Chapter

Physiological, Ecological and Genetic Interactions of Rice with Harmful Microbes

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Abstract

Rice is one of the most important food crops for mankind and suffers significant crop loss annually due to rice diseases. Availability of genome sequences of rice has served as a springboard to utilize its innate immunity to prevent rice diseases. Knowledge on interactions of rice and rice pathogens has rapidly accumulated. Effective resistance genes have been identified from cultivated, weedy species of rice, and wild rice relatives and their roles in plant innate immunity have been uncovered. Presently, rice diseases are being managed using host resistance genes and pesticides in diverse culture systems around the globe. This chapter presents a simple review of interactions of rice with harmful microbes causing the two major damaging diseases, rice blast and sheath blight. The review is written to target new readers in life sciences. Knowledge and critical literatures on physiological, genetic, and ecological aspects of host-pathogen interactions are presented to gain insights leading to sustainable disease management systems.

Keywords: rice, disease, resistance genes, innate immunity, crop protection

1. Introduction

Rice originated 130 million years ago to become the annual cereal crop that provides 20% of the essential calories needed to feed more than one-half of the world population [1]. Major producing countries of rice are China and India; most of the rice produced by both countries is domestically consumed. In the USA, rice has been under cultivation for over three hundred years; however, today less than 2 percent of world rice is being produced in the USA. The rice crop in the USA is known for its high rough rice yield, and excellent milling and cooking quality that occupies the top 10 in the international marketplace. The earliest rice seeds were discovered over 7,700 years ago in the Hangzhou area, eastern China [2] establishing China as the first ancient civilized country to grow rice. The domestication and agronomic improvement of rice began with the exploitation of wild rice and land race varieties during ancient times. Since then, the primary breeding objective has been to increase the yield potential to meet the rapidly increasing demands of human consumption. In the 1950s, the semi-dwarf gene *sd1* was discovered and used to develop semi-dwarf varieties with high grain yield but without lodging. The rapid utilization of this technology began the green revolution in rice. In 1960, the principles and techniques of hybrid production developed by American corn

breeders were adapted for hybrid rice breeding in China [3]. During the 1970s, indica types of hybrid rice were rapidly deployed in major rice producing areas and, currently, hybrid rice is more than 50% of the rice production in China. Hybrid rice production has expanded to other countries and in 2020, occupied approximately 35% of US rice acreage. Globally rice crops have been well protected against diseases; however, throughout this intense agronomic selection for yield enhancing genes, the corresponding genetic diversity needed for effective rice disease control has decreased [4]. Increasing yield through hybrid rice is one way to increase the total rice production. However, rapid extension of hybrid rice worldwide will present a new challenge for the control of rice diseases such as rice blast and sheath blight diseases since limited germplasm can be used for hybrid seed production. An insignificant race of the southern leaf blight fungus *Bipolar maydis* under favorable conditions resulted in 6 billion crop loss when maize hybrids with cytoplasm (cms-T) were heavily deployed in the southern USA (for example, [5]). Understanding mechanisms of interactions of rice with harmful microbes are therefore critical for food security.

Most diseases of rice are caused by harmful fungi such as blast disease caused by [*Magnaporthe oryzae* (anamorph: *Pyriculara oryzae*)], sheath blight [*Rhizoctonia solani* (telomorph: *Thanatephorus cucumeris*)], brown spot [*Cochliobolus miyabeanus* (anamorph: *Bipolaris oryzae*)], false smut (*Ustilaginoidea virens*), kernel smut [*Tilletia barclayana* (*Neovossia horrida*)], Narrow brown leaf spot [*Cercospora janseana* (telomorph: *Sphaerulina oryzina*)], crown sheath rot (*Gaeumannomyces graminis* var. *graminis*), downy mildew (*Phytophthora macrospora*), aggregate sheath spot [*Rhizoctonia oryzae-sativae* (Sawada) Mordue and *R. oryzae* Ryker & Gooch)], eyespot [*Drechslera gigantea* (Heald et Wolf) S. Ito], leaf smut (*Entyloma oryzae*), leaf scald (*Microdochium oryzae*), seedling blight (*Pythium*, *Fusarium*, *Diplodia*, *Rhizoctonia*, and *Penicillium* spp), stem rot (*Phytophthora sojae*), bakanae [*Fusarium moniliforme* (syn. F. verticilloides), teleomorph*: Gibberella fujikuroi* (syn. *Gibberella moniformis*)], respectively. The next most common diseases are caused by harmful bacteria such as leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*X. campestris* pv. *oryzae*), bacterial panicle blight [*Burkholderia glumae* and *Burkholderia gladioli* (Severin)], bacterial leaf streak (*Xanthomonas oryzae* pv. *Oryzicola*), bacterial foot rot (*Dickeya zeae*); sheath brown rot (*Pseudomonas fuscovaginae*), bakanae [*Fusarium moniliforme* (syn. *F. verticilloides*), teleomorph: *Gibberella fujikuroi* (syn. *Gibberella moniformis*)], respectively [6].

Any of the above mentioned rice diseases can result in severe yield loss when ideal circumstances favor disease infection and development. Among them, blast is the most devastating rice disease worldwide [6–8]. Disease symptoms of blast are more pronounced on rice under high nitrogen based nutrients and drought stress conditions, and often seen on rice plants grown on levees and the edges of rice paddies. Blast disease annually significantly reduces the crop in upland and often in flood irrigated rice production as well. Three notable crop damages are 1) the widespread destruction of the cultivar 'Newbonnet' in 1980s in the US; 2) 45% of rice affected by blast in 1993 in Japan; and 3) blast disease caused significant damage in 106 million hectares from 1982 to 2005 in China [9, 10]. Presently, occurrence and severity of blast are becoming more widespread in the Southern US and California. Sheath blight causes damage on all rice cultivars, especially on semidwarfs [11]. Sheath blight disease is the second most damaging disease after blast worldwide. Sheath blight was considered a minor disease for many years but has become more destructive under intensified high input production systems. The damage due to sheath blight was estimated to be from 20–42% in a simulation study [12] and 50% of crop loss under favorable conditions in the USA [13]. To date, sheath blight reduces the crop more than that of rice blast in the USA.

Understanding impacts of rice exposed to the above mentioned harmful microbes under changing climates is a never ending challenge. Rapidly evolving technologies such as genome sequencing, computational biology and genome editing methods have been used to study how nucleotide sequence changes influence the outcomes of host-pathogen interactions. The aim of this chapter is to update the complex interactions between rice plants with the harmful microbes causing diseases. Emphasis is placed on some aspects of the physiological, ecological, and genetic interactions between the rice plant and harmful pathogens such as rice blast (*M. oryzae*) and sheath blight (*R. solani*).

2. Physiological responses of rice plants to pathogens

Rice plants have cuticles, silica and cell walls that often serve as the first passive defense to contain pathogens [14–16]. Subsequent failed active resistance leads to visible pathogen damages. For rice blast disease, typically a diamond shaped symptom on the leaf is called leaf blast, and dark brown on the neck as panicle blast, respectively (**Figure 1**). Both leaf and panicle blast can result in significant yield reduction.

When they encounter rice plants, asexual spores of blast fungus, *M. oryzae* germinate and initiate their life cycle by penetrating the cells with infection pegs from tightly adhered swollen hyphal tips with the highest biological turgor pressures known for a biological organism [17]. During penetration *M. oryzae* absorbs nutrients by producing cutinases to degrade cutin in the cuticle and pectolytic enzymes. *M. oryae* secretes heat labile molecules such as endo ß-1,4-D-xylanase to solubilize rice cell wall fragments to kill cells [18, 19]. After penetration *M. oryzae* develops invasive hyphae that are in direct contact with the membrane of the live cells. Soon after that within approximately 48 hrs *M. oryzae* colonizes the host tissues and releases asexual spores at the end of invasion [20, 21]. *M. oryzae* is thus classified as a hemibiotrophic pathogen.

Figure 1.
Typical symptoms of rice blast disease. A. Leaf blast disease after an artificial inoculation under greenhouse conditions at USDA ARS Dale Bumpers National Rice Research Center, Arkansas, USA, and B. Panicle blast disease in a rice field under natural infections in Puerto Rico (photograph credit: Miss Adriana Rivera).

Figure 2.
Typical sheath blight disease on sheath and leaves in a rice cultivar at the booting stage in Stuttgart, Arkansas, USA.

Sheath blight disease can be found on young rice seedlings but usually does not begin vertical development until the plant is at the reproductive growth stage. Typical symptoms are found on the lower leaf sheaths of rice plants at late tillering or the booting stage (**Figure 2**). The sheath blight disease at flag leaf often results in significant yield reduction.

Sheath blight lesions appear as circular, or ellipsoid, green-gray, water-soaked spots at about 1–3 cm long. As a lesion develops it can enlarge to 2 cm in width and 3–10 cm in length and becomes bleached with an irregular purple-brown border at the center [13]. The fungus *R. solani* grows on the surface of the leaf sheath upward and produces new side branches approximately at 45- and 90-degree angles 5–6 mm from the growing tips of mycelia. The continued growing of side branches results in the formation of an infection cushion that is attached to the epidermis often with mucilage like materials. Penetration peg is then formed from the flattened cells at the base of the cushion. The penetration peg then penetrates the inner epidermal cells to obtain nutrients from the inner and later the outer epidermal cells of the leaf sheath. Within approximately, 48 hrs after inoculation new infection hyphae is produced in an epidermal cell lumen [22, 23]. During infection *R. solani* also produces toxin [24]. Such toxins are called host specific toxins and are pathogenicity factors [25]. Toxins are known to be toxic to plants by inhibiting host defense responses.

3. Ecological interactions of rice plants with pathogens

The three major factors necessary for disease to occur in plants: 1) A pathogen that can cause disease, 2) a host plant that is susceptible to a pathogen, and 3) an environment that favors the pathogen infection. The environment includes temperature, humidity, light intensity, surrounding areas, and/or human intervention. These three-factors referred as the disease triangle contribute to the severity of disease. After diseases occur plant pathogens are typically disseminated within the same field and are often transmitted by wind and/or insects to fields far away from the diseased plants. In nature, rice pathogens find rice and survive with and/or without rice after their infection and amplification [26]. In the tropics the climates are relatively warm year round. Hence, overwintering is not an issue for

M. oryzae. The overwintered conidia and mycelia on alternative hosts and/or rice often serve as a source of primary infection in the disease cycle. *M. oryzae* species is a pathogen of over 50 grass species including crops such as wheat (*Tritium aestivum* L. [27]), Barley (*Hordeum vulgare* L. [28]) and finger millet (*Eleusine coracana*; L. [29]). However, each isolate of *M. oryzae* is often limited to attack a small group of grass species [29]. The causal agent for blast disease in one group of grass species is often different from similar groups that attack other grass species. Cross infections between the species observed under controlled conditions suggest that grass weeds, *Rottboellia exaltata*, *Echinochloa colona*, *Leersia hexandra* and *Alopecurus carolinianus* could be alternative hosts for *M. oryzae* [30, 31].

Even though *M. oryzae* is not a good competitor among saprophytes, rice seeds and diseased rice residues are considered as the primary sources of *M. oryzae* [32, 33]. Infectious *M. oryzae* were purified from foundation, certified and grower seeds in Arkansas (for example, [32]). The spores of *M. oryzae* produced by the contaminated rice seeds infected seedlings from 2 to 4 leaf stages [33]. The infected seedlings are then served as an inoculum for nearby healthy plants that develop blast symptoms later. Often *M. oryzae* infection from booting to flowering/immature panicle results in *M. orzyae* contaminated rice seeds. Diseased residues are considered as another primary source of inocula [34]. Infected rice residues up to 18 months from surface mulch were the sources of *M. oryzae* that caused leaf blast under field conditions (for example, [34]).

R. solani infecting rice belongs to an anamosis subgroup (AG)1-IA [35]. This type of pathogen typically lives on dead tissues, and often changes hosts during alternative growing seasons. Infection of *R. solani* usually begins from sclerotia and mycelia on debris from previous crops in the soil. Sclerotia usually accumulate around rice plants at the water and plant interface. Once sclerotia germinate, they develop mycelia that grow upward on rice plants. Depending upon humidity and moisture conditions, disease development is rapid at early booting to heading and grain-filling stages [36]. Infections often occur near the waterline after the establishment of permanent flood. Lesions on the upper parts of rice plants can be developed to the entire leaves and leaf sheaths (**Figure 2**). Lesions with mycelia on rice plants change from white or gray with brown borders to brown where sclerotia are loosely attached. At maturity, sclerotia are separated from rice plants for overwintering in soil. Under favorable conditions such as high humidity (≥95%) and temperature (28–32°C), *R. solani* spreads rapidly to upper rice plants including rice leaves, grains, and to adjacent plants [13]. *R. solani* causing sheath blight diseases is a broad host pathogen which has multiple anamosis subgroups that specialize on several plant species causing other plant diseases [37].

4. Genetic interactions of rice plants with pathogens

Blast pathogen *M. oryzae* is known to reproduce asexually under field conditions and it has been a challenge to perform sexual crosses under laboratory conditions. The genome sequences of 50 isolates from different times and places showed that they belong to six lineages including isolates from two pandemics on japonica and indica rice [38]. The *de novo* DNA sequences also revealed that these lineages diverged about a millennium ago. Genome sequences of one lineage uncovered evidences of sexual transmission and alleles from multiple lineages. In the USA over the past 6 decades *M. oryzae* races have become more diverse and virulent [39, 40].

Genetic interaction of rice with *M. oryzae* follows the gene for gene theory where a resistance (*R*) gene is effective in preventing pathogen *M. oryzae* strains that contain the corresponding aviruelnce (*AVR*) gene [41, 42]. Presently, 40 *AVR* genes

have been identified, 11 of which have been cloned. *AVR* genes in *M. oryzae* are random secreted molecules predicted to play important roles in pathogenicity and fitness [43]. Rice *R* genes are mutable that may generate more *R* genes. *R* gene polymorphism is thus a significant source of complexity of the interactions of rice with different rice pathogens [44, 45]. Most *R* genes in rice are members of a small gene

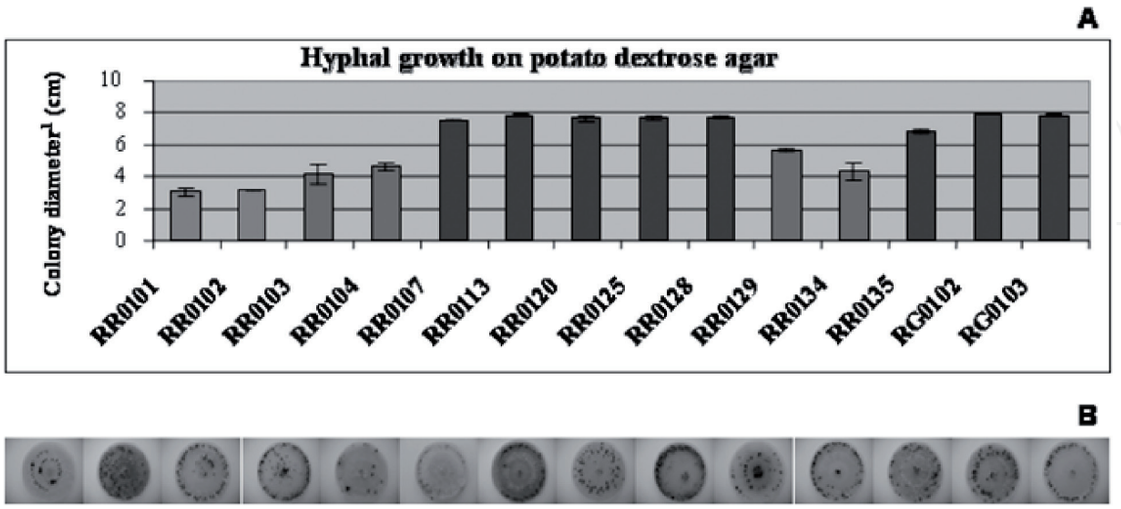

Figure 3.
Physiological characterization of rice sheath blight fungus Rhizoctonia solani. A. hyphal growth on potato dextrose agar of each hyphae from indicated isolates, and B. different morphologies of sclerotia of indicated isolates in a.

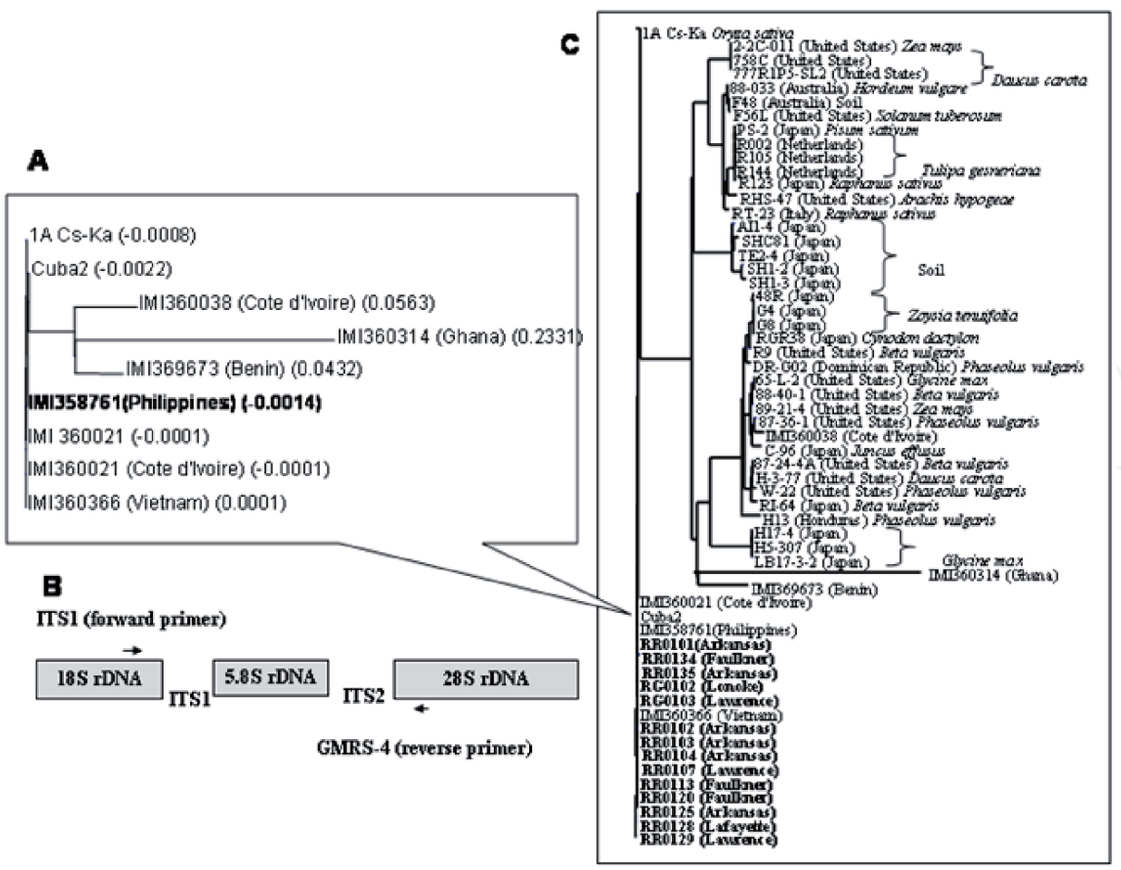

Figure 4.
Molecular characterization of rice sheath blight fungus Rhizoctonia solani. A to C describing region and phylogenetic relations (http://www.ncbi.nlm.nih.gov/nuccore, genbank accession numbers, AY185104 to AY185115 of 14 isolates from indicated counties in Arkansas).

family and are predicted to encode cytoplasmic NLR proteins with nucleotide binding site (NBS) and leucine rich repeats (LRR) [45, 46]. The rice genome (430 Mb) has 480 such NBS-LRR genes that can be the sources of *R* genes to different rice pathogens [47, 48].

Details on the genetic interaction of rice with *R. solani* has lagged far behind that of with *M. oryzae*. A major *R* gene to *R. solani* has not been discovered yet. Minor *R* genes such as *qSHB9–2* in rice cultivar Jasmine 85 have been identified [49, 50]. Further genetic and functional analyses suggest that the *ABC* transporter genes involved in rapid nutrient transportation is responsible for 25% of genetic resistance [51]. *R. solani* (AG)1-IA contains heterogenic multinuclei, and complete genome sequence of *R. solani* (AG)1-IA has been difficult due to the challenges on genome assembly [52]. Differences in morphology, speed of hyphal growth have been noticed and some isolates do display less aggressiveness (**Figure 3**).

These isolates have been recommended for controlled inoculations and genome sequencing [35]. The length of ribosomal DNA internal transcribed spacer (rDNA-ITS) can distinguish subspecies of *R. solani* from *R. oryzae* and *R. oryzae-sativae* [53], minor variation of DNA sequence, hyphal growth on potato dextrose agar and morphology of sclerotia can be seen among the isolates collected. All isolates tested so far in the USA were clustered into one clade, (**Figure 4**) [35, 54].

5. Remarkable features of rice plant innate immunity

Like other plants, rice cannot move to escape from pathogen attack and must evolve an efficient defense system [55]. The plant passive defense system is often initiated by cell wall, and cuticles by releasing pathogen associated molecular patterns (PAMPs, [56]). After sensing these PAMPs plants activate a variety of early defense responses including stomatal closure, transcriptional reprogramming responses and callose deposition that is called pattern-triggered immunity (PTI) [57]. More active defense response is often elicited by NLR *R* gene products. Upon the detection of pathogen *AVR* gene products NLR proteins reorganize and transduce defense signaling often resulting in programed host cell death that is called elicitor triggered immunity (ETI) [58]. Exactly how PTI and ETI lead to effective resistance response is still largely unclear (**Figure 5A**).

R genes are also known to be under fast evolution diversification [58] and have also evolved efficient methods to detect the unstable products of *AVR* genes in *M. oryzae*. A single amino acid of each of major blast *R* genes, *Pi-36*, *Pi-d2*, and *Pi-ta* has been found to determine its efficacy of resistance (for example, [59–61]) (**Figure 5B**). *Pi-d2* is a single copy gene encoding a predicted novel B-lectin receptor kinase with an extracellular domain of a bulb-type mannose specific binding lectin (B-lectin) and an intracellular serine–threonine kinase domain. A single amino acid difference at position 441 Isoleucine to Methionine (R to S) of Pi-d2 distinguishes resistant from susceptible allele. *Pi-d2* was localized in plasma membrane [60]. *Pi-36* is a single copy gene encoding an NBS-LRR protein. A single amino acid at the position 590 Aspartic acid to Serine (R to S) was found to associate with the resistance phenotype [61]. *Pi-ta* encodes an NLR protein with imperfect LRR [59]. Surveys in rice germplasm have identified only one *Pi-ta* allele conferring resistance and thirteen *pi-ta* alleles conferring susceptibility [62–68]. In most cases, a single nucleotide substitution results in a functional polymorphism distinguishing between resistance and susceptibility [59, 67, 68]. All resistant Pi-ta proteins have alanine at position 918 and all susceptible pi-ta proteins have serine at position 918 [62, 65].

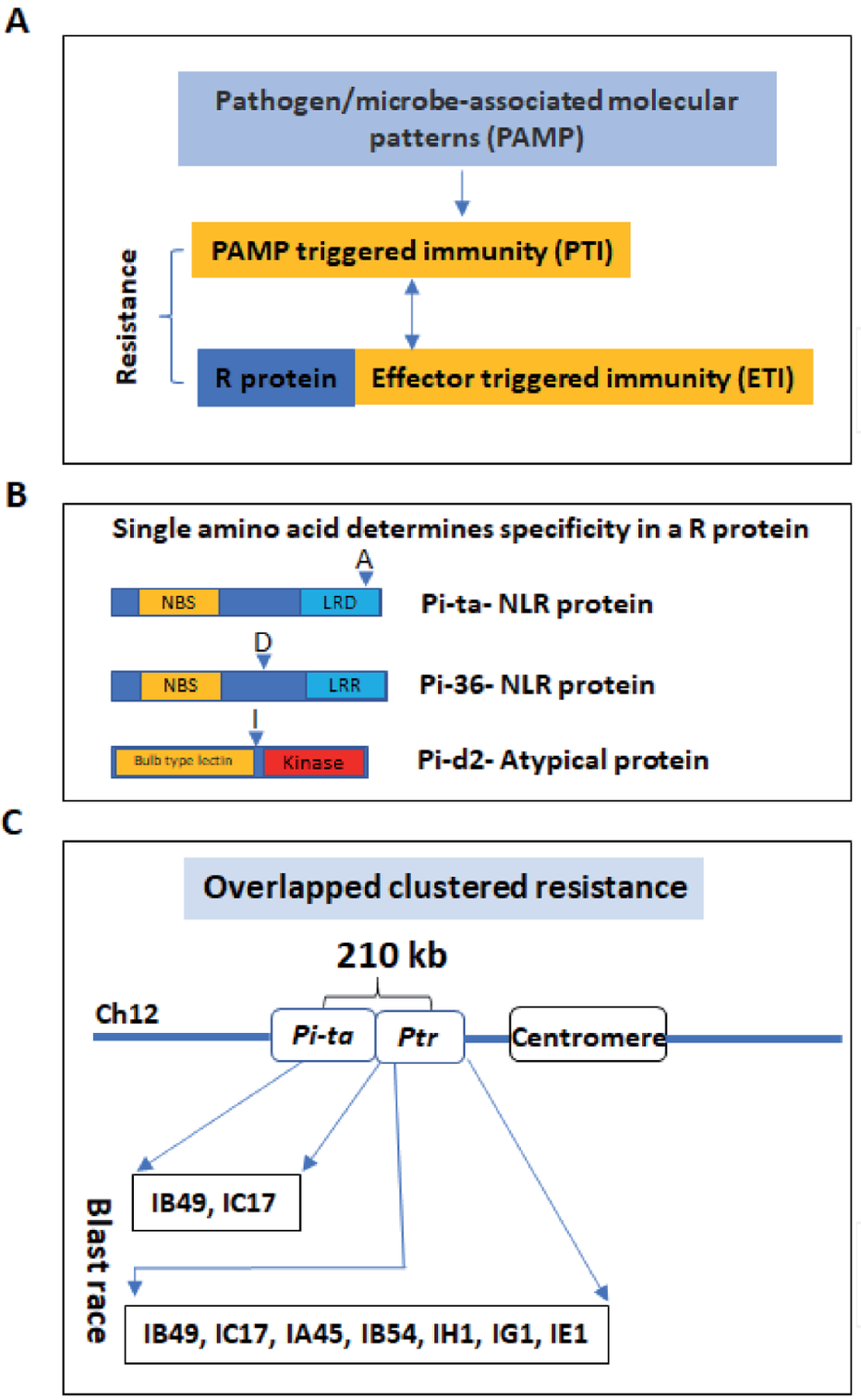

Figure 5.
Diagram shows two significant mechanisms of blast R genes. A. Showing effective resistance is a result of pathogen/microbe-associated molecular pattern triggered immunity (PTI) and effector triggered immunity (ETI) mediated by R protein. B. Showing three blast R proteins with a single amino acid determining recognition specificities. Single letter code was used. C. Showing the location and resistance spectra of blast R genes Pi-ta and Ptr near the centromere of rice chromosome 12. This genomic region has been transferred as a linkage block into diverse rice germplasm due to suppressed recombination. Rice varieties with Pi-ta are resistant to the blast races IB49 and IC17 and with Ptr are resistant to the blast races IB49, IC17, IA45, IB45, IB54, IH1, IG1, and IE1. Graphics were not drawn in proportion.

Another efficient method of plant innate immunity is a plausible failsafe mechanism. *R* genes and helper genes in plant immunity are often found in a short physical interval that can be easily passed on to the next generation (**Figure 5C**).

Rice varieties with *Pi-ta* is resistant to the blast races IB49 and IC17 [63]. *Pi-ta* was predicted to require another gene *Ptr* to be more effective [69]. The *Ptr* gene referred as *Pi-ta2* is 210 kb from *Pi-ta* on chromosome 12 [70, 71]. The *Ptr* gene was identified using a genetic screen of a mutant population created by fast neutrons and was cloned using map-based cloning approach and resistant function was validated by CRISPR-CAS 9 [70–72]. *Ptr* is a broader-spectrum blast *R* gene independent to *Pi-ta* predicted to encode a protein with 4 armadillo repeats [70]. Rice varieties with *Ptr* are resistant the blast races IB49, IC17, IA45, IB45, IB54, IH1, IG1 and IE1. Resistance spectra of both *Pi-ta* and *Ptr* were overlapped for both races IB49 and IC17. Rice genes with armadillo repeats are known to be involved in a wide range of biological functions suggesting that the *Ptr* gene in rice is a failsafe for disease resistance [73]. It remains to be determined the role of *Pi39 (t)/Pi42(t)* (LOC_Os12g1837412), 12 kb from *Pi-ta* and 198 kb from *Ptr* [74, 75] in blast resistance [76]. Resulting knowledge can aid in blast resistant breeding.

6. Genetic improvement of rice for enhanced resistance

Disease resistance has been one of the major breeding strategies in rice breeding programs worldwide. Continued investigation of the mechanism of plant innate immunity can accelerate disease resistant breeding efforts (**Figure 6**).

A donor or several donors for *R* genes are used for crossing or triple crossing, either resistant progeny are selected for further evaluation or these F_2 are advanced 2 to 3 generations and then evaluated for their disease reactions. Early breeding involved the use of field conditions to evaluate the disease reactions of landrace varieties or breeding lines. More recently, rice breeding lines and other germplasm are evaluated for disease reactions under greenhouse conditions for both blast [77, 78] and sheath blight [79]. In many countries evaluations of disease reaction are conducted under field conditions because there often exist conducive environmental conditions (for example, in Colombia, [80]). However, it is difficult to determine if observed resistance is due to any particular *R* gene due to overlapped resistance such as *Pi-ta* and *Ptr* for blast races IB49 and IC17. Marker assisted selection (MAS) uses genetic markers that are linked to the *R* genes or derived from portions of *R* genes [81]. This allows the DNA of progeny to be rapidly examined

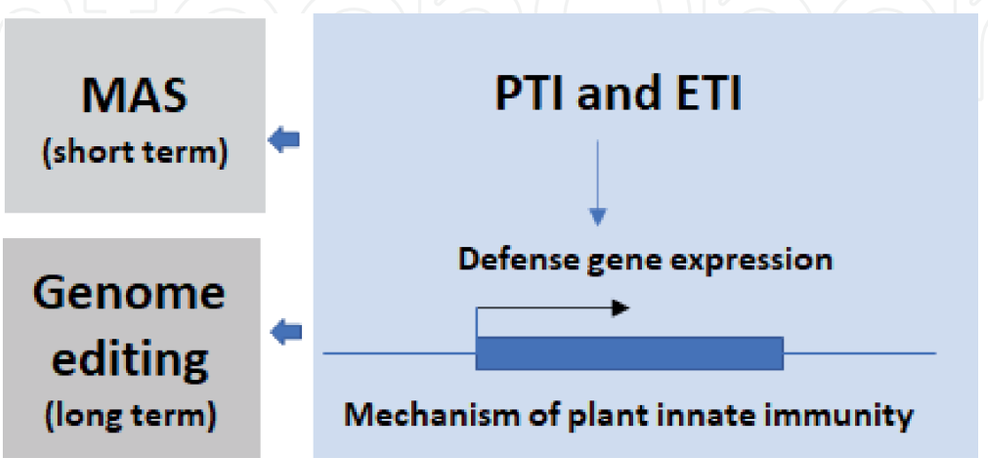

Figure 6.
Understanding interplays of pattern-triggered immunity (PTI) and elicitor triggered immunity (ETI) in activating robust defense gene expression results in a short term benefit - breeding for disease resistance using marker assisted selection (MAS) approach and a long term benefit- engineering durable effective resistance using genome editing.

for the presence of *R* genes without growing the plants to observe their reaction to pathogens. Breeders can monitor these tagged *R* genes during crossing, selection and incorporation [82–86]. MAS is a promising technology for increasing precision of selection for *R* genes and increasing speed of breeding for resistance.

One bottleneck for the use of MAS in classical breeding is the limitation of the number of markers that can be used to accurately tag resistance in any given breeding line because genetic backgrounds of breeding lines are relatively uniform in comparison with most markers developed using diverse genetic crosses of indica with japonica. Thus, the improvements of published markers are needed using local breeding lines. Gene specific DNA markers for *Pi-ta* and *Pi-b* and SSR markers for *Pikm/Piks* and *Piz* were developed based on genomic differences of local breeding lines, and are effective for MAS in the USA [85–88]. Both *Pi-ta* and *Pi-b* markers have also been adapted for blast resistant breeding programs worldwide. Another limitation for MAS is that increased numbers of markers may create a situation in which the segregating population would need to be large enough to ensure the incorporation of all *R* genes to reach the durability of resistance to the pathogens in addition to other breeding objectives. Finally, there often exist linkage blocks where disease resistance genes co-locate with inferior genes involved in quality and productivity [89, 90].

Genetic engineering is a tool that can overcome the above mentioned limitations of classical plant breeding with MAS. This approach will eliminate the inferior genes due to the linkage block in the final products. Any gene should be able to function in any other organism if genetic components for its expression are intact. Genes can be available from any organism, not just from a plant of the same species, as is the case with classical plant breeding with MAS. After genes are added to a genome, their copy numbers, location and their expression may be controlled by other genes. The common method is to insert a new gene into a plant using the natural genetic engineer, bacterium *Agrobacterium tumefaciens* [91]. The plants are regenerated from a single cell because each cell of the engineered plant must contain the new gene. Another method is biolistic transformation using the "gene gun", which bombards protoplasts with metal particles coated with the foreign genes [92]. The gene gun is much less efficient than the Ti plasmid for gene transfer because of multiple copy integrations. In rice, genetic engineering has been demonstrated to improve resistance to sheath blight and blast in various laboratories worldwide [60, 93]. One obvious advantage of genetic engineering is that genes from other organisms might give plants defenses that it never had before. The isoflavone synthase gene is not available in rice and was transferred from soybean into rice conferring an enhanced blast resistance (for example, [94]). Transgenic rice expressing these transgenes can be used to cross with the recurrent parent to produce cisgenic products without marker genes [95]. Each of cloned major *R* genes can be introduced into each susceptible advanced breeding line at the same time to develop improved resistance in as many breeding lines as possible.

7. Promises and challenges of rice crop protection

The most environmentally benign method for human intervention is using host *R* genes integrated with cultural management practices. General effective strategies to protect rice crop are prevention of introduction of pathogen; removal of established pathogen from infected rice plants; prevention of pathogen from infecting susceptible plants by growing rice under unfavorable climate for pathogen; broadening genetic basis; introduction of *R* genes with overlapping resistance spectra. As we better understand exactly how pathogenesis occurs, we can try to interfere with

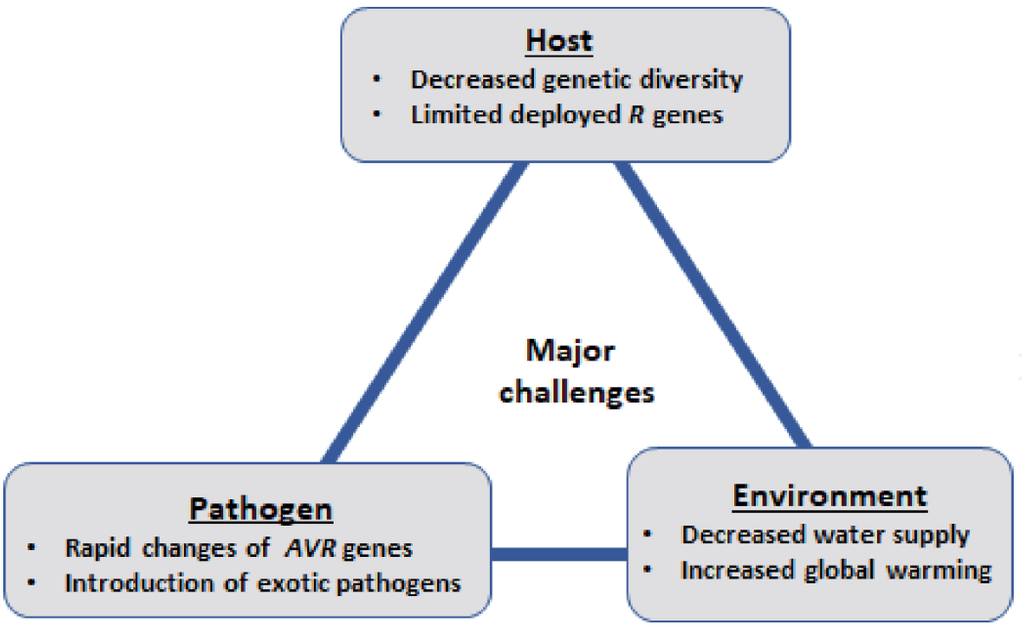

Figure 7.
Major challenges of crop protection imposed by host-pathogen and environment.

how pathogens find their hosts or inactivate important pathogen enzymes or toxins. Major *R* genes are not available to control rice sheath blight, however, sheath blight tolerant cultivars with suitable architecture and plant growth can reduce yield losses [96]. In the absence of *R* genes, fungicides are often used. The reduction in disease progress can be achieved when rice fields are treated with efficacious fungicides at the proper growth stage. A simulation study shows $43 million increase through sheath blight resistant rice production that is enough to feed 1.7 million people in the Mid-south [97].

Presently there still exist at least six major challenges for rice crop protection (**Figure 7**). 1). The intense demand for yield and quality decreases the genetic diversity of cultivated rice needed for basal defenses, such as expansion of hybrid rice that have put rice at a genetic disadvantage relative to the genetic changes of the pathogen. 2). Only limited *R* genes can be deployed locally for preventing diseases due to clonal amplification of the pathogen populations. 3). Pathogen populations can change through time and pathogen genotypes can interact with specific host genotypes leading to the "breakdown" of resistance within very short periods of time and the pathogen can adapt to new environments by rapid alteration of the *AVR* genes to create virulent races of the pathogen [98]. 4). Introduction of unwanted exotic pathogens into rice production areas through seeds. 5). Decreased water supply would increase the incidence and severity of blast disease, and 6) increased global warming not only reduces water supply but also is more dangerous if increased temperature and CO2 concentration create favorable environment for disease and weeds which can be potential sources of alternative hosts.

8. Future perspectives

In the future, the technique of genetic engineering should become easy and inexpensive to use, and social and economic concerns of Genetic Modified Organism (GMO) will be resolved. Genetic engineering of resistance will certainly enhance our capacity to prevent the crop loss due to diseases. Designing rice plants with novel resistance to a wide range of pathogens will be possible using genome editing mediated by CRISPR-Cas system [99]. At the present time, no commercial

GMO rice variety is available, but genome editing is expected to become the breeder's choice since many improved susceptible advanced breeding lines can be engineered with *R* genes in a relatively short time. It is important to monitor if new pathogen genotypes have been introduced into a region and at what frequencies certain pathogen genotypes change over time. On site information of the structure of pathogen populations is useful for the development and implementation of effective disease control strategies, and also provides insights into the evolution of pathogen populations in response to challenges imposed by host *R* genes. Therefore, the study of co-evolutionary mechanisms controlling the interaction of rice and pathogens should allow the application of these discoveries to the construction of more resistant plants. Novel approaches applied to study interactions of rice with *R. solani* has also begun to generate useful knowledge that will lead to the development of improved rice lines through genetic engineering and MAS. New germplasm including weedy species of rice and their adaptive mechanism of resistance will be identified or developed that will be used by rice breeders to incorporate novel sources of resistance into new cultivars [100–102]. Genetic mutants, mutant and mapping populations are available for uncovering important genes to control major rice diseases using methods of forward and reverse genetics [101–104]. More user friendly molecular markers will be identified to accelerate the development of improved rice cultivars through MAS worldwide and more robust *R* genes will be characterized for their deployment either using genetic engineering or MAS. Finally, the development of improved crop management programs to allow increased crop genetic heterogeneity can also be a solution to reduce crop damages due to rice diseases [105].

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