R–AVR Pairs and Interplay in Rice Blast Resistance

Subjects: Plant Sciences

Contributor: Muhammad Usama Younas, Irshad Ahmad, Muhammad Qasim, Zainab Ijaz, Nimra Rajput, Saima Parveen Memon, Waqar UL Zaman, Xiaohong Jiang, Yi Zhang, Shimin Zuo

Rice is a vital component in the diets of many people worldwide, supplying necessary calories for subsistence. Nevertheless, the yield of this crucial agricultural crop is consistently hindered by a range of biotic stresses. Out of these, rice blast, claused mainly by the fungus *Magnaporthe oryzae*, poses a significant menace to worldwide rice cultivation as well as yield. Substantial progress has been achieved in the development of efficient ways to manage rice blast disease. These procedures entail using a variety of rice genetic resources to find, map, clone, and functionally validate individual resistance (*R*) genes and quantitative trait loci (QTLs) that provide long-lasting resistance to rice blast disease. Moreover, the replication and practical confirmation of homologous avirulence (*Avr*) genes in various *M. oryzae* strains have been crucial in comprehending the fundamental molecular mechanisms of host–pathogen interactions.

Keywords: rice ; blast disease ; *Pi-ta* ; *AVR-Pita* ; AVR-Pia ; AVR-Pii ; AvrPiz-t

1. Introduction

Rice (*Oryza sativa* L.) is vital to human existence, particularly in Asia. A substantial portion of the population in this region relies on rice consumption, either directly or indirectly, to meet their caloric needs. For thousands of years, rice has laid the foundations for the establishment of ancient civilizations, cultures, economies, and diets for billions of people. The International Year of Rice was designated by the United Nations Organization (UNO) in 2002, recognizing the significance of rice as a primary cereal crop in ensuring global food security [1]. Rice is a member of the Poaceae family, commonly known as the grass family, and is well-known for its cultivation of essential cereal crops such as wheat, barley, and maize. Rice exists in two forms: wild and domesticated. Two well-known species, *Oryza sativa* and *Oryza glaberrima*, belong to the domesticated rice category. Rice is believed to have originated in tropical and subtropical parts of South Asia and Southeast Africa [2]. The wild type of rice was initially cultivated without an aquatic environment, but later mutations allowed it to specifically grow in submerged conditions. Currently, rice can be grown in diverse environments, but humid, warm, and wet conditions enhance its growth and yield.

Rice blast, caused by the fungus *Magnaporthe oryzae*, is one of the top three most important diseases that impact rice yield worldwide. Annually, it presents a significant menace to rice cultivation, leading to output declines ranging from 10% to 30% in average years and up to a drastic 50% decrease in more severe cases [3]. Rice blast occurs during the reproductive phase of rice and can be classified into many types, such as seedling blast, leaf blast, node blast, panicle blast, and grain blast, depending on when and where the infestation occurs on the rice plant. Panicle blast specifically arises as the most significant menace to rice production [4]. For disease prevention, plants adapt different resistance mechanisms, such as horizontal or vertical resistance mechanisms. Plants with horizontal resistance have a wide, non-specific defense against a range of infections, as this defense heavily relies on several genes for long-lasting protection against diseases [5]. Vertical resistance is powerful, but acts as a transient barrier because viruses may readily overcome it via mutation. It is based on a unique gene-for-gene interaction between the host and the pathogen [6]. The co-evolution of host–pathogen interactions is akin to a continuous arms race in which both sides continuously evolve new offensive and defensive tactics, which results in the generation of new pathogen strains and resistant plant types in a dynamic, evolutionary struggle [7]. Choosing resistant rice types through meticulous selection and sensible application is the most cost-effective and efficient method of blast disease control, in contrast to the disadvantages of using chemical fungicides. Consequently, there has been a shift in focus towards the identification of genes that confer resistance to rice blast disease (*R* genes), which is essential for the production of robust cultivars [8]. Moreover, the use of genetic approaches in rice crop protection provides targeted and sustainable solutions that minimize chemical usage and increase crop resistance to a variety of diseases [9].

2. Pi-ta and AVR-Pita

AVR-Pita and *Pi-ta* from the fungal pathogen *Magnaporthe oryzae* are among the first *R–Avr* interactions to be thoroughly explored [10]. This particular interaction has played a pivotal role in establishing a fundamental understanding of the complex dynamics between plants and pathogens, specifically about disease initiation and the development of resistance mechanisms. The gene *AVR-Pita,* which is located near to telomeres and is responsible for encoding a protein, secretes and possesses a unique domain known as Zn-metalloprotease [11]. The *Avr-Pita* protein attains its mature state as a protease, consisting of a sequence of 176 amino acids located at the C-terminus [12]. *Avr-Pita* is a member of a unique subclass within the *AVR-Pita* gene family, comprising three different genes: *AVR-Pita1*, *AVR-Pita2*, and *AVR-Pita3*. The first two genes mentioned possess functional properties that initiate *Pi-ta*-mediated resistance, whereas the third gene is a pseudogene lacking *Avr* functionality [13]. Moreover, rice plants use their leaf design to change cell structures and modify cuticles, close stomata, and manipulate chemical defenses to impart inherent immunity. A strong defensive system against pathogens is also produced by these physical, chemical, and signaling alterations, which together demonstrate the dynamic interactions between the structural adaptations and biological responses.

The *Pi-ta R* gene counterpart is a conventional NLR (928 amino acids) receptor that is situated in the cytoplasm and generally exhibits constitutive expression [8]. The direct interaction between the leucine-rich domain (LRD) of the Pi-ta protein and the AVR-Pita176 protein leads to the activation of downstream signaling cascades. The utilization of site-directed mutagenesis has facilitated the functional validation of AVR-Pita, leading to the identification of two critical amino acid substitutions, avr-pita176E177D and avr-pita176M178W, which result in the loss of its virulence function. In a similar vein, the presence of a mutated form of the *Pi-ta R* gene, characterized by a single amino acid substitution (LRDA918S), has been observed to reduce the physical interaction between the AVR-Pita176 and Pi-ta LRD proteins. This finding underscores the significance of the interplay between *R–Avr* pairs in the establishment of immunity against *M. oryzae* [14]. Plant gene connections orchestrate a strong defensive network by triggering immunity via pattern recognition, effector sensing, signaling, transcription control, and feedback loops.

3. Pia and AVR-Pia

This is the second class of interaction in which two NLRs, RGA4 and RGA5, interact with a single Avr protein [10][15]. The encoded secretory protein of AVR-Pia contains an N-terminal SP [16]. Different isolates of *M. oryzae* that are resistant to *Pia* genes in rice have different numbers of copies, ranging from one to three; this depends on the isolate. For instance, the avirulent strain Ina168 possesses three copies of *AVR-Pia* genes [17]. The NMR (nuclear magnetic resonance)-determined structure of AVR-Pia reveals a MAX-effector β-sandwich-like structure, while *Pia* is composed of RGA4 and RGA5 protein genes, oriented face-to-face in opposite directions [15]. Furthermore, two isoforms of RGA5 called RGA5-A and RGA5-B are the consequences of RGA5 alternative splicing, in which only RGA5-A mediates *Pia* resistance. According to in vitro experiments, it has been observed that the continuous production of RGA4 leads to the initiation of cell death. However, in the absence of infection, this cell death is suppressed by RGA5 in planta. It is important to note that the NB (nucleotide-binding) domain of RGA4 is essential for the induction of cell death [18][19]. Physical contact between AVR-Pia and the non-LRR C-terminal domain of RGA5 facilitates the inhibition and promotion of RGA4-mediated cell damage.

4. Pii and AVR-Pii

This is the third type of interaction in which the *R–Avr* pair (*Pii* and *AVR-Pii*) mediates the immune response through an indirect interaction with each other [16]. The secreted protein encoded by AVR-Pii belongs to a protein family known as pex33. The protein structure is composed of four homologs with two conserved motifs [20]. Conversely, the protein encoded by Pii is a common NLR consisting of 1025 amino acids [15]. Two different forms (I and II) of AVR-Pii exist in different isolates. Form I is a hybrid of two rice proteins (OsExo70-F2 and OsExo70-F3) and AVR-Pii. Though both rice proteins are required for the immune response, the latter (OsExo70-F3) instead of the former rice protein induces a Pii-mediated immune response. This result implies that OsExo70 serves as a helper protein in the interaction of Pii/AVR-Pii [14].

5. Piz-t and AvrPiz-t

One such form of *R–Avr* contact pertains to the indirect interaction that occurs between *Piz-t* and *AvrPiz-t*, which is a classic example of a plant–pathogen interaction where a single, broad-spectrum *R* gene recognizes and interacts with multiple variants of an *Avr* gene [21]. The AvrPiz-t protein has secretory characteristics akin to those of other well-known *Avr* genes [22]. The structure of *AvrPiz-t* and similar ToXB genes was determined via NMR. AvrPiz-t is composed of a β-

sheet consisting of six disulfide chains from Cys62 to Cys75. Single point mutations on any cysteine residue reduce the toxicity of *AvrPiz-t* [22]. *Piz-t* functions as a broad-spectrum NLR gene. The LRR domain of *Piz-t* exhibits 18 amino acid alterations, which not only determine the activation of resistance but also differentiate *Piz-t* from *Pi-2* [23]. Being a broad-spectrum *R* gene, twelve different interacting proteins of AvrPiz-t (APIPs) interact with AvrPiz-t in different lines of rice. The nature of resistance or immunological response is contingent upon both the specific AvrPiz-t protein and the genetic composition of the rice host harboring the *Piz-t* gene. In the context of *Piz-t*-lacking Nipponbare rice, the suppression of PTI is observed as a result of the interaction between *AvrPiz-t* and PTI. Conversely, in the presence of *Piz-t*, PTI is stabilized when the rice plant is infected by *M. oryzae* [24].

References

1. Gnanamanickam, S.S. Biological Control of Rice Diseases; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2009; Volume 8.
2. Crawford, G.W.; Shen, C. The origins of rice agriculture: Recent progress in East Asia. Antiquity 1998, 72, 858–866.
3. Gao, P.; Li, M.; Wang, X.; Xu, Z.; Wu, K.; Sun, Q.; Du, H.; Younas, M.U.; Zhang, Y.; Feng, Z. Identification of Elite R-Gene Combinations against Blast Disease in Geng Rice Varieties. Int. J. Mol. Sci. 2023, 24, 3984.
4. Wilson, R.A.; Talbot, N.J. Under pressure: Investigating the biology of plant infection by Magnaporthe oryzae. Nat. Rev. Microbiol. 2009, 7, 185–195.
5. Lopez-Moya, F.; Suarez-Fernandez, M.; Lopez-Llorca, L.V. Molecular mechanisms of chitosan interactions with fungi and plants. Int. J. Mol. Sci. 2019, 20, 332.
6. Parlevliet, J.E.; Zadoks, J.C. The integrated concept of disease resistance: A new view including horizontal and vertical resistance in plants. Euphytica 1977, 26, 5–21.
7. Le Pendu, J.; Nyström, K.; Ruvoën-Clouet, N. Host–pathogen co-evolution and glycan interactions. Current opinion in virology. Curr. Opin. Virol. 2014, 7, 88–94.
8. Wu, Y.; Xiao, N.; Yu, L.; Pan, C.; Li, Y.; Zhang, X.; Liu, G.; Dai, Z.; Pan, X.; Li, A. Combination patterns of major R genes determine the level of resistance to the M. oryzae in rice (Oryza sativa L.). PLoS ONE 2015, 10, e0126130.
9. Jones, H.D. Challenging regulations: Managing risks in crop biotechnology. Food Energy Secur. 2015, 4, 87.
10. Wang, L.; Zhao, L.; Zhang, X.; Zhang, Q.; Jia, Y.; Wang, G.; Li, S.; Tian, D.; Li, W.-H.; Yang, S. Large-scale identification and functional analysis of NLR genes in blast resistance in the Tetep rice genome sequence. Proc. Natl. Acad. Sci. USA 2019, 116, 18479–18487.
11. Khang, C.H.; Park, S.-Y.; Lee, Y.-H.; Valent, B.; Kang, S. Genome organization and evolution of the AVR-Pita avirulence gene family in the Magnaporthe grisea species complex. Mol. Plant Microbe Interact. 2008, 21, 658–670.
12. Razzaq, A.; Saleem, F.; Kanwal, M.; Mustafa, G.; Yousaf, S.; Imran Arshad, H.M.; Hameed, M.K.; Khan, M.S.; Joyia, F.A. Modern trends in plant genome editing: An inclusive review of the CRISPR/Cas9 toolbox. Int. J. Mol. Sci. 2019, 20, 4045.
13. Ribaut, J.M. (Ed.) Drought Adaptation in Cereals; CRC Press: Boca Raton, FL, USA, 2006.
14. Hossain, M.A.; Li, Z.G.; Hoque, T.S.; Burritt, D.J.; Fujita, M.; Munné-Bosch, S. Heat or cold priming-induced cross-tolerance to abiotic stresses in plants: Key regulators and possible mechanisms. Protoplasma 2018, 255, 399–412.
15. Mutiga, S.K.; Rotich, F.; Were, V.M.; Kimani, J.M.; Mwongera, D.T.; Mgonja, E.; Onaga, G.; Konaté, K.; Razanaboahirana, C.; Bigirimana, J. Integrated strategies for durable rice blast resistance in sub-Saharan Africa. Plant Dis. 2021, 105, 2749–2770.
16. Martin, G.B.; Bogdanove, A.J.; Sessa, G. Understanding the functions of plant disease resistance proteins. Annu. Rev. Plant Biol. 2003, 54, 23–61.
17. Ribot, C.; Césari, S.; Abidi, I.; Chalvon, V.; Bournaud, C.; Vallet, J.; Lebrun, M.H.; Morel, J.B.; Kroj, T. The Magnaporthe oryzae effector AVR 1–CO39 is translocated into rice cells independently of a fungal-derived machinery. Plant J. 2013, 74, 1–12.
18. Fukuoka, S.; Saka, N.; Koga, H.; Ono, K.; Shimizu, T.; Ebana, K.; Hayashi, N.; Takahashi, A.; Hirochika, H.; Okuno, K. Loss of function of a proline-containing protein confers durable disease resistance in rice. Science 2009, 325, 998–1001.
19. Hayashi, N.; Inoue, H.; Kato, T.; Funao, T.; Shirota, M.; Shimizu, T.; Kanamori, H.; Yamane, H.; Hayano-Saito, Y.; Matsumoto, T. Durable panicle blast-resistance gene Pb1 encodes an atypical CC-NBS-LRR protein and was

generated by acquiring a promoter through local genome duplication. Plant J. 2010, 64, 498–510.

20. Yoshida, K.; Saitoh, H.; Fujisawa, S.; Kanzaki, H.; Matsumura, H.; Yoshida, K.; Tosa, Y.; Chuma, I.; Takano, Y.; Win, J. Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen Magnaporthe oryzae. Plant Cell 2009, 21, 1573–1591.
21. Liu, W.; Liu, J.; Triplett, L.; Leach, J.E.; Wang, G.-L. Novel insights into rice innate immunity against bacterial and fungal pathogens. Annu. Rev. Phytopathol. 2014, 52, 213–241.
22. Sone, T.; Takeuchi, S.; Miki, S.; Satoh, Y.; Ohtsuka, K.; Abe, A.; Asano, K. Homologous recombination causes the spontaneous deletion of AVR-Pia in Magnaporthe oryzae. FEMS Microbiol. Lett. 2013, 339, 102–109.
23. Zhou, B.; Qu, S.; Liu, G.; Dolan, M.; Sakai, H.; Lu, G.; Bellizzi, M.; Wang, G.-L. The eight amino-acid differences within three leucine-rich repeats between Pi2 and Piz-t resistance proteins determine the resistance specificity to Magnaporthe grisea. Mol. Plant-Microbe Interact. 2006, 19, 1216–1228.
24. Fujisaki, K.; Abe, Y.; Ito, A.; Saitoh, H.; Yoshida, K.; Kanzaki, H.; Kanzaki, E.; Utsushi, H.; Yamashita, T.; Kamoun, S. Rice Exo70 interacts with a fungal effector, AVR-Pii, and is required for AVR-Pii-triggered immunity. Plant J. 2015, 83, 875–887.
