

## Phenotypic evaluation of sheath blight resistance using the maize meal sand inoculation method in 3k genome panel subset of rice (*Oryza sativa* L.)

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### ABSTRACT

Sheath blight, caused by *Rhizoctonia solani* Kühn, is a major constraint to rice (*Oryza sativa* L.) yield and grain quality. A subset of 364 rice genotypes from the 3k Rice Genome Panel along with four checks (Swarna, Tetep, HR12 and TN-1) was evaluated for resistance using the maize meal–sand inoculation method. The field experiment was conducted in an Augmented Randomized Complete Block Design with eight blocks and 30-day-old seedlings were transplanted at uniform spacing. A virulent isolate (SB-184) of *R. solani* was revived and used to prepare the inoculum, applied at the maximum tillering stage (30–45 days after transplanting). Disease severity was assessed at 21-day intervals using plant height, lesion length, relative lesion height (RLH) and percent disease index (PDI). RLH and PDI were calculated following standard procedures and disease scoring was done following the 0–9 IRRI SES scale (2013). Significant variation in sheath blight response was observed among genotypes, with several accessions exhibiting low RLH and PDI, indicating potential as donors for breeding resistant cultivars.

**Keywords:** *Maize meal–sand inoculation, Percent Disease Index PDI, 3K Rice Genome Panel, Relative Lesion Height RLH*

Rice (*Oryza sativa* L.,  $2n = 24$ ), a self-pollinated cereal crop belonging to the order Cyperales and family Poaceae, serves as the staple food for more than half of the world's population (Raney, 2004). There are 42 rice-producing countries throughout the world, China and India are the major rice producers (Fuller, 2011). Plants experience continuous exposure to abiotic and biotic stresses throughout their growth cycle. Although crop management practices can mitigate biotic and abiotic stress effects such as temperature extremes, ultraviolet radiation and water imbalance remain dominant yield-limiting factors under field conditions. Among biotic constraints, sheath blight (*Rhizoctonia solani* Kühn), blast (*Magnaporthe oryzae*) and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) are the most destructive diseases affecting rice productivity and grain quality.

Sheath blight is one of the most destructive diseases of rice and is regarded as a globally significant threat, ranking second only to blast disease in prevalence. Sheath blight, *Rhizoctonia solani* Kühn (ShB) of rice was first reported in Japan in 1910 by Miyake and has emerged as a significant disease in

rice growing regions, particularly under conditions of intensive cultivation (Srinivasachary *et al.*, 2011). In India, the first documented occurrence of sheath blight was in Gurdaspur and Punjab, reported by Paracer and Chahal in 1963, followed by a subsequent report from Uttar Pradesh by Kohli in 1966.

Characteristic symptoms of the disease are water-soaked, spherical to oval-shaped or irregularly elongated discoloured, greyish to light brownish lesions with brown margin on the leaf sheath and blades (Molla *et al.*, 2020). The pathogen is soil-borne and can cause severe disease in a variety of crops (Anderson *et al.*, 2017).

Disease onset typically occurs from the late tillering to the internodal elongation stages of crop development, reaching peak severity around the panicle initiation stage. Early symptoms are characterized by the development of lesions on the leaf sheath, which leads to tissue softening, reduced structural integrity, lodging and inhibition of grain filling (Wu *et al.*, 2012). Studies have reported yield losses from 5% to 50%, with severe outbreaks in susceptible cultivars under conducive conditions resulting in losses exceeding 50% (Savary *et al.*, 1995; Ou, 1985). The

pathogen impairs grain filling by damaging the leaf sheath and upper foliage during crucial reproductive stages, reducing photosynthetic efficiency and leading to incomplete grain development, spikelet sterility and plant lodging.

## MATERIAL AND METHODS

### Plant Material and Experimental Design

The study was conducted using 364 rice genotypes along with four check varieties (Swarna, Tetep, HR12 and TN-1). The field experiment was laid out in an Augmented Randomized Complete Block Design with checks repeated in each of the eight blocks. The experimental field measured 21 × 36 m and each genotype was planted in a 0.72 m<sup>2</sup> plot. Thirty-day-old seedlings were transplanted at a spacing of 20 cm × 15 cm with two rows per plot, 12 hills per row and in a row length of 1.8 m.

### Pathogen Culture and Inoculum Preparation

A highly virulent sheath blight isolate of *Rhizoctonia solani* Kühn was used for inoculation. The isolate was stored at “20°C and revived on sterile potato dextrose agar (PDA) plates, followed by incubation at 26°C for seven days to induce sclerotia formation. The maize meal–sand inoculum was prepared by mixing maize meal and sand in a 3:2 ratio, supplemented with 4 g sucrose and 200 ml distilled water per 300 g of mixture. The medium was sterilized by autoclaving on three consecutive days, cooled and inoculated with a quarter portion of a seven-day-old culture plate. The flasks were incubated in the dark at 25–28°C for ten days with periodic agitation to ensure uniform mycelial growth.

### Inoculation Procedure

Inoculation was carried out at the maximum tillering stage (30–45 days after transplanting). Approximately 5 g of the prepared inoculum was placed at the center of the tillers of three randomly selected plants per genotype and the tillers were gently tied to ensure inoculum retention. Disease observations were recorded on the same plants for plant height (PH), lesion length (LL), relative lesion height (RLH) and per cent disease index (PDI).

### Disease Assessment

Disease severity was assessed using the 0–9 scale of the International Rice Research Institute’s

Standard Evaluation System (SES, 2013). Scoring was carried out at 21-day intervals after inoculation. Relative lesion height (RLH) was calculated following Sharma and Teng (1990):

$$\text{RLH \%} =$$

$$\frac{\text{Maximum height at which lesion appears}}{\text{Plant height}} \times 100$$

\*Disease score was given based on RLH% according to SES (2013)

$$\text{PDI \%} =$$

$$\frac{\text{Sum of all ratings}}{\text{Total no. of observations} \times \text{Maximum rating scale}} \times 100$$

## RESULTS AND DISCUSSION

### Population distribution in 3K-RG panel mini-core Plant Height (cm)

Plant height ranged from 39 cm to 171 cm in the present study. The histogram for plant height shows a slightly right-skewed distribution, indicating that most genotypes have moderate to tall plant stature (100–140 cm), while few are shorter or extremely tall. The plant height is inversely proportional to relative lesion height (RLH), suggesting that taller plants tend to exhibit lower lesion development. Since plant height has been reported to influence sheath blight severity (Molla *et al.*, 2020) with taller plants often showing reduced relative lesion height due to reduced canopy humidity near the base. This variation could help in understanding the association between plant height and sheath blight resistance (Fig 1.a).

### Lesion Length (cm)

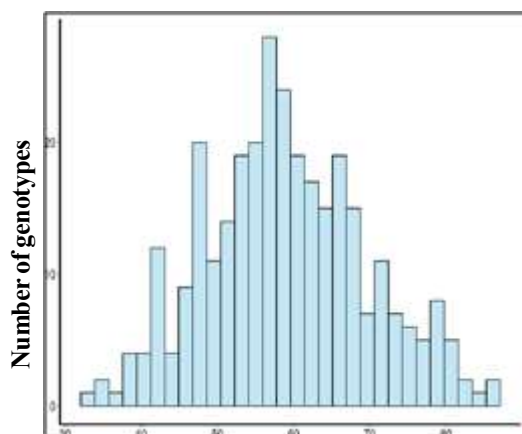
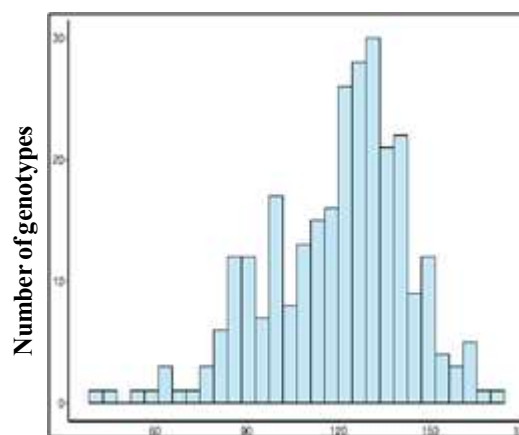
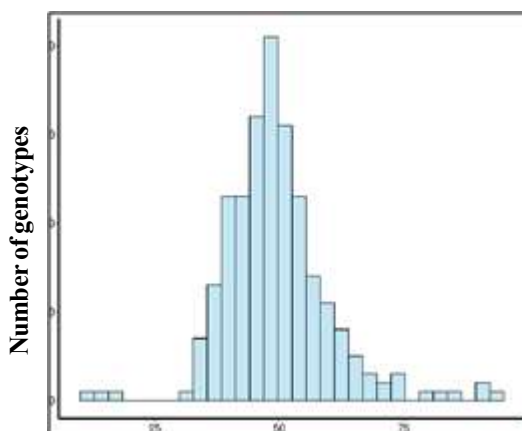
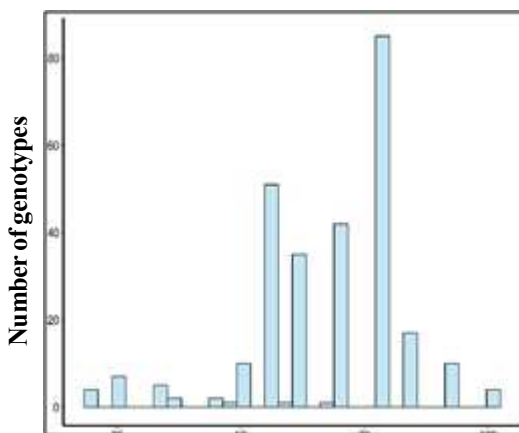
Lesion length ranged from 31.67 to 86.5 cm in the present study. The distribution for lesion length is normal, ranging between 40 to 75 cm, with most genotypes clustered around the mean (59.2 cm). The spread of values implies that the genotypes differ in their susceptibility levels and a few with shorter lesion lengths is considered as less susceptible (Fig 1.b).

### Relative Lesion Height (RLH%)

Relative lesion height ranged from 32.31% to 94.65% in the present study. The histogram for RLH shows a near-normal distribution, with most genotypes concentrated around the mean (H” 45–55%). This indicates continuous variation, suggesting

**Table 1. Standard Evaluation Scale (SES) - IRRI, 2013**

SCALE	RELATIVE LESION HEIGHT (%)
0	No infection
1	Vertical spread of the lesions up to 20% of plant height
3	Vertical spread of the lesions up to 21-30% of plant height
5	Vertical spread of the lesions up to 31-45% of plant height
7	Vertical spread of the lesions up to 46-65% of plant height
9	Vertical spread of the lesions up to 66-100% of plant height

**a) Plant Height (cm)****b) Lesion Length (cm)****c) Relative Lesion Height (%)****d) Per cent Disease Index**

**Fig.1. Histogram plot for disease traits a) Plant height b) Lesion length c) Relative lesion height d) Per cent disease index**

that sheath blight resistance is quantitatively inherited, controlled by multiple genes. A few genotypes exhibited low RLH values, indicating potential sources of partial resistance (Fig 1.c).

#### **Percent Disease Index (PDI%)**

Per cent disease index ranged from 18.52% to 100% in the present study. The PDI histogram

displays an irregular distribution, indicating wide variation among genotypes for disease severity. Some genotypes show low PDI values (indicating moderate resistance), while others show high values (indicating susceptibility). The lack of a smooth normal curve suggests environmental influence or differential genetic response affecting disease expression. Such variation is desirable for association mapping, as it reflects

distinct phenotypic classes ranging from resistant to susceptible types (Fig 1.d).

The population distribution of the 3K-RG panel mini-core revealed substantial phenotypic variability for plant height and sheath blight-related traits, indicating a broad genetic base. The near-normal distributions of plant height, lesion length and relative lesion height suggest quantitative inheritance and continuous variation, while the irregular distribution of PDI reflects strong environmental and genetic interactions influencing disease expression. The presence of genotypes with low lesion length, RLH and PDI values highlights potential sources of partial resistance. Overall, this wide and structured variation is ideal for dissecting trait associations and identifying genomic regions linked to sheath blight resistance through association mapping.

### Subpopulation distribution in 3K-RG panel mini-core

#### Plant Height (cm)

Plant height varied widely among rice subpopulations. The *admix* group showed plant heights ranging from 90 to 137 cm with a mean of 113 cm indicating moderate variability, *aromatic* genotypes exhibited a relatively narrow range (108–161.67 cm) with a lower mean of 109 cm. The *aus* subpopulation displayed wider variation with heights ranging from 63 to 165 cm and a higher mean of 129 cm, *indica* showed the broadest range (49–171 cm) and the highest mean plant height of 130 cm, reflecting high phenotypic diversity and *Japonica* also showed substantial variation, ranging from 39 to 161.5 cm with a mean of 119 cm (Fig 2.a).

#### Lesion Length (cm)

Lesion length showed considerable variation across rice subpopulations. The *admix* group showed lesion lengths ranging from 47 to 74.67 cm with a mean of 63 cm, indicating moderate disease severity, *aromatic* subpopulation exhibited a relatively narrower range (47.33–72.67 cm) with a lower mean lesion length (59 cm) suggesting comparatively better tolerance, *aus* group displayed wider variation, with lesion lengths ranging from 42.33 to 86.5 cm and a higher mean of 66 cm, indicating greater susceptibility, *indica* subpopulation showed a comparatively lower range (31.67–86.33 cm) with the lowest mean lesion length (58 cm), reflecting better resistance, *japonica*

group also showed substantial variation, with lesion lengths ranging from 40.33 to 83 cm and a mean of 62 cm (Fig 2.b).

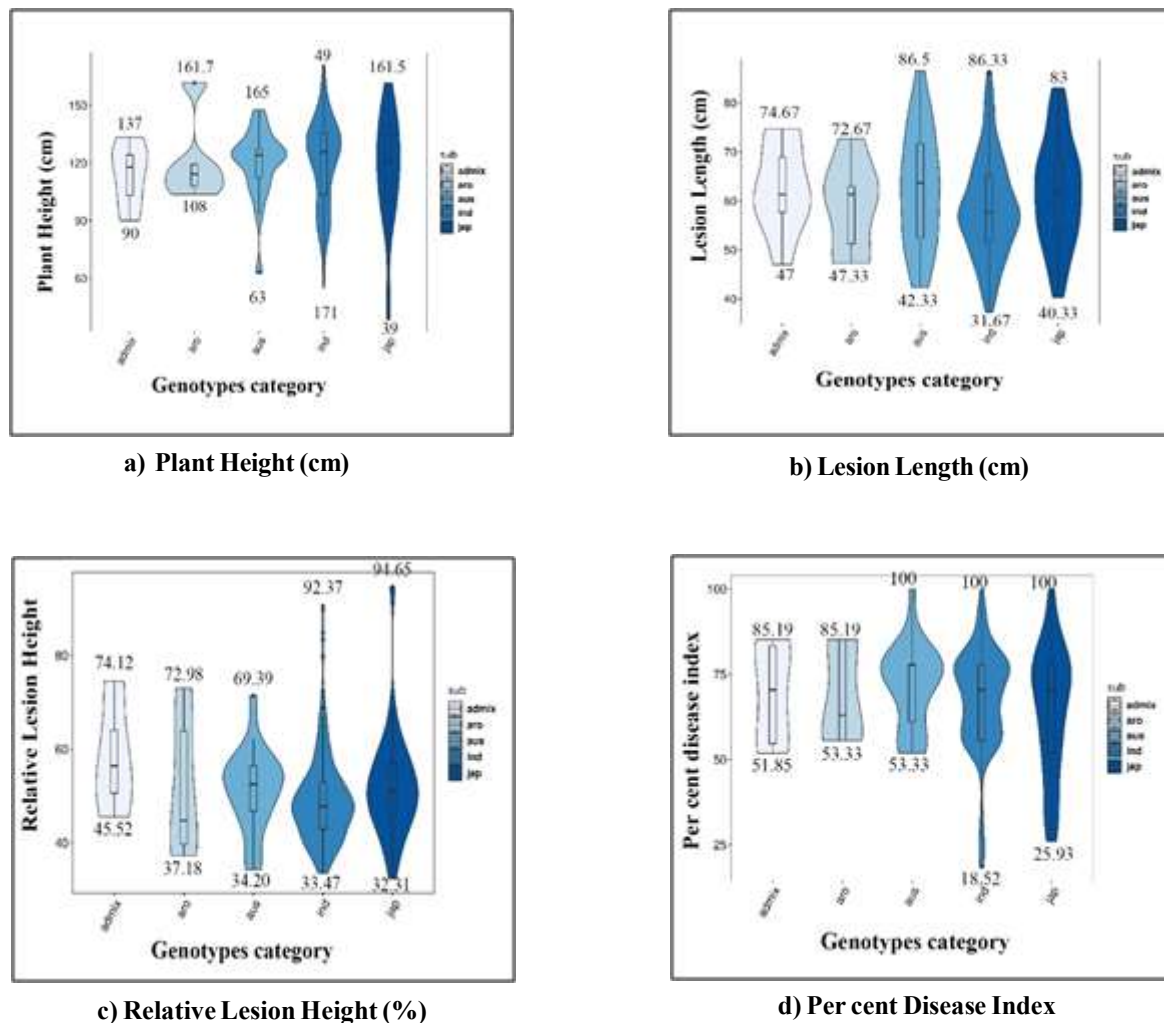
#### Relative Lesion Height (%)

The *admix* group exhibited RLH values ranging from 45.52 to 83 per cent, with a mean of 58 per cent, indicating moderate disease severity, *aromatic* subpopulation showed a comparatively lower range (37.18–72.98%) and the lowest mean RLH (44%) suggesting better resistance, *aus* group displayed moderate variation, with RLH values ranging from 34.20 to 69.39% and a mean of 55%, *indica* subpopulation showed a wide range (33.47–92.37%) with a lower mean RLH (45%), reflecting the presence of relatively resistant genotypes despite high variability and *japonica* group also exhibited substantial variation, with RLH ranging from 32.31 to 94.65 per cent and a mean of 49 per cent (Fig 2.c).

#### Per Cent Disease Index (%)

The *admix* group showed PDI values ranging from 51.85 to 85.19 per cent, with a mean of 69 per cent, indicating moderate to high disease severity, *aromatic* subpopulation exhibited a similar range (53.33–85.19%) with a slightly lower mean PDI (68%), *aus* group displayed wider variation with PDI values ranging from 53.33 to 100 per cent and a higher mean of 73 per cent, reflecting greater susceptibility, *indica* subpopulation showed the broadest range (18.52–100%) with a mean PDI of 70 per cent, indicating the presence of both resistant and highly susceptible genotypes and *japonica* group also showed substantial variation, with PDI values ranging from 25.93 to 100 per cent and a mean of 69 per cent (Fig 2.d).

The subpopulation-wise analysis of the 3K-RG panel mini-core revealed marked differences in plant height and sheath blight-related traits, reflecting distinct genetic backgrounds among rice subpopulations. *Indica* and *japonica* groups exhibited the widest phenotypic variation, indicating the presence of both resistant and susceptible genotypes, while *aromatic* genotypes consistently showed lower lesion length, RLH and PDI, suggesting comparatively better tolerance. The *aus* subpopulation generally recorded higher mean lesion length and PDI, indicating greater susceptibility. Overall, the wide and differential trait distribution across subpopulations provides



**Fig. 2.** Violin plot showing subpopulation distribution for disease traits (a) Plant height (b) Lesion length (c) Relative lesion height (d) Per cent disease index

valuable opportunities for identifying subpopulation-specific sources of sheath blight resistance and for exploiting genetic diversity in association mapping and resistance breeding programs.

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