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BROWN LEAF SPOT OF RICE: PROGRESS IN MOLECULAR UNDERSTANDING

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ABSTRACT: Brown spot disease is one of the most destructive diseases of rice. The development of various molecular techniques has allowed for detailed molecular analyses of the interactions, which could open unexplored avenues to better strategize the prevention and control of the disease. This review article aims to present a synthesis of the current molecular bases of the disease.

KEYWORDS: Rice; Brown Spot Disease; Hormone; Toxins; Innate Immunity.

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1.INTRODUCTION

Rice is the staple food for more than 1.6 billion people of the world. One of the deadliest famines in history, the Bengal Famine of 1943-45, which claimed around 2 million lives, was caused mainly due to the loss of rice crops by the brown spot disease caused by the fungal pathogen *Bipolaris oryzae*(Teleomorph: *Cochliobolus miyabeanus*) [1].In spite of its great economic significance, research on the disease front, especially on the molecular understanding has been far from adequate. The following is a thorough discussion of the recent molecular studies on the disease and points to future directions of research so as to enable us to understand and manage it better.

Silicon Succour

Silicon (Si) fertilizers have emerged as potent sources for managing biotic and abiotic stresses [2]; [3]; [4]. Si, present in the form of silicic acid ($Si(OH)_4$) in the soil, is transported through the root via the xylem to the leaf through various transporters such as Lsi1,Lsi2 and Lsi6. In the leaf, it fortifies the epidermal cells as a stiff barrier made up of subcuticular silica (SiO_2)[2]; [4]; [5]. As the fungal pathogens, unlike their bacterial counterparts, are capable of making their way into the

plant using a combination of physical forces and secreted enzymes, the silicified cell walls provide the first line of defense to the pathogenic invasion in general. Hydroponically grown wild type rice cv. Oochikara treated with Si produced seeds with husks having higher Si content which correlated with lesser infection rates of these seeds by *Bipolaris oryzae* and a higher percentage of the emergence of healthy seedlings, compared to the control seeds or the Si-treated *lsi* mutant lacking the Si transporter. Fungicide treatment of the rice seeds is a common practice to reduce pathogen load or to avoid seedling infection. Fungicide treatment along with Si amendment was found to achieve these goals even better, opening a new door for the management of brown spot of rice [6]. A green house study using diverse rice genotypes grown with Si also showed promising results with the disease severity significantly going down in the treated plants [7].One of the immediate responses the resistant plants shows is the induction of localized death of tissues, which is mediated by a burst of reactive oxygen species (ROS), in the vicinity of the areas of attempted infection, to halt further spread of the pathogen to the healthier regions. While this strategy could prove fruitful against the biotrophic invaders, the necrotrophs like *C. miyabeanus* co-opt programmed cell death (PCD) to their advantage [8]. Indeed, Si induces peroxidase activity in the treated leaf with a concomitant decrease in lipid peroxidation caused by Hydroxyl radicals (•OH), which are highly reactive and generated from hydrogen peroxide (H_2O_2). Therefore it makes complete sense to neutralize it since H_2O_2 is the source of hydroxyl radicals and peroxidase converts the highly oxidizing H_2O_2 to water. Electrolyte leakage, an indicator of cell death, and the amount of dead brown leaf epidermal tissues also decrease following Si treatment. Some of the key ways through which plants resist pathogens include the production of secondary metabolites with antimicrobial properties, cell wall fortification via enhanced lignifications and enzyme mediated dissolution of pathogen cell wall. Si not only elevates the concentration of the total soluble phenolics, but it also raises lignin levels in leaf as well as induce plant chitinases, enzymes that cleave fungal cell wall chitin and the chitin oligomers resulting from the chitinase activity also act as pathogen associated molecular pattern (PAMP), which could further boost immunity [9]. However, it is not clear how Si achieves this. Brown spot of rice infected plants typically exhibit brown lesions surrounded by yellow halos, indicative of impairment of photosynthesis and of localized cell death. Severe disruption of photosynthesis follows senescence in rice, which is accompanied by changes in nitrogen metabolism associated with nutrient mobilization. Microarray based transcriptome studies in rice have, in fact, indicated that *C. miyabeanus* derails primary metabolism by down-regulating several genes governing chlorophyll biosynthesis as well as photosynthesis. On the other hand, the enzymes involved in nitrate reduction saw a reduced expression [10]. Si pre-treatment prior to pathogen inoculation helps to significantly reduce pathogen-induced adverse effects on photosynthesis and nitrate reduction, even though Si treatment induced fewer defense genes in the infected leaves suggesting that it could also play a part in reducing the fitness cost of defense gene

activation while keeping the necessary brakes on the pathogenic success [10]. Again, Si availability in the soil and its interaction with other available nutrients ensure proper uptake and activity. In case of manganese (Mn), at least, it was observed that neither Mn richness nor its deficiency significantly impacts the positive effects of Si in managing brown spot of rice [11].

Curious Case of Ethylene

Hormones are key players in plant-microbe interactions. Among the archetypal defense hormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), SA is implicated in mounting defence against biotrophic pathogens, while the often antagonistic JA-ET pathways resist necrotrophic invaders in Arabidopsis [8]; [12]; [13]. Contrastingly, in rice, ET, although serving a protective function against necrotrophic attackers such as *Rhizoctonia solani*, acts as a boon for *C. miyabeanus*, a fungal necrotroph [14]; [15]. In fact, *C. miyabeanus* synthesizes its ownET as a virulence strategy to hijack the rice defence system. Interestingly, silicon has been shown to boost rice immunity against the brown spot pathogen by cutting down the supply of ET, manufactured by it, to the host [16]. Abscisic acid (ABA) also plays a crucial role by preventing the pathogen from mounting ET mediated virulence. The pathogen also increases the expression of genes involved in ET metabolism in rice [10]. ABA appears to effect this by activating G-protein signalling pathway possibly by directly binding to the rice G-protein coupled receptor (GPCR) [17]; [18].Importantly, ABA treatment and salt stress alike were shown to upregulate the expression of GPCR in Indica rice (*Oryza sativa* cv. Indica group Swarna) [18]. Following the perception of the exogenously applied ABA and G-protein activation, Mitogen Activated Protein Kinase (MAPK) pathway is recruited which ultimately leads to the down-regulation of the ET response gene *EBP89*. The involvement of both G-protein signalling and MAPK pathways was established from the observation that ABA mediated resistance to the pathogen was compromised in the mutant lacking the rice G-protein alpha or where *OsMPK5* gene, which encodes a rice MAPK, was knocked down by RNA interference [17].However, it is yet to be established how G-protein signalling and MAPK pathways crosstalk or how other established components of these two pathways function, to fine tune rice defense against *C. miyabeanus*. Further research should also clarify precisely at what point(s) ABA resistance response intercepts the ET mediated virulence pathway. Since this experiment relied on ABA treatments, one also cannot rule out the possibility of an involvement of Pathogen Associated Molecular Pattern (PAMP) triggered immunity (PTI) whereby the expression of ABA biosynthesis genes might be up-regulated to enhance endogenous levels of the hormone, following the perception of PAMPs, conserved determinants of innate immunity including the chitin, a cell wall component of fungal pathogens, by rice pattern recognition receptors (PRR).

Toxin Trouble

Pathogens frequently employ toxins as virulence factors or even essential weapons to cause disease. Broadly, there are two classes of toxins-host selective toxins (HSTs) and non-host selective toxins

(NHSTs).Tentoxin is a cyclic tetrapeptide non-host selective toxin synthesized non-ribosomally. Tentoxin is generally synthesized by *Alternaria* using non-ribosomal protein synthetases (NPRS). As a virulence factor, the toxin uncompetitively inhibits chloroplast F_1-ATPase to interfere with ATP synthesis and consequently impairs energy metabolism. Additionally, it prevents the transport of the nuclear-encoded polyphenol oxidase into the plastid. One of the crucial symptoms induced by the toxin is chlorosis, yet the mechanism not evident [19]; [20]. Contrary to the prevailing notion that rice is insensitive to tentoxin, a recent study using a deletion mutant in *C. miyabeanus* has indentified tentoxin to be a potent virulence factor in brown spot of rice. The *C. miyabeanus* tentoxin is synthesized by an NPRS called CmNps3and the mutant NPRS was found to be severely compromised in chlorosis development [21]. NPRS also help pathogens to cope with iron deficiency by synthesizing iron-chelting agents called siderophores. A *C. miyabeanus* mutant lacking Ppt1, an Sfp-type 4'-phosphopantetheinyl transferase, necessary for the activation of the NPRS Nps6, is endowed with a hydrophilic surface compared to the hydrophobic coating present in the wild type strain, causing it to be more sensitive to oxidative stress and far less virulent. However, surprisingly, this mutant overproduces the sesquiterpine type NHST ophiobolin A. Although the purpose and the mechanism are not apparent, it is likely that the effect of *ppt1* depends on the perturbation secondary metabolism in general [22].

Innate Immunity

Plant and pathogen are always engaged in a coevolutionary arms race, compelling them to evolve mechanisms that allow have competitive edge. While the plants have surface receptors, the pattern recognition receptors (PRRs) to recognize conserved pathogenic determinants, the pathogen associated molecular patterns (PAMPs) and initiates a normally weak broad-based quantitative defence response termed PAMP-triggered immunity (PTI), successful pathogens deliver proteins, the effectors, to dampen or derail PTI so as to win the race. On the other hand, plants have evolved ways to foil pathogenic designs through another set of receptors, the resistance proteins (R-gene products) that directly or indirectly detect the effectors to mount the second line of usually stronger race-specific qualitative defence response called effector triggered immunity (ETI) [23]. Chitin being present in the cell wall of fungi including *C. miyabeanus*, serves as a PAMP which is recognized by the rice PRR complex made up of the receptor OsCEBiP and the coreceptor OsCERK1, a transmembrane protein which transduces the signal by interacting through its cytosolic domain with the cytoplasmic receptor-like kinase (RLK) OsRLCK185, which is a RLCK-VII family protein, to effect PTI via the MAPK pathway [24]; [25]. Overexpression of BROAD-SPECTRUM RESISTANCE 1 (BSR1), a cytosolic RLK encoded by the *OsRLCK278* gene and belonging to the same protein family as OsRLCK185 have been found to confer resistance against *C. miyabeanus* [26]. It is highly likely that BSR1 acts via the same or a similar pathway, but the molecular connectivity needs to be investigated to arrive at a definitive answer. Other than the PRRs and

Resistance proteins, there are wide arrays of proteins that work in association with them and transduce the signal downstream, which are variously called the Defence Responsive or Defence Related (DR) genes [27]. Overexpression of the DR protein Wall-Associated Kinase 25 (OsWAK25), a transmembrane RLK, causes development of necrotic spots in the rice leaves similar to those formed by *C. miyabeanus* infection and the symptom aggravates following pathogenic challenge, indicating that OsWAK25 is a virulence target the expression of which might be upregulated by as yet identified effectors [27]; [28]. OsWAK25 effects its negative role by NPR1 homolog 1(NH1) mediated expression of pathogenesis related proteins (PR protein) usually triggered by salicylic acid (SA) which, like Gibberellins (GA), has a neutral effect on brown spot rice [28]; [29]; [30]. However, the actual downstream executioners of the lesion mimic or pathogen induced necrosis are yet to be identified. On the pathogen's side, the perception of the host triggers also molecular cascades. Indeed through the disruption of *BMK1* gene, which codes for an MAPK, it was found to be necessary for conidiogenesis, hyphal growth and necrotrophic lesion formation in rice [31]. *C. miyabeanus* has also evolved ways to evade detection and subsequent activation of PTI by rice. As fungal cell wall polysaccharides such as chitin and β-1,3-glucan could be degraded by secreted plant enzymes and chitin by itself and the breakdown products of β-1,3-glucan are capable of triggering PTI, the pathogen masks these polysaccharides by a coat of α-1,3-glucan which the host is unable to digest because they lack the necessary enzyme. In fact, α-1,3-glucan has been shown to be of paramount importance for *C. miyabeanus* infection process in rice [32].

2. CONCLUSION

In spite of the improved understanding of the molecular underpinnings of brown spot of rice, several outstanding questions remain. Even though the environmental factors such as temperature, humidity, light intensity, photoperiod etc have long been recognized to have a bearing on the disease outcome, little is known about the exact molecular and likely epigenetic mechanisms behind it [33]. Again, the fact that *C. miyabeanus* is evolutionarily very successful as a pathogen prompts us to ask whether the pathogen may use sophisticated molecular armours to not only evade host surveillance, but also actively suppress both PTI and ETI. The effectors could, indeed, serve this purpose. Although DNA sequencing analyses have hinted at *C. miyabeanus* effector coding capacity, the effectors are mostly unidentified and consequently nothing is known about how they might function [34].

CONFLICT OF INTEREST

There is no conflict of interest.

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