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# The Effects of WRKY Genes in the Development of Magnaporthe oryzae-induced Blight in Rice

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**Abstract:**

Blast disease induced by *Magnaporthe oryzae* (*M. oryzae*) leads to a reduction in rice yield, and a close relationship between the WRKY gene and *M. oryzae* infection of rice has been found in many studies. However, whether the WRKY gene can be regulated to inhibit M. oryzae from infecting rice is still unclear. Transcriptomic and epigenomic assessments revealed that M. oryzae regulates *WRKY gene*s in rice infection. Overexpression of three OsWRKYs in rice inoculated with *M. oryzae* strains revealed that OsWRKY47 has an important role to play in *M. oryzae* resistance. Furthermore, the interaction of OsIMα1a with OsWRKY62 showed that OsWRKY62.1 plays a negative regulatory role downstream of OsIMα1a in defense against *M. oryzae*. On the other hand, in rice carrying the Pi9 gene, OsWRKY62 also plays a negative role in defense against compatible strains of *M. oryzae*. Finally, rice disease resistance was enhanced by overexpressing oswrky6 and oswrky46 to regulate RIXI. This review aims to stimulate further research on WRKY genes to suppress *M. oryzae* yield damage in rice to assure global hunger and nutritional security.

**Keywords:** WRKY; blast disease; *M. oryzae*.

## 1. Introduction

Rice is one of China's main food crops and major economic sources. With the worldwide population to become 9.7 billion in 2050, current rice cultivation is insufficient to ensure global food and nutritional security. Global analyses suggest that using increased acreage to boost rice production will be affected by the global shortage of arable land [1]. Therefore, approaches to increase productivity and total yield are to avoid losses caused by unfavorable environmental factors and post-harvest losses. Studies in various rice-growing regions of the world have found that the most serious rice disease is blast disease, which is caused by the rice blast fungus *Magnaporthe oryzae* (*M. oryzae*) [1]. The average loss due to this disease ranges from 10 to 30% and may reach 100% in some severe cases. Therefore, it has become imperative to identify effective methods to induce resistance in rice [1].

Rice developed precise signal sensing and defense mechanisms over a long period of evolution with M. oryzae. Six major transcription factor families are affected during defensive signaling, including APETALA2/Ethylene Response Factor (AP2/ERF), basic helix-loop-helix (bHLH), myeloid-associated (MYB), no apical meristematic tissue (NAC), arabidopsis transcriptional activator (ATAF1/2), and cupped cotyledons (CUC2). WRKY and basic leucine zip (bZIP) are committed to the establishment of a regulatory network that activates downstream defense genes [2]. Among them, WRKYTFs have multiple biological features of plant damage resistance, abiotic stress response, nutrient deprivation, senescence, seed and trichome development, embryogenesis, and other developmental and hormonal control processes [3]. To discover the relationship between WRKY genes and *M. oryzae* infection in rice, *M. oryzae*-induced H3K9ac was found to be present in seven WRKY genes by testing rice seedlings inoculated with *M. oryzae* using an antibody to histone H3 lysine 9 acetylation (H3K9ac) followed by RNA-seq analysis. An increase in the presence of *M. oryzae*-induced H3K9ac was found in seven WRKY genes after the induction, and an increase in the presence of H3K9ac in the seven WRKY genes. H3K9ac in the genes increased. This indicates that WRKY can be induced by Magnaporthe [2].

This review summarizes residence to blast disease caused by *M. oryzae* by regulating the WRKY genes.

## 2. Transcriptomic and Epigenomic Assessment Reveals

### 2.1 WRKY Gene Response to Rice Infection by Phytophthora Infectants

In the context of histone acetylation as an active marker

of gene transcription regulating the expression of many developmental and stress-responsive genes, a rice variety was used, which was inoculated with *Oryza sativa ssp*, followed by RNA-seq and data analysis, chromatin immunoprecipitation, microarray-seq and data analysis, Rt H3K9ac responds to genome-wide changes in response to *M. oryzae* inoculation. Seven WRKY genes are induced by H3K9ac. The important role of the immune response in rice has been reported to be played by four WRKY genes, including OsWRKY28, OsWRKY45, OsWRKY62, and OsWRKY76. [4]. Briefly, Transcriptomic and epigenomic assessment found WRKY gene response to rice infection by Phytophthora infectants (Figure 1) [2].

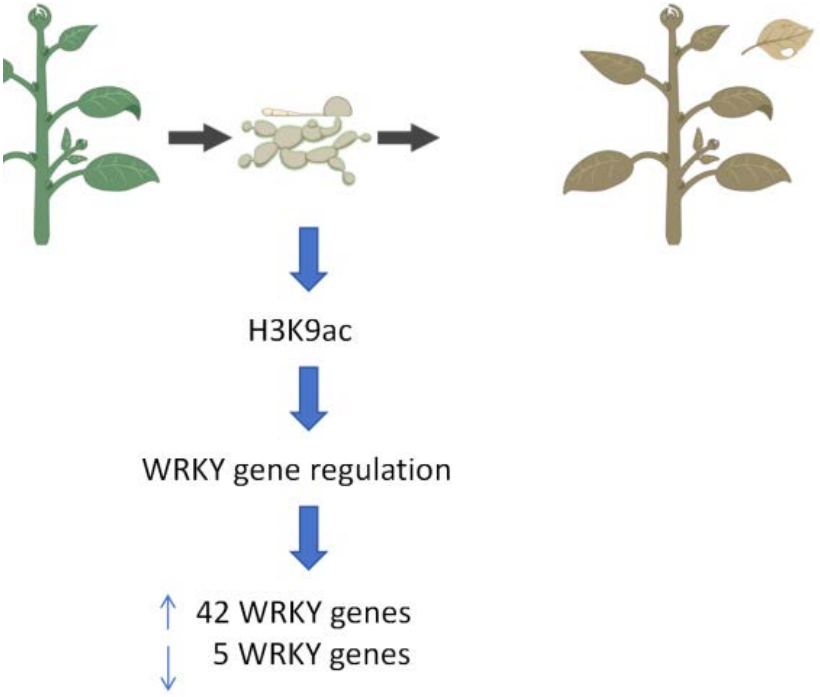


**Fig. 1 WRKY gene regulation induced by M. oryzae infection in rice. H3K9ac, Acetylation on histone H3 lysine 9. Figure credit: original.**

### 2.2 Overexpression of WRKY Enhanced Blast Resistance

By overexpressing three OsWRKYs (OsWRKY47, OsWRKY76, and OsWRKY77) in rice cultivars, the researchers obtained 22 independent OsWRKY47 overexpressing transgenic lines and screened two lines with high expression levels of the OsWRKY47 constitutive phenotype [4]. During the rice blast resistance test on these lines, it was found that seven days after vaccination with the M. oryzae strain, typical spots centered on a grey color appeared and spread in wild-type (WT) rice plants. In contrast, no conspicuous spots were found in the two transgenic lines. Thus, OsWRKY47, with its high level of expression, is resistant to rice blast [4]. OsWRKY47, with a high expression level, greatly enhanced the resistance to rice blast. Moreover, marker gene pathogenicity-related 10 (PR10) was elevated compared to WT, consistent with the enhanced resistance to rice blast. Thus, OsWRKY47 is critical in rice blast resistance (Table 1) [4].

## 3. OsWRKY proteins

### 3.1 OsWRKY67

In order to demonstrate that OsKY67 is a nuclear-localized protein, a transient expression assay using maize leaf nucleus protoplasts has shown that OsKY67GFP can only be found in the nucleus of the maize and that its colocalization with the nuclear marker. Therefore, OsWRKY67 can be a transcription factor that regulates the defense response genes [5]. To evaluate if OsWRKY67 can be an activator or a repressor, transcriptional activity assays in maize protoplasts transiently expressing OsWRKY67 were used [5]. This suggests that OsWRKY67 is a transcriptional activator that activates some of the downstream genes and thus increases the defense response (Table 1) [5].

### 3.2 The role of NPR1 protein

Systemic Acquired Resistance (SAR) is an evitable form of plant protection that produces widespread immunity against secondary infections outside the primary site of

origin. In NPR1-mediated SAR, several WRKY transcription factors are thought to play major roles in Arabidopsis and rice. Previous genomic approaches have identified several WRKYs, such as AtWRKY18, AtWRKY58, and AtWRKY70, to act as regulatory nodes in the Arabidopsis SAR transcriptional network [6]. Additionally, OsWRKY3 and OsWRKY71 were shown to be upstream genes of the rice NPR1 homolog (NH1), whereas OsWRKY45 functions as an isolated regulator in the SA/BTH modulation of the signaling pathway. Subsequently, RNA-seq analyses uncovered differentially expressed genes in transgenic lines and WT barley plants during acquired resistance (AR) [6]. Some WRKYs' expression was closely linked to the expression of NPR1 during the AR event. WRKY genes enhanced platinum resistance, including HvWRKY6, HvWRKY40, and HvWRKY70 [6].

Overall, the NPR1 protein and WRKY can be major regulators of SAR.

### 3.3 OsIMα1a and OsIMα1b

OsWRKY62 and OsWRKY76 are two tightly packed members of the WRKY transcription factors that together act as transcriptional deterrents [7]. The study investigated the interaction of OsWRKY62 and OsWRKY76 with the rice importins OsIMα1a and OsIMα1b in nuclear translocation. The interactions of OsIMα1a and OsIMα1b with OsWRKY62 and OsWRKY76 were found to be predominantly in the nucleus, and OsIMα1a interacted with tandem basic amino acids in the WRKY structural domains of OsWRKY62. A double knockout mutant was also obtained by generating their overexpression and knockout and hybridizing imα1aKO and imα1bKO strains [7]. Through knockout OsIMα1a and OsIMα1b, followed by outcome evaluations, it was shown that both OsIMα1a and OsIMα1b are proactive regulators of resistance to the rice blast pathogen. Since OsIMα1a increased the nuclear localization of OsWRKY62.1, the resistance to *M. oryzae*. was investigated. Thus, OsWRKY62 overexpressed together with OsIMα1a knockout strain was more vulnerable to *M. oryzae* than its parental strains. Thus, OsWRKY62.1 can be a negative regulator of *M. oryzae* defense downstream of OsIMα1a (Table 1) [7].

### 3.4 OsWRKY62

A rice ubiquitin-like structural domain-containing protein (UDP), AvrPi9 -interacting protein 1 (ANIP1), is a direct target of AvrPi9 and also binds to Pi9. It was found to negatively regulate the basal defense of rice against *M. oryzae* [8]. In addition, ANIP1 is physically associated with the rice WRKY transcription factor OsWRKY62, interacting with AvrPi9 and Pi9. With Pi9, ANIP1 can negatively regulate the expression of OsWRKY62, whereas AvrPi9 can increase the abundance of OsWRKY62. Therefore, the knockdown of OsWRKY62 in a non-Pi9 background reduces immunity to *M. oryzae*. Pi9 gene-carrying rice, OsWRKY62, played a negative role in defense against compatible *M. oryzae* strains. Pi9 binds to ANIP1 and OsWRKY62, forming a complex that can contribute to the inactive state of PI9, thereby weakening rice immunity. (Table 1) [8].

### 3.5 Role of OsWRKY6 and OsWRKY46

The RIXI gene is an xylanase inhibitor that can be activated by pathogens. Overexpression of RIXI enhances the resistance against the fungal pathogen M. oryzae. In this study, rice (Oryza sativa cv. Nipponbare) was used as the WT. OsWRKY6 overexpressing transgenic rice was grown by placing the coding regions of OsWRKY6 and OsWRKY46 in strong cauliflower leaves. Transient expression was then confirmed by plasmid constructs and rice transformation, DNA extraction and polymerase chain reaction (PCR) analysis, inoculation with *M. oryzae* G11, real-time quantitative PCR analysis, transient expression in tobacco leaves, chromatin immunoprecipitation PCR assay, measurement of $H_2O_2$ content and antioxidant enzyme activity yielding transient expression confirming that OsWRKY6 and OsWRKY46 bind to the RIXI promoter. During the 14 days of treatment with M. oryzae G11, seedlings were examined [9]. On day 5, the disease resistance of M. oryzaeG11 was increased in OsWRKY6 and OsWRKY46, overexpressing transgenic lines. Notably, overexpression of OsWRKY46 reduced lesion area compared with WT plants, but it was bigger in OsWRKY6 overexpression lines [9]. The number of spots on leaves showed a similar pattern of change in spot size as that of WT plants. The number of spots was highest in WT plants. The expression pattern of RIXI in WT, OsWRKY6, and OsWRKY46 overexpressing transgenic plants was determined. RIXI in the transgenic lines was elevated than that in WT plants before inoculation with *M. oryzae* G11. The expression level of RIXI increased in WT plants as well as transgenic lines at a high level 3 days after inoculation. After infection with *M. oryzae*, the expression level of RIXI was higher in transgenic lines than in WT plants. The expression levels of RIXI in OsWRKY6 overexpression lines were higher than those in OsWRKY46 overexpression lines, both before and after infection [9].

Disease-related genes (PR genes), which are markers of plant defense response, increased rapidly after infection with M. oryzae. Three times after inoculation, PR genes (OsPRla, OsPRIb, and PR4) were higher in transgenic plants than WT [9]. Infection with *M. oryzae* increased RIXI and defense genes in the OsWRKY6 overexpressing transgenic line and the OsWRKY46 overexpressing transgenic line [9]. RIXI genes and defense genes were higher in OsWRKY46 overexpressing transgenic lines, which

were more resistant to the disease.

Briefly, overexpression of OsWRKY6 and OsWRKY46 can increase rice disease resistance by regulating RIXI (Table 1) [9].

**Table 1. The function of OsWRKY families**

| OsWRKYs | Inhibitors or activators | Function or mechanism | Reference |
|---|---|---|---|
| OsWRKY76 | Inhibitors | Overexpression of OsWRKY76 affects transgenic plant vigor | [4] |
| OsWRKY77 | Inhibitors | Overexpression of OsWRKY77 affects transgenic plant vigor | [4] |
| OsWRKY47 | Activator | Overexpression of OsWRKY47 made rice much more resistant to rice blast disease. | [4] |
| OsWRKY67 | Activator | OsWRKY67 is a transcription factor that regulates the genes of defense response. | [5] |
| OsWRKY62 | Inhibitors | OsWRKY62 is a negative defense regulator against rice blast fungus | [7] |
| OsWRKY6 | Activator | OsWRKY6 regulates rixi expression to increase disease resistance in rice | [9] |
| OsWRKY46 | Activator | OsWRKY46 regulates rixi expression to elevate disease resistance in rice | [9] |

## 4. Conclusion

Rice can be effectively protected from infection by Rickettsia spp by regulating WRKY genes. Overexpression of OsWRKY47, oswrky6, and oswrky46 in rice can effectively enhance Rickettsia spp. resistance. AtWRKY18, AtWRKY58, and AtWRKY70 are critical in the plant‘s production of broad-spectrum immunity, which can effectively influence the inhibition of Rickettsia spp. infection in rice. A major role is played by the nuclear transfer of OsHIM1a and OsHIM1b with rice importins oWRKY62 and oWRKY76. OsWRKY62 and OsWRKY76 interacted with the rice importins OsIMα1a and OsIMα1b in nuclear translocation. OsIMα1a and OsIMα1b were positive regulators of resistance to rice blast pathogens. However, OsWRKY62 played a negative role in defense against compatible strains of *M. oryzae* because Pi9 creates a complex by binding to ANIP1 and OsWRK62, resulting in an inactive state of Pi9, thereby weakening rice immunity. Future studies are needed to elucidate the role of WRKY in rice blasts, as well as to seek new genetic approaches based on WRKY-related mechanisms to improve blast resistance.

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