease-management strategies aimed at reducing crop losses and averting outbreaks of epidemics have been developed in the past. Effective chemical control for the management of rice BB is yet to be developed because of the extreme variability of the pathogen population in its sensitivity to antibiotics and chemicals used for control. Resurgence of drug-resistant populations also poses serious threats to efforts directed towards development of effective, long-lasting controls. Though the exploitation of host resistance appears feasible, breeding for BB-resistance with single major gene has proved unsuccessful due to rapid evolution of sub-populations that overcome these resistance genes. Biological control therefore appears to offer an ecology-conscious and cost-effective solution to this serious threat to rice cultivation.

Plant-Associated Bacteria as Biocontrol Agents

Pseudomonas, Bacillus Strains

In preliminary reports from this author's laboratory strains of *P. fluorescens* were shown to inhibit the growth of X. *oryzae* pv. *oryzae* in the laboratory (Sivamani, Anuratha, & Gnanamanickam, 1987). Over the years, however, there has not been a major study on the biological suppression of bacterial blight with bacterial agents. In this situation, twomajor studies were carried out in our laboratory during

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1997–2003. One of them involved *Bacillus* strains (Vasudevan, 2002) and the other involved *Pseudomonas fluorescens* strains that produced 2,4-diacetylphioglucinol (DAPG) (Velusamy, 2003; Velusamy & Gnanamanickam, 2003; Velusamy, Immanuel, Gnanamanickam, & Thomashow, 2006). These studies had the following objectives: 1. to identify efficient strains of *Bacillus and P. fluorescens*, and 2. to establish their role in (a) biological suppression of rice bacterial blight and (b) enhanced growth/yield of rice.

In these experiments, seeds of rice cultivar, IR24 (obtained from S. McCouch, Cornell U.) were used. This cultivar has no known resistance gene(s) for BB resistance and is a universal susceptible check for BB. Rice cultivar, Jyothi, an elite high-yielding variety grown extensively in the state of Kerala in southern India and susceptible to BB was also used. *Bacillus* and *Pseudomonas* strains were isolated from rice rhizosphere samples collected from different locations in southern India and on the basis of dual plate laboratory assays were short-listed if they showed consistent antibiosis to *X. oryzae* pv. *oryzae*.

Among 516 morphologically distinct rice-associated *Bacillus* strains isolated from rhizosphere samples, 42 strains were antagonistic to X. *oryzae* pv. *oryzae* in the dual-plate assays. The zone of inhibition caused by these strains varied from 1.2 to 4 cm in diameter (Fig. 5.1; Table 5.1). Among 637 strains of fluorescent bacteria, 278 strains (44%) showed inhibition of *X. o* pv. *oryzae* in laboratory assays. Twenty-seven of the antagonists produced 2,4-diacetylphloroglucinol (DAPG) and had the characteristic 745-bp fragment amplified by the PCR reaction when the sequence-specific primers developed from the *PhlD* sequence of *P. fluorescens* Q2-67 were used (Raaijmakers, Weller, & Thomashow, 1997; Velusamy & Gnanamanickam, 2003) (Fig. 5.2).

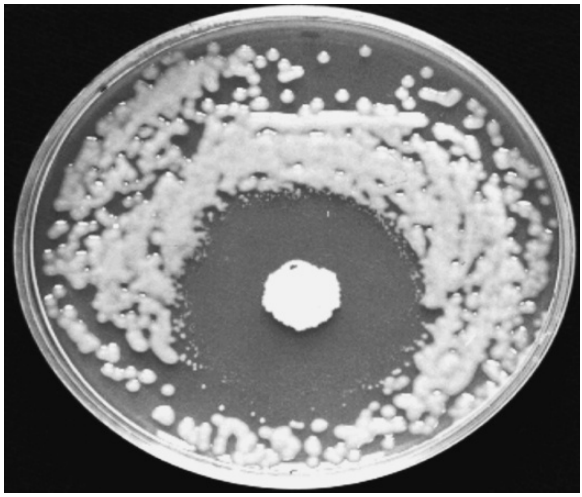

Fig. 5.1 Inhibition of *Xanthomonas oryzae* pv. *oryzae* by *Bacillus lentus* ALP18 in laboratory assays (Vasudevan, 2002)

Table 5.1 Inhibition of growth of *Xanthomonas oryzae* pv. *oryzae* in the laboratory and reduction of bacterial blight (BB) in rice cultivars, IR24 and Jyothi by *Bacillus* strains (Gnanamanickam, Vasudevan, & Velusamy, 2004; Vasudevan, 2002)

*Bacillus*strain	Diameter (in cm) of inhibition zone of *Xanthomonas oryzae* pv *oryzae*	BB reduction in rice (%) from the untreated control			
		1	2	3	4
1. *B. lentus* (ALP 18)	1.2	37.4	55.3	31.4	32.9
2. *B. cereus* (NGC I 15)	4.0	58.8	54.2	21.4	NT
3. *B. circulans* (VY I 18)	2.2	57.7	52.0	56.8	18.3
4. *Bacillus sp*. (CAL 9)	3.0	36.1	52.9	54.6	34.9
5. *Bacillus sp*. (MON 2–17)	2.0	38.5	54.9	45.2	53.9

1 and 2 – Disease reduction in rice cv. IR24 in the net-house and field experiment, respectively. 3 and 4 – cv. Jyothi (net-house and field).

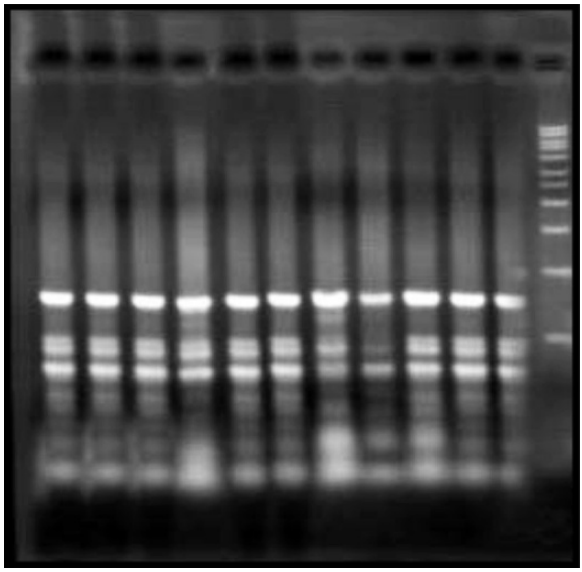

Fig. 5.2 PCR-based detection of 2,4-diacetylphloroglucinol (DAPG) production by *Pseudomonas fluorescens* from the rice rhizosphere of southern India (Velusamy & Gnanamanickam, 2003)

Net-House and Field Experiments

Identification of efficient *Bacillus* strains for bacterial blight suppression.

Bacterial strains were prepared at 10^8 cfu/ml in 1% carboxymethylcellulose and applied as seed treatment before sowing. Each bacterium was also applied as two foliar spray applications on the 35th and 45th day after planting in field plots. Application of 42 bacterial strains to rice cv. JR24 and Jyothi resulted in significant reductions in the mean length of BB lesions in bacteria-treated plants compared to the untreated control in both the net-house and field experiments (Table 5.1).

Disease suppression on cv. 1R24 ranged from 36 to 59% in the net-house experiment, while on cv. Jyothi, disease reductions ranged from 21 to 57%. Disease

suppression of more than 50% was observed in the field experiment with the cultivar 1R24 (Table 5.1). The levels of BB suppression in cv. Jyothi ranged from 18 to 54%.

Five superior strains, namely, ALP 18 *(B. lentus)*, NGC 115 *(B. cereus)*, VY 118 *(B. circutans)*, CAL 9 *(Bacillus* sp.), and MON 2-17 *(Bacillus* sp.) were identified at species level and were selected for further evaluation (Table 5.1).

Evaluation of DAPG-Producing *P. fluorescens* for Suppression of BB

Twenty-seven of the DAPG-producer strains of *P. fluorescens*, also identified as efficient antagonists of *X. oryzae* pv. *oryzae* were evaluated in a field experiment planted with rice cultivar IR24 in Pattambi, Kerala (Table 5.2). Application protocols used were the same as in the earlier experiment conducted with *Bacillus* strains. In addition to the seed treatment and two foliar spray applications, the rice seedlings also received a root-dip in the respective bacterium at 10^8 cfu/ml for 1 hour at transplanting. Mean BB lesion length on rice leaves in bacteria-treated plants ranged from 7.8 to 21.9 cm while in the untreated plants, the average BB lesion length was 22.0 cm (Table 5.2). The untreated plants showed severe BB development with long and spreading blight lesions while the bacteria-treated plants had relatively healthy leaves with shorter BB lesions. The following seven strains that included, IMV14, PTB9, MDR7, KAD7, VEL17, VGP13, and PDY7 reduced BB lesion length by 50-to-64% and were identified as strains of *P. fluorescens*. *P. fluorescens* strain PTB9 afforded the maximum protection of 64%.

Mechanism(s) of BB Suppression

Bacillus

One of the Bacillus strains, ALP 18 produced a heat-resistant (at 121 °C) and pronase-resistant metabolite in culture fluids. The crystalline product of this substance, was produced at the rate of 1.6 mg/ml, and when amended in to peptone-sucrose agar (PSA), inhibited the growth of *X. oryzae* pv. *oryzae*. A careful analysis of its physical properties through FTIR, ^{1}H NMR, and ^{13}C NMR analyses suggested that the heat-resistant antibacterial metabolite is kanosamine (Vasudevan, 2002). Kanosamine production has been reported earlier from other *Bacillus* spp. (Cron et al., 1958; Milner et al., 1996; Umezawa, Umnio, Shibahara, Hamada, & Hashimoto, 1967).

Pseudomonas

To study the relationship between DAPG production and BB suppression by *P. fluorescens* PTB *9*, the most efficient strain and a producer of DAPG (identification made by the PCR method developed by Raaijmakers et al., 1997) (Fig. 5.2).

Table 5.2 Bacterial blight suppression by 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* strains. Field experiment, Pattambi, Kerala, India (Velusamy, 2003)

S. No	Name of bacterial treatment	Mean of BB lesion length (cm)[a, b]	Difference in lesion length from control (cm)	Percent of disease suppression	LSD value	
					5%	1%
1	KAD7	10.15	11.88**	53.93	3.4	4.5
2	IMV14	9.53	12.50**	56.74	3.4	4.5
3	IMV2	14.72	7.31**	33.18	3.4	4.5
4	BGR19	12.62	9.41**	42.71	3.4	4.5
5	PTB9	7.83	14.20**	64.46	3.4	4.5
6	MON1	13.85	8.18**	37.13	3.4	4.5
7	TVM8	16.85	5.18**	23.51	3.4	4.5
8	VEL17	11.07	10.96**	50.75	3.4	4.5
9	VEL10	18.17	3.86*	17.52	3.4	4.5
10	GDY4	12.90	9.13**	41.44	3.4	4.5
11	GDY7	12.05	9.98**	45.30	3.4	4.5
12	TRP5	14.42	7.61**	34.54	3.4	4.5
13	TRP18	11.38	10.65**	48.34	3.4	4.5
14	MDR9	16.90	5.13**	23.29	3.4	4.5
15	MDR7	10.04	11.99**	54.43	3.4	4.5
16	STR7	14.09	7.94**	36.04	3.4	4.5
17	VGP13	11.19	10.84**	51.21	3.4	4.5
18	MDR16	11.87	10.16**	46.12	3.4	4.5
19	PDY7	9.50	12.53**	51.88	3.4	4.5
20	VLB7	20.03	2.00	9.08	3.4	4.5
21	KVR5	13.42	8.61**	39.08	3.4	4.5
22	TNI13	15.82	6.21**	28.19	3.4	4.5
23	KOV8	19.89	2.14	9.71	3.4	4.5
24	RJP31	21.92	0.11	0.50	3.4	4.5
25	KOV3	20.13	1.90	8.62	3.4	4.5
26	PDU1	19.43	2.60	11.80	3.4	4.5
27	PDU9	21.58	0.45	2.04	3.4	4.5
28	Control	22.03	0.00	0.00	0.0	0.0

[a]Each value is a mean of 20 measurements. [b]Mean of normalized lesion length (mean of lesion length in control-lesion length in treatment). *Reduction in lesion length significant at 1% and **Reduction in lesion length significant at 5%.

Extracts of 72 h-grown culture fluids of *P. fluorescens* PTB 9 were analyzed by hplc and the extracts yielded 40 μg of the antibiotic in 1 ml of the culture. When this material dissolved in 65% methanol was assayed for antibiosis towards *X. oryzae* pv *oryzae* in agar (PSA) well diffusion assays, 50–75 μg/ml inhibited the growth of the rice BB pathogen. In control plates which had 65% methanol, there was no inhibition.

Phl-Negative Mutants

In a net-house experiment five Phl$^-$ mutants of *P. fluorescens* PTB9 generated through transpositional mutagenesis-(*Tn5*-Km) were less effective in protecting

Fig. 5.3 Protection of IR24 rice plants from BB infection by *Pseudomonas fluorescens* PTB9 (first leaf from the *left*) and less protection by its Phl⁻ mutants (a,b,c,d, and e) (represented by leaf number 2–6 from the *left*). The last leaf (7th) is from an untreated check and has spreading BB lesion for the entire length of the leaf

IR24 rice plants against BB (Fig. 5.3, Table 5.3). While the wild type strain suppressed BB by 59.5%, the four phl⁻ mutants afforded 19.7, 17.1, 23.8 and 20.8% reductions of BB, respectively.

It can be mentioned that this was the first record of any detailed study on the biological control of rice bacterial blight. *Bacillus* and *P. fluorescens* strains applied as seed treatment and foliar applications afforded significant levels (> 50%) of disease suppression. It is also the first time when 2,4-diacetylphloroglucinol-producing tropical strains of *P. fluorescens* were identified in rice rhizospheres (Velusamy, 2003).

DAPG is a polyketide antibiotic which was known for its key role in "take-all decline" in the United States and for suppression of diseases of tobacco and other crops, has been shown to play a definite role in BB suppression in rice (Bangera & Thomashow, 1999; Defago et al., 1990; Gnanamanickam et al., 2004; Raaijmakers & Weller, 1998; Velusamy et al., 2006).

Enhancement of Rice Growth due to *Bacillus* Treatments

In addition to BB suppression, increases in average plant height were observed in *Bacillus*-treated rice plants against untreated check. Also, mixtures of three and four *Bacillus* strains resulted greater enhancement of plant height than the respective treatments of single strains. Increases in numbers of tillers per hill (as much as 3 fold as that of untreated control) (Fig. 5.4) and grain yields were also observed (Gnanamanickam et al., 2004). Isolation and purification of the

Table 5.3 Evaluation of *Pseudomonas fluorescens* PTB9 and its *Phl*$^-$ mutants for suppression of bacterial blight (BB) in IR24 rice. Greenhouse experiment, Chennai, southern India, July–November, 2003 (Velusamy, 2003; Velusamy et al., 2006)

Bacterial strain	Mean BB lesion length (cm)[a]	Difference in lesion length from control (cm)[b]	Percent BB suppression
Wild type *Pseudomonas fluorescens* PTB9	7.66**	11.27**	59.52
Phl$^-$ mutants	15.21^{ns}	3.72^{ns}	19.65
PTB9a	15.69^{ns}	3.24^{ns}	17.11
PTB9b	14.43^{ns}	4.50^{ns}	23.77
PTB9c	14.99^{ns}	3.94^{ns}	20.81
PTB9d	15.50^{ns}	3.43^{ns}	18.11
PTB9e			
Check	18.93	0.00	0.00

**Reduction in lesion length significant at 1% by LSD method of analysis; ^{ns} = not significant. [a]Mean of three replications. [b]Mean of normalized lesion lengths (mean lesion length in untreated control/check deducted from mean lesion length in bacteria-treated plants).

metabolites produced by the *Bacillus* strains showed production of 3.9–5.5 μg/ml of IAA by the different *Bacillus* strains (Vasudevan, 2002). Some of them also produced GA_3-like substance (Lindow et al., 1998; Tien, Qaskis, & Hubbell, 1979).

Fig. 5.4 Increased tillers in rice cv. IR24 due to *Bacillus* strain Mon2-17 treatment (Gnanamanickam et al., 2004; Vasudevan, 2002)

Lysobacter

Ji, Wei, He, Wu, & Bai (2008) used whole cells or dilutions of culture fluids of a novel strain of *Lysobacter antibioticus* 13-1 isolated from the rice rhizosphere in Yunnan province of China for biological suppression of rice bacterial blight (BB) both in greenhouse and multi-location field trials. In greenhouse experiments, whole bacterial broth culture (WBC) of strain 13-1 afforded up to 69.7% BB suppression. In three field trials, strain 13-1 reduced BB incidence by 73.5%, 78.3%, and 59.1%, respectively. However, disease suppression by strain 13-1 varied significantly among different rice cultivars and also showed variations with pathogen (*Xoo*) strains used. The biocontrol agent outperformed the chemical/antibiotic, zhongshengmycin (1%) that was used as the chemical standard. This is the first report on the use of *L. antibioticus* for rice BB control (Ji et al., 2008). It may be mentioned that *Lysobacter* are gliding bacteria of the family *Xanthomonadaceae* within the gamma *proteobacteria* and the genus has 13 known species. *Lysobacter enzymogenes* (synonym: *Stenotrophomonas maltophilia*) strainC3 is a well known biocontrol agent for several of the fungal pathogens (Giesler & Yuen, 1998; Jochum, Osborne, & Yuen, 2006; Kobayashi, Reedy, Palumbo, Zhou, & Yuen, 2005).

Bacteriocinogenic Strains of X. oryzae pv. oryzae

The avirulent or less virulent strains of the BB pathogen that produce bacteriocins are antagonists of the pathogen *Xoo*. Bacteriocin production was reported to have a role in BB suppression (Sakthivel & Mew, 1991). However, more recent reports do not agree with this claim. Dardick, de Silva, Shen, and Ronald, (2003) found no correlation between in vitro bacteriocin activity and in planta inhibition of the BB pathogen.

Epiphytic Erwinia herbicola

Hsieh and Buddenhagen (1974) and Santhi, Unnamalai, and Gnanamanickam, (1987) observed that epiphytic populations of *E. herbicola* present in rice leaf surfaces lowered the pH of rice leaves by producing an acid and thus suppressed the growth of *X. oryzae* pv. *oryzae*. This appears to be a natural method of BB suppression and has been verified recently by Babu and Thind (2005)

Transgenic Rices for BB Management

The cloning of two of the major genes for BB resistance, *Xa*21 and *Xa*1 are major achievements in plant pathology. *Xa*21 was transferred by IRRI scientists from the wild rice species, *Oryza longistaminata* into a cultivated indica variety IR24.

Pam Ronald and her colleagues at University of California-Davis used map-based cloning method to clone *Xa*21 (Song et al., 1995). These researches showed that its molecular structure represented an uncharacteristic class of plant disease-resistance genes as it coded for a receptor-kinase like protein. Yoshimura et al. (1998) cloned the second BB resistance gene, *Xa*-1 also by using map-based cloning method. Wang, Song, Ruan, Sideris, and Ronald (1996) constructed the first set of japonica transgenic rices of T-309 by incorporating *Xa*21 and showed that it conferred resistance to all pathotypes of *X. oryzae* pv. *oryzae*. Since T-309 was not a commercial variety, Datta and his co-workers at IRRI, Philippines introduced *Xa*21 into several japonica and indica varieties, such as IR72, MH63, and IR51500 (Datta, 2002; Tu et al., 1998; Tu, Datta, Khush, Zhang, & Datta, 2000). The transgenic plants carried a 3.8 kb *EcoRV*-digested DNA fragment corresponding to most of the coding region of *Xa*21 gene. Detailed protocols for generation and assay of the transgenic rices were described by Datta (2002).

In collaboration with Datta (2002) and Narayanan et al. (2004) generated blast and BB-resistant indica varieties, CO39 and IR50. For protocols, please see Chapter 4 of this volume.

Bioassay for Bacterial Blight Resistance

The set of test cultivars IR50, IR24 (susceptible controls), IRBB21 (near isogenic line for *Xa21*), IRBB4 (near isogenic line for *Xa4*), and 20 lines from each transgenic line were sown in plastic trays. Plants were tested against three different races of *Xanthomonas oryzae* pv. *oryzae (Xoo)* to differentiate the genes *Xa21* and endogenous *Xa4* such as *PXO86* (race 2), *PXO99* (race 6) and *PXO341* (race 10). The inoculum of each strain was prepared by incubating the bacteria on Wakimoto's medium (Medium composition: Modified Wakimoto's medium (MF-P): sucrose – 30g, bacteriological peptone – 5g, calcium nitrate – 0.5g, sodium phosphate (dibasic) – 0.82g, ferrous sulphate – 0.05g, agar – 15–17g, pH – 6.0) for 72h at 30 °C, then suspending each pure culture in sterile distilled water and adjusting the inoculum to about 10^9 cells per milliliter. At the maximum tillering stage, each plant was inoculated with the above three strains of *Xoo* using the leaf clipping method at the transgenic greenhouse, IRRI. Reaction of rice plants to each race of *Xoo* was scored 14 days after inoculation.

Resistance to bacterial blight was observed in T_1 plants of IR50 and CO39 (Table 5.4). Bacterial blight lesions of < 2.0 cm length observed in transgenic plants were characteristic resistance reactions. The non-transformed BB-susceptible parent plants showed bacterial blight lesions of > 10.0 cm length.

These transgenic plants will be evaluated in the rice fields in southern India under strict biosafety precautions and their field performances will be monitored carefully. This has not happened yet and efforts are in progress.

In recent years, several transgenic lines/varieties of rices, including Pusa Basmati 1, the aromatic rice, have been constructed and evaluated for BB resistance (Swamy et al., 2006). They are all not described in this volume.

Table 5.4 Reactions of transgenic IR50 plants carrying *Xa21* to races 2 and 6 of *Xanthomonas oryzae* pv. *oryzae* (Narayanan et al., 2002, 2004)

Genotype	Race 2 (PXO86)				Race 6 (PXO99)			
	7d		14d		7d		14d	
	MLL (cm)	Reaction	MLL (cm)	Reaction	MLL (cm)	Reaction	MLL (cm)	Reaction
IR50 (C)	5.3	S	7.8	S	5.8	S	9.4	S
IR24 (C)	13.5	S	15.8	S	14.5	S	17.8	S
IRBB21 (C)	1.3	R	2.4	R	3.4	R	4.7	R
IRBB4 (C)	4.6	R	6.8	S	6.4	S	7.9	S
T 13 R-1[a]	2.6	R	2.6	R	2.8	R	3.8	R
T 13 S-1[b]	5.6	S	7.8	S	12.8	S	15.3	S

[a]Average of 15/20 T_1 progenies showing resistance to BB pathogen. [b]Average of 5/20 T_1 progenies showing susceptible to BB pathogen.
C – non-transformed parental lines; R – Resistant; S – Susceptible; MLL – Mean Lesion Length.

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Chapter 6
Biological Control of Sheath Blight (ShB) of Rice

Pathogen: *Rhizoctonia solani* Kuhn AG1- IA (*Thanetophorus cucumeris*) (Frank) Donk

Over the years rice cultivation practices changed with increased planting of high-canopy forming and nitrogen-responsive, high-yielding, semi-dwarf rice varieties. With these changes, ShB of rice which was a minor disease emerged as a major constraint for rice production in Asia, America and other rice-growing countries. The cost of applying expensive chemicals such as validamycin became prohibitive for resource-poor rice farmers of Asia. The fact that there is inadequate host resistance in rice for this diverse fungal pathogen has compounded the problem of breeding of ShB-resistant rice cultivars. Therefore, biological control of ShB is still an important option. ShB disease and its pathogen *R. solani* have been the target of most attention and focused biological control research. This chapter highlights those that have contributed to further our understanding of how the biocontrol agents work and the underlying mechanisms that are involved.

Biological Control Agents

Bacteria

Seed-Associated Bacteria

Mew and Rosales (1986) made the first published report on the successful use of rhizosphere bacterial strains isolated from rice fields of the International Rice Research Institute in the Philippines for control of rice ShB. In the years that followed, IRRI's effort to train rice researchers from Asian countries built a core group of researchers who were able to isolate and use native strains of plant-associated bacteria as rice inoculants for ShB suppression and thus assist the resource-poor rice farmers of Asia to manage sheath blight (Chen, Yin, Lu, & Li, 1996; Chen et al., 2001; Lai, Nguyen, Phong, Pham, & Mew, 2001). The network effort co-ordinated by IRRI was started in 1990 (when this author was also part of a multi-national Asian Development

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Bank-funded research initiative) and was led by T. W. Mew with active advisory role played by R. J. Cook. In successive years, the effort moved from research to application of rice seed-associated antagonistic bacteria as biological control agents (BCA) to large area of farmers' field in China, Thailand and Vietnam (Mew et al., 2004). This network effort on ShB biocontrol developed a project design that consisted of the following phases: (a) BCA research, (b) Technology development, (c) Pre-commercialization and (d) Commercialization. In the heart of the program was participation by key rice farmers and local county agricultural technicians. The success of the program also had to do with mass-producing the BCA locally in China or in Vietnam based on the area to be treated with BCA. As benefits, significant reductions of ShB and increases in rice yields were realized in multi-location field trials conducted in China and other participating countries (Mew et al., 2004) (Table 6.1). In spite of the successes, the performance by an agent showed inconsistencies between sites and between years. Mew et al., (2004) suggested therefore that applications of BCA alone might not be the best option for biological control of ShB.

Table 6.1 Effect of antagonistic bacterium *Bacillus subtilis* B-916 (commercial product: wenquining) on incidence of *Rhizoctonia* sheath blight in farmers' fields at three sites in Jiangsu province, China (Mew et al., 2004)

Treatment	Rate of application	Percent disease incidence at the site		
1996	(liters/ha)	Jurong	Jiangyan	Wujiang
B-916	3.75–4.50	9.90 b	10.50 b	18.80 b
Jingangmycin (Jm)	3.75–4.50	11.70 b	9.60 b	12.40 c
Check	–	29.40 a	42.8 a	48.80 a
B-916	4.50	8.70 b	–	–
B-916 + Jm	2.25 + 2.25	–	2.10 b	8.06 b
1997				
Jingangmycin	4.50	11.00 b	2.40 b	4.61 b
Check	–	38.50 a	17. 20 a	38.84 a

Each disease incidence value is a mean of 10 replications. Mean in a column within the same year having the same letter are not significantly different from each other (> 0.01) by DMRT.

Plant-Associated Bacteria

In the author's laboratory at the university of Madras in southern India, biocontrol of ShB was studied for a number of years during 1989–2006 and we independently and also in collaboration with IRRI, examined the potential of rice-associated bacteria as microbial inoculants of rice for the biological suppression of blast and sheath blight (ShB) (Gnanamanickam & Mew, 1992; Gnanamanickam, Candole, & Mew, 1992, Gnanamanickam, Valasubramanian, Chatterjee, Chatterjee, & Mew, 1994). *In our studies, basic and applied, we have always tried to discover the mechanism for biological suppression*. Many of these studies were described in our review of the biological control of rice diseases (Vasudevan, Kavitha, Brindha, Babujee, &

Gnanamanickam, 2002). Our earlier studies were directed to the development of bacterial inoculants for rice and in the process, we identified different species of fluorescent pseudomonads and *Bacillus* strains and recorded varying levels of ShB suppression. Valasubramanian (1994) used strain 7–14 of *P. fluorescens* (originally isolated by Gnanamanickam from rice fields at IRRI (Rosales, Thomashow, Cook, & Mew, 1995)) and observed that it controlled leaf blast up to 79% and sheath blight up to 85% in IR50 rice. In detailed genetic analysis of Pf7–14, the phenazine-like antifungal antibiotic appeared to be the primary contributor of disease suppression (Gnanamanickam et al., 1994; Chatterjee et al., 1996).

Subsequently, Thara (1994) identified an effective strain of *P. putia V14-i* (a chitinase producer) which suppressed ShB in IR50 rice by 60–80% in nursery plots (Thara & Gnanamanickam, 1994). This was followed by the studies conducted by Krishnamurthy (1997) who developed biological formulations of methylcellose (mc): talc (1:4) for *P. fluorescens 7–14* and *P. putida V14*-i. *P. putida* suppressed sheath blight up to 60% when used as seed treatment, root dip and as two sprays. When used just as a seed treatment, it afforded only 8% control of the disease (Krishnamurthy & Gnanamanickam, 1998). The *P. putida* strain also induced systemic resistance in rice against ShB (Krishnamurthy & Gnanamanickam, 1997, 1998). Induction of systemic resistance by *P. putida* contributed up to 15–18% reduction of ShB. Kavitha (2002) applied *Bacillus polymyxa VLB16* that produced a heat-tolerant protein and observed that it suppressed ShB up to 67% in field plots planted with IR24 when applied as seed treatment with an additional root dip and foliar sprays (Kavitha, Senthilkumar, Gnanamanickam, Inayathulla, & Jayakumar, 2005).

As part of this biocontrol effort, we also studied the genetic diversity of the pathogen, *R. solani* in Indian rice fields. David (2003) provided a thorough analysis of morphological, cultural and genetic diversity of the pathogen, *R. solani* AG1-IA (Linde, Zala, David Paulraj, McDonald, & Gnanamanickam, 2005; Taheri, Gnanamanickam, & Hofte, 2007). Therefore, in all these earlier studies from our laboratory, efficient strains of *P. fluorescens* or *P. putida* or *Bacillus* with known modes of action were deployed as microbial inoculants of rice to suppress ShB.

In a recent study, we examined the potential of another unique group of fluorescent pseudomonads, the producers of 2, 4-diacetylphloroglucinol (DAPG), for ShB suppression (Immanuel, 2006). The existence of this sub-group of DAPG-producing strains of *P. fluorescens* in Indian soils and their significant role in the biological suppression of the devastating bacterial blight pathogen of rice (adequately described in the previous chapter) became apparent in the last few years (Velusamy & Gnanamanickam, 2003; Velusamy, Immanuel, Gnanamanickam, & Thomashow, 2006).

The emphasis of this study carried out by Immanuel (2006) was on *Pseudomonas* strains that produced this valuable antibiotic called, 2,4-diacetylphloroglucinol (DAPG). Molecular tools were used to provide a good estimation of their biological and genetic diversity in southern India (Immanuel, 2006). Results of the study showed that of the 724 strains of fluorescent pseudomonad strains that were assembled from rice rhizospheres, 268 strains (37%) were antifungal to

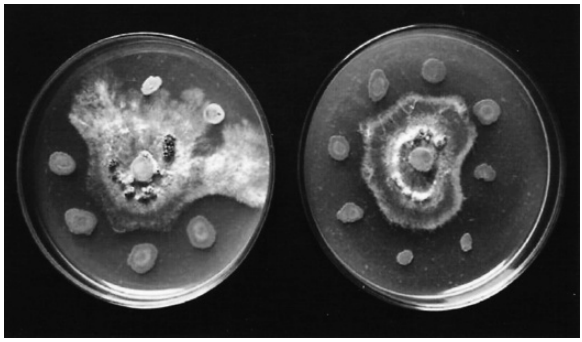

Fig. 6.1 Dual plate *assay for inhibition of Rhizoctonia solani* by strains of *Pseudomonas fluorescens*

Rhizoctonia solani, the sheath blight pathogen of rice (Fig. 6.1) (Immanuel, 2006). A PCR-based screening method which used two batches of primers (batch 1:Phl2a and Phl2b; batch 2: B2Bf and BPR4) located 67 of the 268 strains as producers of 2,4-diacetylphloroglucinol (DAPG) as they had the *PhlD* gene that could be amplified. The first batch of primers amplified a 745 bp fragment while the second batch of primers amplified a 629 bp fragment. On the basis of their consistent inhibition of *R. solani*, 17 of the 67 DAPG-producing strains were short-listed for evaluation in field plots for the biological suppression of sheath blight. There has been no previous report on the involvement of DAPG in the biological control of ShB disease of rice.

On the basis of the fingerprint patterns that emerged in the ribosomal DNA restriction analysis (ARDRA), genetic diversity of twenty-five short-listed strains of *P. fluorescens* (DAPG-producers and antagonists of *R. solani*) was determined. For ARDRA analysis PCR conditions described by Weizburg, Barnes, Pelletier, and Lane (1991) were used. The banding patterns indicated that DAPG production was diverse. Twenty-five strains analyzed formed three major clusters and among three groups. One of these groups had two Indian strains which formed the same cluster as that of the well known DAPG-producer strain CHAO (used as reference) (provided by G. Defago) (Fig. 6.2). This diversity among DAPG-producing Indian strains of *P. fluorescens* was also observed in RAPD and PCR-Southern analyses carried out (Immanuel & Gnanamanickam, 2005).

To assess the potential of these DAPG producing *P. fluorescens* strains for the suppression of sheath blight of rice, seventeen of them were carefully selected and evaluated in a field experiment in Pattambi, Kerala which is a known hot-spot for severe incidences of sheath blight (Table 6.2). In the untreated control plots, IR50 had severe incidence of sheath blight (treated as 100). It is noteworthy that all 17 strains of *P. fluorescens* reduced ShB severity to levels of statistical significance and the disease reductions ranged from 28.9 to 42.6%. Maximum ShB reductions were accomplished by treatments with *P. fluorescens* strain W4 (Fig. 6.4; Table 6.2) (Immanuel, 2006).

In plate tests, it was observed that DAPG concentrations greater than 100 μM completely inhibited the growth of *R. solani* (Fig. 6.3).

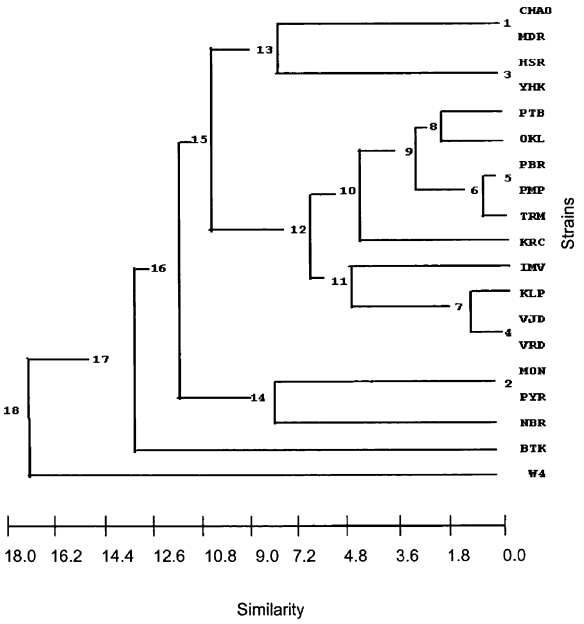

Fig. 6.2 Phylogenetic analysis of *16S rDNA* banding patterns for DAPG-producing *Pseudomonas fluorescens* strains of southern India (Immanuel, 2006)

Table 6.2 Suppression of sheath blight disease in cv.IR50 rice plants due to treatments with 2,4-diacetylphloroglucinol (DAPG) producing bacterial strains in a field experiment (Immanuel, 2006)

S. No	Treatment/bacterial strain	Percent disease incidence[a] (%)	Percent disease suppression[a] (%)
1	KRC6.5	60.53	39.46**
2	KLP2.3	61.57	38.42**
3	MDR7	61.97	38.02**
4	VRD4	68.08	31.91**
5	KRC6.29	66.20	33.79**
6	YHK4.1	67.08	32.91**
7	IMV14	66.23	33.76**
8	YHK11.2	64.08	35.91**
9	PTB9	62.71	37.28**
10	HSR4.1	71.12	28.87**
11	YHK7.3	70.33	29.66**
12	VJD2.2	63.34	36.65**
13	KLP2.7	61.08	38.91**
14	W4	57.44	42.55**
15	KRC1.48	61.72	38.27**
16	PYR10.8	60.34	39.65**
17	YHK8.2	61.05	38.94**
18	Control	100.00	–
LSD:	0.05 = 3.86;	0.01 = 5.13	

[a]Each value is a mean of 50 measurements. Statistical analysis by least significant difference (LSD) test: **Significant at 1%; *Significant at 5%; ns, Not significant.

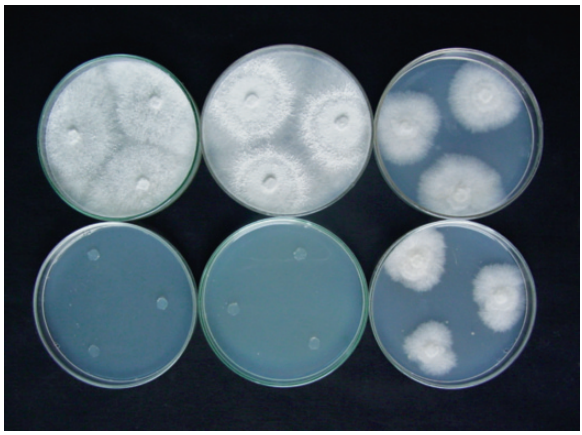

Fig. 6.3 Plate assays show toxicity of 2, 4-diacetylphloroglucinol (DAPG) to *Rhizoctonia solani*. Top left plate is a negative control (without DAPG). Other plates were amended with 1, 10, 50, 100, and 250 μM of DAPG. Complete inhibition of *R. solani* was observed in plates maneded with 100 and 250 μM DAPG

Fig. 6.4 Suppression of sheath blight disease and enhancement of growth in cv. IR50 rice by DAPG-producing *Pseudomonas fluorescens* strainW4 (Immanuel, 2006)

At closer examination, DAPG induced changes in the mycelial tips of *R. solani* (also in some of the other plant pathogenic fungi) which included bulging of the mycelium and bursting of such tips exposed to prolonged incubation. These observations provided circumstantial evidence that *R. solani*, the sheath blight pathogen of rice can be suppressed by DAPG if it is available at the correct stage of the rice crop and at sufficient quantities. Genomic sequence of *P. fluorescens* strains (such

as Pf-5) provide insights about the location of DAPG and other antifungal genes in the genome and reveal their potential as biocontrol agents (Loper, Kobayashi, & Paulsen, 2007).

Another research group at the Tamil Nadu Agricultural University in Coimbatore, southern India made significant contributions to the research on plant-associated bacteria, their formulation and field application for control of rice and other crop diseases. Their efficient *P. fluorescens* strains Pf1 and PfALR2 were formulated into talc-based formulations and applied as seed treatments or sprays for biological control of ShB (Rabindran & Vidhyasekaran, 1996; Vidhyasekaran & Muthamilan, 1999). This group of researchers continue to make valuable contributions on mechanisms of disease suppression. It appears that the antagonistic bacteria have been very well accepted as potential biocontrol agents of rice sheath blight and are being used in most rice-growing countries (Kazempour, 2004).

Fungi

Trichoderma, Gliocladium

Several fungal candidates have been screened or used in preliminary experiments for their potential to suppress the rice sheath blight pathogen. Among them *Trichoderma* and *Gliocladium* species are the most important (Xu, Harman, Wang, & Schen, 1999). *T. hamatum. T. harzianum*, and *T. viride* have been used in most of the studies. Trichoderma elicits suppression of *R. solani* by being mycoparasites as their hyphae often coil around the hyphae of *R. solani* making the host hyphae vacuolated. In the process, *R solani* hyphae collapse and disintegrate. This may also be due to the production of antibiotics and lytic enzymes such as chitinases and glucanases. *Gliocladium* spp are known producers of viridian and gliotoxin antibiotics (Cook & Baker, 1983). It is believed that *Trichoderma* are not as successful in rice as in other crops because of the flooded rice ecology which might not support the competitive nature and growth of an aerobic fungus. There have been numerous studies in rice and often researchers have studied chitinase production as a measure of their biocontrol potential (Krishnamurthy et al., 1999). Researchers at the Tamil Nadu Agricultural University observed that *Trichoderma* neutralized the effects of a host-specific toxin produced by the sheath blight pathogen, *R. solani* (Vidhyasekaran et al., 1999; Krishnamurthy et al., 1999). The integration of *Gliocladium* and *Trichoderma* with soil amendments appears to have the potential for ShB control and is discussed below.

Soil Amendments, AM Fungi and Their Integration

Soil amendments or an integrated method of ShB control has been discussed by several researchers (Baby, 2002; Baby & Manibhushan Rao, 1996). Arbuscular mycorrhizal (AM) fungal populations in agricultural soils are definitely known to be influenced by agricultural practices which affect their frequency and diversity.

Besides their contribution to crop growth, AM fungi are also known to improve plant's tolerance to abiotic stresses such as drought and salinity, and root pathogens. At a time when there was no definite information on the influence of organic substrates on AM development and its possible role on ShB reduction, Baby and Manibushan Rao at the University of Madras (India) conducted a series of studies.

Baby and Manibhushanrao (1996) evaluated the effect of various organic soil amendments on arbuscular mycorrhizal (AM) fungal activity on rice plants and suppression of rice sheath blight in greenhouse and field experiments. They recorded that AM spore density, percent infection, and intensity of infection were increased by the use of organic amendments, while ShB disease decreased. They suggested that green leaf manure when used as an amendment stimulated arbuscule development in rice plants and indicated that selective use of organic amendments may enhance development of native AM fungi and reduce disease (ShB) incidence. In another study, these researchers evaluated fungal antagonists (*Gliocladium virens* and *Trichoderma longibrachiatum*) and two organic soil amendments (gliricidia leaf and neem cake) to control rice sheath blight disease under greenhouse as well as field conditions. Integration of these two systems significantly enhanced the efficacy over their individual effects (Baby & Manibhushanrao, 1993). Baby (2002) reviewed the available information on the biological control of rice ShB through the use of AM fungi, soil amendments and their integration.

Cultural Practices/Soil Conditions

In field experiments conducted at IRRI, bacterial treatments used for biological control of ShB performed better in direct-seeded rice. In greenhouse experiments, it was observed that rice field soil of slightly acidic soil pH (pH 5.0) and soils with boron toxicity were more suitable soil conditions for bacterial treatments to suppress ShB (Gnanamanickam et al., 1992).

Transgenic Rice and ShB Control

More number of transgenic rice plants have been generated for ShB management (Baisakh et al., 2001; Datta et al., 1999; Lin et al., 1995; Uchimiya et al., 1993). Datta (2002) provided an updated review on the evaluation of pathogenesis-related (PR) protein genes which have been introduced with detailed protocols for generating transgenic rice developed in his laboratory at IRRI. At least 12 rice cultivars had been used as starting materials for constructing transgenic rices and these included popular cultivars such as IR72, IR64, CBII, Basmati 122, Swarna, IR58 and others. Two different types of PR genes, PR3-chitinases and PR5-thaumatin-like protein genes have been used as candidate genes. The transformants were examined for inheritance by Southern and Western blot analyses. These transformants synthesized high levels of PR proteins constitutively and exhibited enhanced resistance

when challenged with the sheath blight pathogen, *R. solani*. However, they have not shown enough of field resistance.

In a recent report, Jha, Tank, Prasad, and Chattoo (2008) of Chattoo's group observed that transgenic rice plants expressing a defense gene (defensin genes are small peptides of 45–54 amino acids with a characteristic folding that is stabilized by disulfide-linked cysteines) from *Dalia merckii*, *Dm-AMP1*, showed 84% tolerance to *Magnaporthe oryzae* (blast fungus) and 72% tolerance to *R. solani* in greenhouse assays.

Two aspects of sheath blight make the rice-R. *solani* host-pathogen interactions are important to remember. Rice does not possess adequate levels of host resistance like in the case of blast or bacterial blight. *R. solani* is an aggressive pathogen and unlike other fungal pathogens, under conducive conditions shows explosive abilities to spread from a small or minute lesion. PR proteins can be compared to QTLs and it might take several such genes, if they can be pyramided, to produce durable resistance to sheath blight. Transgenic rices may or may not be the answer for ShB management but in the absence of host-resistance, they have provided us with the best science plant biotechnology can offer.

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Chapter 7
Biological Control of Sheath-Rot and Other Fungal Diseases

Limited amount of research that is available on the biological control of sheath-rot (causal agent: *Sarocladium oryzae*), stem rot (causal agent: *Sclerotium oryzae*) and bakanae (causal agent: *Fusarium moniliforme*) is described in this chapter. These are minor diseases. However, the sheath-rot can be a constraint if it occurs in serious proportions as it causes discoloration of the sheath and affects the marketable quality of rice grains. Vasudevan, Kavitha, Priyadarisini, Babujee, and Gnanamanickam (2002) documented most of the work on the biological suppression of these diseases.

Sheath-Rot (Sh-R)

The only available records are from work that was carried out in the author's laboratory at the University of Madras in India and the International Rice Research Institute in the Philippines.

Sakthivel (1987) carried out detailed studies on sheath-rot suppression with *Pseudomonas fluorescens* strains. A *Pseudomonas fluorescens* strain antagonistic to *Sarocladium oryzae* the sheath rot (Sh-R) pathogen of rice in shown in Fig. 7.1.

The same Pfcp strain caused an inhibition of 2.5 cm (diameter) seen here (in plate on right) also caused in-planta reduction of 54% Sh-R incidence in IR20 rice when it was evaluated in a greenhouse test. Imprints of rice seedlings and a direct-observation technique of staining roots with fluorochromes confirmed the

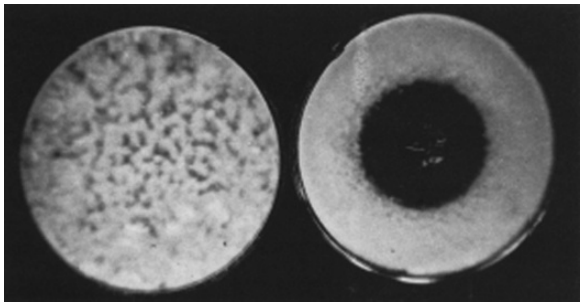

Fig. 7.1 Laboratory dual plate assay shows inhibition of *Sarocladium oryzae* by a *Pseudodmonas fluorescens* strain Pfcp isolated from citrus leaves (plate on the *right*). Plate on the *left* has the uninhibited growth of the pathogen in untreated control (Sakthivel, 1987)

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association of *P. fluorescens* with roots and the ability of the strain to move along shoot tips. In three field tests, treatment with *P. fluorescens* reduced the severity of Sh-R by 20–42% in five rice cultivars. Bacterization of rice cultivars with *P. fluorescens* also enhanced plant height, number of tillers, and grain yields from 3 to 160% (Sakthivel & Gnanamanickam, 1987).

At IRRI, Rosales, Vantomme, Swings, De Ley, and Mew (1993) identified a set of bacterial strains that were assembled from the rice rhizosphere as useful antagonists to different rice pathogens and in the process, identified also antagonists to *Sarocladium oryzae*. Sh-R fungus and its toxin, cerulenin, had unique interactions with other fungal pathogens of rice such as *Magnaporthe oryzae, Rhizoctonia solani, and Sclerotium oryzae*. The growth of these fungal pathogens was inhibited in laboratory tests. Therefore, in a greenhouse experiment, Gnanamanickam and Mew (1991) examined if these inhibitory interactions of *S. oryzae* with other fungal pathogens of rice contributed to dominance of Sh-R and the results suggested that this might be true. However, in the absence of adequate field observations, no further conclusions were arrived.

Sakthivel and his team of researchers at Pondicherry University and a research group in Tamil Nadu Agricultural University in southern India have remained active in elucidating the role of *Sarocladium oryzae* toxins (helvolic acid, cerulenin, and SO-1 toxin) for virulence towards rice in the induction of Sh-R disease symptoms including the discoloration of rice grains (Ghosh, Amudha, Jayachandran, & Sakthivel, 2002; Ayyadurai, Kirubakaran, Srisha, & Sakthivel, 2005; Nandakumar, Babu, Amutha, Raghuchander, & Samiyappan, 2007). While the current status of the disease and Sh-R control strategies has been reviewed, there is no further report on biological control (Sakthivel, 2001).

Stem Rot

Stem rot is also a minor disease of rice and has occurred in serious proportions in California rice. There has been only one study on the use of antagonistic strains of bacteria. They were used as seed treatments for the biological suppression of the stem-rot pathogen, *Sclerotium oryzae* (Elangovan & Gnanamanickam, 1993). Stem-rot infection was reduced by the bacteria treatments which also affected the number of the minute sclerotia of the pathogen formed. Rosales et al. (1993) identified *P. aeruginosa*, *B. subtilis* and *B. pumulus* strains that were effective against *Sclerotium oryzae*.

Bakanae

Biological control of this rice disease caused by *Fusarium moniliforme* has been studied by Mew and his team of researchers at IRRI. Seed treatment of rice with rice-associated antagonistic bacteria produced satisfactory reductions of bakanae symptoms (Rosales & Mew, 1997).

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Chapter 8
Biological Control of Rice Tungro Disease (RTD)

Rice tungro disease (RTD) consists of a spherical virus (RTSV) and a bacilliform virus (RTBV) and the disease is a significant yield constraint in rice-growing areas of South and Southeast Asia. Disease symptoms are caused largely by infection by the rice tungro bacilliform virus (RTBV).

Conventional Biocontrol Agents

Traditional management practices and conventional biological control agents are considered unsuitable or not effective for reduction of RTD. It is understood that RTD is very difficult to control with these practices. Therefore, these methods have not been used in experiments conducted with biocontrol agents. However, in one single study, Ganesan (1999) obtained noticeable differences in RTD development between untreated IR50 rice seedlings that received viruliform green leafhoppers (GLH) and rice seedlings that received a spray inoculation with a strain of *Pseudomonas fluorescens* that showed insect-toxicity, before they received the same number of GLH/seedling. However, this method of biological suppression of RTD by reduction of the GLH vectors has to be verified through further studies.

Transgenic Rice for RTD Management

Apart from conventional breeding for virus resistance, the development of transgenic lines has been considered the most reliable means of curtailing yield losses caused by rice viruses. Vasudevan, Kavitha, Priyadarisini, Babujee, and Gnanamanickam (2002) and Datta (2002) reviewed the efforts to engineer rice for viral resistance. These efforts largely deployed pathogen-derived resistance (PDR) involving the expression of pathogen-derived transgenes such as the coat or capsid protein (CP) genes, in rice plants to interrupt the virus infection cycle (Potrykus et al., 1995; Fauquet et al., 1997; Sivamani et al., 1999).

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Rice Trungro Spherical Virus (RTSV)

For the first time Sivamani et al. (1999) developed transgenic rice plants resistant to the rice tungro spherical virus (RTSV), also perhaps a first example of CP-mediated protection against a virus that contains more than one CP gene. They introduced the coat protein (CP) genes CP1, CP2 and CP3 of rice tungro spherical virus (RTSV) individually or together into indica and/or japonica rice cells by particle bombardment and generated transgenic plants. Plants derived from selfed progeny of the primary transformants were subjected to virus inoculation via leafhoppers, the natural vector of the virus. Sixteen out of the nineteen selected transgenic plant lines, as well as their R1, R2 and/or R3 progeny that contained the target gene, accumulated transcripts of the chimeric CP gene(s) in RNA blot analysis. These researchers obtained evidence of moderate levels of protection to RTSV infection, ranging from 17 to 73% of seedlings that escaped infection and a significant delay in virus replication under greenhouse conditions in plant lines that expressed the RTSV-CP1, CP2 and CP3 genes singly or together.

Rice Tungro Bacilliform Virus (RTBV)

Tyagi, Rajasubramaniam, Venkatrajam, and Dasgupta (2008) applied the concept of RNA-interference (RNAi) for the control of RTBV infection in transgenic rice plants they developed by expressing DNA encoding ORF IV of RTBV, both in sense as well as in anti-sense orientation. This resulted in the formation of double-stranded (ds) RNA. RNA blot analysis of two representative lines indicated specific degradation of the transgene transcripts and the accumulation of small molecular weight RNA, a hallmark for RNA-interference. In the two transgenic lines expressing ds-RNA, different resistance responses were observed against RTBV. In one of the above lines, there was an initial rapid buildup of RTBV levels following inoculation, comparable to that of untransformed controls, followed by a sharp reduction, resulting in approximately 50-fold lower viral titers, whereas the untransformed controls maintained high levels of the virus till 40 days post-inoculation (dpi).

In a more recent breakthrough, Beachy and his team of researchers at the Danforth Plant Science Center in St. Louis, MO have discovered a technology that reduces the spread of the rice virus (Dai et al., 2008). Two host transcription factors, RF2a and RF2b regulate expression of the RTBV promoter and are important for plant development. Expression of a dominant negative mutant of these factors in transgenic rice resulted in phenotypes that mimic the symptoms of RTD, whereas overexpression of RF2a and RF2b had essentially no impact on plant development. Conversely, lines with elevated expression of RF2a or RF2b showed weak or no symptoms of infection after *Agrobacterium* inoculation of RTBV, whereas control plants showed severe stunting and leaf discoloration. These researchers believe that gaining disease resistance by elevating the expression of host regulators provides another strategy against RTD and may have implications for other pararetrovirus infections.

Cultural Practices for RTD Management

Cultural practices that target to reduce the GLH vector populations have been useful for RTD management. Some of these practices include, large scale synchronous planting of rice with a definite fallow period in between cropping seasons, avoidance of late planting, roguing and removal of infected plants and manipulating the rice planting space (Azzam & Chancellor, 2002). A closer planting space, particularly in direct-seeded rice is known to reduce RTD incidence.

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