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Hydrogen peroxide priming-induced lignification in maintaining biomass and reduction of tissue necrosis during *Rhizoctonia solani* infection in rice

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Rice production is severely constrained by the fungal pathogen *Rhizoctonia solani* causing sheath blight disease leading to major yield losses. Priming the plants with defense inducing chemicals, has been found to increase tolerance against diseases. In this study, hydrogen peroxide was used as priming agent to induce tolerance in rice plants against *R. solani*. After priming, and subsequent infection, plants showed healthier appearance with less disease related damage four days post infection. H_2O_2 primed plants also retained more biomass than unprimed plants. Another important parameter to assess disease progression is tissue necrosis, which was found to be significantly less in primed rice plants indicating better preservation of tissue integrity during sheath blight disease progression for four days. Additionally, the deposition of protective compound lignin in greater amount around vascular bundle and epidermal cells of the stem of primed plants provided better structural barrier against the fungal disease. This induced lignification may be one of the parameters contributing to the increased tolerance. Thus, among various control measures reported, hydrogen peroxide priming emerges as an effective method to increase tolerance of rice plants against fungal sheath blight disease.

Keywords : Hydrogen peroxide, lignification, rice, *Rhizoctonia solani*, sheath blight, tissue necrosis with.

INTRODUCTION

Rice (*Oryza sativa L.*) is one of the prime cereal crops in the world, feeding more than half of the global population and India is the second largest producer of rice after China (Almeida *et al.* 2026).Among various biotic and abiotic constraints limiting rice cultivation, the fungus *Rhizoctonia solani* Kuhn AG1-IA severely threaten rice yields causing potential yield loss of up to 50% under favourable conditions (Naveena *et al.* 2025). The soil-borne fungus causes sheath blight disease in rice characterized by lesions on the sheath leading to softness and rotting of the sheath, inhibition of grain filling and ultimately yield loss (Abbas *et al.* 2023).

The conventional crop protection strategies against *R. solani* are limited to the use of synthetic fungicides which can lead to various adverse effects, and the use of natural fungicides or biocontrol agents are often not effective (Shivappa *et al.* 2026).

Priming refers to the pretreatment of plants with an appropriate concentration of chemical or stressor compounds to evoke more robust defense responses against future exposure of stress (Harris *et al.* 2023). Various priming studies have used synthetic chemicals, natural compounds to effectively combat a number of stress factors. Among them, priming plants or seeds with hydrogen peroxide (H_2O_2) has recently gained attention (Choudhury *et al.* 2024). H_2O_2 is a reactive oxygen species (ROS) produced in plants whenever plants are subjected to biotic or abiotic stresses (Jira-Anunkul*et al.* 2021).Many studies have demonstrated that priming with H_2O_2 can enhance abiotic stress tolerance like chilling, heat, drought, salinity, heavy metals by modulating a number of physiological processes (Sako *et al.* 2020; Afrin *et al.* 2019).

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This study aims to decipher the protectiveeffect of H_2O_2 priming against the sheath blight pathogen, *R. solani*. Disease severity in plants are widely determined by the extent of tissue necrosis and reduction in average biomass(Ray*et al.* 2015, Miao *et al.* 2022). Biomass retention is considered as a significant indicator of tolerance of plants against any stressors (Vega-Álvarez *et al.* 2021). In fungal invasion, fortification of cell walls is a defense strategy adopted by plants to restrict hyphal penetration (Lee *et al.* 2019)**.**Among fortifying compounds, lignin is the major one and its deposition around vessel and internal tissues gives better edge to plants to block the hyphal invasion(Zhu *et al.* 2022). This study aims to investigate the above parameters in both unprimed and hydrogen peroxide primed rice plants post infection with *R. solani*which will provide an insight into the efficacy of H_2O_2priming to alleviate detrimental effects of the fungus on the crop plant.

MATERIALS AND METHODS

Growth conditions for rice plants and the fungal pathogen R. solani

Seeds of rice cultivar Ajit, *Oryza sativa* L. var. indica,(Rice Research Station, Chinsurah, West Bengal. India) were grown on commercial soilrite (a mixture of horticulture grade perlite, Irish peat moss and exfoliated vermiculite in 1:1:1 ratio, Keltech, India) and maintained in plant growth chambers (Basu *et al.*2016). Pure culture of AG1-1A isolate of Manisha Thakur and others the fungus *Rhizoctonia solani* Kuhn. (Rice Research Station, Chinsurah, West Bengal. India), was maintained at 28^0C in potato dextrose agar plates.

Priming of rice plants and fungal inoculation

Experiment was set based on previous reports of Gohari *et al.* 2019;Mejía-Teniente *et al.* 2019.Aerial parts of rice plants were treated with a concentration of 0.05μM H_2O_2 solution and sclerotia from 10 days old culture plates of *R. solani* were used to inoculate primed rice plants according to our standardized method (Koley *et al.* 2022). Post inoculation with *R. solani*, experimental plants were photographed separately at 96 hours for comparison of external morphology.

Calculation of plant biomass

After priming and inoculation, plants were maintained in convirons. After 96 hours of disease progression,dry weight of plants were taken and biomass was calculated (Miao *et al.* 2023). The weight of three plants each of three independent experimental sets of each type (primed and unprimed) were obtained for calculation.

Assay of tissue necrosis in rice plants

Rice leaves of control and primed experimental sets were collected at 96hours post-inoculation (hpi). After removal of chlorophyll with acetoethanol solution (glacial acetic acid and ethanol in 1:3 v/v ratio), leaves were stained with lactophenol-trypan blue solution (0.5% trypan blue, lactic acid phenol and water in 1:1:1 v/v/v ratio) to observe blue patches of necrosis, according to our previous report of Ray *et al.* 2015.

Assay of lignin deposition in rice plants

Weisner reagent (1% phloroglucinol in 95% ethyl alcohol) was applied on the sections of the rice stem harvested at different timepoints of 24hpi, 48hpi, 72hpi from both control and primed experimental sets, after infection. After 5 minutes of staining, sections were mounted in 18% HClon glass slides and the localization of lignin was observed as pink to red colouration under compound microscope (Olympus BX-51, Germany) (Zhu *et al.*2022)

Statistical analyses

All experimental data were statistically analysedaccording to our previous publication (Chowdhury*et al.* 2017). All experiments consisted of threeindependent sets with at least three replicates ineach and the values were represented as themean ± SD.

RESULTS AND DISCUSSION

Comparison of biomass of diseased primed and unprimedrice plants

Both the control and H_2O_2 primed rice plants showed sheath blight disease symptoms post inoculation with *Rhizoctonia solani*. Although the control sets were severely affected with chlorosis and wilting, the primed plants showed visibly lesser symptoms even at 96 hpi. This observation also corelated with the biomass of the plants. At the end of 96 hpi, the average biomass of the primed rice plants was about 26% more than the unprimed plants (Fig.1).

Comparison of tissue necrosis of rice leaves post infection

Tissue necrosis was observed following *R.solani* inoculation in detached rice leaves. After staining with trypan blue, resultant blue patches indicating necrosis were observed. Necrosis was significantly lesser in H_2O_2 primed rice plants evident by limited and dispersed blue patches. The percentage of necrosed tissue at 96 hpi was as high as 89.74% in control rice plants, whereas it was limited to 69.13% for the primed plants (Fig.2).

Histochemical detection of lignin deposition in internal tissues of the stems of primed and unprimed rice post R.solani infection

Lignin deposition was observed as pink to red colouration in the vascular bundles post staining with Weisner's reagent. The intensity and distribution of lignin deposition were much higher in stem vascular bundles of primed rice plants whereas in case of control it is scanty. There was a gradual increase in lignification with increasing time-points upto 72hpi. The relative intensity of pink colouration of stained lignin is almost 1.5 times higher in primed rice plants in comparison to control signifying better structural barrier against pathogen (Fig. 3), The present study dealt with impact of *Rhizoctonia solani* infection on rice plants in terms of biomass reduction, tissue necrosis and subsequent defense response of plants by cell wall fortification strategy. In these experiments the effect of priming and subsequent susceptibility to *R. solani* was observed; in order to consider any effect on the priming itself, a control set of plants which has been primed but without any subsequent infection, was used for comparison. Plant biomass indicates the overall health of the plants which predominantly consists of cell walls constituting molecules such as cellulose, hemicellulose, and lignin (Miao *et al.* 2023).Fungal infections cause a drop in plant biomass by causing cellular lysis during entry into the host cells (Li *et al.* 2022).Various fungi such as *Macrophomina phaseolina*, *Fusarium oxysporum* (Hong *et al.* 2022),*Alternaria solani* (Parveen *et al.* 2022) are reported to decrease plant biomass during disease incidence. Lesser reduction of biomass in case of H_2O_2 primed rice plants indicates better tolerance towards the fungal infection.

Percentage of tissue necrosis in peroxide primed and unprimed rice plants follow similar trend. During necrotrophic infection stages, plant tissue degradation is actively induced to derive nutrients for proliferation in the host (Snelders *et al.* 2022) as previously reported in many fungal pathogens like *Magnaporthe oryzae* (Wengler *et al*. 2025), *Sclerotinia sclerotiorum* (Wang *et al.* 2024), *Botrytis cinerea* (Bi *et al.*2023), *Rhizoctonia solani*

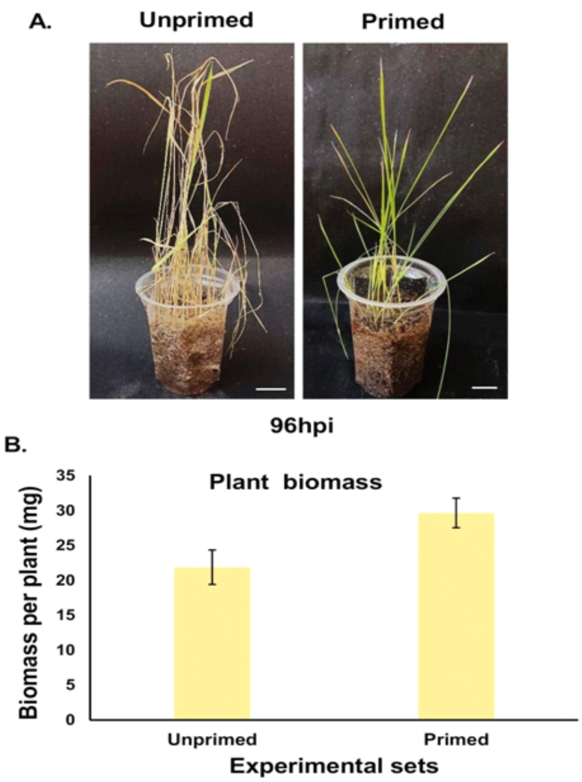

Fig 1 : External morphology and biomass of rice plants in H_2O_2 primed and unprimed sets after *Rhizoctonia solani* infection. **(A)** Primed rice plants show healthier appearance than unprimed rice plants (Bar- 1cm). (**B)** After 96 hours post inoculation (hpi) following *R. solani* infection, primed rice plants maintained its biomass better than unprimed plants. Bars represent mean ± SD of three independent experiments with three replicates.

(Koley *et al.* 2022) and *Alternaria solani* (Ray *et al*.2015).Thus, the extent of tissue necrosis correlates with favourable environment within the host (Ma *et al.* 2019). In our study,lower percentage of cell necrosis in the H_2O_2 primed rice leaves indicated towards hostile environment towards *R. solani*. The induced immunity in primed rice plants limited the proliferation of the pathogen *R. solani* in the host cells resulting in higher cell viability. This corroborates earlier published report from our lab where priming tomato plants with phytohormones yielded similar results in *R. solani* infection (Koley *et al.* 2022).

Lignin deposition followed an opposite trend with comparison to biomass and tissue necrosis. Lignin is a polymerized phenolic molecule that

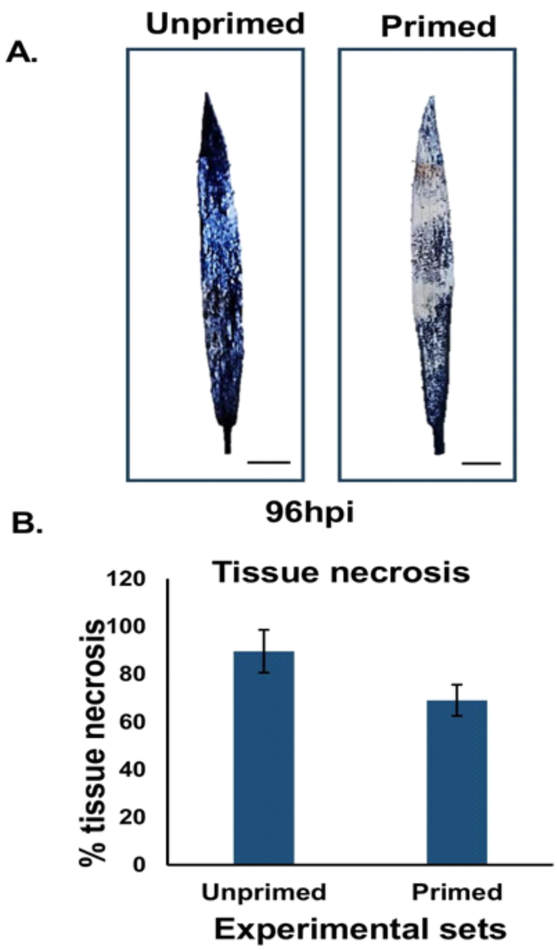

Fig 2 : Assay of tissue necrosis in H_2O_2 primed and unprimed rice leaves post infection with *R. solani*. (A) Infected primed rice leaves show limited tissue necrosis than unprimed sets at 96 hpi (Bar- 3mm). (B) Comparison of tissue necrosis in primed and unprimed rice leaves at 96 hpi. Bars represent mean ± SD of three independent experiments with three replicates.

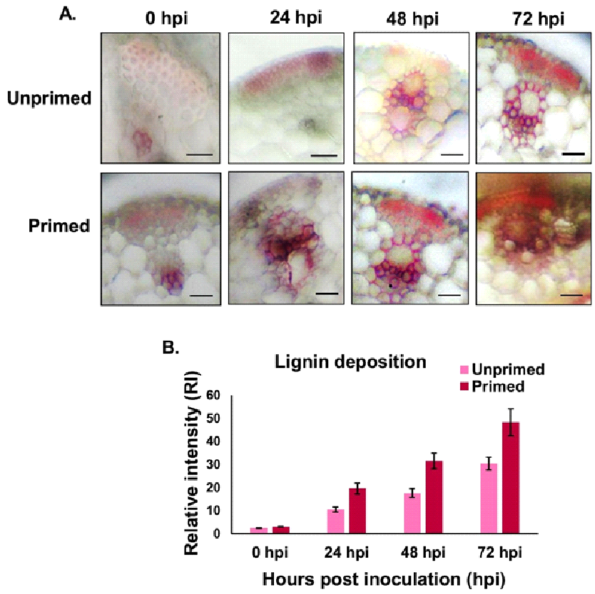

Fig 3 : Comparison of lignin deposition during rice sheath blight disease progression in H_2O_2 primed and unprimed rice plants around vascular bundle and epidermal cells of the stem . (A) TS of stem of primed infected rice plants show increased deposition of lignin than unprimed plants, indicated by development of pink to red colour after staining with Weisner's reagent (Bar-50µm). (B) In every time points upto 72 hpi, primed rice plants show greater relative intensity of pink colouration in TS signifying higher lignin deposition. Bars represent mean ± SD of three independent experiments with three replicates each.

comprises of three hydroxycinnamoyl alcohol monomers and acts as structural barrier against the entry of fungal pathogens (Zhu *et al.* 2025; Lee *et al.* 2019). Role of lignin depositionto restrict the fungal growth had been established against various pathogens like *Alternaria alternata* (Liu *et al.* 2023),*Colletotrichum fruticola* (Li *et al.* 2023), *Rhizoctonia cerealis* (Wang *et al.* 2018), *Fusarium sp* (Funnell-Harris *et al.* 2010).In this study, higher amount of lignin deposition is observed in H_2O_2 primed plants at all timepoints which serve as a barrier against *R. solani* hyphal progression. Elevated deposition of lignin in biotic or abiotic stresses as a result of external priming has been published earlier which strengthen our observation (Hamed *et al.* 2026; Lastochkina *et al.* 2021; Patil *et al.* 2019).Subsequent experiments may be done to study the exact mechanism through which priming alters tolerance of plants to fungal diseases.

CONCLUSION

The present work establishes that hydrogen peroxide priming of rice plants prior to *R. solani* infection contributes to better tolerance against sheath blight disease. During disease progression, the reduction of average plant biomass and tissue necrosis was much lesser in H_2O_2 primed rice plants than unprimed plants contributing to improved plant health. Moreover, deposition of protective compound lignin in higher amounts after priming provided a structural barrier against the fungal pathogen restricting its entry into host tissues. Thus, this study provides useful insights into the efficacy of H_2O_2 priming to control sheath blight disease in rice.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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