

Innate resistance linked to non-enzymatic biochemical alteration of rice plants in response to *Pyricularia oryzae*

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Abstract

Ray, J., Sultana, M. S., Ali, M. Y., Mamun, S. M. A. A., Das, D. & Rezve, M. (2026). Innate resistance linked to non-enzymatic biochemical alteration of rice plants in response to *Pyricularia oryzae*. *Bulg. J. Agric. Sci.*, 32(5), 1125–1138

Blast disease of rice caused by the fungus *Pyricularia oryzae* Cavara is one of the most destructive diseases, which causes about 10% to 30% yield loss. Utilization of innate host resistance has been the best way to manage the disease, for which understanding the innate immunity of our popular rice genotypes is necessary. To measure non-enzymatic biochemical alterations of rice genotypes against *P. oryzae*, an experiment was conducted and repeated once in the next year following a factorial completely randomized design at Plant Protection and Plant Breeding laboratory of Agrotechnology Discipline of Khulna University. BRRI dhan74, BRRI dhan33 and BRRI dhan75 along with IR 64 (resistant check line) showed minimum disease incidence (%) and maximum synthesis of total flavonoids, antioxidants, protein and sugar. These genotypes also maintained a balanced ROS (H_2O_2 ; O_2^-) production at later disease developmental stages (96 and 120 HAI). Pearson correlation revealed that H_2O_2 and O_2^- were positively, whereas antioxidant, flavonoid, protein and sugar was negatively associated with disease incidence. Principal component analysis (PCA) showed 91.7% total variability and indicated that total antioxidant, flavonoid, protein, sugar and H_2O_2 contributed more to the variation. Notably, BRRI dhan74, BRRI dhan33 and BRRI dhan75 sustained higher synthesis levels of resistance-related non-enzymatic compounds with lower disease incidence indicating a robust defense response against *P. oryzae*.

Keywords: rice blast; reactive oxygen species (ROS); antioxidants; flavonoid; disease incidence (%); principal component analysis (PCA)

Introduction

Rice (*Oryza sativa* L.), a staple meal for billions of people, is especially important in South Asia's rapidly growing low-income countries (Simkhada and Thapa, 2022). To satisfy the rising demand for rice, worldwide rice production is projected to need to increase by 38% by 2030 (Samal and Babu, 2018). However, it is hard to grow enough rice to meet the world's rising demand because many biotic and abiotic factors affect yield.

Rice blast, induced by the fungus *Pyricularia oryzae* Cavara, causes a significant and persistent biotic stress, re-

sulting in substantial annual yield losses and posing a global threat to rice production (Younas et al., 2023). The pathogen induces lesions on different plant parts such as leaves, leaf collars, panicle necks and panicles, with variations in color and shape, influenced by varietal resistance, environmental conditions, and age (Asibi et al., 2019).

In ideal conditions, this disease may destroy rice plants in 15–20 days and reduce yields by 100% (Dean et al., 2012). Rice blast is tough to manage because this fungus may survive and grow under severe conditions and spread easily to other fields. Due to new and more virulent fungal strains, varietal resistances have diminished, making management

and control harder (Laha et al., 2017). Understanding the rice blast pathogen and rice defense systems is essential for global food security.

Under normal circumstances, plants use available oxygen for optimal growth and development. During pathogen attack, generation of reactive oxygen species (ROS) occurs through the use of available oxygen (Singla et al. 2019), subsequently resulting in photo-oxidative damage to biomolecules and internal cellular structures (Mittler, 2016). Plants respond to microbial interactions by inducing numerous biochemical changes related to stress signaling, thereby activating their defense pathways. The mechanisms involve the activation of numerous non-enzymatic components, including antioxidants, flavonoids, phenols and phytoalexins as secondary metabolites (Debona et al., 2012). Consequently, plants possess natural biochemical compounds that enable them to manage plant pathogens effectively.

According to Khan et al. (2014), blast was a major problem for the majority of the popular cultivars grown in Bangladesh. As fungicides are costly and harmful to the environment, the most effective method of disease management has been to exploit host resistance, which necessitates the identification of resistance sources (Sharma et al., 2012). The most cost-effective way to control disease and feed the

world is to learn how different pathogens affect the physiological changes in rice and then use that knowledge to breed disease-resistant types. Our popular rice varieties' inherent immunity has been the subject of scant research. Thus, the present study was undertaken to measure the non-enzymatic biochemical alterations of rice plants in response to the stress caused by *P. oryzae*.

Methodology

The experiment was conducted from January to March, 2023, at the Plant Protection Laboratory and the net house of the Plant Breeding Laboratory of Khulna University, and repeated once in the next year (January to March, 2024). Combined mean data were used for analysis (ANOVA of the two years' data was shown in Supplementary Table 1). The approximate geographical location of the laboratory is 22.79904° N and 89.530677° E which belongs to AEZ-13 (Ganges Tidal Floodplain).

Collection of rice seeds

Bangladesh Rice Research Institute (BRRI), Gazipur, provided the seeds of eleven rice genotypes (BRRI dhan33, BRRI dhan31, BRRI dhan39, BRRI dhan75, BRRI dhan87,

Supplementary Table 1. Two-way ANOVA on different biochemical traits of rice genotypes against *P. oryzae* for pot experiments conducted in 2023 and 2024 (*f*-statistics with asterisks denote significance at $p \leq 0.05$)

		H ₂ O ₂ (72 HAI)		H ₂ O ₂ (96 HAI)		H ₂ O ₂ (120 HAI)	
Source	DF	MS	F	MS	F	MS	F
Replication	2	5.6		8.6		13.8	
Genotype	10	1971.13	1064.81*	3198.0	1573.84*	3878.9	1736.42*
Treatment	1	1395.42	756.64*	43784.9	21547.6*	92921.3	41597.4*
Year	1	1.7	0.92	7.9	3.88	7.6	3.41
Geno*Treat	10	25.31	13.72*	314.6	154.81*	703.4	314.89*
Geno*Year	10	1.04	0.57	2.0	0.96	3.0	1.35
Treat*Year	1	0.46	0.25	2.9	1.45	0.6	0.25
Geno*Treat*Year	10	0.55	0.3	2.2	1.07	0.4	0.2
Error	86	1.84		2.0		2.2	

Variable (s)		O ₂ ⁻ (72 HAI)		O ₂ ⁻ (96 HAI)		O ₂ ⁻ (120 HAI)	
Source	DF	MS	F	MS	F	MS	F
Replication	2	0.0002		7.38		2.796	
Genotype	10	0.1259	19.49*	0.278	80.85*	0.338	79.36*
Treatment	1	0.1849	286.17*	1.078	3140.86*	2.02	4745.98*
Year	1	0.00008	0.12	9.28	2.7	9.28	2.18
Geno*Treat	10	0.0027	4.12*	0.012	35.53*	0.022	52.14*
Geno*Year	10	0.0002	0.34	3.014	0.88	2.647	0.62
Treat*Year	1	0.00003	0.04	7.576	0.02	7.576	0.00
Geno*Treat*Year	10	0.0002	0.33	4.091	0.01	2.076	0.05
Error	86	0.0007		3.433		4.257	

Supplementary Table 1. Continued

Variable (s)		Total Flavonoid (72 HAI)		Total Flavonoid (96 HAI)		Total Flavonoid (120 HAI)	
Source	DF	MS	F	MS	F	MS	F
Replication	2	2.65		5.35		2.331	
Genotype	10	61.89	75.75*	92.59	255.64*	153.77	179.78*
Treatment	1	0.1243	0.15	208.81	576.53*	836.39	977.86*
Year	1	0.3873	0.47	0.131	0.36	0.026	0.03
Geno*Treat	10	4.5185	5.53*	28.44	78.51*	108.98	127.42*
Geno*Year	10	6.8345	8.36*	6.39	17.63*	5.32	6.22*
Treat*Year	1	0.006	0.01	0.289	0.8	0.054	0.06
Geno*Treat*Year	10	0.0011	0.00	0.164	0.45	0.429	0.5
Error	86	0.8171		0.362		0.866	

Variable (s)		Total Antioxidant (72 HAI)		Total Antioxidant (96 HAI)		Total Antioxidant (120 HAI)	
Source	DF	MS	F	MS	F	MS	F
Replication	2	2.4		1		6.707	
Genotype	10	5924.7	934.7*	10131	2656.97*	18943.2	3246.94*
Treatment	1	19056.5	3006.44*	122686	32175.14*	1018726	174613.5*
Year	1	19.0	2.99	5	1.42	5.304	0.91
Geno*Treat	10	979.2	154.48*	4122	1081.14*	10701.6	1834.29*
Geno*Year	10	21.6	3.41*	4	1.17	1.3749	0.24
Treat*Year	1	22.8	3.59	17	4.43	0.0322	0.01
Geno*Treat*Year	10	2.7	0.42	7	1.79	1.333	0.23
Error	86	6.3		4		5.834	

Variable (s)		Total Protein (72 HAI)		Total Protein (96 HAI)		Total Protein (120 HAI)	
Source	DF	MS	F	MS	F	MS	F
Replication	2	78.02		24.594		13.81	
Genotype	10	626.02	74.94*	800.176	183.7*	2012.2	307.73*
Treatment	1	2922.93	349.89*	11632.1	2670.48*	43201.1	6606.88*
Year	1	20.59	2.46	18.7	4.29	22.24	3.4
Geno*Treat	10	131.69	15.76*	209.91	48.19*	998.15	152.65*
Geno*Year	10	0.079	0.01	0.077	0.02	0.06	0.01
Treat*Year	1	2.128	0.00	0.118	0.03	1.939	0.00
Geno*Treat*Year	10	0.025	0.00	0.036	0.01	0.015	0.00
Error	86	8.354		4.356		6.539	

Variable (s)		Total Sugar (72 HAI)		Total Sugar (96 HAI)		Total Sugar (120 HAI)	
Source	DF	MS	F	MS	F	MS	F
Replication	2	0.0204		0.0269		0.033	
Genotype	10	0.1497	10*	0.3699	27.3*	0.516	23.47*
Treatment	1	10.727	716.59*	61.568	4544.01*	86.77	3950.02*
Year	1	0.082	5.48	0.084	6.2	0.066	2.98
Geno*Treat	10	0.115	7.69*	0.302	22.26*	0.438	19.94*
Geno*Year	10	0.0013	0.09	0.0028	0.21	0.0013	0.06
Treat*Year	1	0.0015	0.1	0.0025	0.18	0.0046	0.21
Geno*Treat*Year	10	0.0025	0.17	0.0012	0.09	0.0007	0.03
Error	86	0.015		0.0135		0.022	

DF: Degrees of freedom; MS: Mean square; F: *f*-statistics

Source: Authors' own elaboration

BRR1 dhan74, BRR1 dhan28, BRR1 dhan59, BRR1 dhan63, BRR1 dhan88 and IR 64). The genotype IR 64 was selected as a resistant check line.

Cultivation of plants in pot

Prior to seeding, seeds were treated with Carbendazim to control the soil borne disease. After five days of seed setting on petri dish, 20 healthy seedlings were transferred to a plastic tray containing 15 kg of soil (30 cm – 20 cm – 14 cm), and were considered as one replication. The physical and chemical properties of soil were clay loam in texture, pH – 6.6, EC – 1.02, soil organic matter – 1.87%, available N – 1140.5 ppm, available P – 15.04 µg/g, available S- 176.8 µg/g, with an inherent exchangeable K, Ca, and Mg content at 0.65 Cmol kg⁻¹, 16.5 Cmol kg⁻¹, and 6.5 Cmol kg⁻¹ soil, respectively. According to Fertilizer Recommendation Guide-2018 of Bangladesh by Ahamed and Jahiruddin (2018), basal application of MoP (77 kg/ha K), DAP (24 kg/ha P), Gypsum (18 kg/ha S), zinc sulfate (2.6 kg/ha Zn), and Urea (180 kg/ha N) was used as necessary nutrient in all the pots.

Preparation of fungal inoculum and inoculation procedure

A monosporic virulent isolate (RL-17) of *Pyricularia oryzae* stored on silica gel as paper disk was collected from Plant Protection laboratory of Agrotechnology Discipline of Khulna University. Mycelia of *Pyricularia oryzae* was grown initially from paper disk on petri dish containing about 30 ml freshly made oat agar media, and kept on dark at 24°C. Fungal inoculum of *P. oryzae* was prepared and inoculation procedure from spore suspension was done according to Khan et al. (2014). Just after inoculation, the plants were shifted to a growth chamber with 24°C temperature and 90 ± 5% RH for 24-hour dark period. Then, these pots were transferred to the net house and kept there for rest of the experiment duration, where the temperature and relative humidity ranged from 25 to 15 ± 2°C and 95 to 80 ± 5%, respectively. Digital Temperature and Humidity Meter (HTC- 1, China) was used to measure relative humidity and temperature.

Data collection

Samples from the fourth and fifth leaves from the base of each plant were collected at 72, 96 and 120 hours after inoculation (HAI). The leaf samples were kept in liquid nitrogen during sampling and then stored at –80°C until further analysis. Centrifuge (CD-3124R, Phoenix instrument), and UV visible spectrophotometer (T60U, UK) was used for measuring all biochemical variables. The following biochemical variables were taken into consideration during this study:

Hydrogen peroxide (H₂O₂)

Briefly, 1 gm leaf sample was grounded with 5 mL 0.1% (w/v) trichloroacetic acid and centrifuged at 15000 rpm at 4°C for 15 min. collect 0.5 mL supernatant, and followed the procedure of Velikova et al. (2000) to estimate H₂O₂ concentration. Taken absorbance at 390 nm against blank. Based on a standard curve (300 mM/ml H₂O₂ as stock solution), H₂O₂ concentration was estimated and expressed as µmol/kg FW.

Superoxide (O₂⁻)

According to Mir (2014), 1 gm fresh leaf sample was grinded by liquid nitrogen. Then, was added 4 mL dH₂O and waited for 10 minutes. Again added 5 mL dH₂O and centrifuged at 4000 rpm for 15 minutes. 3.5 mL aliquots were taken and added 0.5 mL alkaline mixture (10 mL 0.25 M NaHCO₃ with 10 mL water and 5 mL 0.1 M NaOH solution), kept at room temperature for 50 minutes and read at 415 nm against blank. O₂⁻ concentration was measured by preparing standard curve (Pyrogallol stock solution as standard), and expressed as mmol/kg FW.

Total flavonoid

Total flavonoid content was measured according to Ahmed et al. (2015). Briefly, 3 mg leaf extract was sonicated in 10 mL ethanol for 10 min. 300 µL leaf extract was taken in a test tube, and 3.4 mL aqueous ethanol (30%) was added until to get a clear solution. Then, 150 µL 0.5 M aqueous NaNO₃ solution was added followed by adding 150 µL 0.3 M AlCl₃ solution. After 5 min, 1 mL 1M NaOH solution was added, mixed well and measured its absorbance at 506 nm against a blank. Total flavonoid content was measured by using the standard curve of quercetin (300 µg/mL) and expressed as µg mL⁻¹.

Total antioxidant

Total antioxidant capacity was measured by Prieto et al. (1999), the procedure described by Ahmed et al. (2015) with slight modification. Briefly, 250 µg leaf extract was dissolved in 1 mL ethanol and kept for 5 min. In a test tube, 300 µL plant extract was mixed with 3 mL phosphomolybdate reagent (0.6 M H₂SO₄, 28 mM NaHPO₄ and 4 mM (NH₄)₂MoO₄), covered with aluminum foil and incubated at 95 °C for 90 min. The mixture was then allowed to reach room temperature and taken absorbance at 765 nm against blank. Total antioxidant was measured by a standard curve of ascorbic acid (500 mg/L) and expressed as µg mL⁻¹.

Total protein

Leaves from test plants (1 g) were grinded by using liquid nitrogen and added 1ml of protein extraction buffer [Tris HCl

(pH 8.1) 10mM, EDTA (pH 8.0) 10 mM, β -Mercaptoethanol 5mM and PMSF 0.1 mg/mL] in a cold mortar and pestle. Total soluble protein was determined following Bradford (1976) method, where Bovine Serum Albumin (BSA 2.0 mg/mL) was used as standard. The absorbance was taken at 595 nm against a reagent blank (100 μ l of extraction buffer with 1 mL of Coomassie Blue G dye reagent), to calculate the protein concentration in the extract by comparison with the standard curve for BSA.

Total sugar

Fresh leaves (0.1 g) were added with 5 mL of 80 % ethanol to test tubes and total soluble sugar was determined according to Dubois et al. (1951). The absorbance of final supernatant was read at 490 nm against blank (80% ethanol). Total sugar was determined by using a standard curve of glucose and expressed as mg g⁻¹.

Disease incidence (%)

By observing the typical symptoms, disease incidence (%) was recorded by following the formula

$$D.I.(%) = \frac{\text{Number of infected plant}}{\text{Total number of plant}} \times 100.$$

Experimental design and statistical analysis

The experiment was consisted of two factors. Factor A consisted of eleven rice genotypes and factor B was two statuses of inoculum (control and inoculated). Completely

randomized design (CRD) with three replications was used. Simple effect analysis was done for biochemical variables and means was compared by Tukey's HSD test at 5% level. Different graphs from biochemical variables, Pearson correlation and PCA were made using R programming language software and Microsoft excel.

Results

Disease incidence (%)

Disease incidence (%) was increased with increasing inoculation time and significant difference ($P < 0.05$) was observed among all the rice genotypes (Figure 1). At 120 HAI, DI (%) was lowest in IR 64 (21.67%), which was statistically similar with BRRI dhan33 (21.67), BRRI dhan74 (23.33%), BRRI dhan75 (25%), and highest in BRRI dhan63 (58%) and BRRI dhan31 (55%).

Hydrogen peroxide

H₂O₂ concentration was increased in infected leaf with increasing inoculation time and all genotypes were significantly differed ($p < 0.05$) among themselves from 72 to 120 HAI (Figure 2). The recorded activity of H₂O₂ ranged from 51.35 to 85.52, 51.75 to 86.26 and 52.42 to 86.66 μ mol kg⁻¹ FW at 72, 96 and 120 HAI, respectively, in control leaf sample. In inoculated leaf sample, this activity ranged from 56.9 to 90.57, 74.7 to 138.18 and 85.6 to 160.58 μ mol kg⁻¹ FW at 72, 96 and 120 HAI, respectively. The increasing levels of H₂O₂ after spraying inoculum was higher in BRRI dhan33

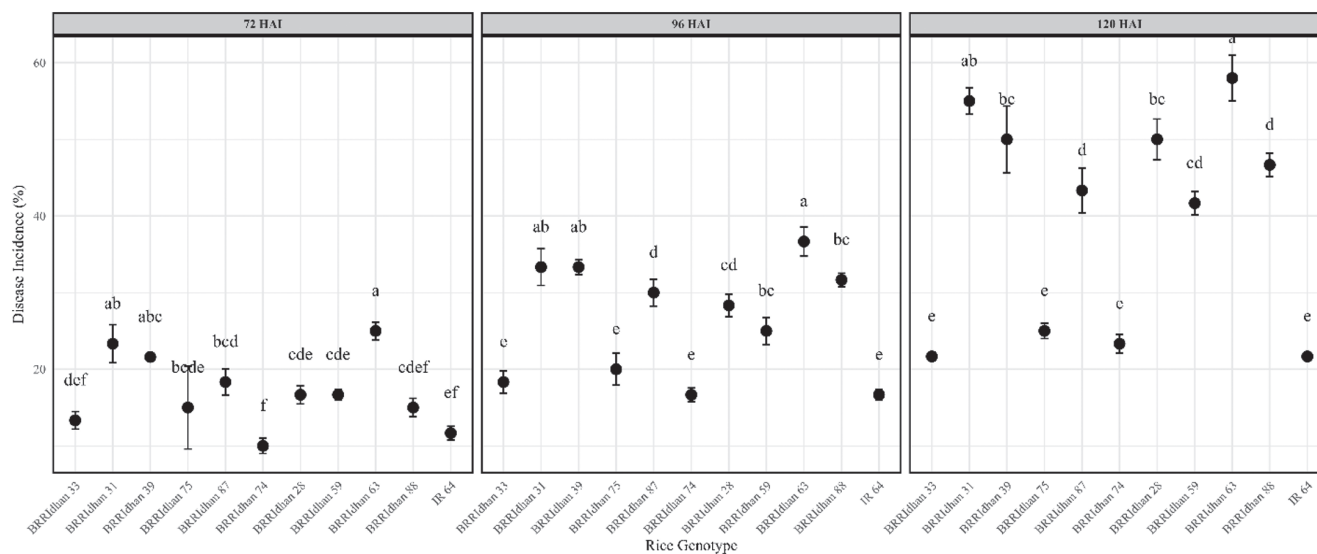


Fig. 1. Blast disease incidence (%) of eleven rice genotypes at 72, 96 and 120 HAI

All values represent the mean $\pm$ standard deviation at $P < 0.05$

Source: Authors' own elaboration

(21%), followed by BRRI dhan74 (15%), BRRI dhan87 (12.13%), IR 64 (12.09%) and lower in BRRI dhan88 (2%), followed by BRRI dhan28 (4%), BRRI dhan59 (6.9%) and BRRI dhan63 (7.15%), compared to control plant at 72 HAI. This increasing trend at 96 HAI was highest in BRRI dhan63 (71.4%), followed by BRRI dhan31 (63.25%), BRRI dhan28 (61.23%) and BRRI dhan88 (57.12%), and lowest was in IR 64 (33.2%), followed by BRRI dhan74 (37.23%), BRRI dhan33 (43.88%) and BRRI dhan59 (45%). At 120 HAI, the increasing trend in inoculated sample compared to control was highest in BRRI dhan63 (100%), followed by BRRI dhan87 (92.57%), BRRI dhan31 (92.16%) and BRRI dhan28 (86.56%), and lowest was in BRRI dhan74 (49.5%), followed by IR 64 (51.32%) and BRRI dhan33 (58.51%).

Superoxide

O_2^- concentration was significantly increased and differed ($p < 0.05$) among genotypes in infected leaf samples from 72 to 120 HAI (Figure 3). The recorded activity of O_2^- ranged from 0.25 to 0.35, 0.28 to 0.36 and 0.29 to 0.36 mmol kg⁻¹ FW at 72, 96 and 120 HAI, respectively, in control leaf sample whereas in inoculated sample, this activity ranged from 0.32 to 0.46, 0.4 to 0.59 and 0.45 to 0.7 mmol kg⁻¹ FW at 72, 96 and 120 HAI, respectively. The increasing levels of O_2^- after spraying inoculum was higher in IR 64 (48%), followed by BRRI dhan74 (40%) BRRI dhan59 (31%), and lower in BRRI dhan31 (9%), followed by BRRI dhan87 (11%), com-

pared to control plant at 72 HAI. This increasing trend at 96 HAI was highest in BRRI dhan63 (90%), followed by BRRI dhan31 (72%) and BRRI dhan28 (71%), and lowest was in BRRI dhan33 (23%), followed by BRRI dhan75 (36%), IR 64 (41%) and BRRI dhan74 (42%). At 120 HAI, the increasing trend in inoculated sample compared to control was highest in BRRI dhan28 (112%), followed by BRRI dhan63 (112%), BRRI dhan31 (106%), and lowest was in BRRI dhan33 (40%), followed by IR 64 (48%) and BRRI dhan74 (49.5%).

Total flavonoid content

The recorded activity of flavonoid ranged from 30.79 to 36.41, 31.02 to 36.23 and 31.02 to 36.53 $\mu\text{g mL}^{-1}$ at 72, 96 and 120 HAI, respectively, in control leaf sample whereas in inoculated sample, this activity ranged from 29.22 to 38.76, 29.46 to 41.8 and 28.67 to 47.86 $\mu\text{g mL}^{-1}$ at 72, 96 and 120 HAI, respectively (Figure 4). Among 11 genotypes, total flavonoid was decreased in five genotypes and increases in six genotypes under inoculated condition at 72 HAI. Maximum decreased was recorded in BRRI dhan31 (-6.88%), and maximum increased was in BRRI dhan74 (6.45%). At 96 HAI, total flavonoid was increased in inoculated sample of all genotypes except BRRI dhan31 (-5.88%) and BRRI dhan63 (-4.75%). Increasing trend was highest in IR 64 (28.58%), followed by BRRI dhan74 (15.04%), BRRI dhan33 (11.49%) and BRRI dhan75 (9.37%). At 120 HAI, relative activity was highest in

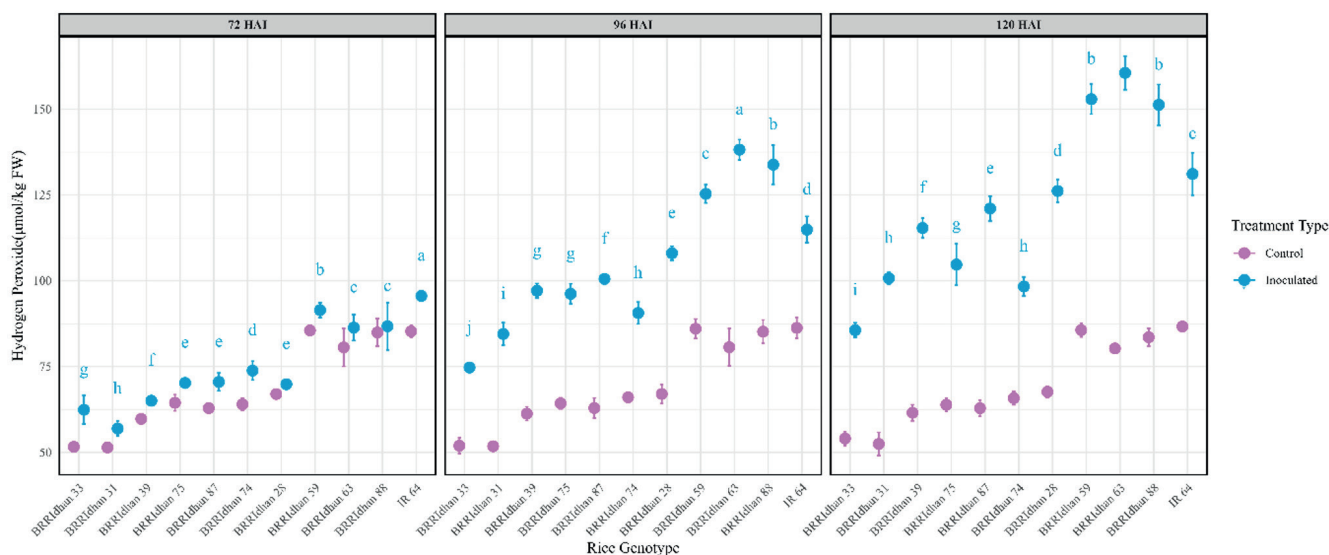


Fig. 2. Hydrogen peroxide (H_2O_2) concentration from control and inoculated leaf of eleven rice genotypes at 72, 96 and 120 hours after inoculation with *P. oryzae*

All values represent the mean $\pm$ standard deviation ($n = 3$) at $P < 0.05$

Source: Authors' own elaboration

IR 64 (47.31%), followed by BRRI dhan33 (37.29%), BRRI dhan74 (29.04%) and BRRI dhan75 (27.97%), and the lowest flavonoid activity was in BRRI dhan63 (-13.49%) and BRRI dhan28 (-2.64%) (Figure 4).

Total antioxidant

The recorded activity of antioxidant showed significant difference ($p < 0.05$) among the genotypes in inoculated sample of all genotypes (Figure 5). Total antioxidants ranged

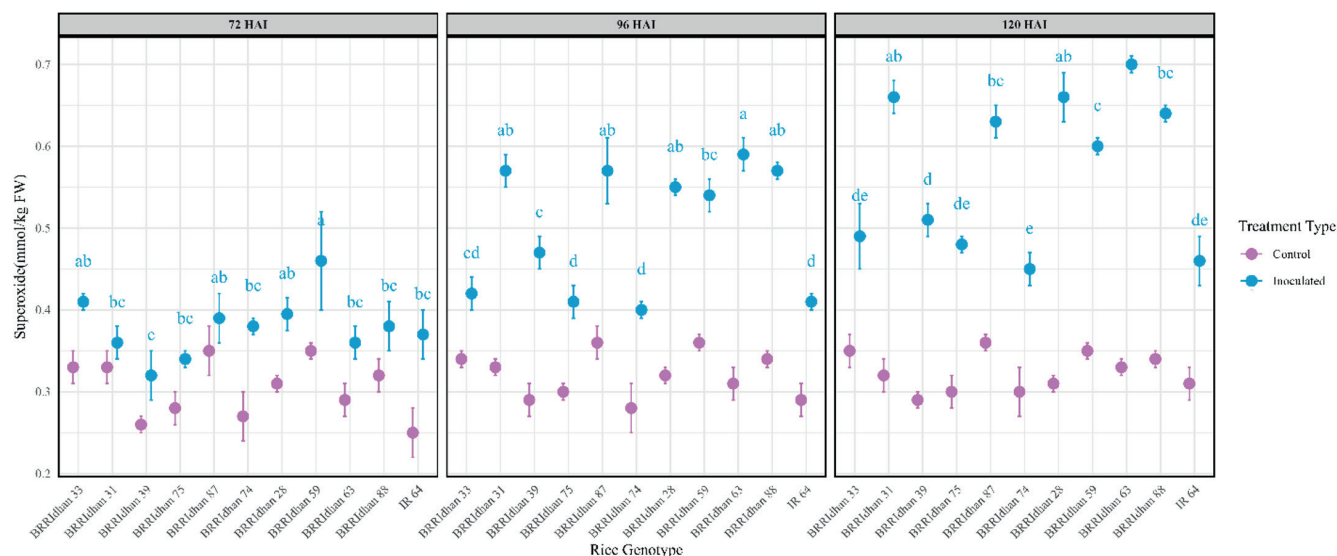


Fig. 3. Superoxide (O_2^-) concentration from control and inoculated leaf of eleven rice genotypes at 72, 96 and 120 hours after inoculation with *P. oryzae*

All values represent the mean $\pm$ standard deviation ($n = 3$) at $P < 0.05$.

Source: Authors' own elaboration

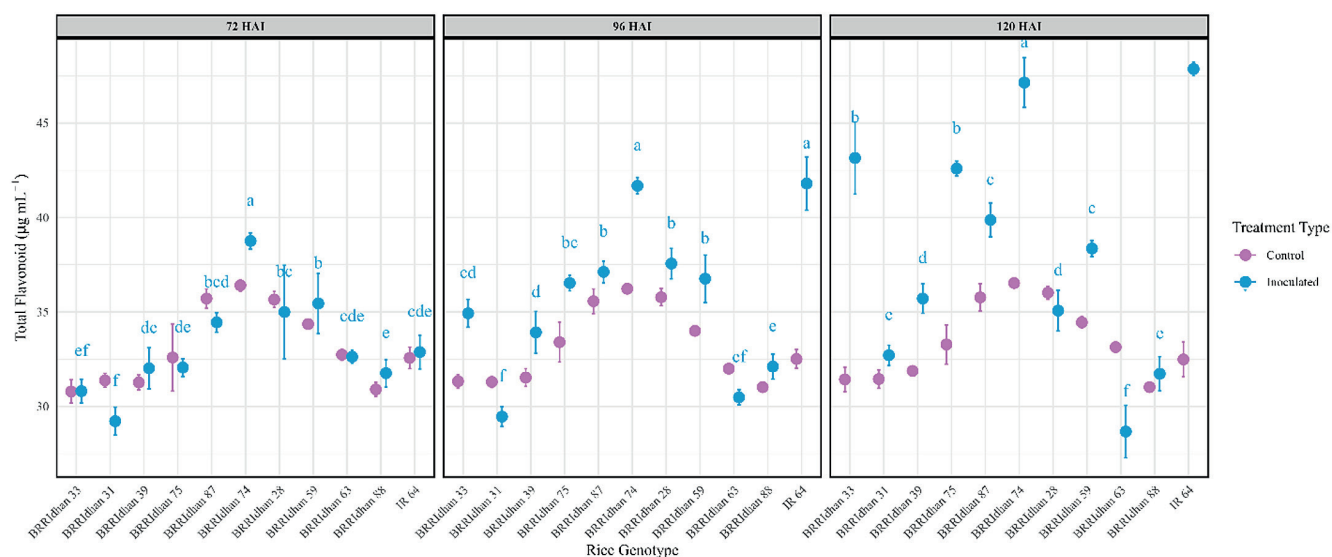


Fig. 4. Total flavonoid content from control and inoculated leaf of eleven rice genotypes at 72, 96 and 120 hours after inoculation with *P. oryzae*

All values represent the mean $\pm$ standard deviation ($n = 3$) at $P < 0.05$

Source: Authors' own elaboration

from 146.21 to 199.56, 148.46 to 199.68 and 148.39 to 200.59 $\mu\text{g mL}^{-1}$ at 72, 96 and 120 HAI, respectively, in control leaf sample whereas in inoculated sample, this activity ranged from 165.61 to 242.14, 190.99 to 324.67 and 284.96 to 490.97 $\mu\text{g mL}^{-1}$ at 72, 96 and 120 HAI, respectively. The increasing levels of antioxidant after spraying inoculum was higher in BRRI dhan74 (32.63%), followed by BRRI dhan33 (27.6%) and IR 64 (21.34%), and lower in BRRI dhan63 (2%) compared to control at 72 HAI. At 96 HAI, this relative antioxidant level was higher in BRRI dhan74 (65.93%), followed by IR 64 (62.6%), BRRI dhan33 (54.48%), BRRI dhan75 (44.13%), and lower in BRRI dhan63 (11.65%) and BRRI dhan31 (13.36%). The increasing levels of antioxidant after spraying inoculum was higher in IR 64 (144.76%), followed by BRRI dhan74 (139.77%), BRRI dhan33 (128.51%), BRRI dhan75 (124.27%), and lower in BRRI dhan63 (54.91%) in compared to control plant at 120 HAI.

Total protein

The recorded total protein differed significantly ($p < 0.05$) among the genotypes in inoculated sample of all genotypes (Figure 6). Total protein ranged from 37.4 to 53.65, 37.53 to 53.96 and 38.21 to 57.26 $\mu\text{g mL}^{-1}$ at 72, 96 and 120 HAI, respectively, in control sample whereas in inoculated sample, this concentration ranged from 39.25 to 74.34, 49.72 to 88.4 and 58.46 to 130.59 $\mu\text{g mL}^{-1}$ at 72, 96 and 120 HAI, respectively. The increasing levels of total protein in inocu-

lated sample was higher in IR 64 (44.8%), followed by BRRI dhan75 (32.7%) and BRRI dhan33 (30.13%), and lower in BRRI dhan28 (8.06%) compared to control plant at 72 HAI. This increasing levels in inoculated sample compared to control was higher in BRRI dhan74 (72.66%), followed by IR 64 (64.96%), BRRI dhan33 (57.64%) and BRRI dhan75 (51.16%), and lower in BRRI dhan63 (18.39%) and BRRI dhan31 (20.88%) at 96 HAI. At 120 HAI, the increasing levels of total protein after spraying inoculum was higher in BRRI dhan74 (128.92%), followed by IR 64 (128.06%) and BRRI dhan33 (122.09%), and lower in BRRI dhan63 (32.5%) in compared to control plant at 120 HAI.

Total sugar

In control sample, no significant difference was recorded among genotypes, but in inoculated sample, significant difference ($p < 0.05$) was recorded among the genotypes at 72, 96 and 120 HAI (Figure 7). Total sugar ranged from 3.00 to 3.25, 3.04 to 3.27 and 3.06 to 3.3 $\mu\text{g mL}^{-1}$ at 72, 96 and 120 HAI, respectively, in control sample whereas in inoculated sample, this concentration ranged from 3.37 to 4.05, 4.1 to 5.15 and 4.2 to 5.45 $\mu\text{g mL}^{-1}$ at 72, 96 and 120 HAI, respectively. The increasing levels of total sugar after spraying inoculum was higher in BRRI dhan75 (26%), followed by BRRI dhan74 (25.57%), BRRI dhan33 (24.8%), IR 64 (24.6%) BRRI dhan39 (20.45%), and lower in BRRI dhan28 (7.67%) BRRI dhan87 (9.78%) in com-

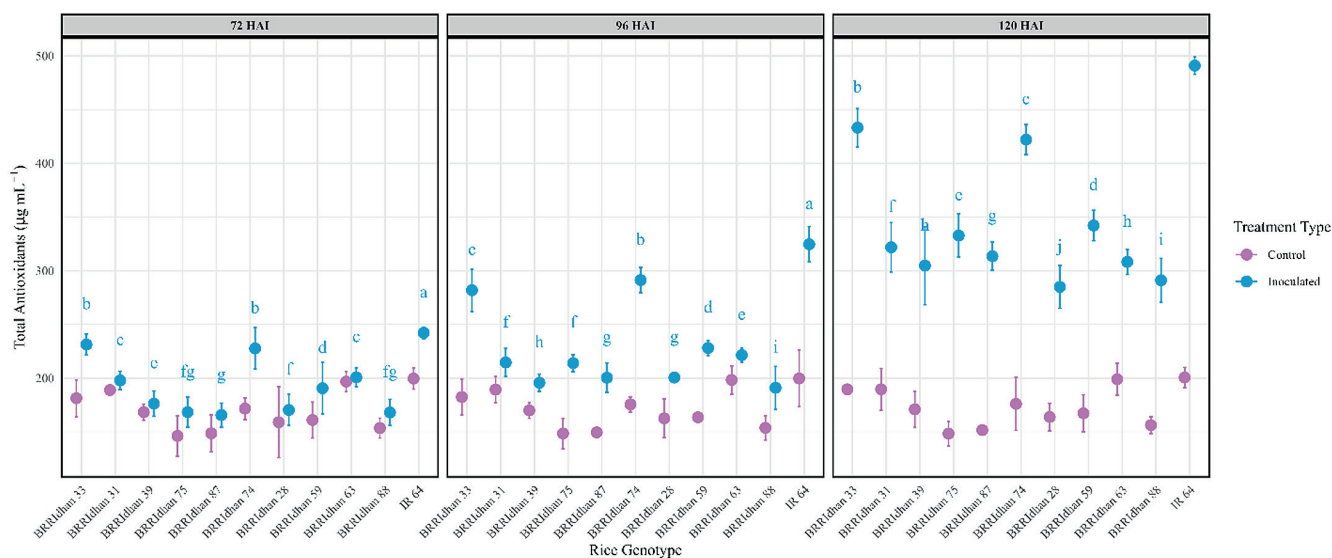


Fig. 5. Total antioxidants content from control and inoculated leaf of eleven rice genotypes at 72, 96 and 120 hours after inoculation with *P. oryzae*

All values represent the mean $\pm$ standard deviation ($n = 3$) at $P < 0.05$

Source: Authors' own elaboration

pared to control plant at 72 HAI. The increasing levels of total sugar after spraying inoculum was higher in BRRI dhan75 (58.55%), followed by IR 64 (57.49%), BRRI dhan74 (51.45%) and BRRI dhan33 (50.16%), and lower in BRRI dhan28 (30.19%), BRRI dhan63 (33.54%), BRRI

dhan31 (34.53%) and BRRI dhan87 (34.7%) compared to control at 96 HAI. At 120 HAI, the increasing levels of total sugar after spraying inoculum was higher in BRRI dhan74 (73.79%), followed by IR 64 (65.15%), BRRI dhan75 (63.73%), BRRI dhan59 (56.69%), BRRI dhan33

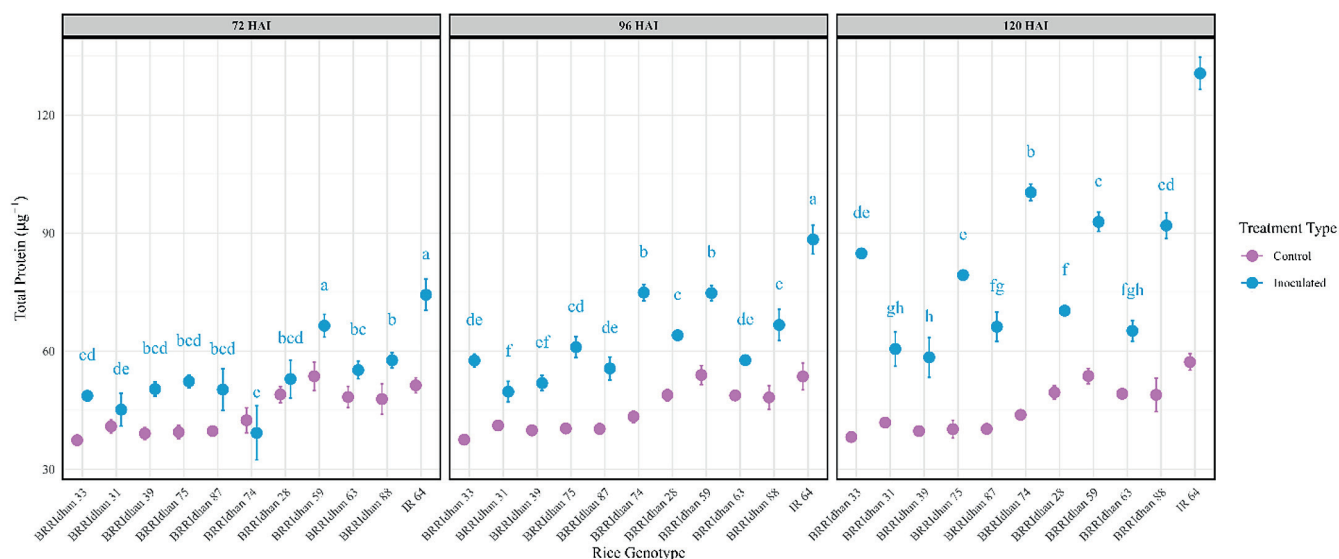


Fig. 6. Total protein content from control and inoculated leaf of eleven rice genotypes at 72, 96 and 120 hours after inoculation with *P. oryzae*

All values represent the mean $\pm$ standard deviation ($n = 3$) at $P < 0.05$

Source: Authors' own elaboration

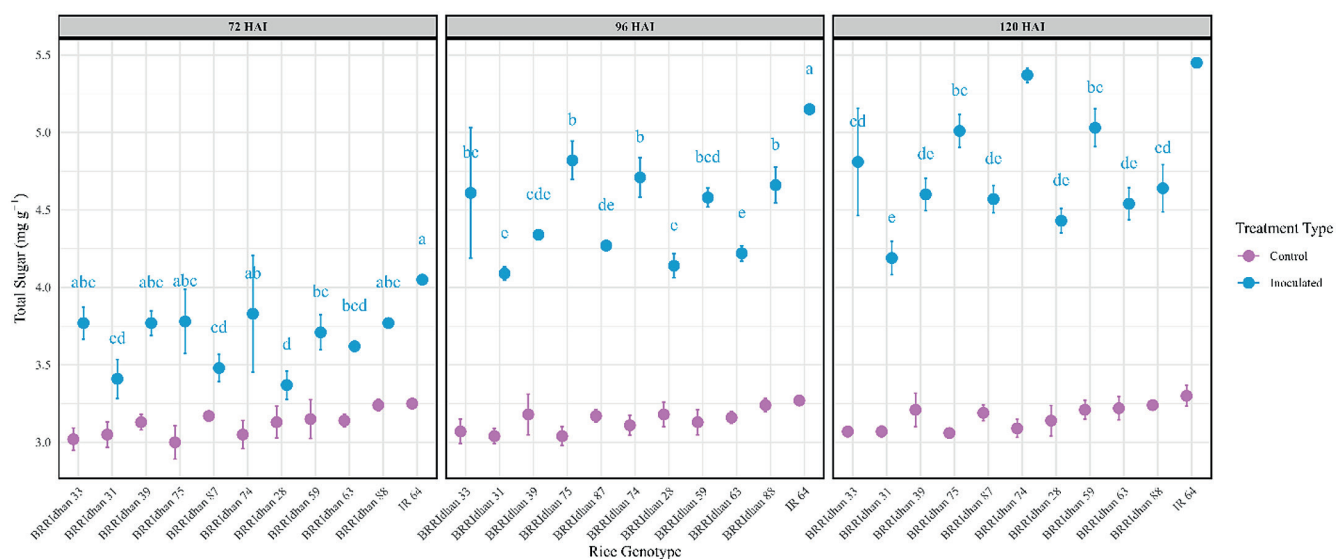


Fig. 7. Total sugar content from control and inoculated leaf of eleven rice genotypes at 72, 96 and 120 hours after inoculation with *P. oryzae*

All values represent the mean $\pm$ standard deviation ($n = 3$) at $P < 0.05$

Source: Authors' own elaboration

(56.68%), and lower in BRRi dhan31 (36.48%) followed by BRRi dhan63 (40.99%) and BRRi dhan28 (41.08%) compared to control.

Correlation between biochemical variables and DI (%)

The estimated correlation coefficient among the biochemical variables and rice blast disease incidence was depicted in Figure 8. Hydrogen peroxide (H_2O_2) and superoxide (O_2^-),

both indicators of oxidative stress, displayed moderate to strong positive correlations with Disease Incidence (H_2O_2 : $r = 0.50$; O_2^- : $r = 0.88$), while strong negative correlation between Disease Incidence (%) and the beneficial biochemical traits, particularly total flavonoid ($r = -0.93$), total sugar ($r = -0.83$), total antioxidants ($r = -0.82$) and total protein ($r = -0.71$) was recorded. Moreover, strong positive correlations were observed among biochemical variables like total

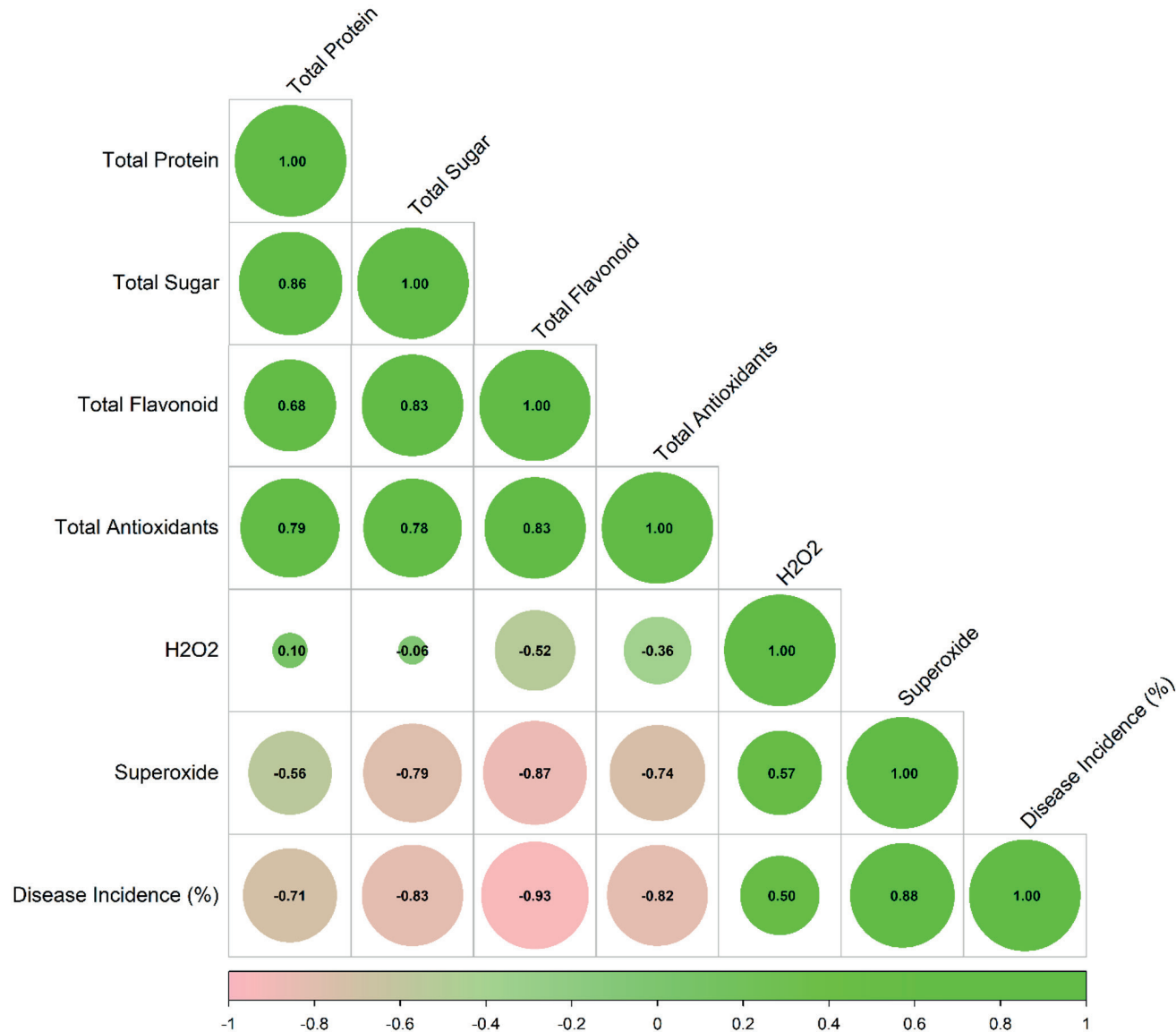


Fig. 8. Pearson correlation among different parameters associated with blast disease of rice genotypes
Color (green: positive correlation; pink: negative correlation) intensity and the size of the circle are proportional to the correlation coefficient.
Source: Authors' own elaboration

protein, total sugar, total flavonoid and total antioxidants, with coefficients ranging from $r = 0.68$ to 0.86 , whereas O_2 showed strong negative correlations with total flavonoid ($r = -0.87$), total sugar ($r = -0.79$) and total antioxidants ($r = -0.74$).

Principle component analysis (PCA)

The PCA biplot generated among disease incidence (%) and different biochemical variables of eleven rice genotypes was presented in Figure 9, where the eigenvalues of the first two principal components (PC1 and PC2) were greater than 1. The first two principal components (PCs) explained a cumulative 91.7% of the total variance, with PC1 accounting for 73.3% and PC2 explaining 18.4%, respectively. The PCA biplot revealed the distribution of variables based on their contribution to each principal component. Total Protein, Total Sugar, Total Flavonoid, and Antioxidants clustered together and pointed in a similar direction along PC1, suggesting a strong positive correlation among these variables. In contrast, H_2O_2 , O_2 , and Disease Incidence (%) were positioned in the opposite quadrant of the biplot, with vectors pointing toward the positive axis of PC2.

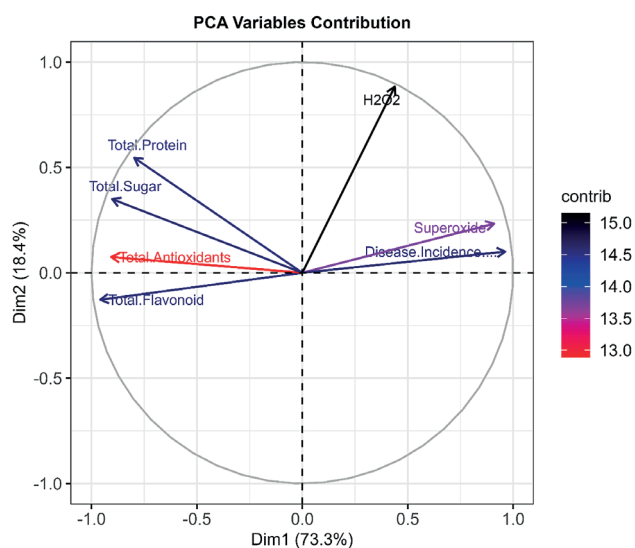


Fig. 9. Principal component analysis of blast disease incidence (%) and different biochemical variables of rice genotypes

The higher contribution is represented by blue and less contribution is represented by red color.

Source: Authors' own elaboration

Discussion

Blast disease caused by *P. oryzae* is the most destructive rice disease, causing 10–30% losses and up to 100% loss

under extreme circumstances (Sharma et al., 2012). Due to the drawbacks of chemical management, developing durable, resistant cultivars is one of the most sustainable, economic, and environmentally beneficial ways to combat rice blasts (Sahu et al., 2022). Due to a lack of genotype information and pathogen population dynamics, resistant cultivars typically become susceptible within a few years. Thus, biochemical profiling of genotypes is required to know the physiological basis of that genotype for developing disease resistant genotype. The objective of the current study was the biochemical characterization of some popular rice genotypes of Bangladesh against *P. oryzae* causing blast disease. It was achieved by determining the activity of two reactive oxygen species (ROS) and four defense related non-enzymatic compounds at seedling stage of rice genotypes upon *P. oryzae* inoculation.

Generation of ROS is crucial for initiating a hypersensitive response in host defense mechanisms. Under stress conditions, cells commonly produce reactive oxygen species (ROS) such as H_2O_2 , O_2^- , $^1O_2$, and OH, which can result in significant oxidative damage, including lipid peroxidation in plant tissues (Di Meo et al., 2016). Maintaining an optimal level of reactive oxygen species (ROS) in cells facilitates appropriate redox biological reactions and regulates various processes critical for plant growth and development (Mittler, 2016). In the present study, H_2O_2 and O_2^- were found to be increased in all the rice genotypes with increasing inoculation time though the increasing pattern differed among genotypes (Figures 2 and 3). Initial assessments indicated higher relative ROS production in BRRI dhan74, BRRI dhan33 and BRRI dhan75 along with check line IR 64. However, over time, these genotypes maintained a balanced ROS production, resulting in a decline in their relative performance. Early H_2O_2 buildup is closely linked to the host plant's lignification, which shields the plant against pathogen penetration, according to Poudel et al. (2019). According to Gupta et al. (2021), resistant rice plants had higher H_2O_2 concentrations at an early stage of infection (48 HAI), but decreased at the disease development stage (96 HAI), whereas susceptible wheat, barley, maize, and swamp rice grass had higher H_2O_2 concentrations that accumulated at the disease developmental stage (96 HAI). Peroxisomal injury during the overall tissue collapse is thought to be the cause of a significant buildup of H_2O_2 at a later stage (Shetty et al., 2007). Plants produce signals to change their metabolism in response to stress, which activates defense genes in the plant sections that are impacted (Gill et al., 2019).

According to Zandalinas et al. (2019), plants have a variety of antioxidant defense mechanisms, which allow them

to control the levels of reactive oxygen species (ROS), and respond well to various environmental stresses. In order to prevent the excessive generation of reactive oxygen species (ROS), antioxidant enzymes and non-enzymatic antioxidants such as phenolic substances, flavonoids, alkaloids, and non-protein amino acids collaborate (Nath et al., 2018; Laxa et al., 2019). Plants synthesize phenols and flavonoids following pathogen attack or UV radiation as part of their active defense response. They are ROS scavengers that can reduce cell organelle damage, according to Agati et al. (2012). In present study, BRRI dhan74, BRRI dhan33 along with check line IR 64 maintained increasing trend as well as higher level of flavonoid from 72 to 120 HAI. Though genotypes like BRRI dhan31 and BRRI dhan87 and BRRI dhan63 synthesized lower quantity of flavonoid compared to control upon infection with *P. oryzae* (Figure 4). The activity of fungus *Verticillium dahliae* was restricted due to increased levels of flavonoids in red cotton genotypes (Long et al., 2019). Metabolomics revealed the accumulation of flavonoid compounds in plants responding to *C. gloeosporioides*, indicating potential targets for resistance breeding through flavonoid biosynthesis (Jiang et al., 2021). Beside antioxidants, proteins are key plant cell components and have various biochemical roles, including disease defense. Many plant pathogenic interactions include protein components in disease resistance (Carvalho, 2006). Sugars, together with protein, are the main substrate for plant defense responses and signal molecules that interact with the hormone signaling network that regulates the plant immune system. High sugar levels in plant tissues boost plant resistance in most fungal pathogen–plant systems (Morkunas and Ratajczak, 2014).

According to this study, higher total antioxidant, total soluble protein and sugar during post infection in rice seedlings with *P. oryzae* compared to control was reported (Figures 5, 6 and 7). BRRI dhan74 (156%), BRRI dhan33 (129%) and BRRI dhan75 (123%) exhibited higher synthesis of total antioxidant compared to control at 120 HAI (Figure 5). Chhabra et al. (2022) also observed higher antioxidant activity and total soluble protein in rice genotypes against *R. solani*, and suggesting this increase may be attributed to the biotic stress created by the pathogen. Ashfaq et al. (2021) recorded that the higher concentration of total protein in rice inversely correlated with disease incidence. The relationship between total protein levels and plant defense mechanisms against fungi and bacteria has been documented in various studies, including rice (Kumawat et al., 2008), oat (Fink et al., 1988), maize (Nasser et al., 1990), and barley (Høj et al., 1989). Biswas et al. (2003) reported an increase in protein content in resistant wheat plants

against spot blotch disease compared to controls, indicating immunized wheat seedlings. The increase in protein content in tolerant cultivars is attributed to the accumulation of pathogenesis-related proteins (PRs), which are encoded by the host plant and are associated with systemic acquired resistance to subsequent infections by the disease. Giberti et al. (2011) reported that sugar significantly contributes to resistance against fungal pathogens by stimulating phenylpropanoid metabolism. Chhabra et al. (2022) revealed that there was decline in sugar content of seedlings after *Fusarium fujikuroi* infection in both the resistant and susceptible genotypes, but the decreasing rate was higher in susceptible genotype compared to resistant one.

From present investigation, BRRI dhan74, BRRI dhan33 and BRRI dhan75 maintained higher synthesis of total flavonoid, antioxidant, protein, sugar and balanced production of H_2O_2 and O_2^- with comparatively lower DI (%), with increasing inoculation time by *P. oryzae* stress. On contrary, genotypes like BRRI dhan31, BRRI dhan63 and BRRI dhan28 with higher DI (%), reduced the synthesis of non-enzymatic compound with increasing of inoculation time. These results were reflected in Pearson correlation and PCA (Figures 8 and 9), which indicates that most of the biochemical variables contributed significantly for making variability, and DI (%) was positively associated with H_2O_2 and O_2^- , while others biochemical variables were negatively correlated with it. Together, the PCA and correlation analysis highlights antagonistic relationship between biochemical resilience and disease vulnerability (e.g., high ROS and disease incidence), reinforcing the hypothesis that metabolic quality and stress markers are crucial indicators of plant health. These findings are very important for planning plant breeding programs, stress physiology studies, and developing integrated disease management strategies emphasizing on the enhancement of natural defense compounds.

Conclusion

Balanced activity of ROS (H_2O_2 , O_2^-) and increased activity of defense related non enzymatic compound correlated with lower levels of blast disease incidence in BRRI dhan74, BRRI dhan33 and BRRI dhan75, suggesting that these biochemical variables could be used as biochemical markers for developing disease-resistant rice genotypes.

Conflict of interest

The authors affirm that this work is their original research and is not under consideration for publication. In addition, no conflict of interest is declared by all the authors.

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