



Unraveling defense strategies: gene expression dynamics in blast-resistant vs. susceptible aromatic rice landraces

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Abstract

In this study, efforts were made to understand the defence responses in resistant and susceptible aromatic landraces against the rice leaf blast pathogen, *Magnaporthe oryzae*. Four resistant genotypes (Benugopal, Manas, Karpurkali, and Kalazeera) and two susceptible genotypes (Kalikati 2 and Kalikati 1) underwent biochemical analysis in relation to blast disease. The results of the biochemical analysis revealed increased enzyme activity levels, namely peroxidase, phenylalanine ammonia-lyase, polyphenol oxidase, superoxide dismutase, and catalase, in the resistant genotypes (Benugopal (AC44184) and Karpurkali (AC44155)) in comparison to the susceptible ones [Kalikati 2 (AC44236) and Kalikati 1 (AC44156)]. The same trend has been seen in the assays for β -1,3-glucanase and total phenols. RT-qPCR evaluation has been performed to establish the expression levels of PO, PAL, PPO, SOD, and CAT in the four resistant genotypes and the two susceptible ones after inoculation with *M. oryzae* (RLB 06) at various time intervals. Noteworthy variations in defence gene expression were noted among the genotypes. The expression of defence genes was upregulated in the resistant genotypes [Benugopal (AC44184) and Karpurkali (AC44155)] compared to their susceptible ones. Gene expression studies result indicated that the relative defence genes expression levels related to the spectrophotometric data.

Keywords Aromatic landraces · Blast · Resistance · Defence genes · RT-qPCR

Introduction

Rice, as the primary staple food crop for a significant proportion of the worldwide population, holds paramount importance. Notably, China and India have emerged as the leading rice-producing nations on a global scale (Singh et al. 2019). Considering the projected population growth, with an anticipated reach of 9.7 billion individuals by 2050, the urgency to augment rice and other cereal crop production becomes imperative. In the Indian context, rice cultivation takes place across diverse agro-ecological circumstances, encompassing a vast expanse of 43 million hectares with an average yield of 2.7 tonnes per hectare (Naveenkumar et al. 2022). However, the cultivation of rice faces significant challenges due to the

impact of diseases, which act as major impediments. Rice diseases contribute to an estimated annual loss of 10–15% in global rice production, posing a serious threat to global food security (Shivappa et al. 2021). Among the numerous diseases affecting rice, rice blast caused by *Magnaporthe oryzae* is particularly destructive and leads to substantial losses for rice growers (Ou 1985). *M. oryzae* (anamorph: *Pyricularia oryzae*) is a haploid filamentous fungus classified within the phylum *Ascomycota*. It produces pear-shaped conidia and specialized appressoria for host penetration, and thrives in high humidity and temperatures of 24 °C to 28 °C. It is estimated that rice blast disease alone causes a reduction in rice yield ranged from 10–30% annually (Skamnioti and Gurr 2009). The blast pathogen can infect more than 50 cultivated plant species, making all stages of the rice crop susceptible to its detrimental effects (Ou 1980; Yadav et al. 2019a).

Various plant protection methods are available to manage blast disease, including the cultivation of resistant varieties, the use of chemical fungicides, and biological control. While chemical fungicides are effective in disease

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control, concerns surrounding environmental pollution, pesticide residue, and human health issues arise due to their usage. Biological control serves as an alternative to chemical methods, but its practical application in agriculture often exhibits inconsistent performance. An eco-friendly and economically viable approach to controlling this disease is to identify resistant sources within the germplasm and develop resistant varieties (Susan et al. 2019; Yadav et al. 2019b). Odisha boasts a rich diversity of aromatic landraces and wild rice cultivars, which offer potential solutions for combating biotic and abiotic stresses, despite their lower yield potential compared to high-yielding varieties. However, there has been limited and insufficient exploration of aromatic landraces in Odisha for identifying sources of resistance to blast disease. Finding indigenous rice landraces with comprehensive resistance genes and comprehending the defence mechanism is essential for reducing the frequency and severity of rice blasts.

Many researchers have documented the improved activities of defence-related and antioxidant enzymes in response to pathogen infection in resistant varieties of rice (Chandrakanth et al. 2018; Naveenkumar et al. 2022). Phenylalanine ammonia-lyase (PAL) plays a significant role in the activation of the phenylpropanoid pathway, indicating the biosynthesis of lignin monomers, phenolics, and certain phytoalexins that contribute to increased plant resistance (Polle et al. 1994). Furthermore, polyphenol oxidase (PPO) and peroxidase (PO) participate in the polymerization of proteins and lignin, forming a physical barrier within plant cell walls that hinders pathogen penetration and movement (Bradley et al. 1992; Avdiushko et al. 1993; Mohapatra et al. 2016). ROS (Reactive oxygen species) are frequently involved in plant defence mechanisms, acting as protective agents against insect pests and plant pathogens (He et al. 2011). However, excessive levels of ROS could cause substantial damage to cellular structures. To mitigate the toxic impacts of ROS-induced oxidative harm, plants activate ROS scavengers like catalase (CAT) and superoxide dismutase (SOD) to regulate cytotoxicity within plant cells (Naveenkumar et al. 2022). Several types of research have registered a positive relationship among enhanced activities of PPO, PO, CAT, PAL, and SOD and enhanced plant resistance.

Though multiple authors have documented the response of defence enzymes to different diseases, there is currently a lack of research specifically focused on elucidating the defence mechanisms employed by resistant and susceptible aromatic landraces against blast disease in Odisha. Considering this knowledge gap, the objective of this study is to gain a comprehensive understanding of the defence mechanism underlying resistance in aromatic landraces against *M. oryzae*.

Material and methods

Plant material and pathogen

Four resistant genotypes, viz., Benugopal (AC44184), Manas (AC44175), Karpurkali (AC44155), and Kalazeera (AC44200), and two susceptible genotypes, viz., Kalikati 2 (AC44236) and Kalikati 1 (AC44156), were selected for this study. The seeds have been gathered from the Gene Bank at ICAR-National Rice Research Institute (NRRI), located in Cuttack, India. The virulent strain (RLB 06) of the rice blast pathogen was attained from the Plant Pathology department at ICAR-NRRI. The pathogen has been sub-cultured on the medium of PDA (Potato Dextrose Agar) and preserved at a temp of 4 °C for future utilization.

Pot experiment

The experimental trial has been performed under net house conditions at ICAR-NRRI, Cuttack. Seeds of rice aromatic landraces were subjected to surface sterilization using a 1% sodium hypochlorite solution for a duration of 2 min. They have been after then rinsed 3 times with sterile distilled water to remove any residual disinfectant. After air-drying, the seeds have been sown in pots (45 × 60 cm) filled with sterile clay loam soil. 25 days after sowing, the seedlings have been uprooted with care and transplanted into pots (45 × 60 cm) at a rate of 2 seedlings/hill, with 3 hills/pot. The experiment followed a completely randomized design with 3 replications, and each replication consisted of five pots. Adequate fertilizer was applied to the pots. At 45 days after planting, the virulent isolate RLB 06 spore suspension (1×10^6 spores per ml) was sprayed onto the plants. Humidity was maintained using sprinklers. Control plants without pathogen inoculation were maintained for comparison. Leaf samples were collected at specific time points, including 0, 24, 48, 72, 96, and 120 h after pathogen spray. The collected leaf samples were immediately transferred to liquid nitrogen and subsequently kept at a temp of -80 °C until enzyme extraction.

Peroxidase (PO) assay

For Peroxidase (PO) assay, 1 g of the leaf was placed in a pre-chilled pestle & mortar and submerged in liquid N₂. The sample was then ground to a fine powder. The powdered sample was homogenized in 2 ml of 0.1 M (Sodium Phosphate Buffer) Na-PB (pH 7.0). The homogenates were subsequently centrifuged at 12,000 rpm for 25 min at a temperature of 4 °C. The upper aqueous solution obtained after centrifugation has been gathered and utilized as a crude

enzyme. The PO assay (EC 1.11.1.7) activity followed the steps outlined by Hammerschmidt et al. (1982). For the assay, a reaction mixture (2.5 ml) was prepared, which comprised of 0.25 percent (v/v) guaiacol in 0.1 M Na-PB, 0.1 M H_2O_2 , and 0.1 ml of the crude enzyme. The blank was the boiled enzyme. The rise in absorbance at 470 nm per minute per milligram of protein was used to calculate the peroxidase activity.

Polyphenol oxidase (PPO) assay

The Polyphenol oxidase (PPO) (EC 1.10.3.1) activity has been established according to the process provided by Mayer et al. (1965). A leaf sample weighing 1 g has been homogenized in a pre-chilled pestle and mortar using 2 ml of 0.1 M Na-PB (pH 7.0). The homogenate has been then subjected to centrifugation at the speed of 13,000 rpm for 10 min at a temperature of 4 °C. For the activity assay, the reaction mixture was prepared by combining 1.5 ml of 0.1 M Na-PB (pH 6.5) with 200 μ l of the enzyme extract. To initiate the reaction, 200 μ l of 0.01 M catechol has been added. The activity of PPO was quantified by measuring the variation in absorbance at 420 nm per min/ml of the protein.

Phenylalanine ammonia-lyase (PAL) assay

The Phenylalanine ammonia-lyase (PAL) activity determination has been conducted following the methodology explained by Qin and Tian (2005). To initiate the assay, 0.5 g of rice leaf sample has been homogenized in a pre-chilled pestle along with the mortar using 5 ml of 50 mM L^{-1} sodium borate buffer (pH 8.8) comprising 5 mmol β -mercaptoethanol and 0.5 g of polyvinyl pyrrolidone. Subsequently, the homogenate was subjected to the process of centrifugation at 13,00 rpm for 20 min at a temp of 4 °C, and the resulting supernatant has been utilized for the assay. In order to conduct the assay, a sample containing 0.1 ml of the enzyme extract has been then incubated for 60 min at temperature of 37 °C with 2 ml of 50 mM L^{-1} sodium borate buffer (pH 8.8) and 0.5 ml of 20 mM L -phenylalanine. The reaction was halted by adding 0.1 ml of 6 M HCL. PAL activity was quantified by measuring the synthesis of cinnamate using a spectrophotometer at 290 nm. On the basis of fresh weight, the enzyme activity has been expressed as nmol trans-cinnamic acid per minute per milligram of the sample.

Superoxide dismutase (SOD) assay

To prepare the enzyme extract, 1 gm of leaf tissue has been homogenized in 2 ml of 0.2 M citrate phosphate buffer (pH 6.5) at a temperature of 4 °C. The homogenate has been then subjected to centrifugation at 15,000 g for 30 min at

a temperature of 4 °C. The following supernatant has been utilized as the source of enzyme for determining SOD (superoxide dismutase) activity (EC 1.15.1.1) based on its capability to reduce the NBT (nitroblue tetrazolium) photochemical reduction. For the assay, a mixture of 3 ml was prepared, consisting of 2 μ M riboflavin, 50 mM Na-PB (pH 7.8), 75 μ M NBT, 13 mM methionine, and 0.1 mM EDTA. Additionally, 100 μ l of the enzyme extract was added, and riboflavin has been added in end. The tubes have been shaken and after then it placed under a 40-W fluorescent lamp" at a temperature of 25 °C. The light was turned on to start the reaction, and it was turned off to stop it. Parallel to the assay tubes, identical non-illuminated samples were used as the blank for computing the absorbance at 560 nm. The percentage inhibition of NBT photo-reduction has been computed by subtracting each extract from the corresponding blank, dividing the resultant mathematical difference by the blank, and then multiplying the result by 100. The activity of SOD has been measured in SOD units per milligram of tissue, with one unit representing 50 percent NBT inhibition (El-Moshaty et al. 1993).

Catalase (CAT) assay

The Catalase (CAT) activity has been assayed following the process explained by Chaparro-Giraldo et al. (2000). The reaction mixture (3 ml) comprised of 100 mM potassium phosphate buffer (pH 7.5) and 2.5 mM H_2O_2 and 0.1 ml of enzyme extract. The reaction mixture has been shaken well & value of absorbance has been observed immediately at 240 nm over 1 min using UV-visible spectrophotometer. The activity has been calculated by monitoring the H_2O_2 degradation. The activity has been computed by utilizing the extinction coefficient ($\epsilon_{240nm} = 40 \text{ mM}^{-1} \text{ cm}^{-1}$) for H_2O_2 and expressed in $\mu\text{mol min}^{-1} \text{ mg}^{-1}$ of plant tissue.

β 1,3-glucanase assay

To establish β -1,3-glucanase activity in rice leaf tissue, 1 gm of the tissue has been homogenised in 5 ml of 0.05 M sodium acetate buffer with a pH of 5.0. The resultant homogenate has been centrifuged for 10 min at the temperature of 4 °C at 12,000 rpm, and the enzyme was extracted from the supernatant. The assessment of β -1,3-glucanase activity has been conducted following the laminarin-dinitrosalicylic acid approach as outlined by Pan et al. (1991). The reaction mixture comprised of 62.5 μ l of 4% laminarin (Sigma) and 62.5 μ l of the enzyme extract. The reaction took place at a temp of 40 °C for a duration of 10 min. To terminate the reaction, 375 μ l of dinitrosalicylic acid were added, followed by heating for the 5 min in boiling water. After vortexing the mixture, the absorbance at 500 nm was determined. The enzyme activity has been quantified and expressed as $\mu\text{g glucose released min}^{-1} \text{ g}^{-1}$ of fresh tissue.

Total phenol content

The Zieslin and Ben-Zaken procedure was followed in order to estimate the phenol content (1993). Using a pestle and mortar, one gramme of rice leaf tissue was homogenised in 10 ml of 80percent methanol before being centrifuged for fifteen minutes at the temperature of 70 °C. Next, 5 ml of distilled water, 250 µl of Folin Ciocalteu reagent (1N), and 1 ml of the methanolic extract were combined. The mixture has been then kept at the temperature of 25 °C. One millilitre of a saturated sodium carbonate solution and one millilitre of distilled water were added after three minutes, and the reaction mixture has been then incubated at 25 °C for an hour. With a UV–Visible Spectrophotometer, the resultant blue colour absorbance has been computed at 725 nm (Varian Cary 50, Victoria, Australia). By comparing the total soluble phenol content to a standard curve created using a phenol solution (C₆H₆O) and a Folin–Ciocalteu reagent, the content was calculated and expressed in milligrams of catechol equivalents per tissue weight.

RT-qPCR analysis

Leaf samples weighing 100 mg were collected from both resistant and susceptible genotypes at various time points (0 h, 24 h, 48 h, 72 h, 96 h, and 120 h) after pathogen spray to extract overall RNA. The RNeasy® Plant Mini kit (QIAGEN, Germany) was utilized for RNA isolation. The QuantiTect® Reverse Transcription Kit was then used to create complementary DNAs (cDNAs) from 1 µg of the overall RNA in a 20 µl reaction mixture (QIAGEN). A 1:50 ratio was used to dilute the cDNA with distilled water. For the PCR reaction, 10 µl of the diluted cDNA template was mixed with 5 µl of 2X buffer SYBR Green (QIAGEN, Germany), 0.5 µl each of forward as well as reverse primers, and 3 µl of sterilized nanopure water. RT-qPCR has been performed using the BIORAD CFX96 Real-Time System. Supplementary Table 1 comprises a list of gene-specific primers. The 18S along with the 25S rRNA genes have been utilized to normalise each and every gene expression (Sawant et al. 2023). The PCR was conducted under the following conditions: 2 min of initial denaturation at temp of 95 °C, denaturation 40 cycles at temperature of 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at temperature of 72 °C for 20 s. The relative change in gene expression has been calculated by utilizing the 2^{-ΔΔCT} approach.

Statistical analysis

The International Rice Research Institute in the Philippines' Biometric Unit developed the IRRISTAT v.92–1 programme, which was used to analyse the data. An

analysis of variance (ANOVA) was performed on the data. For comparing the means of the treatments, Duncan's multiple range test (DMRT) was employed (Gomez and Gomez 1984).

Results

Four resistant genotypes (Benugopal, Manas, Karpurkali and Kalazeera) and two susceptible genotypes (Kalikati 2 and Kalikati 1) were subjected to biochemical analysis against blast disease (Fig. 1). The estimation of defence-related biochemical compounds was performed, and a comparison was made between the resistant and susceptible phenotypes. The enzyme accumulation levels exhibited significant differences among all four resistant genotypes. The differences in the expression of defence enzymes indicate the stimulation of distinctive biochemical pathways in each and every genotype exhibiting resistance.

Peroxidase (PO) activity

The PO activity results indicated that the highest increase in PO activity occurred at 72 h after pathogen inoculation, followed by a subsequent decline at 96 and 120 h. Significant differences in peroxidase enzyme accumulation were observed between the resistant and susceptible genotypes. The PO activity ranged from 1.32 to 2.67 (EU mg⁻¹ FW) in the resistant genotypes, whereas the susceptible genotypes showed a range of 0.499 to 1.22 (EUmg⁻¹ FW). Among the genotypes, Benugopal exhibited the highest PO activity at 72 h (2.85 EUmg⁻¹ FW), followed by Karpurkali (2.67 EUmg⁻¹ FW) and Kalazeera (2.53 EUmg⁻¹ FW). The susceptible genotypes Kalikati 2 and Kalikati 1 showed the lowest accumulation of PO (Fig. 2a; Supplementary Table 2).

Polyphenol oxidase (PPO) activity

The activity of PPO varied between 1.45 and 3.25 (EU mg⁻¹ FW) in the resistant genotypes, while the susceptible genotypes exhibited a range of 0.34 to 0.90 (EU mg⁻¹ FW). The highest PPO accumulation has been seen in Benugopal (3.25 EU mg⁻¹ FW), followed by Karpurkali (3.15 EU mg⁻¹ FW) at 72 h after pathogen inoculation, after which the activity gradually declined. However, there was no major variation in PPO accumulation among the resistant genotypes Benugopal and Karpurkali. The lowest PPO levels were recorded in susceptible genotypes Kalikati 2 and Kalikati 1. The PPO accumulation in the resistant genotype Benugopal (3.25 EU mg⁻¹ FW) was 3.96 times higher than that in the

Fig. 1 Resistant and susceptible aromatic rice landraces against *M. oryzae*. The symptoms were observed 15 days after pathogen inoculation



Highly Resistant genotypes
A. Karpurkali (AC - 44155)
B. Benugopal (AC - 44184)
C. Kalazeera (AC - 44200)
D. Manas (AC-44175)

Susceptible genotypes
A. Kalikati 1 (AC - 44156)
B. Kalikati 2 (AC - 44236)

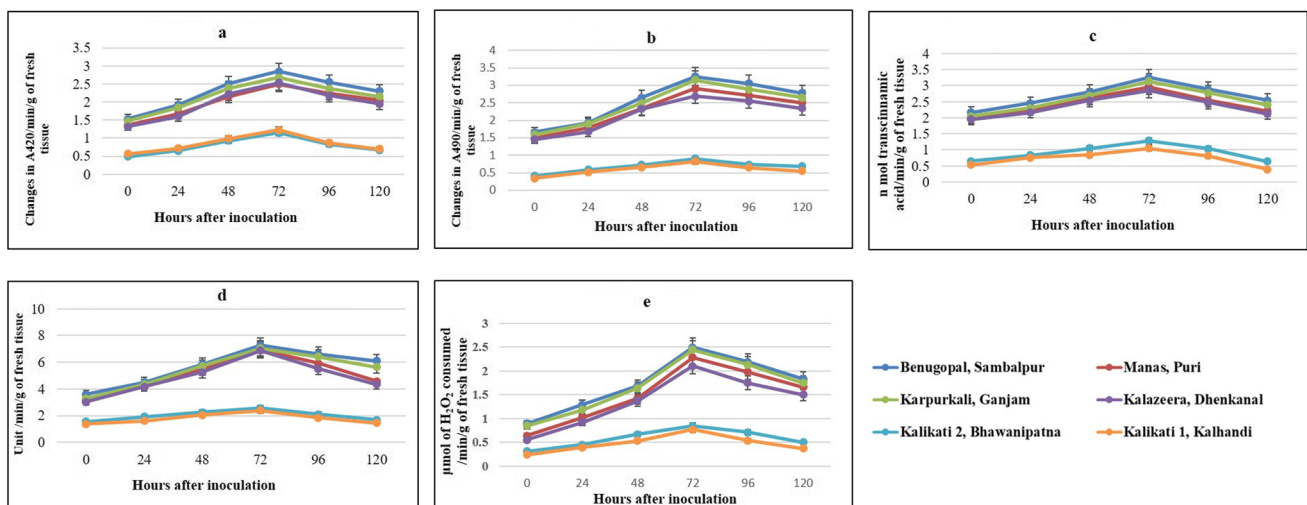


Fig. 2 Changes in defense enzymes activity in resistance and susceptible genotypes against leaf blast disease in aromatic rice landraces. **a** peroxidase (PO) activity; **b** polyphenoloxidase (PPO) activity; **c** Phe-

nylalanine ammonialyase activity; **d** Superoxide dismutase activity; **e** Catalase activity

highly susceptible genotype Kalikati 1 (0.82 EU mg⁻¹ FW) (Fig. 2b; Supplementary Table 3).

Phenylalanine ammonialyase (PAL) activity

No major variations have been seen in the accumulation of PAL activity among the resistant genotypes up to 48 h after pathogen inoculation. However, significant differences were recorded at 72, 96, and 120 h following pathogen infection. The PAL activity ranged from 1.95 to 3.25 (EU mg⁻¹ FW) in the resistant genotypes. The highest accumulation was

noted after 72 h of pathogen inoculation in Benugopal (3.25 EUmg⁻¹ FW), followed by Karpurkali (3.13 EU mg⁻¹ FW), Manas (2.96 EUmg⁻¹ FW), and Kalazeera (2.85 EUmg⁻¹ FW). In contrast, the susceptible genotypes showed lower PAL accumulation ranging from 0.53 to 1.28 (EUmg⁻¹ FW) (Fig. 2c; Supplementary Table 4).

Superoxide dismutase (SOD) activity

For the activity of SOD, the highest increase occurred at 72 h after pathogen inoculation, followed by a subsequent

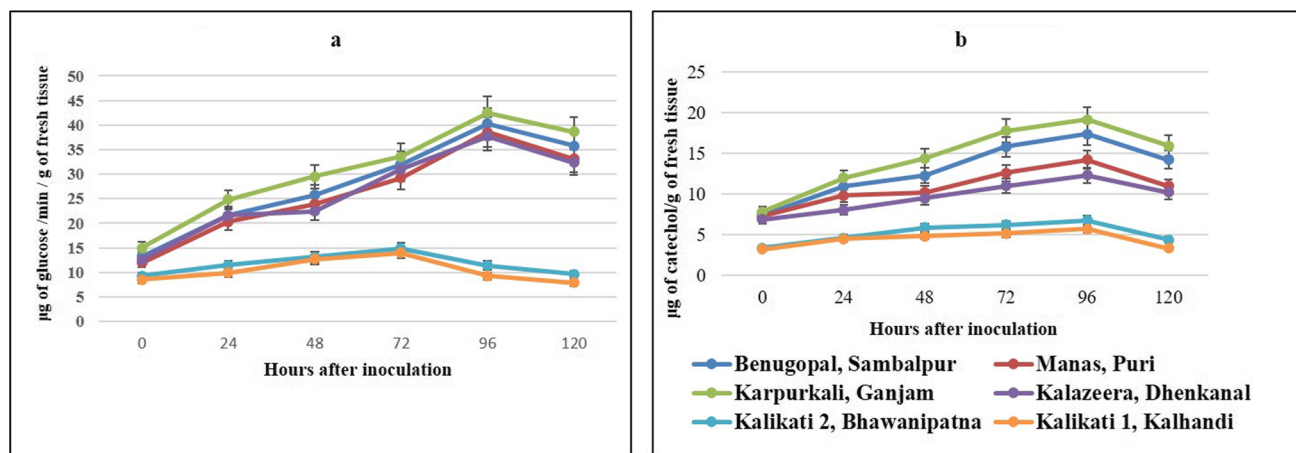


Fig. 3 Changes in **a** β -1,3-glucanase activity and **b** total phenol content in resistant and susceptible genotypes against leaf blast disease in aromatic rice landraces

decline at 96 and 120 h. A major variation has been seen among the resistant and susceptible genotypes. However, among the four resistant genotypes, there were no significant variations in SOD accumulation. The enzyme accumulation in Benugopal (7.25 EU mg⁻¹ FW) was 3.04 times higher compared to the highly susceptible genotypes Kalikati 1 (2.38 EU mg⁻¹ FW) (Fig. 2d; Supplementary Table 5).

Catalase activity

The CAT enzyme activity varied between 0.55 to 2.49 (EU mg⁻¹ FW) in the resistant genotypes. More accumulation was observed in Benugopal (2.49 EU mg⁻¹ FW) followed by Kalazeera (2.44 EU mg⁻¹ FW). Conversely, lower CAT activity has been noted in the susceptible genotypes Kalikati 2 (0.84 EU mg⁻¹ FW) and Kalikati 1 (0.77 EU mg⁻¹ FW) (Fig. 2e; Supplementary Table 6).

Assay of β -1, 3-glucanase

The results of β 1, 3 glucanase activity indicated the highest increase at 96 h after pathogen inoculation, followed by a subsequent decline at 120 h. Among the genotypes, Karpurkali exhibited the highest accumulation (42.45 EU mg⁻¹ FW), followed by Benugopal (40.28 EU mg⁻¹ FW). The β 1, 3 glucanase lowest activity has been recorded in susceptible genotype S2 (9.25 EU mg⁻¹ FW) (Fig. 3a; Supplementary Table 7).

Total phenols

The resistant genotypes exhibited a significantly higher total phenol content compared to the susceptible genotype. In

contrast to the susceptible genotype Kalikati 1, which had phenol expression levels of 5.64 EU mg⁻¹ FW, the resistant genotype Karpurkali had much higher levels at 19.15 EU mg⁻¹ FW, with the phenol expression in the susceptible genotype being 3.39 times lower (Fig. 3b; Supplementary Table 8).

RT-qPCR analysis

RT-qPCR has been employed to analyse the expression levels of PPO, PO, CAT, PAL and SOD in four resistant rice genotypes (Benugopal, Manas, Karpurkali, and Kalazeera) and two susceptible ones (Kalikati 2 and Kalikati 1) following inoculation with *M. oryzae* (RLB 06) at time points of 0, 24, 48, 72, 96, and 120 h. Notable variations in peroxidase gene expression were observed, with the highest relative fold change of 82.89 in the resistant genotype Benugopal at 72 h after inoculation. Conversely, the peroxidase gene expression level was notably lower in the susceptible genotype Kalikati 1. The expression of the PPO gene exhibited substantial upregulation in the resistant genotype Benugopal, with a remarkable 74.54-fold increase in relative expression at 72 h after inoculation to time zero of the resistant genotype. This pattern was also evident in the genotype Karpurkali, showing a 63.41-fold rise at 48 h and a 73.49-fold rise at 72 h after inoculation. Conversely, both susceptible genotypes did not show a significant increase in PPO expression levels. The Phenylalanine Ammonia Lyase (PAL) gene exhibited significant upregulation, reaching its peak expression level of 93.58-fold at 72 h after inoculation, specifically in the resistant genotype Benugopal, surpassing the other three resistant genotypes. Notably, the PAL gene's expression remained elevated up to the 72-h time point in both resistant and susceptible genotypes. However, after this period, there was a notable decline in its expression across

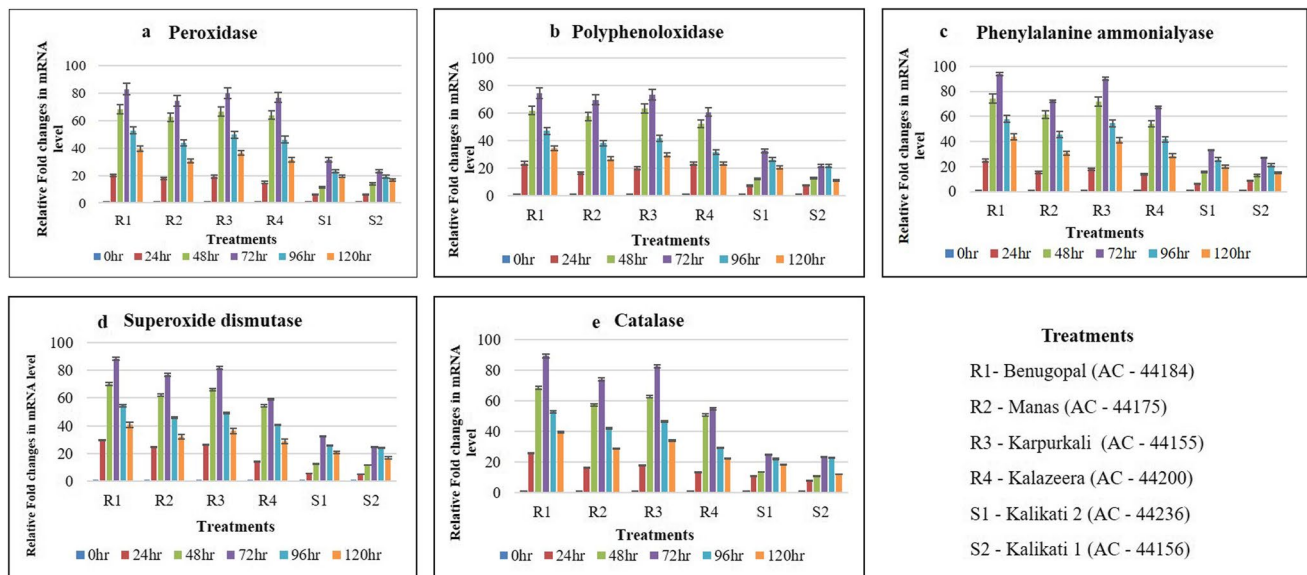


Fig. 4 Gene expression analysis of defense genes in resistant and susceptible genotypes of aromatic rice leaf against *M. oryzae* by qRT-PCR

all genotypes. The Superoxide dismutase (SOD) gene exhibited notably high expression levels and upregulation in the Benugopal genotype, with transcript levels reaching 70.18-fold at 48 h and peaking at 88.49-fold at 72 h, surpassing the expression levels in the susceptible genotypes (Kalikati 2 and Kalikati 1). Following this, the resistant germplasm Karpurkali exhibited notable relative expression levels at 48 h (66.03-fold) and 72 h (81.73-fold). The expression of the SOD gene exhibited a gradual increase up to the 72-h time point, after which it decreased significantly. The catalase gene displayed upregulation in all the resistant genotypes, with the highest expression observed in the Benugopal genotype, reaching 68.45-fold at 48 h and peaking at 89.31-fold at 72 h. Subsequently, after the 72-h time point, the gene's expression gradually decreased. In contrast, the susceptible genotypes displayed significantly lower expression levels of the CAT gene (Fig. 4).

Discussion

Plants hold latent defence genes that activate when attacked, using aromatic compounds like phenols to trigger crucial defence and antioxidant systems (Surendra et al. 2015). This response involves phenolic mixtures, flavonoids, lignins, and key enzymes (e.g. PAL, PPO, as well as antioxidant enzymes like CAT, peroxidases (POX), SOD, and glutathione reductase (GR)), resulting in tannins and phytoalexins (Van Loon et al. 2008). Nevertheless, the precise regulatory mechanisms governing this process are not fully elucidated (Mohapatra et al. 2016). Understanding these defence genes could reveal insights into how rice interacts

with the *M. oryzae* pathogen. The current study set out to characterise rice germplasm with resistant and susceptible phenotypes against *M. oryzae*, which causes rice blast disease, biochemically. The accomplishment was attained through the assessment of five defence-related enzymes during the seedling phase subsequent to inoculation with a virulent isolate of *M. oryzae*.

The current research demonstrates a notable increase in PO and PPO activities up to 72 h post-pathogen inoculation in resistant rice genotypes (Benugopal, Manas, Karpurkali, and Kalazeera) compared to susceptible ones (Kalikati 2 and Kalikati 1). This enhancement is also confirmed at the mRNA level by qPCR analysis. These outcomes are reliable with the outcomes of Chandrakanth et al. (2018), highlighting distinct activity variations of PO and PPO in both resistant along susceptible rice strains upon *M. oryzae* inoculation, indicating their involvement in rice blast resistance. The roles of peroxidase and PPO in catalysing lignin formation in plants are crucial for bolstering pathogen resistance (Lee et al. 2019).

PAL, an enzyme weighing between 77 to 83 kDa and forming a tetramer, has been extensively researched (Jendresen et al. 2015). It acts as the key catalyst in the phenylpropanoid metabolism pathway, crucial for producing secondary metabolites like phenolic derivatives, phenols, and lignin (Bhattacharjee et al. 2017). PAL, along with chitinase, is suggested to aid plant resistance against fungal pathogens. In our research, resistant genotypes displayed the highest PAL enzyme activity and gene expression, contrasting the lower levels seen in susceptible genotypes. Anushree et al. (2016) similarly found elevated PAL activity in resistant genotypes exposed to *M. oryzae*. Vanitha et al.

(2009) observed increased PAL activity, providing resistance against bacterial wilt in tomato plants during pathogen onset.

Upon pathogen invasion, plants trigger the production of ROS such as H₂O₂, superoxide anion (O₂⁻), and hydroxyl radical (OH). ROS serve as vital signalling molecules in plants, influencing defence-related genes and various signalling pathways (Chen et al. 2009; Schieber and Chandel 2014). Excessive ROS accumulation can lead to heightened cell death, increasing plant susceptibility. Antioxidant enzymes like CAT and SOD actively scavenge ROS, aiding in the breakdown of O₂⁻ into harmless substances (Alscher et al. 2002). In our study, resistant rice varieties (Benugopal, Karpurkali, Manas, Kalazeera) displayed increased SOD and CAT activity in their leaves compared to susceptible genotypes (Kalikati 2 and Kalikati 1) when exposed to *M. oryzae*. Similar trends were observed in real-time PCR results, aligning with our quantitative findings. Studies by Mahatma et al. (2011), Debona et al. (2012) and Mohapatra et al. (2016) echoed these results, showcasing elevated CAT and SOD activity in resistant plant genotypes against various pathogens.

The enzyme β -1,3-glucanase stands as a crucial component within a plant's natural defence, fortifying its resilience against diverse fungal infections (Ebrahim et al. 2011). Our research noted elevated β -1,3-glucanase activity in resistant genotypes in comparison to susceptible ones for up to 96 h post-*M. oryzae* inoculation. This observation underscores the enzyme's pivotal role in imparting resistance within rice plants. A rise in phenolic content in plants is often related to enhanced resistance to biotic stress (Naveenkumar et al. 2022). Phenolic compounds, acting as antioxidants, safeguard cellular organelles along with organic molecules like membrane lipids, proteins, RNA, and DNA from oxidative damage (Kulbat 2016). It is commonly known that resistant plants show a higher rate of phenol or polyphenol accumulation than susceptible plants. In the present investigation, genotypes with resistance showed higher accumulation of phenolic compounds than genotypes with susceptibility. This enhanced concentration may strengthen the resistance against blast disease in rice. These findings support the findings of Gupta et al. (2012), who observed elevated levels of total phenolic content in resistant transgenic rice plants even after pathogen inoculation compared to susceptible ones.

Conclusion

In conclusion, the study revealed that the increased activity of defence enzymes like PPO, PAL, CAT, PO, β -1,3-Glucanase, superoxide dismutase, and elevated total phenols, supports the assertion that the resistant aromatic landraces possess a robust defence mechanism against blast disease. This finding underscores the promising potential of harnessing these resistant landraces' genetic traits to fortify

blast disease resistance in rice cultivars, offering a valuable direction for future breeding strategies.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13313-024-00983-7>.

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Authors contribution U. Keerthana: Conceptualization, Methodology and Writing—original draft. A. K. Senapati and Manas K. Bag: Writing—review & editing. S. R. Prabhukarthikeyan and Shraddha B. Sawant: Data analysis, Data curation and Software. M. K. Mishra and S. D. Mohapatra: Data validation and Resources.

Data availability statement The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval Not Applicable.

Competing interests All authors declare that they have no conflict of interest.

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