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Research Article

Biochemical and Morpho-Physiological Responses of Rice to *Pyricularia oryzae*

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ABSTRACT

Rice blast, caused by *Pyricularia oryzae*, poses a significant threat to global rice production. Understanding biochemical and morpho-physiological defense responses is essential for enzyme profiling and the development of resistant varieties through marker-assisted breeding. Two experiments were conducted to compare the biochemical, morpho-physiological, and yield-related responses of BRRI dhan29 (high-yielding but susceptible variety) and IR 64 (resistant check) against *P. oryzae*. Biochemical analysis showed lower increases in SOD (9.64 U mg^{-1} protein) and PAL (4.57 U mg^{-1} protein) in BRRI dhan29 compared to IR 64 (11.06 and 5.18 U mg^{-1} protein, respectively) at 120 h after inoculation (HAI), while LOX and CHT activities were statistically similar. BRRI dhan29 accumulated more H_2O_2 (196.72 µmol kg^{-1} FW), whereas IR 64 exhibited higher levels of total phenolics (15.5 µg mL^{-1}), flavonoids (46.29 µg mL^{-1}), antioxidants (443.21 µg mL^{-1}), soluble sugars (4.75 mg g^{-1}), and proteins (124.44 µg g^{-1}) at 120 HAI. Although both genotypes showed reductions in plant height, tiller number, biomass, leaf area, and yield after inoculation, BRRI dhan29 suffered greater losses, with a 32% yield reduction compared to 10.96% in IR 64. At 20 days after inoculation, BRRI dhan29 showed a higher Percent Disease Index (PDI) of 80%, while IR 64 had a PDI of 50%, indicating partial resistance and superior physiological performance. These results highlight IR 64's enhanced resistance to *P. oryzae* and its potential as a donor line in breeding programs. Enhancing blast resistance in BRRI dhan29 is vital for stable yields and food security in affected regions.

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Introduction

Rice (*Oryza sativa*) plays a vital role in global food security, serving as a staple food for more than half of the world's population. In Asia, where a large proportion of the population lives in poverty, rice contributes up to 50% of the daily caloric intake, highlighting its critical importance (Muthayya et al., 2014).

Rice blast disease, caused by the fungal pathogen *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*), poses a major threat to rice production, resulting in significant yield losses and affecting millions who rely on rice for sustenance. The disease infects all aerial parts of the rice plant, including leaves, nodes, and panicles, thereby disrupting photosynthesis and nutrient translocation

(Shahriar et al., 2020). Neck and node blast cause necrotic lesions that severely reduce yield, while neck blast in particular inhibits grain filling, potentially leading to total crop failure (Rahman and Uddin, 2017). Outbreaks can devastate nurseries and field crops, resulting in large-scale epidemics (Khan et al., 2014). Environmental conditions such as high humidity and moderate temperatures exacerbate blast severity, leading to yield losses ranging from 10% to 90% (Hossain et al., 2017).

Plants deploy a range of defense mechanisms in response to pathogen invasion, including physical barriers, the production of secondary metabolites, and the activation of defense-related enzymes. Key enzymes such as superoxide dismutase (SOD), phenylalanine ammonia-lyase (PAL), and chitinase (CHT) play essential roles in mitigating oxidative stress, synthesizing phenolic compounds, and degrading fungal cell walls, respectively (Prasannath, 2017). In addition, enzymes like lipoxygenase (LOX) and β-1,3-glucanase contribute to pathogen defense by facilitating signaling cascades and hydrolyzing pathogen cell wall components. During infection, plants can rapidly generate reactive oxygen species (ROS) at the site of attack, triggering localized cell death (hypersensitive response) and activating systemic acquired resistance (Nisha et al., 2012). To mitigate the harmful effects of ROS, plants utilize both enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants such as SOD and catalase (CAT) detoxify excess ROS, while non-enzymatic antioxidants, including vitamins and flavonoids, contribute to cellular protection against oxidative stress (Fahad et al., 2019).

Rice blast caused by *P. oryzae* results in substantial productivity losses in rice-producing countries. Management strategies include the use of resistant cultivars, fungicidal treatments, and optimized agronomic practices. Although over 100 resistance (R) genes have been identified, single-gene resistance is often short-lived, typically breaking down within 3-5 years due to the high adaptability of the pathogen (Sharma et al., 2012). BRRI dhan29, a widely cultivated mega-variety in Bangladesh, is highly susceptible to blast disease. However, comprehensive studies on its biochemical, enzymatic, and physiological defense responses are limited in the context of Bangladesh. Such investigations are essential for developing sustainable management strategies to combat rice blast and ensure food security in Bangladesh and other blast-prone regions.

The current study, therefore, was undertaken to evaluate and compare the biochemical, morphological, physiological, and yield-related responses of BRRI dhan29 and IR64 under *P. oryzae* infection.

Materials and Methods

Experimental materials and cultivation procedure

Two separate experiments were conducted in the Plant Protection Laboratory and the net house of the Plant Breeding and Biotechnology Laboratory, Agrotechnology Discipline, Khulna University, Khulna, Bangladesh. The geographical coordinates of the experimental site are 22º84' N latitude, 89º54' E longitude, and an elevation of 9 m above sea level. Seeds of rice cultivars BRRI dhan29 and IR64 (used as a resistant check) were obtained from the Bangladesh Rice Research Institute (BRRI), Gazipur. A virulent monosporic isolate of *P. oryzae* (strain RL-17) was used in the experiments.

The experimental soil was clay loam in texture, with a pH of 6.6, electrical conductivity (EC) of 1.02 dS m^{-1}, and 1.87% organic matter. Nutrient concentrations were as follows: nitrogen (N) 1140.5 ppm, phosphorus (P) 15.04 µg g^{-1}, sulfur (S) 176.8 µg g^{-1}, and exchangeable potassium (K) 0.65 Cmol kg^{-1}, calcium (Ca) 16.5 Cmol kg^{-1}, and magnesium (Mg) 6.5 Cmol kg^{-1}.

Seeds were treated with Mancer (0.3% w/w) and germinated on moist filter paper at 28°C in the dark for 5 days. For biochemical analyses, 25 seedlings were transplanted into pots measuring 30 × 20 × 14 cm, each containing 15 kg of soil. For morpho-physiological analyses, 25 seedlings were sown in pots measuring 30.5 × 30.5 × 74 cm with 20 kg of soil. Each pot was treated as an independent replication.

Culture and Inoculation of *P. oryzae*

The *P. oryzae* strain was cultured on oat agar medium and incubated in darkness at 23°C for 14 days. Mycelial mats were gently scraped using sterilized toothbrushes and then exposed to continuous light at 24°C for 72 h to promote sporulation. Following microscopic confirmation, the spores were harvested, filtered, and adjusted to a concentration of 3 × 10^5 spores ml^{-1} using Tween 20 to facilitate adhesion. Thirty-five-day-old rice seedlings were inoculated by fine mist spraying. After inoculation, trays were placed in a growth chamber (24°C, dark) for 24 h before being transferred to the net house.

Experimental design and duration

Both experiments were laid out in a completely randomized factorial design. The biochemical study (conducted from December 2023 to January 2024)

involved two factors: Factor A (rice genotypes) and Factor B (sampling times at 0, 72, 96, and 120 h post-inoculation), with three replications. The morpho-physiological study (December 2023 to April 2024) also involved two factors: Factor A (rice genotypes) and Factor B (inoculation status: inoculated vs. non-inoculated), with five replications.

Determination of defense-related enzyme activity in rice plants

Superoxide dismutase

SOD activity was determined by measuring the inhibition of nitroblue tetrazolium (NBT) photoreduction, following the method of Del Longo et al. (1993) with minor modifications. Fresh leaf tissue (0.5 g) was homogenized in 100 mM potassium phosphate buffer (pH 7.0), and the homogenate was centrifuged. The reaction mixture consisted of 40 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, and 100 µl of crude enzyme extract. Absorbance was measured at 560 nm after 30 min of light exposure. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT photoreduction.

$$\text{SOD} = \frac{(\text{Abs blank} - \text{Abs sample})}{\text{Abs blank}} \times \frac{\text{Volume of reaction mixture}}{\text{time} \times 0.01}$$

Phenylalanine ammonia-lyase

PAL activity was assayed by homogenizing 1 g of fresh leaf tissue in sodium borate buffer (pH 7.0) containing 2-mercaptoethanol and polyvinylpolypyrrolidone (PVPP), followed by centrifugation at 16,000 × g for 15 min (Shamim et al., 2018). The reaction mixture, consisting of the enzyme extract, borate buffer (pH 8.8), and L-phenylalanine, was incubated at 30°C for 30 min. The formation of trans-cinnamic acid was quantified by measuring absorbance at 290 nm.

Lipoxygenase

LOX activity was determined spectrophotometrically by measuring the increase in absorbance at 235 nm. Fresh leaf samples were homogenized in potassium phosphate buffer (pH 6.3) and centrifuged at 10,000 rpm for 20 min at 4°C (Ben-Aziz et al., 1970). The reaction was initiated by the addition of 250 µM arachidonic acid (AA), and enzyme activity was expressed as µmol of hydroperoxide produced per min per mg of protein.

$$\text{Enzyme Activity} = \frac{\text{Volume of reaction mixture} \times \text{absorbance difference}}{\varepsilon \times \text{volume of enzyme}}$$

Where, ε = 27,500.

Chitinase (CHT)

Chitinase activity was determined following the method described by Byrne et al. (2001). Fresh leaves (4 g) were homogenized in sodium acetate buffer (pH 5.0), and the homogenate was centrifuged. The resulting supernatant was used as the enzyme source. The reaction mixture was incubated at 25°C, terminated by the addition of HCl, cooled, centrifuged, and the absorbance was measured at 550 nm.

β-1,3-glucanase

β-1,3-glucanase activity was estimated according to the method of Ippolito et al. (2000). Fresh leaf samples were homogenized in sodium acetate buffer (pH 5.0) and centrifuged. The supernatant was incubated with laminarin at 37°C for 1 h. After incubation, DNS reagent was added, and the absorbance was recorded at 540 nm.

Determination of defensive biochemicals in rice plants

Hydrogen peroxide (H_2O_2) content

H_2O_2 content was determined following the method of Velikova et al. (2000). Fresh leaf tissue (500 mg) was homogenized in 0.1% trichloroacetic acid (TCA), and the homogenate was centrifuged at 12,000 rpm for 15 min. A 0.5 ml aliquot of the supernatant was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide (KI). The absorbance was recorded at 390 nm, and H_2O_2 concentration was calculated using a standard curve and expressed as µmol kg^{-1} fresh weight.

Superoxide content

Superoxide content was estimated according to the modified method of Mir (2015). Fresh leaf samples (5 g) were ground in liquid nitrogen, suspended in distilled water, and centrifuged at 4,000 rpm for 15 min. A pyrogallol stock solution and an alkaline mixture were prepared separately. For the assay, 3.5 ml of the extract was mixed with 0.5 ml of the alkaline solution, incubated in the dark for 40 min, and absorbance was measured at 415 nm.

Total phenolic content (TPC)

TPC was measured using the Folin-Ciocalteu method described by Slinkard and Singleton (1977). A 300 µl aliquot of the plant extract was mixed with methanol, distilled water, and Folin-Ciocalteu reagent. Sodium carbonate was added, and the mixture was incubated at 40°C for 30 min. Absorbance was recorded at 765 nm, and TPC was calculated using a gallic acid standard curve and expressed as µg gallic acid equivalents (GAE) per ml of extract.

Total flavonoid content

Total flavonoid content was quantified according to the method of Ahmed et al. (2014). Plant extract (300 µl) was mixed with 30% aqueous methanol, followed by

sodium nitrite and aluminum chloride. After incubation, sodium hydroxide was added, and the absorbance was measured at 506 nm. Flavonoid content was determined using a rutin standard curve and expressed as µg rutin equivalents (RE) per ml.

Total antioxidant capacity (TAC)

TAC was evaluated using the phosphomolybdate assay, as described by Ahmed et al. (2015). A 300 µl aliquot of plant extract was mixed with 3 ml of phosphomolybdate reagent and incubated at 95°C for 90 min. Absorbance was measured at 765 nm.

Total sugar content

Total soluble sugar content was determined using the anthrone method (Plummer, 1990). Samples were hydrolyzed with 6 N hydrochloric acid (HCl) at 80°C for 1 h, diluted, and reacted with anthrone reagent. The absorbance was measured at 620 nm, and sugar content was quantified using a glucose standard curve and expressed as µg glucose equivalents.

Protein content

Protein content was estimated following the Bradford assay (Bradford, 1976). Leaf samples (1 g) were homogenized in protein extraction buffer and centrifuged at 12,000 rpm for 20 min at 4°C. Supernatants (5 µl and 20 µl) were mixed with Bradford dye reagent, and absorbance was recorded at 595 nm. Protein concentration was determined using a bovine serum albumin (BSA) standard curve and expressed as µg protein.

Morpho-physiological data collection

Morpho-physiological parameters were recorded during the experimental period and included: plant height (cm), total number of tillers per plant, number of effective tillers per plant, leaf number, leaf area (m^2), fresh weight (g), dry weight (g), root length (cm), leaf greenness (measured using SPAD, Soil Plant Analysis Development), disease severity (%), panicle length (cm), number of panicles per plant, number of sterile grains per panicle, number of filled grains per panicle, grain length (mm), grain width (mm), 1000-grain weight (g), and grain yield (t ha^{-1}).

Percent disease index (PDI)

PDI was assessed at 10- and 20-days post-inoculation by scoring typical blast symptoms (elliptical lesions with gray centers and brown margins) based on the Standard Evaluation System (SES) for rice developed by the International Rice Research Institute (IRRI). The leaf blast scoring scale is provided in Table 1.

The PDI was calculated using the formula described by James (2003) as given below:

$$\text{PDI} = \frac{\sum \text{of all numerical ratings}}{\text{Total number of leaves or plants observed} \times \text{Maximum disease score}} \times 100$$

Relative Performance (RP)

RP between control and inoculated treatments was calculated using the formula described by Asana and Williams (1965) as mentioned below:

$$\text{RP} = \frac{Variables\ measured\ under\ inoculated\ condition - Variables\ measured\ under\ control\ condition}{\text{Variables measured under control condition}} \times 100$$

Statistical analysis

The data were analyzed using Analysis of Variance (ANOVA), and treatment means were compared using Tukey's Honest Significant Difference (HSD) test at a 5% significance level. All analyses were performed using Statistix 10 software. Result values were expressed as means ± standard deviations (SD).

Table 1. Standard scoring system for leaf blast based on predominant infection type.

Lesion type (score)	Description	Reaction
0	No lesion observed	Resistant (R)
1	Small brown specks of pinpoint size or larger brown specks without sporulating center	R
3	Small, roundish to slightly elongated necrotic sporulating spots, about 1-2 mm in diameter with a distinct brown margin or yellow hallow	R
5	A narrow or slightly elliptical lesion, 1-2 mm in breadth, more than 3 mm long with a brown margin	Moderately Resistant (MR)
7	A broad spindle-shaped lesion with a yellow, brown, or purple margin	Susceptible (S)
9	Rapid coalescing small, whitish, grayish, or bluish lesions without distinct margins	S

Results

Determination of resistance-related enzyme activities in rice leaves

Under *P. oryzae* stress, the activity of several defense-related enzymes increased in both rice cultivars, with variation in magnitude and timing.

SOD activity

SOD activity increased significantly with inoculation time in both rice cultivars, peaking at 120 h after inoculation (HAI), with values of 9.64 and 11.06 U min^{-1} mg^{-1} protein for BRRI dhan29 and IR 64, respectively (Figure 1a). At 120 HAI, IR 64 exhibited a 65.32% increase in SOD activity compared to the control, whereas BRRI dhan29 showed a 57.26% increase.

PAL activity

PAL activity in BRRI dhan29 showed a slight increase over the course of infection, ranging from 4.34 to 4.57 µM min^{-1} mg^{-1} protein. In contrast, IR 64 demonstrated a significant increase from 4.32 to 5.18 µM min^{-1} mg^{-1} protein. At 120 HAI, PAL activity increased by 5.3% in BRRI dhan29 and by 19.91% in IR 64 compared to their respective controls (Figure 1b).

LOX activity

LOX activity increased significantly over time in both genotypes (Figure 1c). However, no statistically significant difference in LOX activity was observed between BRRI dhan29 and IR 64 at any time point.

CHT activity

CHT activity showed a marked increase over time in both cultivars (Figure 1d). At 120 HAI, IR 64 exhibited a 27.66% increase in CHT activity, while BRRI dhan29 showed a 14% increase, compared to their respective controls.

β-1,3-glucanase activity

β-1,3-glucanase activity increased progressively in both rice cultivars, reaching peak levels at 120 HAI (Figure 1e). Notably, BRRI dhan29 consistently exhibited significantly higher β-1,3-glucanase activity than IR 64 at all-time points, as confirmed by statistical analysis.

Determination of resistance-related non-enzymatic compounds in rice leaves

H_2O_2 accumulation

The levels of H_2O_2 increased progressively in both rice genotypes over time. Initially, no significant difference was observed between BRRI dhan29 and IR64; however, at 96 and 120 HAI, BRRI dhan29 exhibited significantly higher H_2O_2 levels compared to IR64. At 120 HAI, BRRI dhan29 recorded a 153.08% increase, while IR64 showed a 106.65% increase (Table 2).

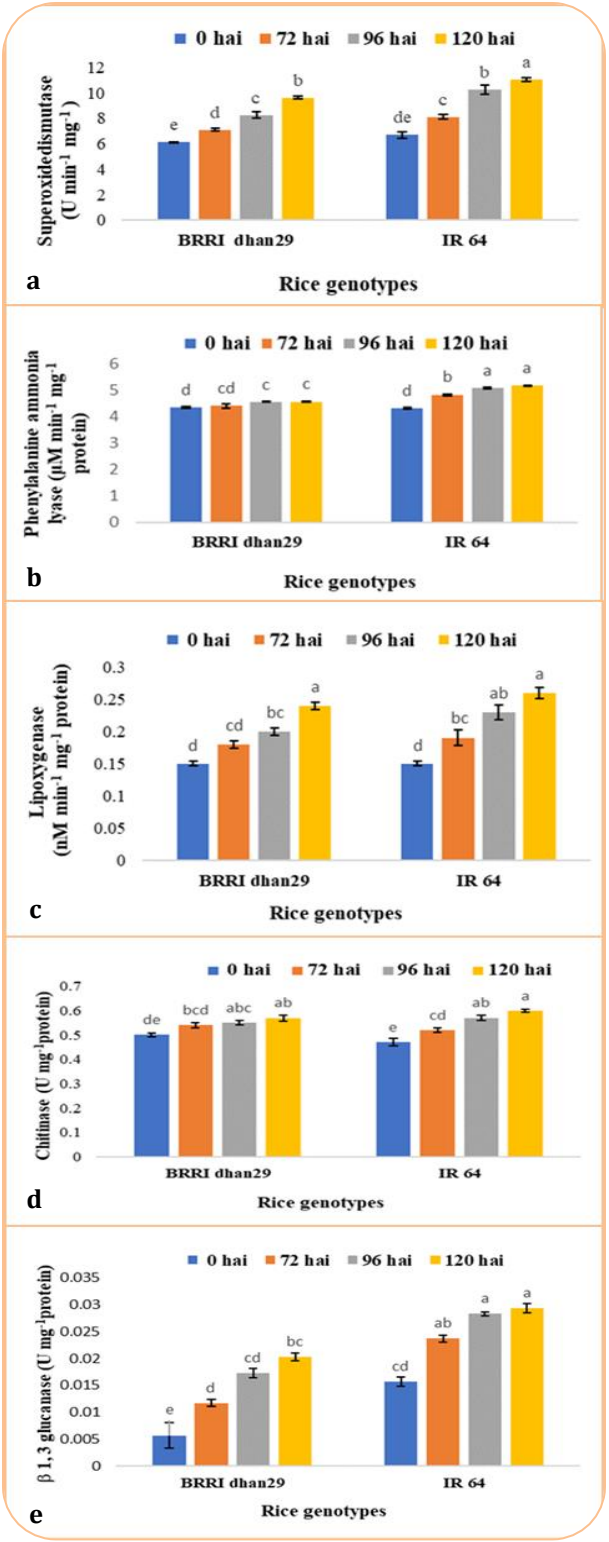

Figure 1. Activity of innate resistance-related defensive enzymes in BRRI dhan29 and IR64 under *P. oryzae* stress. All values represent the mean ± standard error (n = 3), with significance determined at $P < 0.05$ using Tukey's HSD All-Pairwise Comparisons Test.

Superoxide content

Superoxide levels also rose with time in both genotypes. Although BRRI dhan29 demonstrated a more robust response, the statistical differences between genotypes were modest. At 96 and 120 HAI, superoxide content levels were significantly higher in BRRI dhan29, which exhibited a 59.79% increase, compared to a 20.66% increase in IR64 at 120 HAI (Table 2).

Phenol content

An overall increase in phenolic content was observed in both genotypes throughout the experimental period. IR64 showed a more substantial increase of 29.38% at 120 HAI, whereas BRRI dhan29 exhibited a relatively smaller increase of 10.29% (Table 2).

Flavonoid content

Flavonoid concentrations progressively increased in both genotypes. IR64 displayed a notable rise of 32.41% at 120 HAI, in contrast to the 12.16% increase observed in BRRI dhan29, indicating a stronger secondary metabolite response in IR64 (Table 2).

Total antioxidant activity

Total antioxidant activity was significantly higher in IR64, with a 39.64% increase at 120 HAI, suggesting enhanced oxidative stress mitigation capacity. In comparison, BRRI dhan29 showed a 14.92% increase under similar conditions (Table 2).

Protein content

Protein concentration increased substantially in both genotypes. However, IR64 exhibited a more pronounced rise of 121.38% at 120 HAI, while BRRI dhan29 recorded a 73.20% increase, indicating relatively lower metabolic activity (Table 2).

Total soluble sugar content

Total soluble sugar levels increased in both genotypes, reflecting enhanced carbohydrate metabolism. IR64 showed a significant rise of 18.75% at 120 HAI, whereas BRRI dhan29 exhibited a comparatively lower increase of 5.70% (Table 2).

Morpho-physiological responses of BRRI dhan29 and IR64 to *P. oryzae* infection

Plant growth parameters such as plant height, number of tillers, fresh weight, and dry weight were significantly reduced ($P < 0.05$) in both rice genotypes following infection with *P. oryzae*. However, BRRI dhan29 exhibited greater susceptibility compared to IR64 (Table 3).

Specifically, IR64 showed a 5.19% reduction in plant height compared to the control, whereas BRRI dhan29 exhibited a more pronounced reduction of 13.38%. In terms of tiller number, BRRI dhan29 showed a substantial decrease of 25%, indicating a severe impact on vegetative growth, while IR64 experienced a relatively minor reduction of 4%.

For biomass accumulation, IR64 recorded a 7.23% reduction in fresh weight, whereas BRRI dhan29 exhibited a greater reduction of 14.62%. Similarly, the decline in dry weight was more pronounced in BRRI dhan29 (19.95%) than in IR64 (13.42%), further emphasizing the higher vulnerability of BRRI dhan29 under blast disease pressure (Table 3).

In contrast, the number of effective tillers and root length in IR64 showed no significant changes under infection. However, BRRI dhan29 exhibited reductions of 26.67% and 30.62% in effective tiller number and root length, respectively.

Table 2. Activity of resistance-related non-enzymatic compounds in BRRI dhan29 and IR 64 under *P. oryzae* stress.

Rice genotypes	Treatment (HAI)	Hydrogen peroxide (µmole kg^{-1} FW)	Superoxide (mmole kg^{-1} FW)	Total phenol (µg ml^{-1})	Total flavonoids (µg ml^{-1})	Total antioxidant (µg ml^{-1})	Total soluble sugar (mg g^{-1})	Total soluble protein (µg g^{-1})
BRRI dhan29	0	77.73 e	0.97 f	12.63 ef	38.44 d	300.08 d	3.13 f	39.7 d
	72	121.91 d	1.17 e	13.23 de	41.27 c	313.41 d	3.34 e	55.7 cd
	96	169.03 b	1.44 b	13.49 cd	42.38 bc	337.69 c	3.29 e	63.82 bc
	120	196.72 a	1.55 a	13.93 bcd	43.42 b	344.87 c	3.31 e	68.76 bc
IR 64	0	86.72 e	1.21 de	11.98 f	34.96 e	317.38 d	4 d	56.21 cd
	72	122.21 d	1.29 cd	14.06 bc	39.67 d	351.14 c	4.18 c	76.57 b
	96	148.78 c	1.38 bc	14.37 b	45.28 a	401.6 b	4.44 b	107.88a
	120	179.21 d	1.46 ab	15.5 a	46.29 a	443.21 a	4.75 a	124.44a
CV (%)		3.89	2.50	1.84	1.07	1.87	1.27	8.22

Values followed by different letters are significantly different at the 5% probability level according to Tukey's HSD All-Pairwise Comparisons Test.

Table 3. Effects of *P. oryzae* on morphological and physiological parameters of rice genotypes IR 64 and BRRI dhan29.

Genotype	Treatment	Plant Height (cm)	No. of Tillers	No. of Effective Tillers	Fresh weight (gm)	Dry weight (gm)	Root length (cm)
IR 64	Control	90.4 a	25 a	25 a	99.95 a	42.24 a	33.38 a
	Inoculated	85.7 b	24 b	23 a	92.72 b	36.57 b	29.64 a
BRRI dhan29	Control	86.82 b	16 b	15 b	73.18 c	37.4 b	30.18 a
	Inoculated	75.2 c	12 c	11 c	62.48 d	28.94 c	23.02 b
CV (%)		1.10	4.74	7.24	0.15	1.23	11.05

Values followed by different letters are significantly different at the 5% probability level according to Tukey's HSD All-Pairwise Comparisons Test.

Yield-contributing responses of BRRI dhan29 and IR 64 to *P. oryzae* stress

No significant differences were observed in panicle number, grain length, or grain width between the control and *P. oryzae*-inoculated plants in either genotype. Although panicle length remained unaffected in IR 64, BRRI dhan29 exhibited a notable reduction of 28.88% (Table 4).

Under inoculated conditions, both genotypes showed significant reductions ($P < 0.05$) in unfilled grain number, filled grain number, 1000-grain weight, and total yield. Specifically, the number of unfilled (sterile) grains increased by 34.62% in IR 64 and by 51.89% in BRRI dhan29. The reduction in filled grain number was less severe in IR 64 (11.45%) than in BRRI dhan29 (44.73%). Regarding grain weight, IR 64 maintained relative stability with a 12.5% reduction in 1000-grain weight, whereas BRRI dhan29 experienced a more substantial decline of 37.60%, indicating better grain weight stability in IR 64 under biotic stress.

Furthermore, total yield in IR 64 decreased by only 10.96%, compared to a significant 32% reduction in BRRI dhan29. These findings collectively suggest that IR 64 exhibits greater tolerance to *P. oryzae* infection than BRRI dhan29.

Effects of *P. oryzae* stress on leaf traits and PDI in rice genotypes

Leaf area, leaf number, and leaf greenness significantly decreased ($P < 0.05$) in both IR 64 and BRRI dhan29 under *P. oryzae* stress, with BRRI dhan29 exhibiting greater reductions compared to IR 64 (Figure 2). IR 64 maintained a larger leaf area, experienced smaller reductions in leaf number, and retained higher leaf greenness. Furthermore, IR 64 (10% and 50% approximately) recorded significantly lower PDI values than BRRI dhan29 (20% and 80% approximately) at 10 and 20 days after inoculation respectively, indicating greater resistance to P. oryzae (Figure 2).

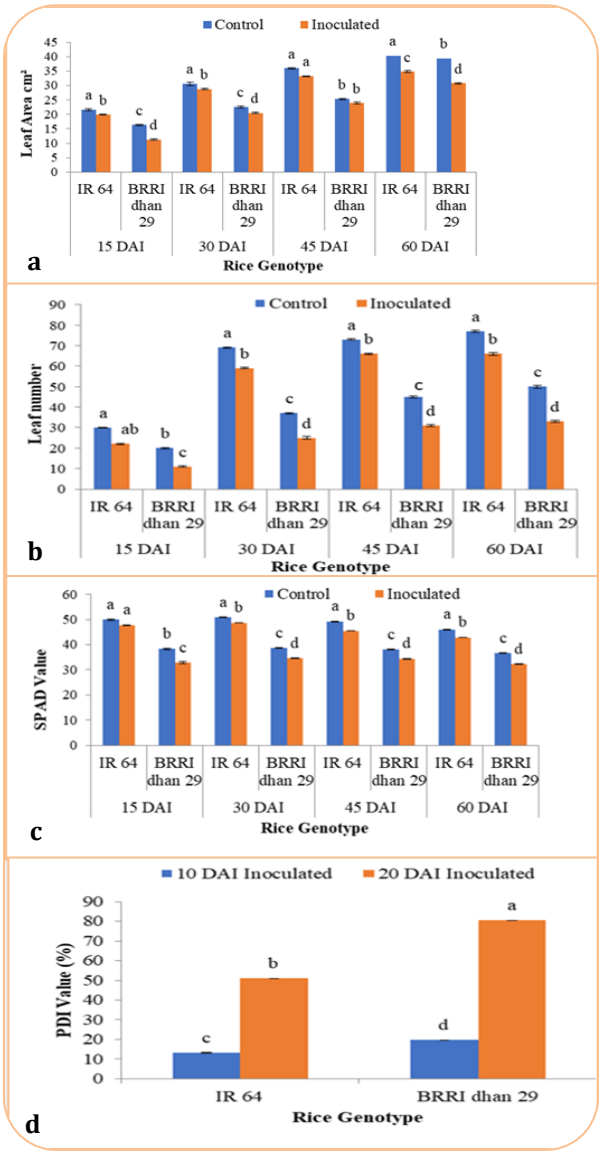

Figure 2. Effects of *P. oryzae* on leaf area (a), leaf number (b), leaf greenness (c), and PDI (d) in IR 64 and BRRI dhan29. Values are presented as mean ± standard error (n = 3), with significant differences determined at $P < 0.05$ using Tukey's HSD all-pairwise comparisons test.

Table 4. Effect of *P. oryzae* on yield-contributing parameters of IR 64 and BRRI dhan29.

Genotype	Treatment	Panicle Length (cm)	Panicle No.	Unfilled Grain	Filled Grain	Thousand Grain Weight	Grain Length (mm)	Grain Width (mm)	Total Yield (t ha^{-1})
IR 64	Control	34.56 a	35 a	290 c	1310 a	3.2 a	5.7 a	2.3 a	8.76 a
	Inoculated	30.2 a	32 a	391 b	1160 b	2.8 ab	5.08 a	2 a	7.8 b
BRRI dhan29	Control	30.4 a	23 b	395 b	760 c	2.34 b	4.02 b	1.4 b	7.5 b
	Inoculated	21.62 b	19 b	600 a	420 d	1.46 c	3.7 b	1.17 b	5.1 c
CV (%)		10.28	8.77	1.13	.29	13.33	10.28	14.12	6.15

Values followed by different letters differ significantly at the 5% probability level according to Tukey's HSD All-Pairwise Comparisons Test.

Discussion

In Bangladesh, the winter rice-growing season experiences more substantial yield losses due to blast disease compared to the pre-monsoon and monsoon seasons. This highlights the critical need to enhance winter rice productivity to meet rising food demands (Mainuddin and Kirby, 2015). Rice blast has significantly reduced the yield of BRRI dhan29, a widely cultivated mega variety in Bangladesh that accounts for 40% of the winter season area due to its high amylose content and yield potential (Mahmud and Hossain, 2018). Improving BRRI dhan29 requires integrating knowledge of innate immune responses, plant-pathogen interactions, and the physiological attributes of the genotype.

This study demonstrated distinct immune responses of BRRI dhan29 compared to the resistant check variety IR 64 under *P. oryzae* infection. IR 64 exhibited higher activities of SOD (65.32%), PAL (19.91%), and CHT (27.66%) compared to BRRI dhan29 (57.2%, 5.3%, and 14%, respectively), indicating a more robust oxidative stress response and stronger activation of defense pathways (Figure 1). The progressive increase in SOD and PAL activities aligns with findings by Keerthana et al. (2024), emphasizing their roles in ROS detoxification and phenylpropanoid pathway activation.

Chitinase, a key hydrolytic enzyme induced in response to pathogen infection, was elevated in inoculated plants, contributing to fungal cell wall degradation (Sels et al., 2008). Bai et al. (2021) reported similar increases in chitinase activity in wheat against *Puccinia striiformis* f. sp. *tritici*, conferring broad-spectrum resistance. LOX activity also increased in both genotypes post-infection, although the difference between them was not statistically significant. This aligns with Singh et al. (2022), who emphasized the role of LOX in jasmonic acid-mediated defense signaling.

Enhanced β-1,3-glucanase activity was observed in inoculated samples of both genotypes (Figure 1), corroborating earlier reports by Anushree et al. (2016). Notably, BRRI dhan29 demonstrated higher relative β-1,3-glucanase performance despite lower absolute values (0.02) compared to IR 64 (0.029). This suggests that although defense enzymes were activated, they may have been insufficient to restrict *P. oryzae* proliferation effectively. The elevated β-1,3-glucanase activity in the susceptible BRRI dhan29 may reflect a delayed, non-specific, or dysregulated defense response. Van Loon et al. (2006) noted that elevated pathogenesis-related (PR) protein levels alone are inadequate for resistance if they are activated late, circumvented by the pathogen, or unsupported by effective resistance genes (Datta et al., 1999). Such ineffective defense, sometimes referred to as an abortive response, has been reported in other susceptible genotypes (Devanna et al., 2022). Excessive β-1,3-glucanase activity may also degrade host cell wall components, inadvertently facilitating pathogen colonization (Prasannath, 2017).

In contrast, IR 64 displayed a more coordinated defense response, with simultaneous activation of SOD, PAL, LOX, and chitinase, suggesting a well-integrated network that restricts pathogen progression effectively (Singh et al., 2022; Yang et al., 2024).

In this study, BRRI dhan29 showed a rapid accumulation of H_2O_2 and superoxide compared to IR 64 (Table 2), likely due to inefficient ROS scavenging, as reported by Aver'yanov et al. (2015). Excess ROS accumulation in BRRI dhan29 may reflect an uncontrolled oxidative burst, leading to host cell damage and enhanced pathogen ingress rather than effective signaling for defense (Velikova et al., 2000). Thus, the defense of BRRI dhan29 appears quantitatively intense but qualitatively misregulated, while IR 64's moderate, well-coordinated response supports greater resistance.

IR 64 also exhibited higher levels of secondary

metabolites such as phenols, flavonoids, and antioxidants, consistent with Toan et al. (2017), suggesting improved redox balance and defense signaling. Increased protein and sugar contents in IR 64, observed by Yang et al. (2024), further indicate enhanced metabolic adaptation under pathogen stress.

P. oryzae significantly impacted growth and physiological parameters in both rice genotypes (Table 3). Reductions in plant height and tiller number under infection were attributed to fungal colonization and damage to meristematic tissues, restricting growth and tillering capacity (Khan et al., 2014).

Similarly, yield components such as panicle length, panicle number, and grain filling were adversely affected (Table 4). Blast infection reduced panicle fertility and grain development, increased sterility, and shortened panicles (Khan et al., 2014; Li et al., 2022). BRRI dhan29 exhibited greater yield losses, while IR 64 maintained relatively better yield traits under disease pressure due to its partial resistance mechanisms.

Significant reductions in leaf area were recorded in both genotypes under *P. oryzae* stress (Figure 2), with BRRI dhan29 being more affected. This decline reflects tissue damage and disrupted cellular processes due to pathogen infection (Shahriar et al., 2020). IR 64 retained more leaf area and number, indicating partial resistance and sustained photosynthetic capacity (Devanna et al., 2022). The reduced leaf number in both genotypes resulted from blast-induced lesions and necrosis, which hinder leaf expansion (Miah et al., 2013). SPAD values decreased over time, with greater reductions in inoculated plants, reflecting chlorophyll degradation and resource diversion for defense (Acharya et al., 2019). These variations highlight genotypic differences in tolerance to blast infection.

BRRI dhan29 exhibited the highest PDI at 20 DAI, reaching approximately 80 (Figure 2), indicating heightened susceptibility. In contrast, IR 64 maintained lower PDI values, particularly at 10 DAI, reflecting reduced disease progression. These findings are consistent with Neupane and Bushal (2021), who emphasized the importance of infection timing in disease severity.

The higher susceptibility of BRRI dhan29, reflected in a 32% yield loss and increased grain sterility, poses a serious challenge to sustainable rice production in Bangladesh. These findings align with earlier reports by Hossain et al. (2017) and Rahman and Uddin (2017), who documented similar disease severity under field conditions. The results emphasize the importance of enhancing defense mechanisms in BRRI dhan29 through targeted breeding approaches, as proposed by Sharma et al. (2012) and Miah et al. (2013). Complementary disease management strategies, including timely fungicide applications and improved agronomic practices, may also help mitigate blast-related losses (Shahriar et al., 2020). The moderate resistance and yield stability of IR 64 likely stem from the presence of partial resistance genes and coordinated defense mechanisms.

Conclusion

This study highlights the contrasting responses of rice genotypes IR 64 and BRRI dhan29 under *P. oryzae* stress. IR 64 exhibited superior defense responses through enhanced enzymatic (SOD, PAL, CHT) and non-enzymatic (phenols, flavonoids, proteins, sugars) mechanisms, maintaining better growth, physiology, and yield attributes. In contrast, BRRI dhan29 displayed weaker biochemical defenses, higher susceptibility, and greater yield losses. These results emphasize the importance of utilizing resistant cultivars like IR 64 in blast-endemic regions.

Future research should focus on proteomic and metabolomic profiling of secondary metabolites, pyramiding of resistance genes, gene-editing approaches such as CRISPR/Cas9, detailed defense gene expression studies, and multi-season, multi-location evaluations. Integrating these tools with sustainable crop management and Integrated Pest Management (IPM) strategies will be essential for improving the long-term resistance and productivity of BRRI dhan29.

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Authors' Contributions

SB, FCC and TT conducted the experiments and collected data; AAM analyzed and arranged the data; MSS, YA and JR conceived, designed, and supervised the study; SB and TT wrote the initial manuscript; MSS, YA AAM and JR edited the manuscript; JR finalized the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

References

Acharya, B., Shrestha, S.M., Manandhar, H.K., Chaudhary B., 2019. Screening of local, improved and hybrid rice genotypes against leaf blast disease (*Pyricularia oryzae*) at Banke district, Nepal. Journal of Agriculture and Natural Resources 2(1), 36-52.

Ahmed, D., Fatima, K., Saeed, R., 2014. Analysis of phenolic and flavonoid contents, and the anti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of *Carissa opaca* roots and its fractions in different solvents. Antioxidants 3(4), 671-683.

Ahmed, D., Khan, M.M., Saeed, R., 2015. Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from *Adiantum caudatum* leaves. Antioxidants 4(2), 394-409.

Anushree, P.U., Naik, R.M., Satbhai, R.D., 2016. Activity profile of defence-related enzymes in rice genotypes (*Oryza sativa L.*) against rice blast (*Magnaporthe oryzae*). Archives of Phytopathology and Plant Protection 49(7-8), 167-181.

Asana, R.D., Williams, R.F., 1965. The effect of temperature stress on grain development in wheat. Australian Journal of Agricultural Research 16, 1-3.

Aver'yanov, A.A., Pasechnik, T.D., Lapikova, V.P., Romanova, T.S., Baker, C.J., 2015. Systemic reduction of rice blast by inhibitors of antioxidant enzymes. Russian Journal of Plant Physiology 62, 586-594.

Bai, X., Zhan, G., Tian, S., Peng, H., Cui, X., Islam, M.A., Goher, F., Ma, Y., Kang, Z., Xu, Z-S., Guo, J., 2021. Transcription factor BZR2 activates chitinase Cht20.2 transcription to confer resistance to wheat stripe rust. Plant Physiology 187(4), 2749-2762.

Ben-Aziz, A., Grossman, S., Ascarelli, I., Budowski, P., 1970. Linoleate oxidation induced by lipoxygenase and heme proteins: A direct spectrophotometric assay. Analytical Biochemistry 34(1), 88-100.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2), 248-254.

Byrne, N.D., Duxbury, M., Sharpe, N., 2001. The determination of chitinase activity of grapes: An introductory enzyme assay. Biochemistry and Molecular Biology Education 29(4), 144-146.

Datta, K., Velazhahan, R., Oliva, N., Ona, I., Mew, T., Khush, G.S., Muthukrishnan, S. and Datta, S.K., 1999. Over-expression of the cloned rice thaumatin-like protein (PR-5) gene in transgenic rice plants enhances environmental friendly resistance to *Rhizoctonia solani* causing sheath blight disease. Theoretical and Applied Genetics 98(6), 1138-1145.

Del Longo, O.T., González, C.A., Pastori, G.M., Trippi, V.S., 1993. Antioxidant defences under hyperoxygenic and hyperosmotic conditions in leaves of two lines of maize with differential sensitivity to drought. Plant and Cell Physiology 34(7), 1023-1028.

Devanna, B.N., Jain, P., Solanke, A.U., Das, A., Thakur, S., Singh, P.K., Kumari, M., Dubey, H., Jaswal, R., Pawar, D., 2022. Understanding the dynamics of blast resistance in rice-*magnaporthe oryzae* interactions. Journal of Fungi 8(6), 584.

Fahad, S., Rehman, A., Shahzad, B., Tanveer, M., Saud, S., Kamran, M., Rahman, M. H., 2019. Rice responses and tolerance to metal/metalloid toxicity. In Advances in rice research for abiotic stress tolerance (pp. 299-312). Woodhead publishing.

Hossain, M., Ali, M.A., Hossain, M.D., 2017. Occurrence of blast disease in rice in Bangladesh. American Journal of Agricultural Science 4(4), 74-80.

Ippolito, A., Ghaouth, A.E.I., Wilson, C.L., Wisniewski, M., 2000. Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharvest Biology and Technology 19(3), 265-272.

James, W., 2003. Assessment of plant diseases and losses. Annual Review of Phytopathology 12, 27-48.

Keerthana, U., Senapati, A.K., Bag, M.K., Prabhukarthikeyan, S.R., Sawant, S.B., Mishra,

M.K., Mohapatra, S.D., 2024. Unraveling defense strategies: gene expression dynamics in blast-resistant vs. susceptible aromatic rice landraces. Australasian Plant Pathology 53, 335-343.

Khan, M.A.I., Bhuiyan, M.R., Hossain, M.S., Sen, P.P., Ara, A., Siddique, M.A., Ali, M.A., 2014. Neck blast disease influences grain yield and quality traits of aromatic rice. Comptes Rendus Biologies 337(11), 635-641.

Li, H., Xu, Q., Li, C., Zhang, J., Wang, Q., Xiang, H., Liu, Y., Wei, H., Qin, Z., 2022. Dual role of acid rain and *pyricularia oryzae* on growth, photosynthesis and chloroplast ultrastructure in rice seedlings. Agronomy 12(3), 567.

Mahmud, H., Hossain, I., 2018. Efficacy of BAU-Biofungicide, chemical fungicides and plant extracts on rice (*Oryza sativa* L.) diseases and yield. Journal of Plant Physiology and Pathology 6(2), 1-8.

Mainuddin, M., Kirby, M., 2015. National food security of Bangladesh to 2050. Food Security 7, 633-646.

Miah, G., Rafii, M.Y., Ismail, M.R., Puteh, A.B., Rahim, H.A., Asfaliza, R., Latif, M.A., 2013. Blast resistance in rice: a review of conventional breeding to molecular approaches. Molecular Biology Report 40(3), 2369-2388.

Mir, S.A., 2015. Optimized pyrogallol based superoxide scavenging assays for testing antioxidants. International Journal of PharmTech Research 7(2), 266-274.

Muthayya, S., Sugimoto, J.D., Montgomery, S., Maberly, G.F., 2014. An overview of global rice production, supply, trade, and consumption. Annals of the New York Academy of Sciences 1324, 7-14.

Neupane, N., Bhusal, K., 2020. A review of blast disease of rice in Nepal. Journal of Plant Pathology and Microbiology 11, 528.

Nisha, S., Revathi, K., Chandrasekaran, R., Kirubakaran, S.A., Sathish-Narayanan, S., Stout, M.J., Senthil-Nathan, S., 2012. Effect of plant compounds on induced activities of defense-related enzymes and pathogenesis related protein in bacterial blight disease susceptible rice plant. Physiological and Molecular Plant Pathology 80, 1-9.

Plummer, D.T., 1990. An Introduction to Practical Biochemistry, Third Edition. McGraw-Hill College, 179.

Prasannath, K., 2017. Plant defense-related enzymes against pathogens: a review. AGRIEAST: Journal of Agricultural Sciences 38-48.

Rahman, M.H., Uddin, M.J., 2017. Blast: a threat to cereal crops in Bangladesh. Barisal University Journal Part 1 4, 237-260.

Sels, J., Mathys, I., De Coninck, B.M.A., Cammue, B.P.A., De, B.F.C., 2008. Plant pathogenesis- related (PR) proteins: a focus on PR peptides. Plant Physiology and Biochemistry 46 (11), 941-950.

Shahriar, S.A., Imtiaz, A.A., Hossain, M.B., Husna, A., Eaty, M., Khatun, N., 2020. Rice blast disease. Annual Research and Review in Biology 35(1), 50-64.

Shamim, M., Siddiqui, W., Khan, N., Srivastava, D., Kumar, D., Kumar, M., Kumar, S., Jha, V., Singh, K., 2018. Comparative biochemical analysis of enzymatic scavengers and defence signaling molecules after *R. solani* infection in rice and barley. International Journal of Current Microbiology and Applied Science 7, 4476-4487.

Sharma, T.R., Rai, A.K., Gupta, S.K., Vijayan, J., Devanna, B.N., Ray, S., 2012. Rice blast management through host-plant resistance retrospect and prospects. Agricultural Research 1, 37-52.

Singh, P., Arif, Y., Miszczuk, E., Bajguz, A., Hayat, S., 2022. Specific roles of lipoxygenases in development and responses to stress in plants. Plants 11(7), 979.

Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. American Journal of Enology and Viticulture 28, 49-55.

Toan, N.P., Ha, P.T.T., Xuan, T.D., 2017. Effects of rice blast fungus on phenolics, flavonoids, antioxidant capacity in rice. International Letters Natural Science 61, 1-7.

Van Loon, L.C., Rep, M., Pieterse, C. M., 2006. Significance of pathogenesis-related proteins in plant defense. Annual Review of Phytopathology 44, 135-62.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants protective role of exogenous polyamines. Plant Science 151, 59-66.

Yang, X., Yan, S., Li, G., Li, Y., Li, J., Cui, Z., Sun, Y., 2024. Rice-*Magnaporthe oryzae* interactions in resistant and susceptible rice cultivars under panicle blast infection based on defense-related enzyme activities and metabolomics. Plos One 19(3), e029999.