



RESEARCH ARTICLE

Assessment of sheath blight resistance in rice landraces using disease progression metrics and machine learning

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Abstract

Sheath blight (ShB), caused by *Rhizoctonia solani*, is a major constraint in rice (*Oryza sativa* L.) production, leading to considerable yield losses. The objective of the present study was to assess sheath blight resistance in rice landraces based on the disease progression parameters and machine learning methods. Field screening was conducted under inoculated conditions and disease progression was monitored from 10–70 days after inoculation using disease severity (DS), percent disease index (PDI) and area under disease progress curve (AUDPC). Machine learning models - linear regression, decision tree, random forest and gradient boosting were employed to predict DS of sheath blight in rice landraces based on disease progression parameters and their predictive performances were compared using R^2 , root mean square error (RMSE) and classification-based evaluation metrics. Among the landraces screened, NLR 33892 and Swarna Swoubhagya showed resistance against *R. solani*, while the majority were susceptible or highly susceptible. Both DS and PDI increased progressively over time and showed a strong positive correlation across assessment stages, confirming consistent disease development. Linear regression performed the best with the highest coefficient of determination ($R^2 = 0.8178$) and the lowest RMSE = 3.852, followed by decision tree ($R^2 = 0.8077$), random forest ($R^2 = 0.8010$) and gradient boosting ($R^2 = 0.7899$). The analysis of confusion matrices further supported the better consistency of classification of linear regression.

Keyword: disease severity; machine learning; percent disease index; rice landraces; sheath blight

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide, serving as a staple food for more than half of the global population and providing approximately 20 % of the total dietary calories consumed globally, particularly across Asia and sub-Saharan Africa (1). About one-third of the world's population roughly 3.5 billion people, depend on rice as their staple food. (2, 3). India produces 129 mt of rice annually with a productivity of about 3.86 t ha⁻¹ on the rice cultivation area of 44 mha (4). The rice area in Karnataka is 1.4 mha with production of approximately 3.5 mt and productivity of 2.5 t ha⁻¹. Rice productivity is limited by several biotic stresses, among which sheath blight is one of the most destructive fungal diseases. The sheath blight fungi, *Rhizoctonia solani*, can cause 4–45 % yield loss which is equivalent or less than the crop growth stage, infection time and environment (5, 6). After blast disease, sheath blight is widely regarded as one of the most economically damaging diseases of rice in terms of loss in

seasonal production of rice (7, 8). It has been a significant constraint in production in high-yielding-fertiliser-responsive-hybrid varieties and highly tillered varieties in high-yielding-fertiliser-responsive-hybrid rice production systems (9, 10).

Thanatephorus cucumeris (teleomorph) and *R. solani* Kuhn AG1-IA (anamorph) are facultative saprotrophs and parasites that can be found in the soil. As the pathogen comes into contact with the parts of the plant, e.g. tillers and leaves, it is spread quickly through sclerotia that can remain in the soil and water up to three years (11, 12). The disease affects different parts of the plants such as the sheaths of leaves, the upper leaves and even the panicles and its first signs are realised at approximately the late tillering stage. It intensifies at the panicle differentiation stage (13). One or more broad, rectangular or irregularly elongated lesions on the leaf sheath tend to help in the identification of the disease (14). The sclerotia that the fungal pathogen has when exposed to unfavourable environmental conditions combined with the broad host range makes the disease hard to control. Chemical fungicides

are the main tool of dealing with the disease. Nevertheless, these fungicides are not commercially and environmentally viable in many instances. Losses of sheath blight (ShB) in rice can be reduced by breeding resistant cultivars because the rice landraces are reported to be genetic wealth that is highly vigorous and sources of invaluable sheath blight resistance (15). However, the development of resistant cultivars has achieved limited success because complete resistance to sheath blight has not yet been identified in cultivated rice germplasm (16–19). Resistance to *R. solani* is quantitatively inherited and controlled by multiple polygenic quantitative trait loci (QTLs), most of which exhibit small to moderate effects and are strongly influenced by environmental conditions (20, 21). In addition, the pathogen possesses high genetic variability and ecological adaptability, enabling long-term survival through sclerotia under diverse environmental conditions. Consequently, resistant germplasm remains rare and stable field-level resistance has been difficult to achieve across different environments. Therefore, screening rice landraces for sheath blight resistance may help identify novel resistance sources and support the development of high-yielding rice cultivars with improved resistance to sheath blight. By screening popular landraces against resistance, new genes and pathways in defence response against *R. solani* could be identified. The information attained in these studies can be used in forming the high-yielding rice varieties that are more resistant to sheath blight. To aid the solution to these issues, scientific methods of using data to combine phenotypic, environmental and molecular data to define resistance and aid in breeding decisions have gained focus. Random forests (RF) and artificial neural networks (ANN) machine learning (ML) models have shown great potential in the classification of resistant and susceptible lines and prioritisation of candidate loci. Their approaches complement the traditional QTL mapping and multi-omics research as it is possible to determine more valuable genetic material faster and more precisely. The ML models are also deemed to be useful decision support in the pipelines of breeding since they are able to handle non-linear and complex data (22). Sheath blight resistance has never been completely developed in rice; most varieties have demonstrated partial resistance. Random forest and ANN are not only computational methods that open new opportunities to comprehend the quantitative properties of sheath blight resistance, but also to integrate multi-environmental data and create potential genotypes to be further bred. This is the reason behind the present research which evaluates RF and ANN models to categorise sheath blight resistance and sorts rice genotypes to be utilised in improvement programs (23). This study evaluated 43 rice landraces for sheath blight resistance under field conditions and examined disease progression patterns using epidemiological metrics and machine learning models for predictive classification of disease response.

Materials and Methods

Isolation of the pathogen

Sheath blight-infected rice samples showing typical symptoms were collected from Ranadevi & Baghusala farms of Centurion University of Technology and Management during the kharif season of 2023. A total of 21 diseased rice samples were collected from different experimental and farmers' fields for pathogen isolation. Sheath blight-infected rice samples were initially washed with distilled water to clean the samples from dirt and other extraneous materials. Pieces (2–3 cm long) containing the diseased and healthy tissues

were excised and sterilised by exposing them to 0.1 % mercuric chloride solution for 2 min. The sterilised pieces were then rinsed three times with sterile distilled water to eliminate any residual mercuric chloride. The sterilised tissue pieces were then aseptically placed at the centre of the potato dextrose agar (PDA) in Petri plates. The Petri plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 3–5 days for the growth of the fungus. The emerging fungal colonies were sub-cultured to obtain pure cultures.

Molecular identification and phylogenetic analysis of *Rhizoctonia solani*

Following molecular characterisation, the causative pathogen was processed for molecular detection. The cetyltrimethylammonium bromide (CTAB) DNA extraction method was used to confirm the molecular detection of isolate R.S-1. Because of the large internal transcribed spacer (ITS) data collection for fungi, the extremely variable nature within highly conserved ribosomal coding sequences and the high popularity of studies dealing with fungal identification and phylogenetic relationships (24), fungal molecular identification was performed using sequencing of ITS regions. The ITS region proved to be a suitable barcoding identifier in Mucorales (25). The primer pair ITS-1 and ITS-4 were used to amplify the internal transcribed spacers of the ribosomal DNA of the R.S-1 isolate (26). Amplification was performed with BIORAD My Cycler™ thermal cycler (Bio Rad, USA). Polymerase chain reaction (PCR) amplification was followed through standard cycling protocol with the denaturation temp. 95°C for 1min (1 cycle), Annealing temp. 55°C for 60 s (30–35 cycle), elongation temp. 72°C for 2 min with the final extension of 72°C for 2 min (1 cycle). The final reaction mixture (25 μL total volume) was formulated with 2.5 μL of $10\times$ Taq buffer consisting of 15 mM MgCl_2 , 1.0 μL of ITS1 primer ($5\text{ pmol}\cdot\mu\text{L}^{-1}$), 1.0 μL of ITS4 primer ($5\text{ pmol}\cdot\mu\text{L}^{-1}$), 1.0 μL of 25 mM dNTP mix, 1.5 U of Taq polymerase ($3\text{ U}\cdot\mu\text{L}^{-1}$), 16 μL sterile distilled water and 3 μL of genomic DNA template. *Rhizoctonia* DNA isolation requires an optimal yield of 100–300 ng/ μL (minimum 20 ng/ μL for PCR), quantified using NanoDrop. *Rhizoctonia* DNA isolation requires an optimal yield of 100–300 ng/ μL (minimum 20 ng/ μL for PCR), quantified using NanoDrop. Purity is assessed spectrophotometrically, with A260/A280 ratio of 1.8–2.0 confirming absence of protein or phenol contamination and A260/A230 ratio of 1.8–2.2 ensuring freedom from polysaccharides, CTAB, or salts common contaminants in *Rhizoctonia* due to its melanin and polysaccharide-rich mycelium. Gel quality is verified by 0.8 % agarose gel electrophoresis at 80–100 V for 30–45 min, where high-quality DNA appears as a single, sharp, high-molecular-weight band ($> 20\text{ kb}$) with no smearing or RNA contamination. Phylogenetic analysis was performed through neighbour-joining tree by MEGA X. The evolutionary history was inferred using the neighbour-joining method (27). The bootstrap consensus tree inferred from 1000 replicates (28) is taken to represent the evolutionary history of the taxa analysed (28).

Pathogenicity test

Pathogenicity test was conducted by taking healthy rice leaves which were cut into equal-sized pieces ($\approx 5\text{--}7\text{ cm}$) and sterilised. The pieces were placed in sterile Petri plates containing moist filter paper. A small injury was made on the sheath surface using a sterilised needle. Mycelial discs (5 mm diameter) containing actively growing hyphae and sclerotia were placed on wounded sheath tissues to facilitate active infection. The plates were incubated at $28 \pm 2^\circ\text{C}$ and the observations were made at different time intervals (2, 4, 6, 8 and 10 days after inoculation). Potted rice plants were

maintained in the greenhouse rice plants were grown in 129 plastic pots under controlled conditions. Each germplasm was maintained in 3 replications, with each pot containing 3–5 plants. The inoculation was done at the tillering stage a slight injury was made on the sheath near the water level using a sterilised needle. Mycelial bits colonised by *R. solani* were inoculated at the injured part of the bottom portion of the plant sheath just above the water level. Moisture was maintained by keeping water in pots and wrapping the inoculated part with wet cotton (if needed). Plants were kept at 25–30 °C temperature with 85–90 % relative humidity and observations on disease symptom appearance, lesion development, disease progression and sheath blight symptom expression were recorded regularly.

Experimental design and experimental site

The field experiment was conducted at Centurion University of Technology and Management, Ranadevi farm, Parlakhemundi, Odisha, during 2023–25 cropping season. The experiment was laid out in a randomized block design (RBD) with 3 replications. The trial was conducted in 43 germplasms, 3 replications and 1 check (Swarna (MTU 7029)). The field was ploughed twice in cross direction by tractor-drawn harrow and weeds were removed. The nursery bed was prepared separately for rising seedlings. Each experimental plot measured 4 × 2 m and plots were separated by 0.5 m irrigation channels. Thirty-days-old seedlings were transplanted into the main field at a spacing of 20 cm between rows and 10 cm between plants, accommodating a total of 200 plants per plot. Standard agronomic practices were followed throughout the crop growth period.

Experimental materials

A total of 43 rice landraces along with one susceptible check were evaluated for sheath blight resistance. Germplasm was collected from Agricultural research station, Ragolu, Andhra Pradesh, Baghusala farm and Ranadevi farm of Centurion University of Technology and Management, Parlakhemundi, Odisha (Table 1).

Artificial inoculation

A total of 43 rice germplasms were artificially inoculated with sclerotia of *R. solani* to evaluate their reaction against sheath blight disease. The pathogen was cultured on PDA medium and 5–7 day old actively growing mycelial bits along with mature sclerotia were used as inoculum (29). Inoculation of healthy rice plants was done at maximum tillering stage. A slight injury was made on the leaf sheath near the water level using a sterilised needle and mycelial bits with sclerotia were placed on the injured portion of the sheath. Approximately two sclerotia inoculum per tiller was used for uniform infection. Inoculated area was covered with moist cotton to keep moisture wrapped with polyethylene film to maintain high humidity to achieve successful infection. After symptoms appeared, lesion length, lesion spread and relative lesion height were used to assess disease severity (DS).

Disease assessment

Disease severity is defined as the “zone of a sample unit (plant surface) infected by disease as a percentage or fraction of the total area”. Disease severity data were started taking from the emergence of disease; data is taken for 10 days interval of time up to 9 observations. The disease score was noted on 0–9 scale based on lesion character and area, where 0 represented no lesion characteristics and 9 represented lesions in more than 75 % leaf area. Disease severity was estimated by the formula of Shrestha

and Mishra (1994).

$$\text{Disease severity (DS)} = \frac{\text{Total lesion length}}{\text{Total length of sheath}} \times 100$$

$$\text{Percent disease index (PDI \%)} = \frac{\text{Sum of all individual disease ratings}}{\text{Total no. of plant assessed} \times \text{maximum ratings}} \times 100$$

Area under disease progress curve (AUDPC)

The AUDPC is a widely used method to combine multiple observation of disease development into one value. Our research reveals that this method significantly underestimates the impact of the first and final observations (30).

$$\text{AUDPC} = \sum n-1[(x_i + x_{i+1})/2] \times (t_{i+1} - t_i)$$

The AUDPC is a quantitative indicator of disease intensity across time. It is used in plant pathology to identify and compare disease resistance levels among crop cultivars.

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where,

n is the total number of observation time

y_i is the disease percentage at the i^{th} observation

y_{i+1} is the disease percentage at the next observation

t_i is the time in days at the i^{th} observation

$t_{i+1} - t_i$ is the time duration between successive observation

Among all the 43 landraces of rice that includes one check variety Swarna, were screened under field epiphytotic conditions for occurrence of disease development of sheath blight.

Screening of resistance cultivar through field trial

The natural field test was conducted in the form of field trial for the screening of rice cultivar against sheath blight disease. Cultivars were rated into five classes as resistant (R) of scale 1, moderately resistant (MR) of scale 2, moderately susceptible (MS) of scale 3, susceptible (S) of scale 4 and highly susceptible (HS) of scale 5 based on the severity of the disease. The trial was conducted in RBD with 3 replications and standard agronomic practices were followed in the growing season (kharif 2022–24). Disease progress was recorded every 10 days up to 70 days after inoculation and the AUDPC was calculated. The cultivars were grouped according to AUDPC values into R (0–250 %), MR (250–300 %), MS (300–422 %), S (422–712 %) and HS (above 712 %) categories (31). This categorisation was helpful in identifying cultivars with varying resistance to sheath blight of rice.

Statistical analysis

Dataset and preprocessing

All statistical analyses were performed using SPSS Statistics (version 26.0; IBM Corp., USA) and Python (version 3.10) in Google Colab. The data set in the current study included the yield as the dependent variable and some independent variables, such as DS, grains per panicle (G/P), 1000-grain weight, plant height (PH) and PDI, which were chosen on the basis of their effect on crop productivity and their role in disease influence. One-way ANOVA was performed to test for significant differences among rice

Table 1. Germplasm used for the screening and their place of collection

Sl. No.	Varieties	Varietie name	Place of collection
1	V1	Swarna swoubhagya	Agricultural research station, Ragolu, Andhra Pradesh
2	V2	NLR 33892	Agricultural research station, Ragolu, Andhra Pradesh
3	V3	MTU 1156	Agricultural research station, Ragolu, Andhra Pradesh
4	V4	CR 411	Ranadevi farm, CUTM, Paralkhrmundi
5	V5	CR 311	Agricultural research station, Ragolu, Andhra Pradesh
6	V6	MTU 1016	Agricultural research station, Ragolu, Andhra Pradesh
7	V7	Govindbhog	Baghusala farm CUTM, Paralkhemundi
8	V8	CR 1017	Ranadevi farm, CUTM, Paralkhrmundi
9	V9	MTU 1318	Agricultural research station, Ragolu, Andhra Pradesh
10	V10	MTU 1075	Agricultural research station, Ragolu, Andhra Pradesh
11	V11	RGL 2537	Agricultural research station, Ragolu, Andhra Pradesh
12	V12	KNMM 1638	Agricultural research station, Ragolu, Andhra Pradesh
13	V13	MTU 1061	Agricultural research station, Ragolu, Andhra Pradesh
14	V14	MTU 5204	Agricultural research station, Ragolu, Andhra Pradesh
15	V15	RGL 1880	Agricultural research station, Ragolu, Andhra Pradesh
16	V16	MTU 1121	Agricultural research station, Ragolu, Andhra Pradesh
17	V17	GRGL 11226	Baghusala farm CUTM, Paralkhemundi
18	V18	RGL 2538	Agricultural research station, Ragolu, Andhra Pradesh
19	V19	NLR 34449	Agricultural research station, Ragolu, Andhra Pradesh
20	V20	MTU 1156	Agricultural research station, Ragolu, Andhra Pradesh
21	V21	IR 64	Agricultural research station, Ragolu, Andhra Pradesh
22	V22	BPT 2782	Baghusala farm CUTM, Paralkhemundi
23	V23	MTU 1224	Agricultural research station, Ragolu, Andhra Pradesh
24	V24	BPT 3082	Agricultural research station, Ragolu, Andhra Pradesh
25	V25	MTU 1210	Agricultural research station, Ragolu, Andhra Pradesh
26	V26	RNR 15048	Agricultural research station, Ragolu, Andhra Pradesh
27	V27	NLR 3354	Agricultural research station, Ragolu, Andhra Pradesh
28	V28	CR1	Baghusala farm CUTM, Paralkhemundi
29	V29	Marvel 1011	Agricultural research station, Ragolu, Andhra Pradesh
30	V30	CR 315	Baghusala farm CUTM, Paralkhemundi
31	V31	RNR Marvel	Ranadevi farm, CUTM, Paralkhrmundi
32	V32	MTU 101	Agricultural research station, Ragolu, Andhra Pradesh
33	V33	Soubhagya	Ranadevi farm, CUTM, Paralkhrmundi
34	V34	CR Dhan 308	Ranadevi farm, CUTM, Paralkhrmundi
35	V35	Mohani	Ranadevi farm, CUTM, Paralkhrmundi
36	V36	CR Dhan 319	Agricultural research station, Ragolu, Andhra Pradesh
37	V37	MTU 1010	Baghusala farm CUTM, Paralkhemundi
38	V38	RGL 2624	Agricultural research station, Ragolu, Andhra Pradesh
39	V39	Manipuri local	Baghusala farm CUTM, Paralkhemundi
40	V40	RNR 15048	Agricultural research station, Ragolu, Andhra Pradesh
41	V41	Juvraj Dhano	Ranadevi farm, CUTM, Paralkhrmundi
42	V42	Puja	Ranadevi farm, CUTM, Paralkhrmundi
43	V43	Lal Basmati	Ranadevi farm, CUTM, Paralkhrmundi

CUTM: Centurion University of Technology and Management.

landraces across all parameters and where ANOVA was significant, Tukey's HSD post hoc test was applied for pairwise comparisons. Pearson's correlation coefficients (Spearman's where normality was violated) were computed among variables at each assessment time point. For machine learning, the dataset comprising yield (dependent variable) and five predictors-DS, PDI, G/P, 1000-grain weight and PH was cleaned by mean imputation for missing values, standardised using StandardScaler (zero mean, unit variance) and split into training (80 %) and testing (20 %) subsets (random state = 42). Model performance was evaluated using R^2 , RMSE and mean absolute error (MAE) on the held-out test set and generalisation was assessed by 5-fold cross-validation with GridSearchCV-based hyperparameter tuning for ensemble models.

Model development

Linear regression: With the aim of establishing a linear relationship between the predictor variables and the yield, a multiple linear regression was developed. Parameters included in the implementation of the model were: fit intercept true, which included the intercept term in the model and the other parameters were all at default values. Linear regression assumptions such as linearity, independence, homoscedasticity and normality of residuals were taken into consideration when developing the model.

Random forest: An ensemble learning method was used to enhance the accuracy of prediction and minimise overfitting, which is the random forest regression model. A model was built based on 100 decision trees (estimators = 100) in which squared error was used as a splitting criterion. The rest of the parameters that were used were max depth = none, min samples split = 2, min samples leaf = 1, use bootstrap sampling = on and fixed random state = 42 to guarantee reproducibility. The model is a combination of predictions by several trees constructed based on random subsets of data and features.

Decision tree: A decision tree regression model was also used to model non-linear relationships between the variables. Squared error was the criterion of split quality in the model with the best splitter decision being chosen. Max depth, min samples split, min samples leaf and random state were set to unrestricted 2, 1 and 42 respectively. The model operates on the principle of dividing the

dataset into smaller subsets through recursive partitioning of the data using the feature values to reduce the prediction error.

Gradient boosting: A gradient boosting regression model was used to improve the predictive performance by sequential learning. The model was trained on 100 estimators (nestimators = 100) and a learning rate of 0.1 and a maximum depth of 3. The other parameters were min samples split: 2, min samples leaf: 1 and subsample: 1.0 and random-state: 42. This is done sequentially, with each next model correcting the residual errors of the previous model. Coefficient of determination was used to measure the performance of all the models as well as RMSE. The goodness of fit and prediction accuracy of each model was measured using these metrics. The comparison bar graph was created to compare R^2 values of various models.

Results

Molecular detection and phylogenetic analysis of the pathogen

Amplified PCR products were resolved on a 1.5 % agarose gel in 1X Tris-acetate-EDTA (TAE) buffer using ethidium bromide staining and visualised on a Bio-Rad gel documentation unit (USA). The PCR amplification of the ITS region produced a single clear amplicon of approximately 681 bp, consistent with the expected fragment size for *R. solani* as shown in Fig. 1. Sequence analysis of the 681 bp ITS-rDNA fragment showed 100 % similarity with reference *R. solani* sequences (accessions PV916039 and ON974837), confirming species identity as shown in the Fig. 2. Phylogenetic analysis using 16 nucleotide sequences showed the evolutionary connections between the investigated taxa, in which sequences were clustered according to their similarity. Results revealed that isolate RS-1 (*R. solani*, India) submitted under the GenBank accession number-PZ485856 which clustered together with *T. cucumeris* isolates from Brazil (GU570166), Japan (AB195928), Vietnam (EF429214) and *R. solani* from China (KU696484), forming Cluster 1 as because they are conspecific on the same branch node. The isolate exhibited a close phylogenetic relationship with Cluster 1 taxa's and is more closely related to *R. solani* from Sweden (LR994100). This grouping was strongly supported by a 99 % bootstrap value. In contrast,

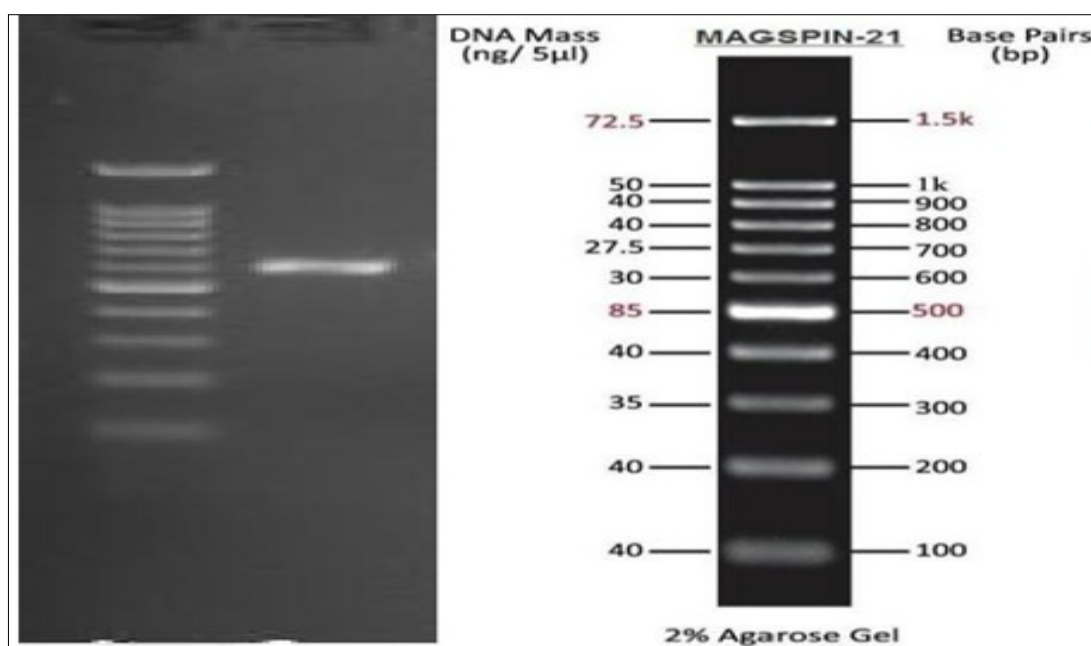


Fig. 1. 1.8 % agarose gel showing single 681 bp of 18S amplicon.

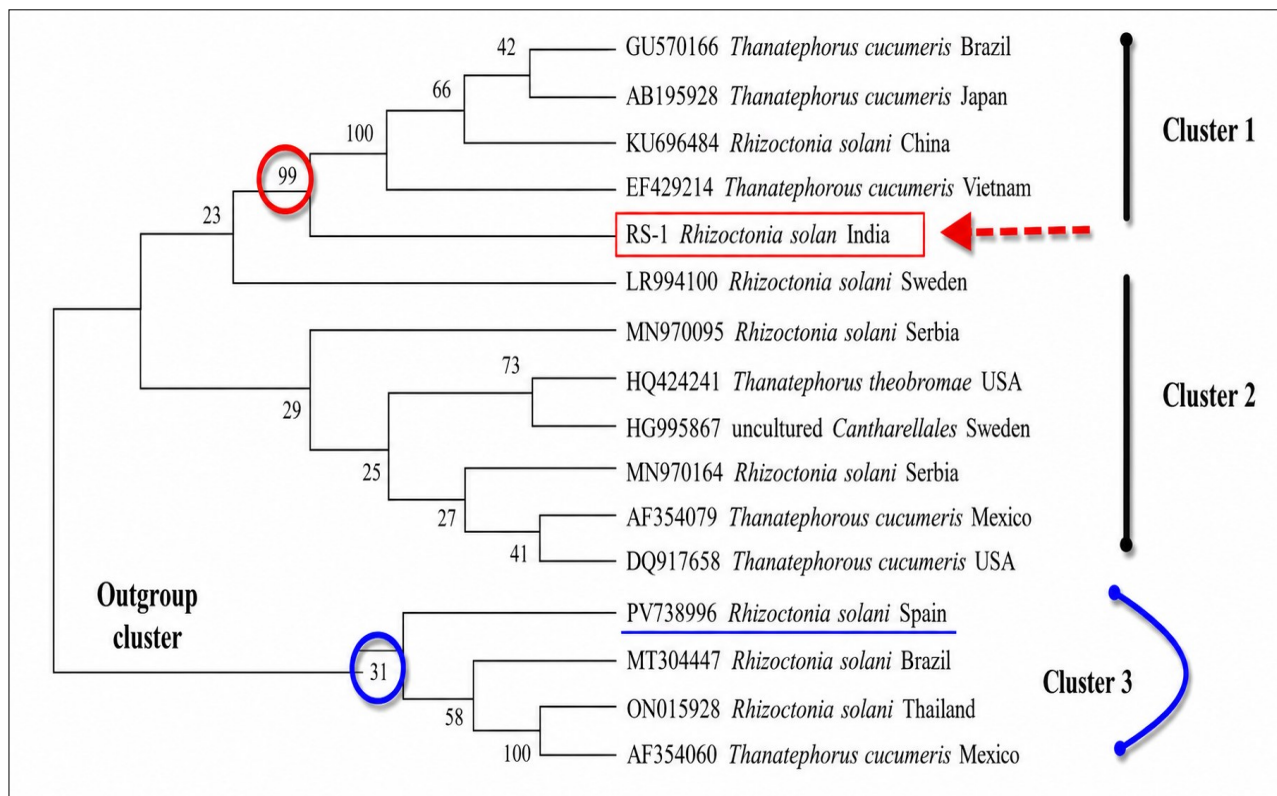


Fig. 2. Molecular phylogenetic analysis by maximum likelihood method.

isolates belonging to Cluster 3, including *R. solani* from Spain (PV738996) formed a distinct outgroup cluster, separated from the RS-1 clade with a bootstrap support of 31 % as shown in the Fig. 2.

Symptomatic characterisation

Field effects were systematically monitored and photos of sheath blight symptoms were taken. Rice sheath blight lesions initially appeared as small, water-soaked, oval to irregular spots on the leaf sheath near the water level. As the disease progressed, the lesions enlarged and developed greyish-white centre with brown margins. The lesions gradually extended vertically along the sheath and spread to the leaf blades. In severe infections, multiple lesions coalesced to form large necrotic areas, resulting in drying of the sheath and leaves, followed by wilting and lodging of the plants as shown in the Fig. 3. Those are the symptoms of sheath blight caused by *R. solani*.

Pathogenicity test

The pathogenicity of the test fungus was confirmed by performing *in vitro* and *in vivo* experiments. In the detached leaf test, the control did not show any symptoms, but the first infection symptoms appeared in the inoculated leaf after 1–3 days. The lesions gradually progressed, from water-soaked lesions to typical necrotic lesions. After 5–7 days, typical sheath blight symptoms including brown lesions and tissue disintegration were observed along with sclerotia formation, indicating successful pathogen penetration as shown in the Fig. 4.

Similar symptoms were evident in greenhouse-inoculated rice plants. The disease started from the wounded sheath area at the water level and progressed upwards. Sheath blight symptoms, such as long lesions and rotting of the sheath tissues, occurred at high humidity (Fig. 5). The reproduction of symptoms in the two tests indicated the pathogenicity of the *R. solani* isolate.

Screening of a resistance cultivar

Field screening of rice germplasm to sheath blight resistance during three consecutive seasons (2023–25) of the kharif showed a significant variation in the disease response depending on PDI, AUDPC, disease rating and disease score class, as shown in Table 2. In the kharif 2023 season, the PDI ranged from between 23.07 to 44.14 and AUDPC between 131.67 to 1265.69 and just a few genotypes exhibited a resistant reaction with most being susceptible to highly susceptible. In kharif 2024 PDI values were between 10.47 to 21.43, but AUDPC between 195.41 to 1170.38, only a few genotypes were stable regarding their resistance and most of them were in moderately and highly susceptible ranges. The wider variation was in kharif 2025 with 3.04–26.77 PDI and 126.8–1412.7 AUDPC values with few entries recording resistance and very low disease intensity, but a high proportion again recorded susceptible and highly susceptible responses with very high AUDPC (> 722). The expression of resistant or moderately resistant was consistently conferred by a few genotypes across seasons, whereas many showed variations indicating a role of the environment in the expression of the disease. On the whole, AUDPC changed gradually to highly susceptible categories, which proves its validity as a cumulative measure of disease progression and the findings point to the lack of consistent sources of resistance.

Correlation analysis

Correlation analysis as shown in Fig. 6, revealed that the DS and PDI showed a strong and positive correlation at all stages, with coefficients of $r \approx 0.48$ – 0.95 and $r \approx 0.63$ – 0.94 respectively, which illustrates a consistency in disease progression. Regarding agronomic characteristics, early-stage disease was positively related to yield (PDI10; $r \approx 0.35$), which implied a small effect in the early stages. Nevertheless, later at the later stages, both DS and PDI showed negative correlation with yield, specifically, PDI60 ($r \approx -0.34$).

Table 2. Screening of rice sheath blight resistant under field condition of season 2023–25

Sl. No.	Genotypes	Kharif 2023				Kharif 2024				Kharif 2025			
		PDI	AUDPC	DR	S	PDI	AUDPC	DR	S	PDI	AUDPC	DR	S
1	Swarna swoubhagya	30.22	131.67	R	1	18.2	195.41	R	1	6.16	281.7	MR	2
2	NLR 33892	25.45	169.34	R	1	17.58	195.51	R	1	3.42	126.8	R	1
3	MTU 1156	36.47	334.19	MR	2	19.8	310.68	MR	2	13.99	712.1	S	4
4	CR 411	37.38	313.19	MR	2	15.78	312.57	MR	2	25.10	1280.4	HS	5
5	CR 311	23.98	318.19	MR	2	21.21	326.21	MR	2	8.43	418.8	MS	3
6	MTU 1016	34.04	252.73	MR	2	16.96	295.78	MR	2	7.38	255.6	MR	2
7	Govindbhog	35.7	354.94	S	4	15.08	365.56	S	4	13.09	539.15	S	4
8	CR 1017	29.26	438.84	S	4	17.62	386.09	S	4	3.04	162.15	R	1
9	MTU 1318	44.14	408.26	S	4	15.81	381.78	S	4	12.58	611.1	S	4
10	MTU 1075	31.84	407.07	S	4	19.58	404.6	S	4	15.71	616.1	S	4
11	RGL 2537	42.03	356.73	S	4	20.19	406.57	S	4	10.58	422.55	MS	3
12	KNMM 1638	37.93	378.38	S	4	17.63	423.29	S	4	20.19	1028.6	HS	5
13	MTU 1061	30.88	406.31	S	4	13.78	428.75	S	4	14.23	643.95	S	4
14	MTU 5204	37.98	439.8	S	4	14.93	416.33	S	4	14.74	563.85	S	4
15	RGL 1880	39.97	420.3	S	4	19.71	399	S	4	14.28	700.4	S	4
16	MTU 1121	34.16	377.74	S	4	10.47	385.74	S	4	9.88	414.4	MS	3
17	GRGL 11226	29.64	608.28	MS	3	16.94	610.79	MS	3	11.67	562.85	S	4
18	RGL 2538	42.73	619.6	MS	3	19.26	613.28	MS	3	11.32	565.55	S	4
19	NLR 34449	23.84	589.77	MS	3	14.33	541.22	MS	3	12.13	551.1	S	4
20	MTU 1156	31.42	641.29	MS	3	21.43	656.86	MS	3	18.83	917.7	HS	5
21	IR 64	36.64	636.4	MS	3	13.25	623.96	MS	3	25.82	1334.7	HS	5
22	BPT 2782	28.31	458.62	MS	3	16.76	575.17	MS	3	12.30	592.1	S	4
23	MTU 1224	38.75	708.64	MS	3	18.94	537.3	MS	3	23.87	1209.9	HS	5
24	BPT 3082	27.55	688.22	MS	3	10.66	589.03	MS	3	13.79	714.75	S	4
25	MTU 1210	36.31	608.42	MS	3	14.19	599.76	MS	3	23.20	1191.3	HS	5
26	RNR 15048	23.07	624.67	MS	3	14.12	636.55	MS	3	25.47	1330.2	HS	5
27	NLR 3354	40.6	1265.69	HS	5	16.61	601.38	MS	3	22.97	1172.8	HS	5
28	CR	41.08	1156.26	HS	5	15.55	621.89	MS	3	20.63	975.8	HS	5
29	Marvel 1011	38.5	1066.98	HS	5	19.15	1032.29	HS	5	13.68	721.5	HS	5
30	CR 315	26.77	917.51	HS	5	17.45	824.88	HS	5	19.69	1068	HS	5
31	RNR Marvel	26.81	916.21	HS	5	12.72	1137.82	HS	5	20.29	1118	HS	5
32	MTU 101	38.81	968.59	HS	5	18.75	839.51	HS	5	24.56	1280.4	HS	5
33	Soubhagya	41.72	1053.41	HS	5	18.94	950.92	HS	5	21.52	1034.6	HS	5
34	CR Dhan 308	32.05	905.93	HS	5	14.69	1101.23	HS	5	23.90	1194	HS	5
35	Mohani	37.85	1221.26	HS	5	19.41	872.35	HS	5	19.46	998.2	HS	5
36	CR Dhan 319	28.57	1044.43	HS	5	14.92	898.59	HS	5	21.82	1132.3	HS	5
37	MTU 1010	41.25	814.5	HS	5	18.93	986.52	HS	5	20.88	1122.5	HS	5
38	RGL 2624	26.26	1141.71	HS	5	15.15	1007.54	HS	5	26.77	1412.7	HS	5
39	Manipuri local	33.44	946.1	HS	5	15.32	901.11	HS	5	21.43	1041.5	HS	5
40	RNR 15048	40.52	879.1	HS	5	18.92	1170.38	HS	5	25.60	1337.1	HS	5
41	Juvraj Dhano	31.17	892.77	HS	5	20.01	1099.14	HS	5	20.92	1001.7	HS	5
42	Puja	31.2	1158.61	HS	5	15.99	944.46	HS	5	26.10	1370.4	HS	5
43	Lal Basmati	25.97	996.82	HS	5	11.92	1017.03	HS	5	16.95	817.8	HS	5

PDI: percent disease index; AUDPC: area under the disease progress curve; DR: disease rating; S: susceptible.

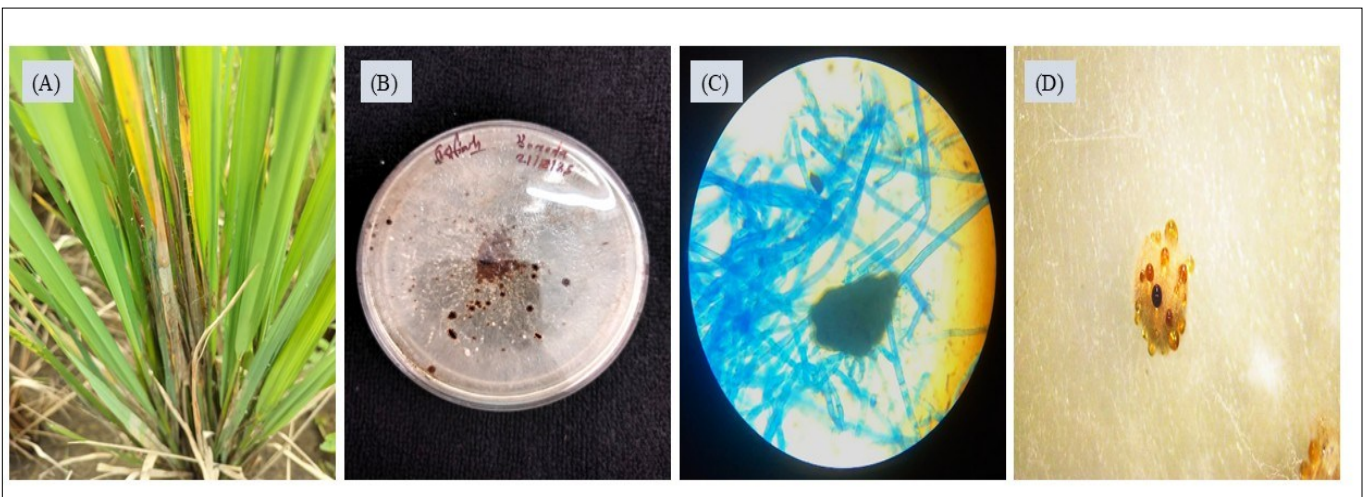


Fig. 3. Symptoms and morphological characterisation of sheath blight pathogen (*Rhizoctonia solani*) in rice. A: Typical sheath blight symptoms on rice plants under field conditions; B: Fungal colony growth on culture medium; C: Microscopic view of fungal hyphae; D: Stereoscopic view of sclerotia shows the exudates.

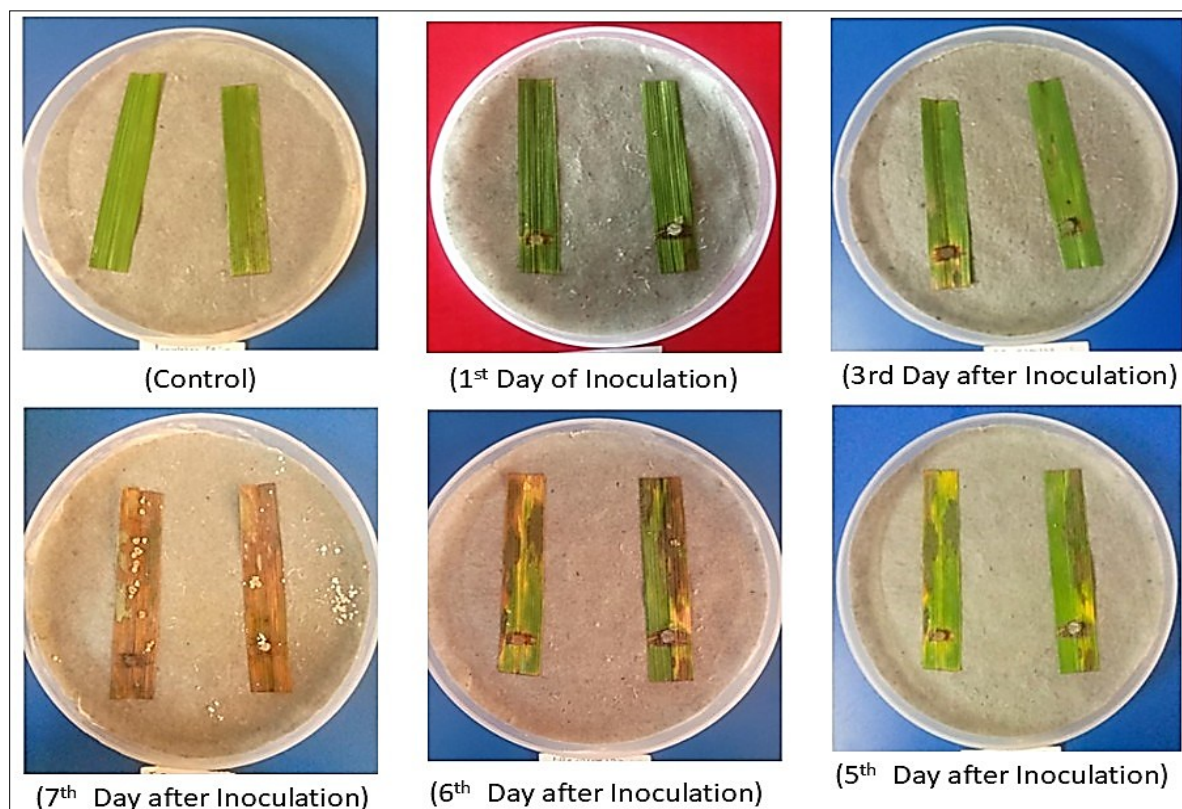


Fig. 4. Pathogenicity test of *Rhizoctonia solani* on *Oriza sativa* by detach leaf assay. 1st (days after inoculation) DAI- Initial infection starts at the point of inoculation, 3rd DAI- Turn light green appearance with increased lesions find, 5th DAI- Gradual increase of infection locus, 6th DAI- Gradual increase of infection locus, 7th DAI- Totally destroyed tissue along with sclerotia formation.



Fig. 5. Pathogenicity of *Rhizoctonia solani* on rice plants under pot conditions (Symptoms start appearing on the sheath near the waterline).

and PDI70 ($r \approx -0.38$) and productivity decreased with the progression of the disease. Likewise, the number of grains per panicle ($r \approx -0.23$) and the mass of 1000 grains ($r \approx -0.09$) were significantly correlated with increased disease levels but plant height showed no significant correlation ($r \approx 0.03$) as shown in Fig. 6. All in all, these findings indicate that more severe disease at advanced stages of growth has a great impact on yield and its related characteristics.

Performance evaluation model with R^2 and root mean square error.

Linear regression

Among all evaluated models, linear regression achieved the highest predictive performance, recording the greatest coefficient of determination ($R^2 = 0.8178$) and the lowest prediction error (RMSE = 3.852), reflecting strong agreement between observed

and predicted values (Fig. 7).

Decision tree

The decision tree model also showed good predictive capacity ($R^2 = 0.8077$), although prediction residuals were more dispersed, resulting in a higher RMSE (4.242) compared with Linear Regression (Fig. 8).

Random forest

Random forest produced stable predictive performance ($R^2 = 0.8010$; RMSE = 4.148), though its explanatory power was marginally lower than linear regression and decision tree models (Fig. 9).

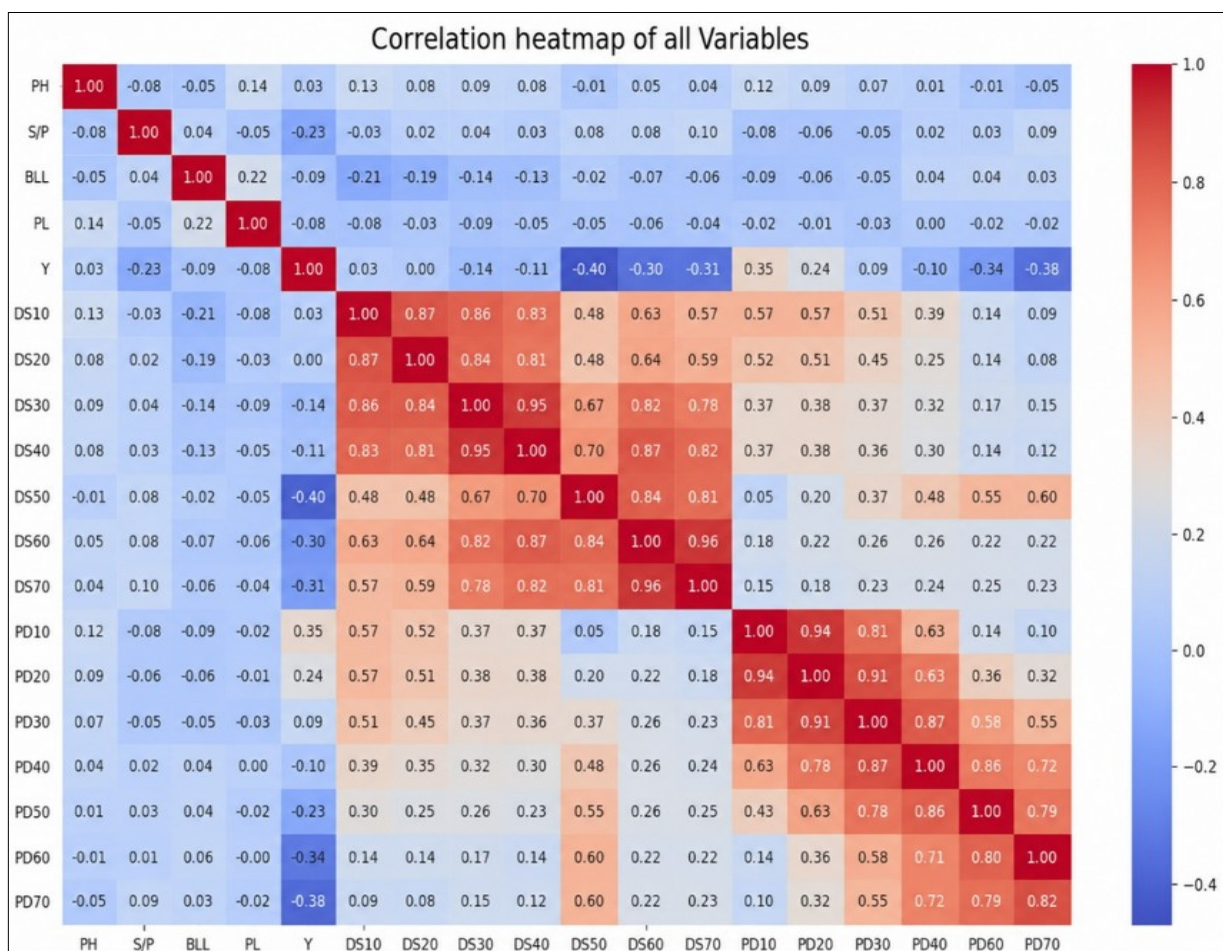


Fig. 6. Correlation heatmap of agronomic traits, disease severity, percent disease index and yield variables.

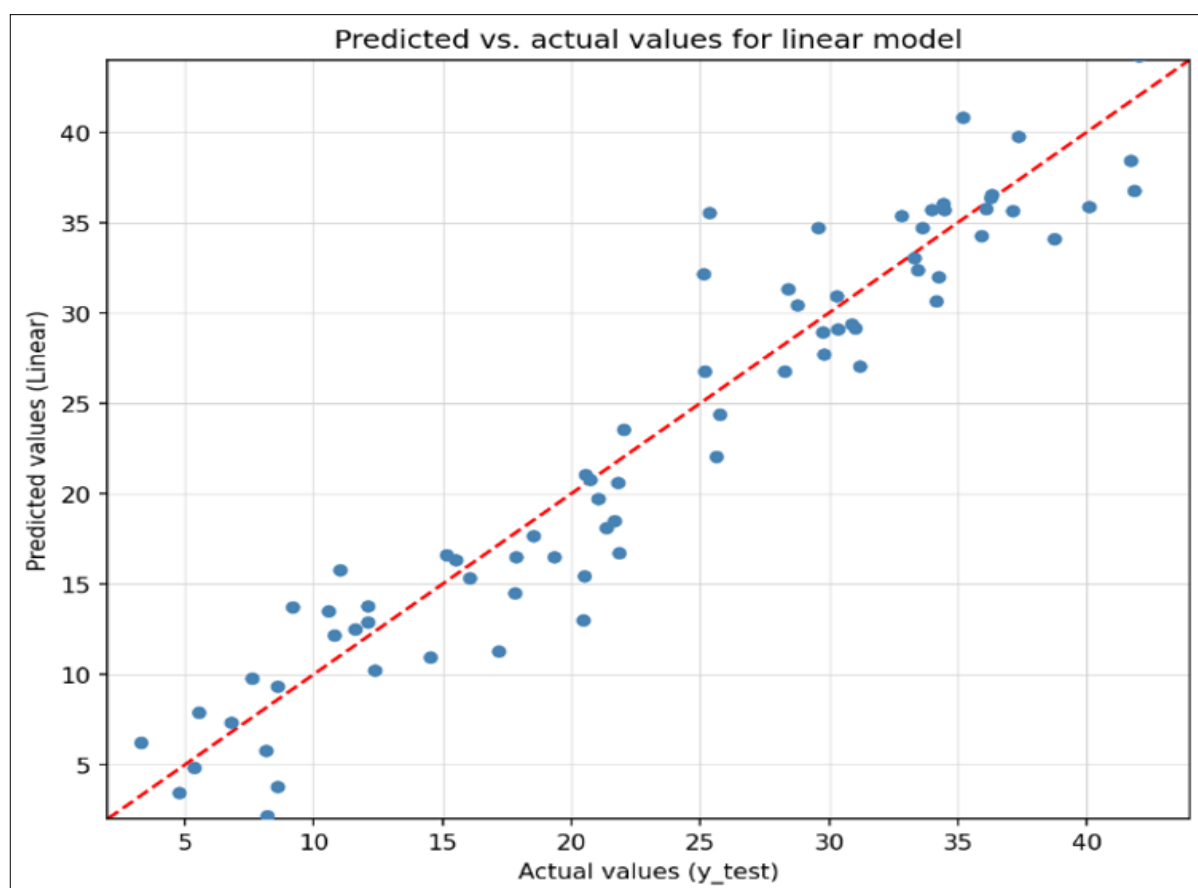


Fig. 7. Predicted vs. actual values for linear regression model.

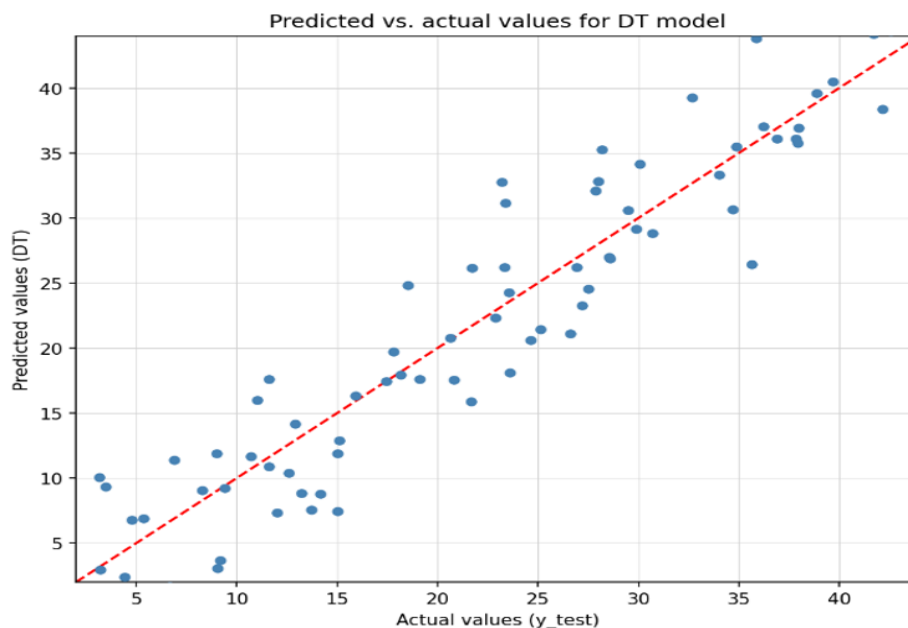


Fig. 8. Predicted vs. actual values for decision tree.

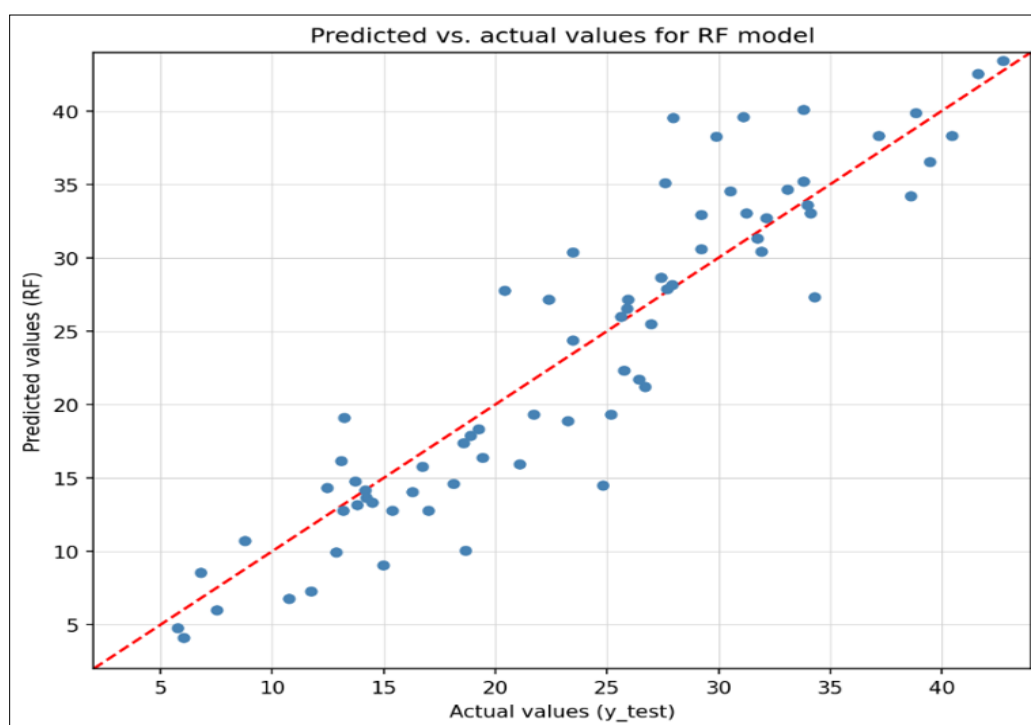


Fig. 9. Predicted vs. actual values for random forest model.

Gradient boosting

The spread in predictions in gradient boosting model is relatively wider. It had the weakest R^2 of 0.7899 and RMSE of 4.141, which means it was the least predictive model compared to the rest of the models (Fig. 10).

Overall interpretation

Of all the models, linear regression is the best in making predictions with the highest R^2 and lowest RMSE which is also graphically proved by the fact that the clustering of the points along the ideal fit line is the closest. The other models though showing an increased dispersion and less explanatory power are

capable as shown in the Fig. 11. The superior performance of linear regression suggests a predominantly linear association between disease progression metrics and yield-related responses in the evaluated germplasm set.

Model performance comparison

Linear regression confusion matrix

The confusion matrix of the linear regression model shows that it is an effective model of categorising disease severity. The model exhibits good performance with regards to predicting the HS class with 12 instances being correctly classified and only a small number misclassified. Likewise, the MS category is fairly well

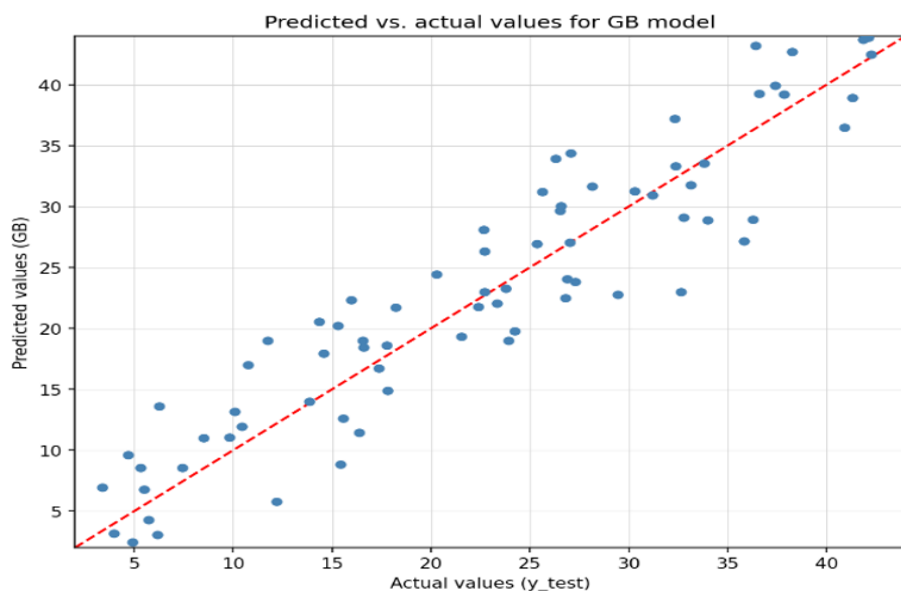


Fig. 10. Predicted vs. actual values for gradient boosting model.

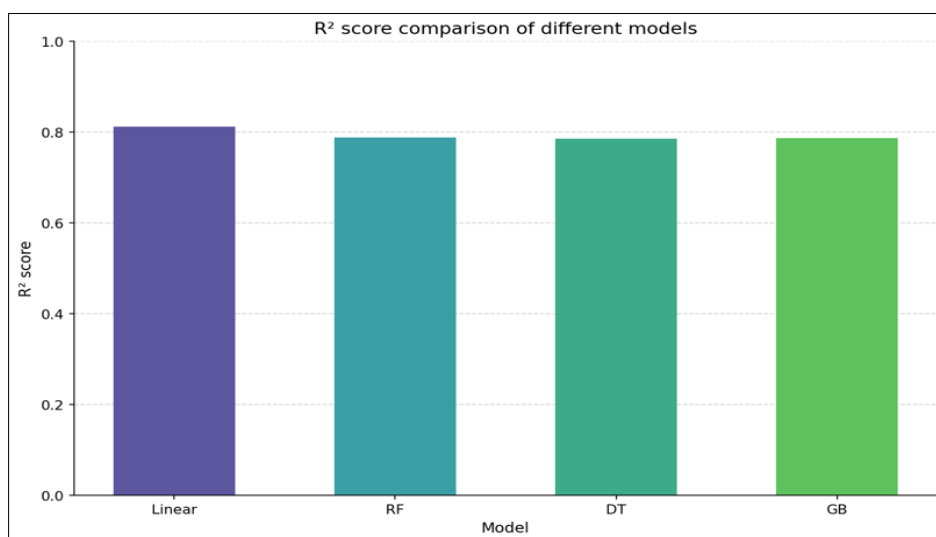


Fig. 11. Comparative analysis of R^2 scores across four predictive models (linear regression, random forest, decision tree and gradient boosting), highlighting the highest predictive accuracy achieved by the linear regression model.

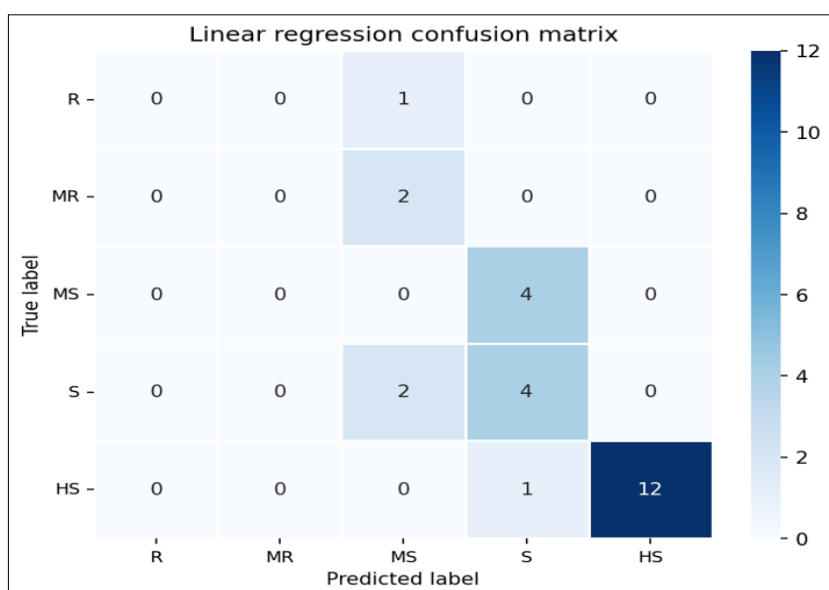


Fig. 12. Confusion matrix of the linear regression model showing classification performance across disease severity categories (resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), highly susceptible (HS)).

forecasted, having 4 right classifications. The S group has average performance showing 4 correct classifications, but it has some confusion with the other similar categories like MS. It has, however, been observed that the model is less precise with the R and MR categories with most of the cases being misclassified as the MS, as shown in the Fig. 12. Irrespective of these shortcomings, the model exhibits a fairly stable pattern of classification, which justifies its appropriateness to predict disease severity.

Random forest confusion matrix

According to the confusion matrix of the random forest model, the bias is very significant with regards to certain classes, especially to HS and S classes. The HS class was perfectly classified and 13 instances were correctly predicted and none misclassified. Likewise, all the observations on the S class and also on the MS, MR and R classes, were largely grouped under the S category. Although this means that dominant classes are highly accurate, it also points to a serious drawback of this model conflicting with the desire to classify the level of different degrees of disease severity. The lack of accurate predictions of MR, MS and R classes indicates low sensitivity to intermediate and less severe classes as shown in Fig. 13. Generally, the random forest model has a low generalisation potential, since it is prone to overfitting particular classes instead of the entire variability in the data set.

Discussion

Molecular identification, pathogenicity confirmation and multi-season phenotyping collectively established a robust framework for evaluating sheath blight resistance in rice landraces. The pathogenicity of the isolate was evaluated by inoculating it on rice plants *in vitro* and in the greenhouse, resulting in the development of typical sheath blight symptoms, thus satisfying Koch's postulates similar results were reported by early researchers (32). The emergence of symptoms 1–3 days post inoculation and subsequent lesion enlargement and sclerotia development by 5–7 days post inoculation demonstrates the pathogen's aggressiveness. Similar symptoms under pot conditions further validated the pathogenicity of the isolate under conducive environmental conditions, including

high humidity and temperature (25–30 °C), which are conducive for disease development. In the field, a total of 43 rice genotypes were screened for three consecutive kharif seasons (2023–25) and a wide range of response was observed. The PDI ranged from 23.07–44.14 (2023), 10.47–21.43 (2024) and 3.04–26.77 (2025), while AUDPC values varied widely from 131.67 to 1412.7, indicating differential disease progression across genotypes and seasons. A few genotypes (NLR 33892 and Swarna Swoubhagya showed consistent resistance reactions while most of the genotypes were S and HS, similar results were reported by previous researchers (33). The rising AUDPC values (>722 in many entries) reflect the cumulative pathogen load and the suitability of AUDPC to measure disease progression. Variation in resistance expression across seasons indicates that disease development and host-pathogen relationship is highly influenced by the environment, similar results were reported by previous researchers (34). The correlation analysis also showed positive correlations between the DS and PDI ($r \approx 0.48$ – 0.95 and 0.63 – 0.94 , respectively) at different stages of growth, which indicated progressive disease development. Yield was positively correlated at early stages (PDI10; $r \approx 0.35$) and negatively correlated at later stages (PDI60: $r \approx -0.34$; PDI70: $r \approx -0.38$) of growth, suggesting that disease severity is more pronounced in later stages of growth. Likewise, grain number per panicle ($r \approx -0.23$) and 1000-grain weight ($r \approx -0.09$) showed a negative correlation with disease severity, suggesting yield reduction due to infection. Plant height had a weak association ($r \approx 0.03$), indicating that it is less affected by sheath blight. Predictive modelling using machine learning showed that the linear regression model showed the highest performance with the highest ($R^2 = 0.8178$) and lowest RMSE (3.852), suggesting it is the best predictor with the least error. Decision tree ($R^2 = 0.8077$; RMSE = 4.242) and random forest ($R^2 = 0.8010$; RMSE = 4.148) models also performed well, but with increased variability. The worst-performing model was gradient boosting ($R^2 = 0.7899$; RMSE = 4.141), implying a lower level of prediction in this dataset. Moreover, confusion matrix data indicated that the linear regression model provided equivalent classification for HS and MS classes while random forest exhibited a bias towards major classes with a higher proportion (particularly HS), suggesting overfitting and poor discrimination of intermediate classes. In summary, the use of molecular and pathogenicity studies,

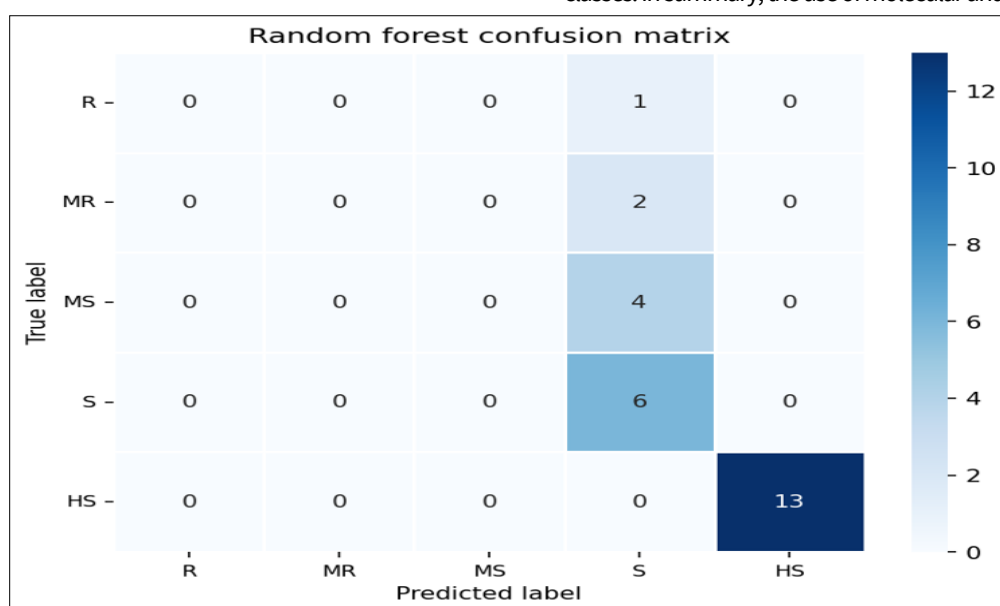


Fig. 13. Confusion matrix of the Random Forest model illustrating classification performance and class prediction distribution across disease severity categories (resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), highly susceptible (HS)).

multi-season field screening and predictive modelling offers an overall picture of the sheath blight disease and resistance variability to the disease in rice. Combining epidemiological phenotyping with predictive modelling offers a scalable framework for identifying stable resistance donors and accelerating sheath blight resistance breeding in rice improvement programmes.

Conclusion

Marked seasonal variation in disease response was observed among rice landraces, although genotypes such as NLR 33892 and Swarna Swoubhagya showed comparatively stable resistance across seasons. The disease had high correlation with yield loss in later plant growth stages. Among predictive models, linear regression provided the strongest predictive performance ($R^2 = 0.8178$), outperforming tree-based ensemble approaches. This study stresses the need for genetic stability in resistant rice varieties and the importance of area under disease progress curve (AUDPC) and predictive models in disease control. Furthermore, the identification of stable resistant genotypes provides valuable genetic resources for rice breeders to develop sheath blight-resistant cultivars through resistance breeding programs, thereby contributing to sustainable rice production.

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Authors' contributions

KAB conducted the field trial, conceptualised the study, and prepared the original draft. SD contributed to conceptualisation, study framing, analysis, and approved the final draft. NR, RLM, SB, CB, and SD performed data analysis and reviewed the manuscript draft. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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