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Research Article

Expression Pattern of Pi2 and Pi54 Resistance Genes (R-Genes) in Rice in the Presence or Absence of Exogenous Abscisic Acid during *Magnaporthe oryzae* Colonisation

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ABSTRACT

Magnaporthe oryzae is a fungal pathogen that causes one of the most devastating diseases called rice blast. This study investigates the expression pattern of Pi2 and Pi54 resistance genes in a rice sample treated with exogenous abscisic acid, followed by inoculation of the rice sample with *Magnaporthe oryzae* using the a wet inoculation method. The treatment of the rice plant started at the fifth leaf stage, and the real-time gene expression analysis was done seven days after pathogen inoculation. The results of this study confirmed that treatment of plants with exogenous abscisic acid favours colonisation by the pathogen and increases the severity of blast diseases, as sample B treated with both abscisic acid and *Magnaporthe oryzae* showed 100% of the infections compared to 80% in Sample A infected with *M. oryzae* alone. Samples A and D (control samples) exhibit no infection. Gene expression analysis (RT-qPCR) revealed that both Pi2 and Pi54 were significantly overexpressed to provide protection against infected samples. with the highest expression levels observed in sample B(Pi2: 14.05-fold, Pi5: 12.30-fold). A significant positive correlation (r>0.5) was found between the expression of the genes and infection severity. The minimal gene expression in sample C (treated with only abscisic acid) and high expression in sample B (inoculated only with *M. oryzae* and treated with abscisic acid) suggest that ABA does not suppress Pi2 and Pi54 but rather enhances disease susceptibility. Additionally, blast early symptoms can appear within four days under favorable conditions, emphasizing the need for effective disease management.

Keywords: Abscisic Acid; Blast diseases; *Magnaporthe oryzae;* Resistance genes; Rice; RT-qPCR

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INTRODUCTION

Rice (*Oryza sativa*) is a basic crop that provides roughly one-fifth of all calories consumed by humans (Spence *et al*., 2015), and over 3.5 billion people receive their daily energy from it (Muthayya *et al*., 2014).

Despite the economic significance of rice, crop loss from blast disease, which is brought on by the hemibiotrophic fungal pathogen *Magnaporthe oryzae*, is one of the biggest issues affecting rice production (Spence *et al*., 2015). The pathogen possesses both necrotrophic and biotrophic traits. Hemi-biotrophs usually grow as biotrophs at first, but eventually switch to necrotrophic growth, which results in the death of the host tissues (Perfect and Green 2001).

Abscisic acid (ABA) is a tiny signaling molecule that is involved in a wide range of plant processes, such as the start of stress adaptive responses to different environmental cues, as well as seed dormancy and development (Spence *et al*., 2015). Though little is known about the underlying processes, ABA has recently become a key player in the integration and control of plant immune responses (Xu *et al*., 2013). The action of ABA is complicated and tightly controlled at various phases since it is involved in multiple, overlapping activities (Spence *et al*., 2015).

Studies have indicated that an excess of ABA in Rice increases rice susceptibility to *Magnaporthe oryzae* colonisation (Ton *et al*., 2009). On the other hand, rice's resistance to blast disease was increased

when its synthesis of abscisic acid was reduced or when its signalling was disrupted (Kazumi *et al*., 2012). Furthermore, it has been observed that when rice plant senses the presence of *Magnaporthe oryzae* it shut down its ABA Biosynthesis in order to confer resistance to the pathogen (Kazumi *et al*., 2012).

In order to enhance its own pathogenicity, undermine host resistance, and promote colonization, the pathogen subsequently devised a strategy of up-regulating the expression of the rice NCED3 (nine-cis-epoxycarotenoiddioxygenase 3) gene, which is responsible for rice Abscisic acid biosynthesis (Spence *et al*., 2015).

The mechanism of how Abscisic acid, used by *Magnaporthe oryzae*, suppresses rice immunity is still unclear. To pave a way toward understanding this mechanism we used real-time quantitative PCR (qRT-PCR) to investigate the expression levels of pi54 and pi2 resistance genes (R-genes) in rice during *Magnaporthe oryzae* colonisation and in the presence of exogenous ABA.

MATERIALS AND METHODS

Rice preparation

The rice cultivar known locally as Jamila was obtained from Dutsinma market and used in this study, it is known to have a moderate vulnerability to rice blast diseases. The methods of Xu *et al*. (2013) were followed in treating the rice seeds. Ten seeds were placed in each of the polythene bags, which held around five kilograms of autoclaved soil. A, B, C, and D were written on the polythene bags. The plants received natural sunshine and were cultivated in a screen house. Sample A was inoculated with *M. oryzae*, Sample B was inoculated with *M. oryzae* and treated with ABA. Sample C was foliar sprayed with ABA and Sample D is a control sample.

Rice Abscisic Acid Treatment

Abscisic acid was prepared and applied to the rice sample 72 hours prior to the *Magnaporthe oryzae* inoculation following the procedure of Xu *et al*., (2013). Every seedling received treatment at week 4 when they had five leaves (Zhou *et al*., 2020).

Pathogen Preparation and Inoculation

Magnaporthe oryzae pure isolates was obtained from Universiti Putra Malaysia (UPM) and sub-cultured on PDA at 25^{o}c. The fungi was inoculated into rice plants in accordance with Islam *et al*. (2016) method.

Quantitative analysis of pi2 and pi54 resistance genes

The leaves of the sample were collected seven days after inoculation and the expression of Pi2 and Pi54 resistance genes was detected using Real-time Quantitative Polymerase Chain Reaction (RT-qPCR) following the RT-qPCR kits manufacturer's instructions Bioneer®..

Table 1: Pi54, Pi2 and eEf1a (Housekeeping gene) Primer Sequences

Primer name	Sequences (5' to 3')	References
Pi54 MAS	F:CAATCTCCAAAGTTTTCAGG R:GCTTCAATCACTGCTAGACC	Ramkumar *et al*.(2011)
Pi2 AP22	F:GTGCATGAGTCCAGCTCAAA R:GTGTACTCCCATGGCTGCTC	Xiaoyuan *et al*.(2012)
eEF1a (HKG)	F: TTTCACTCTTGGTGTGAAGCAGAT R:GACTTCCTTCACGATTTCATCGTAA	Jain *et al*.(2006)

Data Analysis

Data obtained from Real-time PCR in this study was recorded and Relative gene expression was calculated from obtained Cycle threshold (Ct) values in Microsoft excel. Data analyses were performed using descriptive statistics and inferential statistics accordingly.

RESULTS

Results of the percentage infection of rice with the blast disease are presented in Figure 1. Rice blast disease was first observed on the 4th day of *M. oryzae* inoculation. White small lesions were observed appearing on the leaves of the infected samples four days after inoculation.

Plate 1 Sample A showing early blast symptoms

Plate 2. Sample B showing aggressive symptoms of blast

Sample A which was inoculated with only *M. oryze* had four of its five leaves have blast disease symptoms scoring 80% infection. Sample B treated with both Abscisic acid and *Magnaportheoryzae* was observed to have blast symptoms more than the other samples scoring 100% leaf blast infection, all Sample B leaves are carrying blast lesions. Sample C and D are healthy with no blast symptoms, therefore 0% infection with blast as shown on Figure 1 below.

Expression Levels of Pi2 and Pi54 resistance genes

The leaves of the Rice samples were investigated for Relative expression and Relative Fold change of Pi2 and Pi54. The difference between the expression pattern of the two genes were insignificant ($p>0.05$) using two way Anova followed by Tukey post hoc test. The sample factor is statistically significant ($p=0.0029$), meaning the gene expression levels significantly differ across the samples.

Figure 2 presented expression levels of Pi2 and Pi54. Sample A treated with *M. oryzae* has 6.835 relative expression of Pi54 and 5.157 relative expression of Pi2. Pi2 and Pi54 resistance genes in Sample B treated with both Abscisic acid and *M. oryzae* expressed highest than in other samples, scoring 14.05 and 12.304 respectively. Both the Pi2 and Pi54 expressed the least in Sample C and D with $2^{\wedge-\Delta\Delta CT}$ value of 1 each. However, the positive relative fold change results indicate upregulation of the resistance genes in both the Samples.

Correlation analysis between Percentage and Gene Expression levels

Pearson correlation analysis was conducted to examine the relationship between percentage infection and gene expression levels as shown on figure 3 Percentage infection showed high correlation with pi54 gene expression ($r=0.98$) and Pi2 gene expression ($r=0.894$). Additionally, Pi54 and Pi2 gene expression levels were highly correlated ($r=0.965$), suggesting a coordinated response to infection.

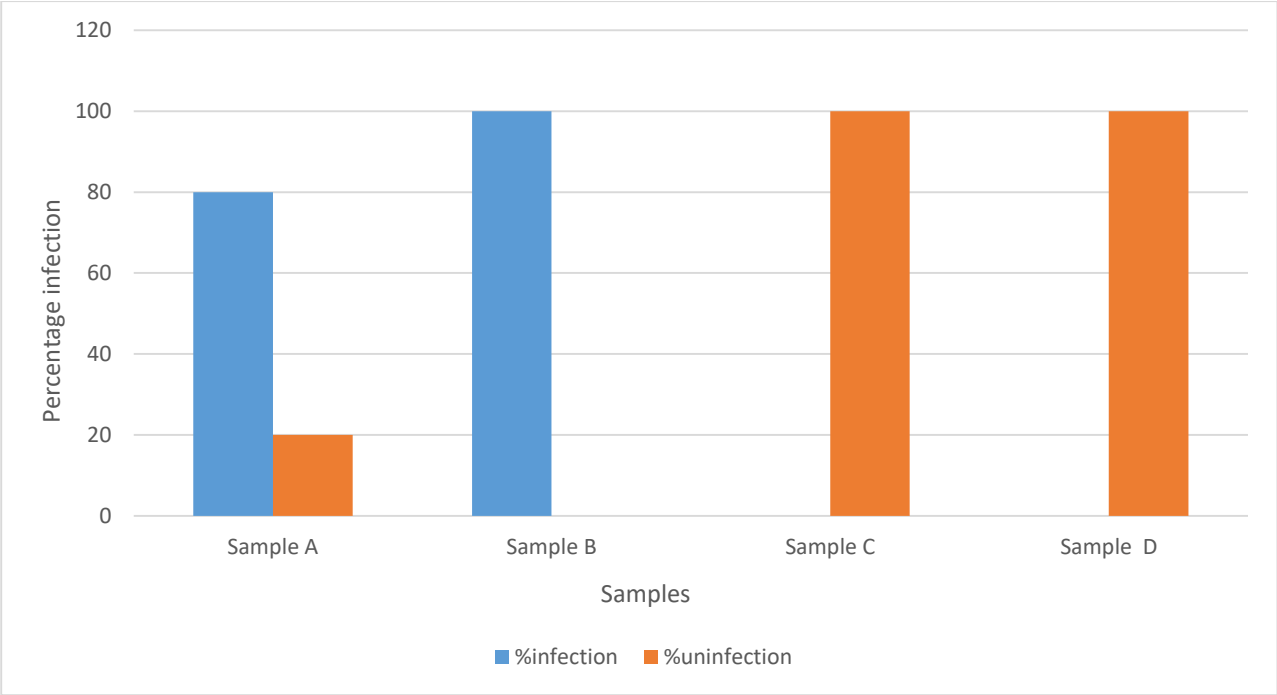

Fig 1. Percentage infection and un-infection of Blast diseases

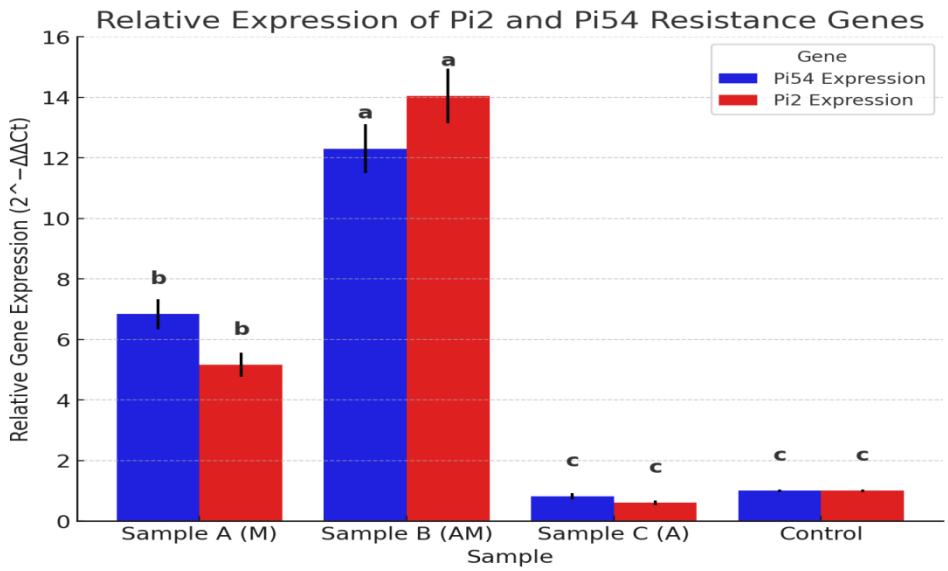

Fig 2. Relative expressions of Pi2 and Pi54 Resistance genes

Keys:

Letter a, b c, show how the mean differs; a is highest mean followed by b and c based on Tukey's post hoc test at p<0.05 following two way Anova. Group sharing the same letter are not statistically different.

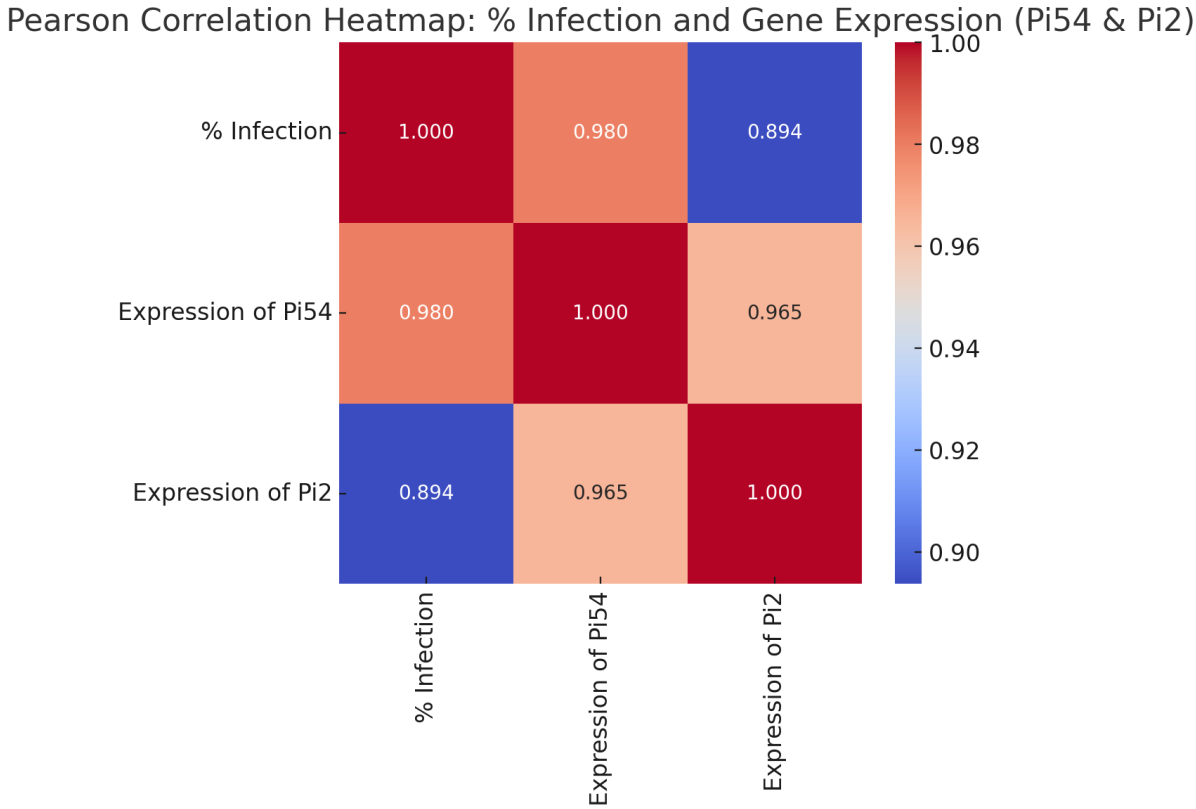

Fig 3. Correlation heatmap between blast disease percentage infection and genes expression

DISCUSSION

A strong positive correlation was observed between the expression of Pi2 and Pi54 resistance genes and the percentage of infection in the samples. The more severe the infection is, the higher the expression of these resistance genes. This finding aligns with previous studies, such as that of Yingzhong et al. (2010), which demonstrated that resistance (R) proteins quickly trigger the expression of pathogenesis-related genes upon sensing the presence of pathogens.

It is well-documented that Pi2 and Pi54 provide broad-spectrum resistance against different races of *Magnaporthe oryzae* (Xiao *et al.*, 2020).

Consistent with this, both genes were expressed in response to blast disease in this study. Additionally, Zhou *et al*., (2006) reported that the Pi2 resistance gene, which encodes a nucleotide-binding site leucine-rich repeat (NBS-LRR) protein, plays a crucial role in regulating rice blast disease. Similarly, Sharma *et al*., (2005) found that the Pi54 resistance gene confers strong resistance to blast disease in the previously susceptible rice cultivar *Tetep*.

Although abscisic acid (ABA) is known to promote susceptibility to rice blast disease (Ton *et al*., 2009), this study suggests that ABA does not directly affect the expression of Pi2 and Pi54, as both genes were expressed in rice samples treated with ABA. However, previous research has shown that ABA can interfere either directly or indirectly with the salicylic acid (SA)-jasmonic acid (JA)-ethylene (ET) defense signaling pathway (Fan *et al*., 2009). ABA has been observed to disrupt SA-mediated defence responses, which are crucial for resistance against biotrophic pathogens, while also inhibiting JA and ET signaling, which play a major roles in plant defense against necrotrophic pathogens (Pieterse *et al*., 2012). This interference could compromise the plant's ability to mount an effective immune response against diverse pathogens.

Additionally, ABA has been reported to counteract gibberellic acid (GA)-controlled defenses by stabilizing proteins that inhibit GA signaling (Grant *et al*., 2009). This suggests a potential regulatory balance between ABA and GA in plant growth-defence trade-offs, where increased ABA levels may not only enhance pathogen susceptibility but also suppress GA-dependent growth responses. Further study have linked ABA to cytokinin (CK)-mediated stress responses (Peleg *et al*., 2011). As CK plays an essential role in promoting plant immunity. The interaction between ABA and CK could impact the plant's ability to adapt to environmental stressors, further influencing disease susceptibility.

A major challenge in rice breeding is the risk of sudden loss of resistance in rice varieties carrying multiple blast resistance genes (Xiao et al., 2020). The positive fold change observed in infected samples (A and B) indicates the upregulation and overexpression of Pi2 and Pi54. However, excessive expression of resistance genes can weaken or even kill rice cells (Yingzhong et al., 2010). Therefore, strategic gene cloning approaches are needed to enhance resistance without compromising plant health.

Samples C and D showed minimal expression of Pi2 and Pi54. This may be due to environmental conditions favoring pathogen establishment. These samples were planted in August 2023, during the rainy season, which is characterized by high humidity and low temperatures—conditions that promote blast infection. Additionally, Sample C was treated with exogenous ABA, which may have further facilitated pathogen colonization. Yuan and Gary (2016) reported that under conditions favorable for blast disease, resistance genes are only slightly expressed to provide minimal protection against potential pathogens.

CONCLUSION

This study found that both Pi2 and Pi54 resistance genes were expressed in rice to combat blast disesase. However, treatment with Abscisic acid increased the severity of *Magnaporthe oryzae* infection, as observed in Sample B, which showed 100% disease severity. The minimal genes expression in sample C (treated with only ABA) and high expression in sample B (inoculate with both ABA and *M. oryzae*) suggest that ABA does not suppress Pi2 and Pi54 but rather enhances diseases susceptibility. Additionally, blast early symptoms can appear within four days under favorable conditions, emphasizing the need for effective disease management.

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