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PATHO-PHENOTYPING OF A BREEDING POPULATIONDERIVED FROM A NOVEL RICE SHEATH BLIGHT DISEASE TOLERANT DONOR

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ABSTRACT

Sheath blight disease, caused by the fungus *Rhizoctonia solani* (AG1-IA), is a prevalent infection that poses a significant threat to rice plants, resulting substantial economic losses. So far, no complete resistance has been identified for this destructive disease. The primary method of managing sheath blight is through chemical control, which, while cost-effective, can have adverse effects on the environment. In this regard, a population of 330 Recombinant Inbred Lines (RILs) was developed by cross between Improved Samba Mahsuri (ISM) and INGR21112. ISM has been noted for its resistance to Bacterial Leaf Blight (BLB) but highly susceptible to sheath blight, having medium slender grains. INGR21112 is a novel sheath blight tolerant landrace from North East India. 330 RILs were evaluated against sheath blight through artificial inoculation by standard typha bit method under field during *Kharif*-2017. Among 330 RILs 27 were identified as a resistant with 3 score, 188 were identified as a moderately resistant, 87 were as susceptible and 28 were as highly susceptible. Identified resistant/tolerant RILs will be used for the sheath blight resistant breeding programs.

Key words: Rice, sheath blight, *Rhizoctonia solani*, tolerant

Introduction

Rice accounts for 29% of the world's total cereal production and is one of the major staple foods consumed by 70% of the world's population and occupies one-fifth of the total land covered under cereal crops. India is the second largest rice producing country, grow rice in an area of about 47.5 million ha and produces around 136.7 million tonnes 2873 with productivity of Kg/ha (UPAG, 2023). Rice occupies an important position in the food security of India and would continue to remain so due to its broader adaptability to diverse agro-ecology. This important crop is highly susceptible to various diseases caused by fungi, bacteria, viruses, nematodes, and physiological disorders. These factors contribute to an annual loss of 12 to 25 percent of total production, with fungal diseases alone accounting for 12 to 20 percent of the damage (Gupta *et al*., 2014). Among the fungal diseases affecting rice, sheath blight (ShB) disease caused by *R. solani* is a significant concern. ShB caused by *Rhizoctonia solani* (AG1-IA) Kühn (Teleomorph: *Thanatephoruscucumeris* (Frank) Donk) has a wide spread impact on rice production worldwide (Li *et al*., 2021). *R. solani*, a soil-borne semi-saprophytic fungus, was first identified as a parasite of potato plants by Kühn in 1858 (Almasia *et al*., 2008). It has a wide host range, infecting over 33 plant families of 188 genera (Srinivasachary *et al*., 2011; Sattari *et al*., 2014). It was first reported in Gurdaspur, Punjab, India by Paracer and Chahal in 1963. This pathogen infects the sheaths of rice plants, leading to the production of circular to oval necrotic lesions from the sheaths to the flag leaf. In severe cases, the emergence of panicles is hindered, resulting in yield losses and economic impacts on rice production.

Yield losses due to this disease ranged from 1.2 to 69.0 percent (Naidu, 1992). The disease causes a yield reduction ranging from 20 to 50% depending on the severity of infection (Groth and Bond, 2007; Margani and Widadi, 2018). Various strategies are being implemented to combat this disease. While the use of chemicals is often considered the best approach, their adverse effects on the ecosystem and high costs make it challenging to rely on them for disease management (Yellareddygari *et al.*, 2014). Additionally, the excessive use of fungicides can lead to the development of fungicidal resistance in pathogens (Dath, 1990). Presently, ShB occurs frequently in all seven rice growing zones and fifteen agro-climatic zones of India (Prakasam *et al.*, 2013).

The fungus's genetic variability, wide host compatibility, and ability to persist through the formation of dormant sclerotia from one crop season to the next have further complicated control efforts (Singh *et al.*, 2019). Limited information is available about the donors having high degree of resistance and inheritance of disease resistance. Cultivars with desired level of resistance are not available for commercial cultivation throughout the world (Prakasam *et al.*, 2021). To meet these goals, urgent need to identify resistant or tolerant donors from various rice breeding materials, landraces, and wild rice accessions. This method is environmentally safe and reliable. Keeping these things in view, the present study, a set of 330 RILs were tested their resistance potential against sheath blight during *Kharif*-2017.

Materials and Methods

Rice RIL population

A population of 330 Recombinant Inbred Lines (RILs) was developed at the ICAR-Indian Institute of Rice Research, Hyderabad (17.32010° N latitude and 78.39390° E longitude). This population was developed through a cross between the female parent Improved Samba Mahsuri (ISM) and male parent INGR21112 (Wazuhophek) a novel sheath blight resistant/tolerant landrace from the North Easternpart of India.

Improved Samba Mahsuri (RP BIO-226) was developed from a cross between Sambha Mahsuri (BPT-5204) and SS113 (NIL's of PR 107) and released at Central Variety Release Committee (CVRC) during 2007 (Sundaram *et al.*, 2008). ISM has been reported as resistant to Bacterial Leaf Blight (BLB) but highly susceptible to sheath blight, with medium slender grains. ISM (RP BIO-226) is a high-yielding, fine-grain rice variety developed by incorporating genes resistant to bacterial blight, a serious disease affecting rice crops. This variety retains the desirable agronomic traits of its parent, Samba Mahsuri, such as medium-slender grains, excellent cooking quality, and aroma, while significantly enhancing resistance to bacterial blight through the incorporation of three resistant genes (*xa21, xa13, and xa5*). Improved Samba Mahsuri matures in about 140 days, has a moderate plant height, and performs well in irrigated conditions, making it suitable for cultivation in multiple rice-growing regions. It is particularly favoured for its superior grain quality, high market value, and reduced need for chemical inputs, promoting sustainable rice cultivation.

INGR21112 is a landrace of rice from the north-eastern part of India, classified under the indica subspecies of *Oryza sativa*. It flowers at around 117 days and reaches a medium plant height of 125 cm. The variety typically produces 14 to 17 tillers per plant, with 11 to 13 of these being productive panicle-bearing tillers. The panicles themselves are about 25 cm long, and the flag leaves extend to around 30 cm. Yield per plant ranges from 37 to 39 grams, and the grains are medium bold in type. It is noted for its tolerance and promise against sheath blight disease, and it shows moderate resistance to neck blast and leaf blast. It has shown a moderate resistance to sheath blight in three successive seasons screened at the ICAR-Indian Institute of Rice Research

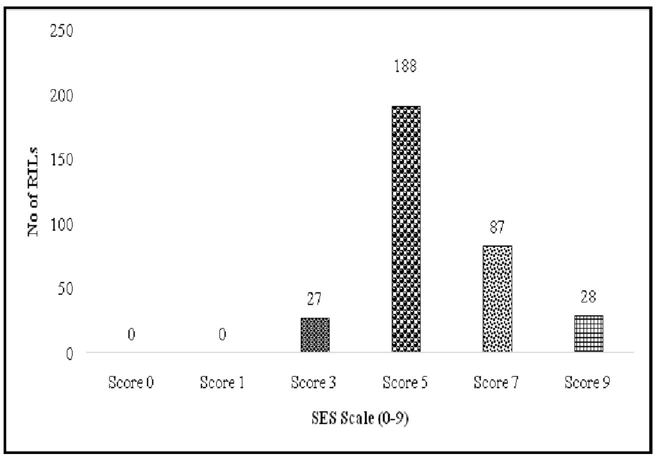

Fig. 1: Response of 330 RILs against sheath blight of rice, K-2017.

Table 1: Standard Evaluation System (SES) (IRRI 2014) for sheath blight of rice.

Disease score	Disease reaction	Description based on relative lesion height –RLH (%)
0	Immune	No infection
1	Highly Resistant	Vertical spread of lesion up to 20% of plant height
3	Resistant	Vertical spread of lesion up to 21-30% of plant height
5	Moderately Resistant / Moderately Susceptible	Vertical spread of lesion up to 31-45% of plant height
7	Susceptible	Vertical spread of lesion up to 46-65% of plant height
9	Highly susceptible	Vertical spread of lesion up to 66-100% of plant height

(Dey *et al*., 2016), as well as in multi-location testing in different agro-climatic zones of India through the All-India Co-ordinated Research Project on Rice (AICRPR) during 2017 and 2018. It was registered at ICAR-National Bureau of Plant Genetic Resources, New Delhi as highly tolerance to sheath blight and low Phosphorus.

Phenotypic screening of the 330 RILs, along with the parents, against sheath blight was conducted in IIRR farm during *Kharif*-17, at F8 generation. Planting was carried out by using 25-day old seedlings in three rows for each RIL, with a spacing of 20 × 20cm. The field experiment was conducted using augmented design.

Pathogen

The present study used highly virulent local Rajendranagar isolate (VP-15) of the rice sheath blight pathogen *R. solani* AG 1-IA from ICAR-Indian Institute of Rice Research, Hyderabad. The isolate was mass multiplied in Potato Dextrose Agar (PDA) plates by sub culturing from 3 dayold pure culture, which were then placed in a BOD incubator at 27±1°C for 4-7 days until complete mycelium and sclerotia growth was observed. The same culture plate was utilized for mass production of inoculum.

Mass multiplication of fungus

Typha (*Typha angustifolia*), an aquatic weed growing in moist environments, is harvested when it reaches a height of about 150 cm and flowering stage. The stems were cut into pieces having length of 6-8 cm and soaked in a solution containing 20 grams of peptone, 1000 ml of water, and 20 grams of sucrose for 2-5 minutes, then drained. These nutrient-rich typha bits were placed in autoclavable plastic bags or conical flasks and sterilized twice in an autoclave at 121°C for 30 minutes on two consecutive days. After sterilization, the typha bits were inoculated with fresh mycelium discs and sclerotia from a seven-day old *R. solani*. The inoculated bags were then incubated in a BOD incubator at 27±1°C about 15-20 days, allowing the mycelium to spread throughout the bag and promoting sclerotial production.

Artificial inoculation of pathogen

In order to induce sheath blight infection, mass multiplied *R. solani* is artificially inoculated in 45 days old plants from the day of transplanting. This is achieved by strategically placing 5-6 colonized typha bits between the hills just above the water line, and securing them tightly by wrapping with rubber bands (Bhaktavatsalam, 1978).

Table 2: Sheath blight disease reaction of 330 RILs after 30 days after artificial inoculation during *Kharif*-2017.

Sheath blight score	Disease reaction	Frequency of RIL's	Recombinant inbred lines (RILs)
0 (0%)	Immune	-	-
1 (=<20%)	Highly Resistant	-	-
3 (21-30%)	Resistant	27	5, 13, 25, 35, 36, 38, 45, 47, 50, 78, 88, 104, 124, 126, 130, 150, 158, 210, 243, 256, 262, 264, 289, 297, 299, 302 and 311.
5 (31-45%)	Moderately resistant /Moderately susceptible	188	1, 6, 12, 15, 17, 21, 26, 27, 28, 29, 30, 37, 39, 40, 41, 42, 43, 44, 46, 48, 49, 52, 55, 56, 57, 58, 59, 63, 65, 66, 67, 68, 69, 70, 71, 72, 75, 76, 77, 79, 80, 81, 82, 85, 86, 87, 89, 96, 100, 102, 103, 106, 107, 108, 109, 111, 113, 114, 115, 119, 120, 121, 122, 123, 125, 127, 128, 129, 131, 134, 135, 137, 138, 140, 143, 144, 145, 146, 147, 151, 155, 156, 157, 161, 163, 166, 168, 169, 171, 172, 173, 174, 175, 176, 177, 178, 179, 181, 183, 185, 186, 188, 189, 193, 194, 196, 197, 199, 200, 202, 203, 206, 208, 209, 211, 212, 213, 214, 218, 219, 220, 226, 228, 231, 237, 240, 242, 244, 245, 247, 248, 249, 250, 251, 252, 254, 255, 257, 258, 259, 260, 261, 265, 266, 268, 269, 273, 275, 276, 277, 278, 279, 280, 284, 287, 288, 291, 293, 294, 295, 298, 300, 303, 304, 305, 306, 307, 308, 309, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329 and 330.
7 (46-65%)	Susceptible	87	2, 3, 4, 7, 9, 10, 11, 14, 16, 19, 20, 22, 23, 31, 32, 33, 51, 54, 64, 74, 83, 90, 91, 92, 93, 94, 98,101, 105, 110, 112, 116, 117, 118, 132, 133, 136, 139,141,149, 152, 154, 159, 160, 162, 164, 165, 180, 182, 184, 190, 191, 192, 195,198, 201, 205, 207, 215, 216, 217, 221, 222, 223, 224, 225, 227, 229, 230, 233, 234, 235, 236, 238, 239, 241, 246, 253, 263, 267, 271, 274, 282, 283, 292, 296 and 301.
9 (>65%)	Highly susceptible	28	8, 18, 24, 34, 53, 60, 61, 62, 73, 84, 95, 97, 99, 142, 148, 153, 167, 170, 187, 204, 232, 270, 272, 281, 285, 286, 290 and 310.

The water level is carefully maintained at 5-8cm from the base of the plant, creating a microclimate that is conducive to the pathogen initiating the infection process. In each Recombinant Inbred Line (RIL), five individual plants were inoculated along with the parents.

Data evaluation

Phenotypic data for plant height and lesion height are crucial factors in evaluating sheath blight disease. Two consecutive observations were taken at an interval of 15 to 30 days after inoculation. The vertical relative lesion height is calculated based on plant height and lesion height to assess the host's response. RILs were categorized into various groups using the standard evaluation scale (SES) provided by IRRI (2014). The Relative lesion height (RLH %) was calculated by the following formula.

$$\text{RLH} = \frac{\text{Lesion height}}{\text{Plant height}} \times 100$$

Results and discussion

Sheath blight disease reaction in the 330 RIL population derived from the cross of Improved Samba Mahsuri (ISM) and INGR21112 have displayed a significant frequency distribution (Table 2). It was previously reported as tolerant (Dey *et al*., 2016), exhibited a similar resistant reaction, while Improved Samba Mahsuri showed a highly susceptible reaction. Two observations were recorded from the day of inoculation, with disease pressure gradually increasing by the end of the second observation at a 30-day interval period. The relative lesion height (RLH), which is calculated as the height of the highest disease lesion divided by plant height (PH) (Savary and Mew, 1996), serves as the standard measurement to reflect disease intensity.

A phenotypic score ranging from 0 to 9 was assigned to the population based on the relative lesion height. The population was then categorized into immune, resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible groups. Among the 330 RIL population, none exhibited complete resistance or immune reaction to sheath blight during the screening. Out of 330 RILs, 27 RILs showed resistant reaction, 188 showed a moderately susceptible/ moderately resistant reaction, 87 RILs showed a susceptible reaction and 28 showed a highly susceptible reaction.

Singh and Borah (2000) also screened 60 local upland rice cultivars in Assam and reported that only one variety *i.e.*, Chingdar was found to be resistant. Also reported that seven cultivars were moderately resistant and rest 52 were susceptible. Numerous rice germplasm has previously been evaluated for resistance to sheath blight through both natural and artificial inoculation methods by various researchers (Dubey *et al*., 2014; Chandra *et al*., 2016; Kumar *et al*., 2017; Pavani *et al*., 2018, Kapoor *et al*., 2022). However, none of the genotypes exhibited immunity or resistance to the disease. Although there has been significant research conducted on the origins of sheath blight resistance in rice plants, only a limited number of rice varieties, breeds, or closely related wild species have been able to develop effective vertical resistance. According to Nadarajah *et al*., (2014) and Lore *et al*., (2015) vertical resistant observed in rare instance. However, in the present study also RILs not showed immune or highly resistant reaction against ShB. However, significant number (27) of RILs showed resistant reaction. Resistant nature of identified RILs may be due to combination of genetic and physiological factors. The identified resistant RILs will be used for the sheath blight resistant breeding programs in elite cultivars in India.

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