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Improving blast disease tolerance in rice plants by enhancing antioxidant defense with tebuconazole and trifloxystrobin fungicides

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Abstract. The fungus *Magnaporthe oryzae* (Mo) is the causal agent of rice blast disease, one of the most common and serious diseases affecting cultivated rice. Developing effective techniques to manage this disease is currently a crucial issue. Therefore, this study was designed to investigate the protective role of two fungicides, tebuconazole (TEB) and trifloxystrobin (TRI), in enhancing the tolerance of rice seedlings to blast disease. Ten-day-old hydroponically grown seedlings were inoculated with a Mo spore suspension, and after three days, fungicide was sprayed. In 15-day-old seedlings, numerous blast symptoms were observed, which hindered their growth. Compared with the control, Mo inoculation increased the malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content by 249.37 and 118.29%, respectively. In addition, Mo inoculation also increased electrolyte leakage (EL) in both shoots and roots and caused a reduction in leaf relative water and photosynthetic pigment contents. However, exogenous application of TEB and TRI reduced the content of MDA, H_2O_2, and EL. Fungicide application also increased Chl *a*, Chl *b*, Chl (*a*+*b*), and the carotenoid content by 28.16, 41.61, 33.67, and 26.29%, respectively. Consequently, increased photosynthetic pigment and relative water contents, along with increased rice seedling biomass and growth, were observed. These findings suggest that exogenous administration of TEB and TRI is a promising technique for improving blast tolerance in rice plants.

Keywords: blast, *Magnaporthe oryzae*, rice seedling, antioxidant defense, tebuconazole, trifloxystrobin

1 Introduction

The estimated global population is projected to increase from 7.7 billion in 2019 to 10.9 billion in 2100, with the predicted growth mainly concentrated in low-income, underdeveloped nations where the majority of people rely on agriculture for their livelihood [1]. Crop yields are expected to increase by up to 110% to feed the 9.7 billion individuals projected to inhabit the planet by 2050 [2]. Meeting this demand will be challenging because of several issues that can influence food production. Rice is an essential part of the diet for over half of the global population. Estimated 480 million metric tons of milled rice are produced worldwide each year [3]. Rice production is essential to the country's economy, contributing over 20% of GDP and producing almost one-sixth of Bangladesh's total national revenue [4]. According to FAO, the harvested rice crop area, yield, and production in Bangladesh were 331,471 ha, 29,354 kg·ha^{-1}, and 973,000 tons, respectively [5]. Numerous biotic and abiotic factors negatively affect rice production, leading to substantial losses for farmers. One of them is disease. In Bangladesh, blast is a primary rice disease and is predicted to cause a yield loss of up to 98% at the most significant disease severity. Because of infestation, various physiological metabolisms undergo changes in plants. Naturally, plants create a wide range of reactive oxygen species (ROS) that comprise non-radical molecules, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), and free radicals, for example, superoxide ($O_2^{\bullet -}$) and hydroxyl ($^{\bullet}OH$) radicals [6]. Conversely, excessive amounts of ROS are produced by stressed plant cells, which disrupt plant metabolisms and seriously harm vital cellular constituents, including proteins, lipids, carbohydrates, and DNA.

Triazole fungicides are the cornerstone of global disease control programs, applied as foliar sprays or seed treatments to crops such as cereals, vegetables, decorative plants, and wineries [7]. Their broad range of effectiveness against primary pathogens of these crops accounts for their widespread use, which is expected to increase. Fungal growth is inhibited by triazole fungicides because of lanosterol 14-α-demethylase, which is necessary for the production of ergosterol, a crucial element found in the membranes of fungi [8]. The impacts of triazole fungicides have not received as much attention as those of the insecticides, as indicated from data in the literature. Strobilurins are another group of fungicides that bind to the quinol oxidation (Qo) site, or also known as the ubiquinol site, of cytochrome *b* in the mitochondria. They prevent electron transfer between cytochrome *b* and cytochrome *c*, which, in turn, stops the production of ATP and reduced nicotinamide adenine dinucleotide oxidation [9]. The fungus finally dies as a result of the energy generation ceasing.

The exogenous use of chemical fungicides can effectively inhibit microbial growth. A foliar spray of strobilurin and triazole fungicides has been advised as a means of preventing the plant disease [10]. Strobilurin and triazole fungicides modify molecular, physiological, and biochemical processes related to both biotic and abiotic stresses, thereby improving growth and stress tolerance. These regulators typically affect the levels of important plant hormones and substances, such as gibberellins, cytokinins, auxins, abscisic acid, and ethylene [11]. It is necessary to develop a management strategy that not only suppresses the pathogens but also encourages plant growth. Therefore, the primary objective of the present study is to investigate the mechanisms by which tebuconazole (TEB, triazole group) and trifloxystrobin (TRI, strobilurin group) fungicides enhance blast disease tolerance in rice plants by improving physiological responses and antioxidant defense systems.

2 Materials and methods

2.1 Pathogen and plant material collection

Rice seeds (*Oryza sativa* L. cv. BRRI dhan 28) were provided by the Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur, Bangladesh. The fungus *Magnaporthe oryzae* (Mo) was obtained from the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. After collection, the fungus was preserved in the potato dextrose agar (PDA) media (Fig. 1).

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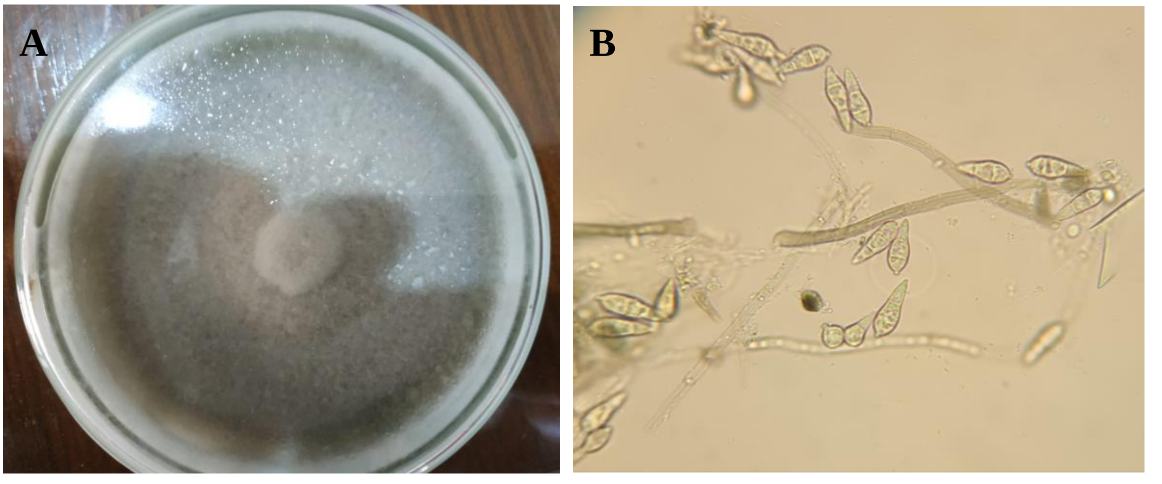

Fig. 1. Pure culture of *Magnaporthe oryzae* in PDA media (A) and conidia under compound microscope (B)

2.2 Growing conditions for rice plants and application of treatments

The rice plants were grown hydroponically. The seeds were disinfected with 70% ethanol and steeped in distilled water (dH_2O) for 24 hours. After soaking, the seeds were sown in plastic pots (8 cm in diameter and 250 mL in volume) and placed in a net house. During the growing period, the seedlings were given half-strength Hoagland solution as a nutrient. This solution was replaced every 5 days. On the 10th day, the seedlings were sprayed with a Mo spore suspension (10 mL per pot) adjusted to 2×10^4 conidia·mL^{-1}, and the pots were covered with plastic to maintain humidity. After three days of inoculation, the seedlings were foliar-sprayed with TEB (0.3 g·L^{-1}) and TRI (0.15 g·L^{-1}) (Nativo 75 WG, Bayer, which contained 50% TEB and 25% TRI). After 15 days, disease symptoms appeared, and the physiological and biochemical attributes were observed. The experiments were conducted with a completely randomised design, with three replications. The treatment combinations are as follows: C (control or plants were treated with sterile water); F (plants were treated with TEB (0.3 g·L^{-1}) and TRI (0.15 g·L^{-1}); P (plants were inoculated with *M. oryzae*); and F+P (plants were inoculated with *M. oryzae* and treated with TEB (0.3 g·L^{-1}) and TRI (0.15 g·L^{-1}).

2.3 Percentage of plant infection

The number of infected plants was counted manually, and the average number of infected plants per pot was estimated. The percentage of plant infection was calculated as follows:

$$\text{PPI} = \frac{\text{Number of infected plants in pot}}{\text{Total number of plants in pot}} \times 100\ (\%)$$

2.4 Number of lesions per plant and their size

The number of lesions per plant was determined and averaged. The lesion size was measured with a measuring scale (mm scale) and averaged.

2.5 Growth parameters

The shoot and root length and the plant height (cm) of rice seedlings were measured with a scale, and the average values were calculated. After harvesting, the fresh weight (FW) of the shoots, roots, and entire plants was determined with a precision scale. After 48 hours of drying at 70 °C, the dry weight (DW) was also determined.

2.6 Leaf relative water content

The relative water content (RWC) of the leaves was calculated according to the method described by Barrs and Weatherley [12]. Leaf FW, DW, and turgid weight (TW) were determined. The following formula was used to calculate RWC: RWC (%) = [(FW − W)/(TW − DW)] × 100.

2.7 Malondialdehyde and H_2O_2 content

The malondialdehyde (MDA) content was determined to observe the lipid peroxidation level according to the method described by Heath and Packers [13]. Trichloroacetic acid (TCA, 5%) was used to extract the fresh leaves, and the samples were then centrifuged at 11,500×g. After adding 4 mL of thiobarbituric acid (TBA) to 1 mL of the supernatant, the final mixture (5 mL) was incubated for 30 minutes in a hot water bath. The process was then abruptly stopped by rapidly cooling the mixture, and the optical absorbance was measured at 532 nm and adjusted to 600 nm. The amount of malondialdehyde was calculated, as follows:

$$\mathrm{MDA} = \frac{\text{Absorbance} \times \text{reaction mixture} \times \text{extration mixture}}{\text{Plant sample weight} \times \text{extinction coefficient}} \times 1000\ (\mathrm{n\,mol/gFW})$$

The H_2O_2 content was estimated according to the method slightly modified by Mohsin et al. [14]. After homogenising the leaves with 5% TCA and a reaction reagent, such as potassium phosphate (K-P, pH 7.0) buffer, and potassium iodide (1M) were added. H_2O_2 was used to build a standard curve, which was then used to observe the optical absorbance at 390 nm.

2.8 Histochemical detection of membrane damage, hydrogen peroxide, and superoxide generation

To determine the generation of $O_2^{\bullet-}$, H_2O_2, and membrane damage, we used 0.01% acidic nitroblue tetrazolium chloride (NBT), 3,3'-diaminobenzidine (DAB), and Evans blue [14]. The leaves were incubated at 25 °C for 24 h after being submerged in separate solutions. Consequently, to remove the Evans blue, DAB, and NBT solution, we first cleaned the leaves in 70% ethanol and then in dH_2O. The leaves were then heated at 70 °C until distinct dark blue, brown, and light-blue spots appeared. These spots indicate the presence of membrane damage, H_2O_2, and $O_2^{\bullet-}$ production.

2.9 Shoot and root electrolyte leakage

Electrolytic leakage (EL) was detected with the method described by Dionisio-Sese and Tobitas [15]. Small fragments of leaf tissue were placed in test tubes with dH_2O and heated for 1 h at 40 °C. After the test tubes were cooled to ambient temperature, a CON 700 conductivity meter (Eutech Instruments, Singapore) was used to evaluate the primary electrical conductivity (EC1). Following another autoclave heating and cooling period, the test tubes' final electrical conductivity (EC2) was measured. The formula for electrical leakage is EL = EC1/EC2 × 100 (%).

2.10 Photosynthetic pigment content

The photosynthetic pigment (carotenoid and chlorophyll, Chl) was determined according to Lichtenthaler [16]. 0.1 g of leaves was cut into small pieces and placed in tiny test tubes filled with 100% ethanol. After being heated to 60 °C for 30 minutes in a hot water bath, the samples were chilled. Spectrophotometric measurements of absorbance were conducted at 470, 648, and 664 nm.

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2.11 Statistical analysis

All data were analysed with the R software (Version 4.4.1) through one-way analysis of variance. The means were compared with Fisher's Least Significant Difference (LSD) test, and the difference was considered significant when $p \leq 0.05$. Besides, Pearson's correlation analysis and graphical presentations were performed to quantify the relationships between the analysed variables.

3 Results and discussion

3.1 Phenotypic appearance and disease symptoms in rice seedlings

Inoculation with the Mo spore suspension hampered rice seedling growth. As seen from the characteristic blast symptoms on the leaves of the seedlings treated with Mo, the majority of the leaves began to wither after 20 days of inoculation. TEB and TRI, as exogenous fungicides, reduced blast infection and enhanced the phenotypic appearance of rice seedlings (Fig. 2). After inoculation with Mo, a large number of plants became infected and exhibited blast symptoms on their leaves. The number of infected plants per pot decreased by 59.04% compared with the Mo-treated plants (Table 1). Numerous plants were infected and had blast symptoms on their leaves after the Mo inoculation. The number of infected plants per pot decreased by 46.75% compared with the corresponding Mo-treated plants.

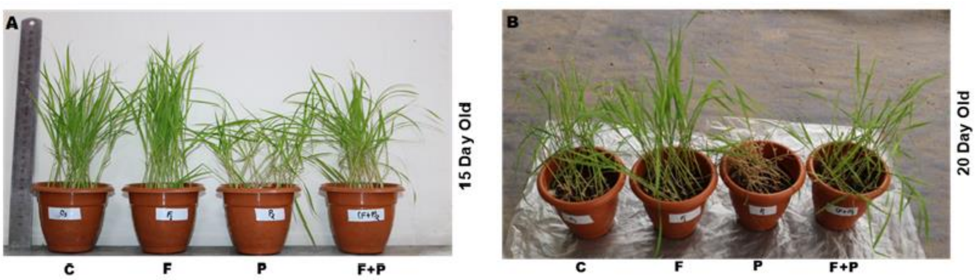

Fig. 2. In *vitro* evaluation of TEB and TRI on visual difference in 15-day-old (A) and 20-day-old (B) hydroponically grown rice seedlings under blast disease conditions, for five days; C (control or plants were treated with sterile water); F (plants were treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}) fungicide; P (plants were inoculated with *M. oryzae*); F+P (plants were inoculated with *M. oryzae*, and treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}) fungicide

Table 1. Effects of TEB and TRI on 15-day-old hydroponically grown rice seedlings under blast disease conditions

Treatments	Number of infected plants per pot	Percent plant infection (%)	Number of lesions per plant	Size of the lesion (mm)	Shoot length (cm)	Root length (cm)	Total plant height (cm)
C	–	–	–	–	21.25 ± 0.65 a	16.45 ± 0.32 b	37.69 ± 0.78 b
F	–	–	–	–	22.95 ± 0.93 a	17.74 ± 0.69 a	40.68 ± 1.32 a
P	27.66 ± 1.15 a	92.22 ± 3.84 a	2.56 ± 0.15 a	1.76 ±0.13 a	15.90 ± 1.66 c	15.39 ± 0.34 c	31.29 ± 1.65 c
F+P	11.33 ± 1.52 b	37.77 ± 5.09 b	1.36 ± 0.16 b	0.90 ± 0.15 b	18.75 ± 0.65 b	16.77 ± 0.35 b	35.51 ± 0.77 b

C (control or plants were treated with sterile water); F (plants were treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}); P (plants were inoculated with *M. oryzae*); F+P (plants were inoculated with *M. oryzae*, and treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}). Values with different letters are significantly different at $p \leq 0.05$, according to Fisher's LSD test.

Additionally, the percentage of contaminated plants per pot decreased by 48.77% (Table 1). Many plants were infected and had blast symptoms on their leaves after the Mo inoculation. The number of diseased plants per pot decreased with the application of fungicide. After fungicide application, the percentage of contaminated plants per pot decreased by 48.77% (Table 1).

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In our study, blast disease infection substantially reduced the growth of rice seedlings. However, TEB and TRI enhanced its phenotypic look by lowering the infection of this disease. When applied with Mo, rice seedlings exhibited a higher number of lesions. In contrast, fungicide application resulted in fewer diseased plants, significantly reducing the number of lesions per plant and the size of the lesions. Mohiddin et al. [17] found that azoxystrobin + difenoconazole and azoxystrobin + tebuconazole were the most efficient fungicide combinations, with a leaf blast incidence of 9.19 and 10.40%, respectively.

3.2 Oxidative stress markers and histochemical detection

The MDA content in the blast disease-exposed seedlings increased by 249.37% compared with the control. In comparison with the blast-infected plant alone, the fungicide application resulted in a 29.80% reduction in MDA concentration (Fig. 3A). Seedlings exposed to blast disease produced a higher H_2O_2 content, with an increase of 118.29% compared with the control. The application of fungicide reduced the H_2O_2 level by 29.82% compared with blast-infected plants alone (Fig. 3B). Blast disease infection enhanced electrolyte leakage in both shoots and roots, with a 163.98 and 97.71% increase, respectively, compared with the control. Fungicide application reduced shoots and root electrolyte leakage by 39.53 and 29.36%, respectively (Fig. 3C and 3D). Histochemical analysis revealed that the blast-infected rice seedling leaves exhibited membrane damage (blue spots), the production of H_2O_2 (brown spots), and the presence of $O_2^{\bullet-}$ (light blue spots). However, fungicide application reduced the membrane damage and the production of H_2O_2 and $O_2^{\bullet-}$ in rice seedlings (Fig. 4).

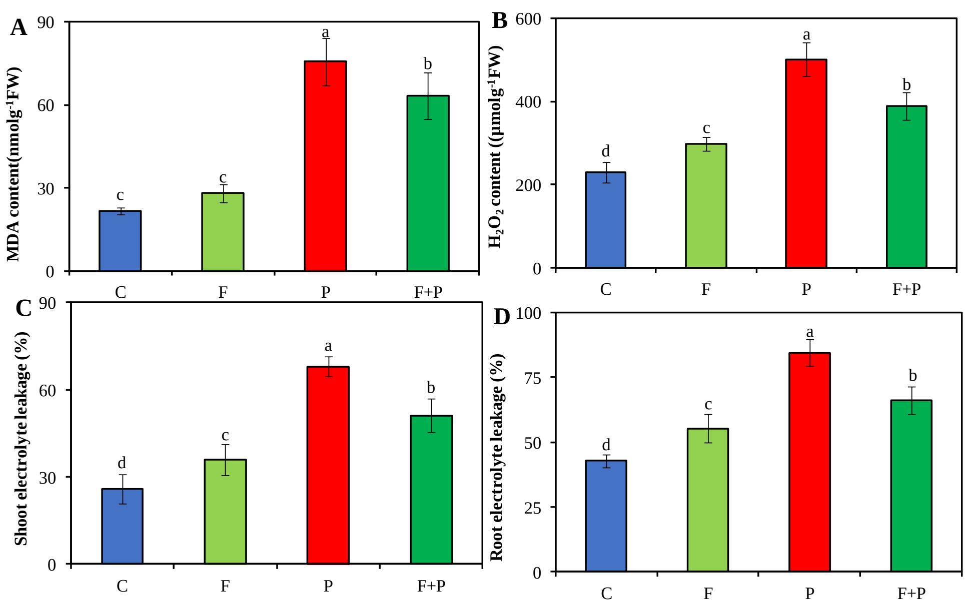

Fig. 3. In *vitro* evaluation of TEB and TRI on MDA (A), H_2O_2 (B), shoot EL (C), and root EL (D) of 15-day-old hydroponically grown rice seedlings under blast disease conditions, for five days; (control or plants were treated with sterile water); F (plants were treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}) fungicide; P (plants were inoculated with *M. oryzae*); F+P (plants were inoculated with *M. oryzae*, and treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}) fungicide

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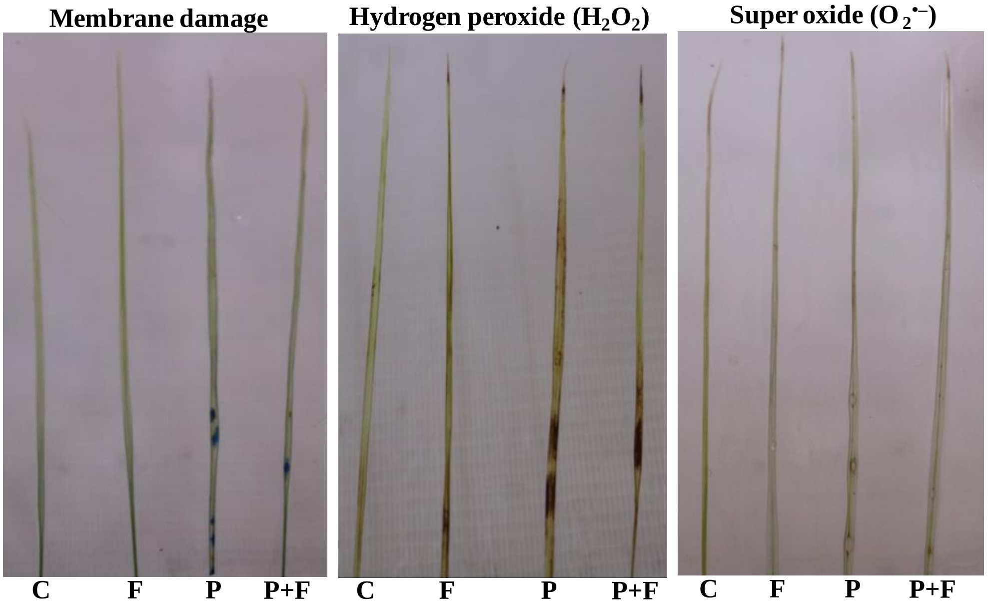

Fig. 4. In *vitro* evaluation of TEB and TRI on histochemical detection of membrane damage, H_2O_2, and $O_2^{•-}$ of 15-day-old hydroponically grown rice seedlings under blast disease conditions, for five days; C (control or plants were treated with sterile water); F (plants were treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}) fungicide; P (plants were inoculated with *M. oryzae*); F+P (plants were inoculated with *M. oryzae*, and treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}) fungicide

One way to measure the level of oxidative stress and damage to the plasma membrane is to determine the amount of MDA, a chemical generated by membrane lipids in reaction with ROS [18]. In this study, higher MDA contents result from blast disease infection, which causes stress by increasing the levels of ROS in the plants. Research on how plants respond to biotic and abiotic stresses has revealed that the plant MDA level increases with damage. In other words, plants generate ROS in response to abiotic or biotic stresses, impeding the production of macromolecules such as proteins, lipids, and nucleic acids. This initiates the increase in plasma membrane permeability and MDA content, which results in cell vasation [19]. However, comparatively lower MDA content was found after the application of TEB and TRI in the Mo-inoculated rice plants. Yuzbasioglu [20] reported that foliar application of fungicide enhanced the plant's physiological mechanism, which lowers H_2O_2 and MDA concentrations.

Several metabolic processes that take place at different locations inside a plant cell frequently result in the formation of ROS in plants. According to Anjum et al. [21], excessive amounts of ROS can cause significant harm to proteins, DNA, and lipids, thereby impairing regular cellular processes and potentially leading to irreversible metabolic malfunction and plant mortality. Some of these generate H_2O_2 directly, whereas others only do so by using stronger reactivity intermediaries (such as $O_2^{•-}$ or $^1O_2$). The content of H_2O_2 increases rapidly because of the oxidative burst associated with a portion of the hypersensitive response to pathogens [22]. Following inoculation, the plant $O_2^{•-}$ and H_2O_2 concentrations increased; as a result, plants are under additional stress. The application of TEB and TRI significantly reduced the plant state of stress. Wu and Von [23] also observed that $O_2^{•-}$ in leaves was significantly decreased as a result of the fungicides strengthening the plant's antioxidative system. Electrolytes, including K^+

ions, leak out of a dying cell when the cell membrane becomes compromised. Therefore, the number of electrolytes that leak out of a tissue is what causes the tissue's cell death. Measuring the increase in electrolytic conductivity of the water containing the tissue with dying cells is a simple way to assess the quantity of electrolytes that leak from a tissue. In plant tissues, this electrolyte leakage test has been used to quantify the percentage of cells that die as a result of biotic and abiotic stressors, including pathogen infection, insect herbivory, injuries, UV radiation, oxidative stress, drought, high salinity, cold, and heat stress [24]. In this study, a substantial rate of electrolyte leakage in both the shoot and the root was observed following pathogen inoculation, indicating pathogenic infection. However, the application of TRI and TEB enhanced the root and shoot growth, similar to the effect observed with fungicide treatment, while also reducing electrolyte stress.

3.3 Leaf relative water photosynthetic pigment content

The relative water content of rice cultivars decreased when Mo was applied (Table 2). Compared with the control, the plants with blast disease infection reduced the RWC by 32.12%. However, the plants treated with fungicide improved RWC by 4.07% compared with the diseased plant. The photosynthetic pigment content decreased in Mo-inoculated rice seedlings (Fig. 5A–5D). Compared with the control, seedlings with blast disease infection reduced the content of Chl *a*, Chl *b*, Chl (*a*+*b*), and carotenoids by 37.76, 43.97, 40.46, and 31.02%, respectively. In contrast, the plants treated with fungicide showed an increase in the Chl *a*, Chl *b*, Chl (*a*+*b*), and carotenoid content by 28.16, 41.61, 33.67, and 26.29%, respectively, in comparison with the diseased plants.

Table 2. In *vitro* evaluation of TEB (0.3 g L^{-1}) and TRI (0.15 g L^{-1}) on growth parameters in 15-day-old hydroponically grown rice seedlings under blast disease conditions

Treatments	Shoot FW (g)	Root FW (g)	Total plant FW (g)	Shoot DW (g)	Root DW (g)	Total plant DW (g)	Leaf RWC (%)
C	0.47 ± 0.0 b	0.06 ± 0.004 b	0.53 ± 0.021 b	0.074 ± 0.004 a	0.014 ± 0.001 a	0.088 ± 0.005 a	94.69 ± 1.85 a
F	0.57 ± 0.03 a	0.08 ± 0.006 a	0.65 ± 0.023 a	0.075 ± 0.003 a	0.014 ± 0.002 a	0.089 ± 0.002 a	90.84 ± 2.70 a
P	0.34 ± 0.03 c	0.04 ± 0.004 c	0.38 ± 0.032 c	0.056 ± 0.003 a	0.010 ± 0.002 b	0.066 ± 0.002 b	64.28 ± 4.14 c
F+P	0.44±0.04 b	0.05 ± 0.008 b	0.50 ± 0.036 b	0.071 ± 0.007 b	0.011 ± 0.001 b	0.082 ± 0.008 a	77.76 ± 5.54 b

C (control or plants were treated with sterile water); F (plants were treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}); P (plants were inoculated with *M. oryzae*); F+P (plants were inoculated with *M. oryzae*, and treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}). Values with different letters are significantly different at $p \leq 0.05$, according to Fisher's LSD test.

Regarding the physiological effects of cellular water deficiency, RWC is the best indicator of plant water status. Regarding cellular hydration, RWC is a suitable measure of plant water status when considering the potential influence of both osmotic adjustment and leaf water potential [12]. The current study found that, in comparison with severely stressed plants, plants under zero stress conditions maintained higher RWC. Following the management of TRI and TEB, there was demonstrated improvement in development and better maintenance of greater RWC, ensuring better hydration and more favorable internal water relations of tissue, with a potentially larger pressure potential. The process of photosynthesis, which is widely understood, is how plants convert carbon dioxide into organic molecules and utilise that chemical energy to produce light energy for growth and development [25]. The level of pigments used in photosynthetic reactions varied among treatments in the current study. The inoculation of Mo leads to a decrease

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in the rate of photosynthetic pigment production, which hinders growth. Stressful environments damage the chloroplast ultrastructure, resulting in reduced photosynthetic activity because of a decline in the chlorophyll content [26]. Compared with the plant control, rice seedlings exhibited a greater reduction in blast disease infection and Chl *a* content. Compared with the diseased plant, the use of TEB and TRI enhanced the plant's chlorophyll content.

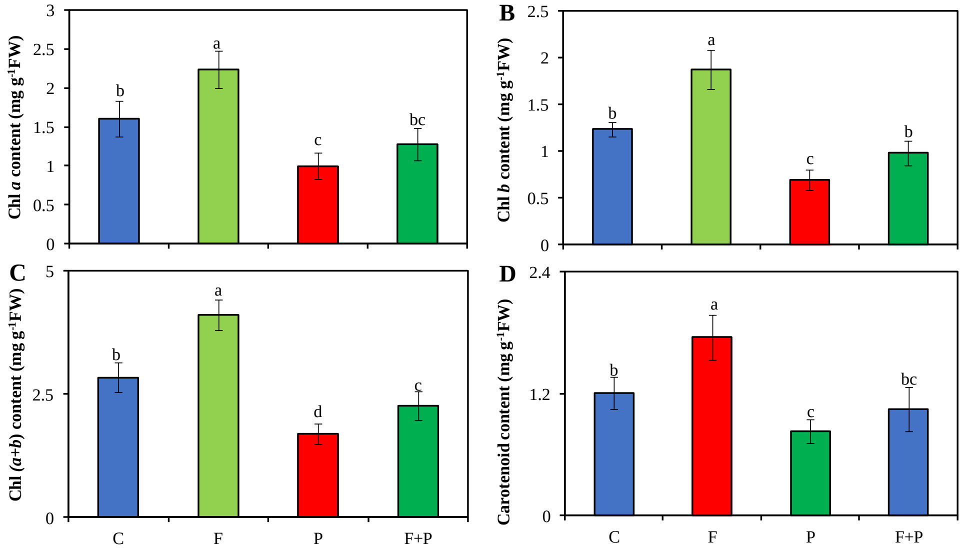

Fig. 5. In *vitro* evaluation of TEB and TRI on Chl *a* (A), Chl *b* (B), Chl *(a+b)* (C), carotenoid (D) of 15-day-old hydroponically grown rice seedlings under blast disease conditions, for five days; C (control or plants were treated with sterile water); F (plants were treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}) fungicide; P (plants were inoculated with *M. oryzae*); F+P (plants were inoculated with *M. oryzae*, and treated with TEB (0.3 gL^{-1}) and TRI (0.15 gL^{-1}) fungicide

3.4 Plant growth and biomass

Blast disease infection reduced the height of the entire plant as well as the length of the roots and shoots (Table 1). Total plant height, shoot length, and root length of the blast disease-infected plant decreased by 17, 25.16, and 6.45%, respectively. In contrast to blast-infected plants alone, fungicide application increased shoot and root length, total plant height, and the corresponding percentages of 17.90, 8.97, and 13.51%, respectively. However, compared with the control plant alone, the application of fungicide was the only factor that increased shoot and root length as well as overall plant height by 8, 7.84, and 7.93%, respectively. In comparison with the control, the blast disease-infested plants reduced shoot FW, root FW, and total plant FW by 27.62, 32.79, and 28.20%, respectively. In contrast to blast-infected plants alone, the application of fungicide increased shoot FW, root FW, and total plant FW by 19.67, 35.52, and 21.46%, respectively (Table 2). Blast disease infection reduced the shoot DW, root DW, and total plant DW by 24.66, 26.19, and 24.91%, respectively, compared with the control. In comparison with the blast-infected plant alone, the fungicide application increased shoot DW, root DW, and total plant DW by 90, 2.38, and 1.13%, respectively (Table 2).

Plants are unable to maintain their regular growth because of their interaction with harmful blast pathogens. Consequently, both the root and the shoot become shorter than usual. In comparison with the control plant, the length of the shoots, roots, and entire plant decreased. The addition of TEB and TRI resulted in improved shoots, roots, and overall plant length compared with blast-infected plants. The FW and DW of the rice seedlings' shoot, root, and entire plant varied significantly. Because of the Mo infection, the shoot FW and DW were substantially decreased. The treatment of TEB and TRI successfully raised the shoot and root FW, as well as the overall plant weight. The impact of stress decreased the DW of the roots, shoots, and entire plants. TEB and TRI were effective in increasing the DW. As a result, fungicide interactions with stressed plants had a considerable impact on overall rice plant growth. The evaluation of different fungicide combinations was carried out, and it was found that fungicides TRI 25% and TEB 50% performed better with the least disease incidence compared with other chemicals [27].

3.5 Relationship between various parameters

The relationships between various parameters of rice grown under blast disease conditions, both with and without the application of fungicide are illustrated on Fig. 6. As can be seen that oxidative stress markers, such as MDA, shoot EL, and root EL, showed a positive correlation with the percentage per infection, number of infected plants, number of lesions, and lesion size. On the other hand, MDA and shoot and root EL were negatively correlated with photosynthetic pigments (Chl *a*, Chl *b*, Chl (*a+b*), and carotenoid), growth parameters (shoot length, root length, shoot FW, shoot DW, root FW, and root DW) and RWC that point out blast disease disrupts the antioxidant defense systems of rice seedlings, affecting their growth and physiological processes.

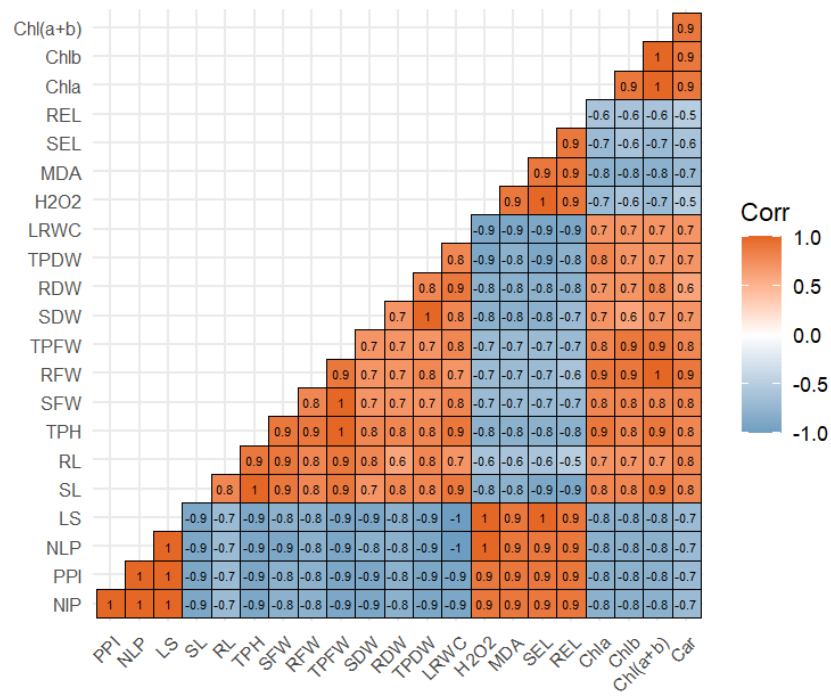

Fig. 6. Pearsons correlation between various attributes of hydroponically grown fifteen-day-old rice seedlings under blast disease conditions, for five days. PPI (percent per infection); NIP(number of infected plants); NLP (number of lesion plant^{-1}); LS (lesion size); SL (shoot length); RL (root length); TPH (total plant height); SFW (shoot fresh weight); RFW (root fresh weight); TPFW (total plant fresh weight); SDW (shoot dry weight); RDW (root dry weight); TPDW (total plant dry weight); LRWC (leaf relative water content); H_2O_2 (hydrogen peroxide); MDA (malondialdehyde); SEL (shoot electrolyte leakage); REL (root electrolyte leakage); Chl (chlorophyll); Car (carotenoid)

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4 Conclusion

This study revealed that the physiological and biochemical processes of rice seedlings were disrupted by blast disease. According to the characteristic blast symptoms on the leaves of the seedlings in response to Mo, the majority of the leaves began to wither. Consequently, more diseased plants and leaf lesions were found. Mo inoculation resulted in enhanced oxidative damage, as evidenced by increased MDA, shoot and root EL, and the formation of H_2O_2 and $O_2^{\bullet-}$. By lowering the RWC and photosynthetic pigment concentrations in leaves, injuries from blast-induced oxidative stress prevented rice seedlings from growing normally, which consequently reduced plant biomass and development. However, TEB and TRI fungicide applications increased blast disease tolerance by mitigating the disease's harmful effects. The results of this study show that the fungicides TEB and TRI protect rice plants by controlling plant growth and enhancing their resistance to blasts. These results may also help in the development of the rice types resistant to blast. Additionally, information was provided regarding the fungicides TEB and TRI as phytoprotectants for managing biotic stressors. Finally, additional field research on various rice cultivars is required for establishing the mediated plant resistance mechanisms in the blast disease environment.

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Author contributions

MF designed, conceived, and executed the experiments and drafted the manuscript; FMA, MRI, DQT, and MSMC edited and reviewed the manuscript; AH and JJJ actively participated in the experiments; MF edited and reviewed the manuscript; SMM supervised the experiments, analysed the data, and edited and reviewed the manuscript.

References

1. De Wrachien D, Schultz B, Goli MB. Impacts of population growth and climate change on food production and irrigation and drainage needs: A worldwide view. Irrig Drain. 2021;70:981-995.
2. Ray DK, Mueller ND, West PC, Foley JA. Yield trends are insufficient to double global crop production by 2050. PloS One. 2013;8(6):1-8.
3. USDA. World Agricultural Production. 2018; Available: https://www. fas.usda.gov/data/world-agricultural-production.
4. 4. Timothy TS, Mainuddin K, Chiang C, Rahman A, Haque A, Islam N, Quasem S, Sun Y. Agriculture and adaptation in Bangladesh: Current and projected impacts of climate change. Intl Food Policy Res Inst. 2013;01281:1-63
5. FAO. Food and Agriculture Organization of the United Nations. 2020;
Available:
https://www.fao.org/faostat/en/#data/QCL.
6. Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M, Fotopoulos V. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants. 2020;9(8):1-52
7. Poole NF, Arnaudin ME. The role of fungicides for effective disease management in cereal crops. Can J Plant Pathol. 2014;36:1-11.
8. Cycon M, Piotrowska-Seget Z, Kaczynska A, Kozdrój J. Microbiological characteristics of a sandy loam soil exposed to tebuconazole and l-cyhalothrin under laboratory conditions. Ecotoxicology. 2006;15:639-646.
9. Brandt U, Haase U, Schaegger H, Jagow G. Species specificity and mechanism of action of strobilurins. Dechema Monogr. 1993;129:27-38.
10. Kohli MM, Mehta YR, Guzman E, De Viedma L, Cubilla LE. Pyricularia blast – a threat to wheat

cultivation. Czech J Genet Plant Breed. 2011; 47:130-134.

11. Mohsin SM, Hasanuzzaman M, Bhuyan MHMB, Parvin K, Fujita M. Exogenous tebuconazole and trifloxystrobin regulate reactive oxygen species metabolism toward mitigating salt-induced damages in cucumber seedling. Plants, 2019;8(10):1-17.

12. Barr HD, Weatherley PE. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust J Biol Sci. 1962;15:413-428.

13. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 1968;125:189-198.

14. Mohsin SM, Hasanuzzaman M, Parvin K, Morokuma M, Fujita M. Effect of tebuconazole and trifloxystrobin on *Ceratocystis fimbriata* to control black rot of sweet potato: processes of reactive oxygen species generation and antioxidant defense responses. World J Microbiol Biotechnol. 2021; 37(9):1-16.

15. Dionisio-Sese ML, Tobita S. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 1998;135:1-9.

16. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Packer L, Douce R (eds). Methods in Enzymology. Cambridge: Academic Press; 1987. P. 350-382

17. Mohiddin FA, Bhat NA, Wani SH, Bhat AH, Ahanger MA, Shikari AB, Sofi NR, Parveen S, Khan GH, Bashir Z, Vachova P. Combination of strobilurin and triazole chemicals for the management of blast disease in mushk budji-aromatic rice. J Fungi. 2021;7(12):1-13.

18. Morales M, Munne-Bosch S. Malondialdehyde: Facts and artifacts. Plant Physiol. 2019;180:1246-1250.

19. Kong W, Liu F, Zhang C, Zhang J, Feng H. Non-destructive determination of malondialdehyde (MDA) distribution in oilseed rape leaves by laboratory scale NIR hyperspectral imaging. Sci Rep. 2016;6:1-8.

20. Yuzbasioglu E. Effect of fungicide pretreatment on lipid peroxidation, antioxidant enzyme systems and proline accumulation in tomato (Lycopersicon esculentum Mill.) leaves under high temperature stress. Turkish J Bot. 2020;44:604-617.

21. Anjum NA, Sofo A, Scopa A, Roychoudhury A, Gill SS, Iqbal M. Lipids and proteins-major targets of oxidative modifications in abiotic stressed plants. Environ Sci Pollut Res. 2015;22:4099-4121.

22. 22. Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ. 2010;33:453-467.

23. Wu YX, Von TA. Impact of fungicides on active oxygen species and antioxidant enzymes in spring barley (*Hordeum vulgare* L.) exposed to ozone. Environ Pollut. 2002;116:37-47.

24. Demidchik V, Straltsova D, Medvedev SS, Pozhvanov GA, Sokolik A, Yurin V. Stress-induced electrolyte leakage: the role of K+-permeable channels and involvement in programmed cell death and metabolic adjustment. J Exp Bot. 2014;65:1259-1270.

25. 25. Simkin AJ, Faralli M, Ramamoorthy S, Lawson T. Photosynthesis in non-foliar tissues: implications for yield. Plant J. 2020; 101:1001–1015.

26. Sidhu GPS, Singh HP, Batish DR, Kohli RK. Alterations in photosynthetic pigments, protein, and carbohydrate metabolism in a wild plant *Coronopus didymus* L. (Brassicaceae) under lead stress. Acta Physiol Plant. 2017;39:1-9.

27. Pramesh D, Nataraj K, Guruprasad GS, Mahantashivayogayya K, Reddy BGM. Evaluation of a new strobilurin group of fungicide for the management of blast disease of paddy. Am J Exp Agric. 2016;13:1-6.