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Identification of superior hybrids and parents for blast resistance in rice (*Oryza sativa* L.)

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Abstract
Blast disease is one of the major disease of rice causing substantial yield losses. Host plant resistance is the best means of combating the blast disease in rice. The aim of the present investigation was to identify the superior hybrids and their parents with blast resistance by adopting Uniform Blast Nursery (UBN) at Rice Research Center, ARI, Rajendranagar during Rabi, 2017-18. The experiment was carried out with 12 parents, 32 hybrids along with susceptible check (TN1). The disease scoring was done by using 0-9 scale of Standard Evaluation System (SES) developed by IRRI, 2013. Accordingly based on the disease scoring, the genotypes were categorized as resistant, moderately resistant/ susceptible and susceptible types.

Keywords: Superior hybrids, parents, blast resistance, rice, *Oryza sativa* L.

Introduction
Rice crop is affected by about 36 fungal, 21 viral and 6 bacterial diseases (Ou, 1985) [5]. The rice blast *Pyricularia oryzae* B. Couch [formerly *Magnaporthe grisea* (Hebert) Bar] is one of the important fungal diseases effecting considerable loss in rice production. It is a widespread and damaging disease of cultivated rice and around 50 per cent of production may be lost in a field moderately affected by blast infection. Each year the fungus affects rice enough to feed an estimated 60 million people (Zeigler *et al*., 1994) [9]. In India, it was first recorded in Thanjavur (Tanjore) delta of South India by Mc Rae in 1918. Seven epidemics of blast happened between 1980 and 1987 in the states of Himachal Pradesh, Andhra Pradesh, Tamil Nadu and Haryana resulting in huge yield losses (Sharma *et al*., 2012) [7]. With the objective of identification of superior parents and hybrids for blast resistance, blast screening investigation was carried out at Rice Research Centre (PJTSAU), Rajendranagar, Hyderabad.

Material and methods
Plant Material: The hybrid seed material generated by crossing selective parents with four male sterile lines viz., CMS 23A, CMS 59A, CMS 64A, and JMS 13A was screened along with parents during *Rabi*, 2017-18 by adopting the Uniform Blast Nursery method. The variety TN1 used as susceptible check in screening experiments. The genotypes were sown as a separate replication and screening data was not subjected to statistical analysis.

Methods
The following method of isolation and maintenance of blast cultures described by Prasad *et al*. (2011) [6] was used throughout the study as mentioned here under: Isolation and maintenance of blast cultures – Scraping method. The blast infected leaf bits were surface sterilized in 0.1% mercuric chloride and later washed in sterile distilled water for 3-4 times. Then, the infected leaf bits were kept on leaf extract agar medium in a petri plate under aseptic condition and were incubated at 27 °C for 3-4 days till mycelial growth was observed. The fungal mycelium was scraped from the infected leaf bit and transferred to a fresh petriplate containing sterile leaf extract agar. The petriplates were incubated at 27 °C for further growth. After sufficient growth the fungus was subsequently transferred to test tubes, containing sterile leaf extract agar for culture establishment. carried out with both sterilized (S) and unsterilized (US) seeds. Seed simply washed with distilled water were used as unsterilized (US) seeds.

Tapping method
The blast infected leaf bits were surface sterilized in 0.1% mercuric chloride with 4 to 5 repeated washes in sterile distilled water. The infected leaf bits were then placed in sterile petri plates containing sterile moistened filter paper, small insertions were made in the filter paper

placed in the upper part of lid and the infected leaf bits are inserted in the insertions made in the upper portion of the moister paper in the upper lid. Then the petri plates were incubated at 27 °C for 24-48 hrs to initiate the sporulation, then the petri plate lid containing infected leaf bits were transferred to fresh petiplates containing sterile leaf extract agar, and the lid was gently tapped to dislodge the spores on to the under lying leaf extract medium. The plates were incubated at 27 °C for 5-7 days till fungal growth was observed. The fungus was subsequently transferred to test tubes containing sterile leaf extract agar for culture establishment.

Mass multiplication on sorghum seeds

About 50 gm of jowar (Sorghum vulgaris) seeds were boiled in distilled water containing yeast extract powder (2 gm/l) for about 30 minutes. Then the seeds were sterilized in an autoclave at 121 °C (15 lbs pressure) for 20 minutes. Seven days old culture was transferred to conical flasks aseptically along with agar block to the sterilized sorghum seeds and incubated at 28 °C for 7 days till appropriate growth was observed.

Mass multiplication on oat meal agar

The blast fungus was also multiplied on oat meal agar medium. Seven days old pre inoculated fungal agar block was aseptically transferred to sterile Oat meal agar containing petriplates and these plates were incubated at 28 °C for 7 days till sporulation was observed. From the above sporulation methods mass multiplication on oat meal agar method was adopted for this study because among the different media explained above oat agar meal agar media was supported maximum growth and sporulation of rice blast isolates (Khadka *et al*., 2012) [4]. Average number of days for sporulation of rice blast isolates was 9 days in this media. (Jia, 2009) [3].

UBN (Uniform Blast Nursery) method of screening for blast disease resistance

Method adopted for screening of leaf blast resistance was Uniform Blast Nursery (UBN) Method. UBN was a 10 x 1 m bed and the soil was pretreated with FYM and recommended dose of fertilizers. Later commercial sulphuric acid was added to the beds before sowing. Local susceptible variety TN 1 was sown as border rows on all sides of the bed. The susceptible check variety was sown after every twenty test entries. This helped in spreading the inoculum. Test material was sown in 50 cm rows perpendicular to the border rows. Relative humidity was maintained with water sprinklers. The beds were covered with polythene sheets during night to maintain high humidity and to increase the disease pressure on the entries.

Inoculation

Spore suspension was prepared from 7-day-old blast culture grown on oat meal agar. The mycelium was scraped in 10 ml of distilled water and the solution was filtered through two fold cheese cloth to remove the fungal debris. The spore concentration was adjusted to l x $l0^5$ conidia per ml using heamocytometer. The spore suspension containing Tween-20 (0.2%) was sprayed uniformly over the 15-day-old seedlings using hand-held low volume automizer. The inoculum was sprayed in the evening hours till the entire plant surface became wet with conidial suspension and left overnight. Water was sprayed three to four times one day after inoculation during day time to maintain high humidity.

Table 1: Disease scoring is done using 0-9 SES scale (IRRI, 2013) [2] for blast nursery

Score	Description of symptom
0	No lesions
1	Small brown specks of pin head size without sporulating centers
2	Small round to slightly elongated, necrotic grey spots, about 1-2 mm in diameter with a distinct brown margin region and lesions were mostly found on the lower side
3	Lesion type was the same as in scale 2, but significant number of lesions were on the upper side 4 Typical sporulating blast lesions, 3 mm or longer, infecting less than 2% of leaf area.
5	Typical sporulating blast lesions, infecting 2 -10% of the leaf area
6	Blast lesions infecting 11-25% of the leaf area
7	Blast lesions infecting 26-50% of the leaf area
8	Blast lesions infecting 51-75% of the leaf area
9	More than 75% leaf area affected

Scoring was done after 10-15 days of post infection depending on the severity of the infection on each entry and susceptible check using Standard Evaluation System (SES, IRRI, 2013) [2]. Based on leaf blast scores recorded, the genotypes were categorized as resistant (0-3.0), moderately susceptible (3.1- 5.0) and susceptible (5.1- 9.0).

Results and discussion

The results indicate that, among the testers, JMS 13B showed resistant reaction while other three testers showed susceptible reaction. Among the eight lines five lines showed moderately susceptible reaction while lines JGL 18047 and IET 26274 showed resistant reaction. Among the hybrids, four hybrids viz., JMS 13A x JGL 18047, CMS 23A x RNR 11450, JMS 13A x IET 26264 and JMS 13A x IET 26274 were resistant to screening reaction and 11 hybrids were moderately susceptible to the blast disease. It was observed that, most of the parents showing susceptible or moderately susceptible reaction, gave rise to moderately susceptible natured hybrids. It was also observed, that certain resistant parents, gave rise to moderately susceptible hybrids. Similar field screening experiments were conducted for identification of location specific blast resistant lines and results were reported by Hosagoudar and Jairamamadabade (2017) [1] and Vinayak Turaidar *et al*. (2017) [8].

Table 2: Screening of rice genotypes for leaf blast resistance during Rabi, 2017-18

S. No	Entry	Reaction to leaf blast (sore 0-9 scale)	Disease reaction
Testers			
1	CMS 23B	7	S
2	CMS 59B	7	S
3	CMS 64B	7	S
4	JMS 13B	3	R
Lines			
5	RNR 26060	5	MS
6	WGL 14	5	MS
7	JGL 18047	3	R
8	JGL 11118	5	MS
9	RDR 1140	7	S
10	RNR 11450	5	MS
11	IET 26264	5	MS
12	IET 26274	3	R
Checks			
13	JGL 11470	7	S
14	MTU 1001	3	R
15	US 312	7	S
16	HRI 174	7	S
Hybrids			
17	CMS 23A x RNR 26060	7	S
18	CMS 59A x RNR 26060	7	S
19	CMS 64A x RNR 26060	7	S
20	JMS 13A x RNR 26060	7	S
21	CMS 23A x WGL 14	7	S
22	CMS 59A x WGL 14	5	MS
23	CMS 64A x WGL 14	5	MS
24	JMS 13A x WGL 14	7	S
25	CMS 23A x JGL 18047	5	MS
26	CMS 59A x JGL 18047	5	MS
27	CMS 64A x JGL 18047	9	S
28	JMS 13A x JGL 18047	3	R
29	CMS 23A x JGL 11118	7	S
30	CMS 59A x JGL 11118	9	S
31	CMS 64A x JGL 11118	7	S
32	JMS 13A x JGL 11118	5	MS
33	CMS 23A x RDR 1140	9	S
34	CMS 59A x RDR 1140	5	MS
35	CMS 64A x RDR 1140	5	MS
36	JMS 13A x RDR 1140	9	S
37	CMS 23A x RNR 11450	3	R
38	CMS 59A x RNR 11450	5	MS
39	CMS 64A x RNR 11450	5	MS
40	JMS 13A x RNR 11450	7	S
41	CMS 23A x IET 26264	5	MS
42	CMS 59A x IET 26264	7	S
43	CMS 64A x IET 26264	5	MS
44	JMS 13A x IET 26264	3	R
45	CMS 23A x IET 26274	7	S
46	CMS 59A x IET 26274	7	S
47	CMS 64A x IET 26274	5	MS
48	JMS 13A x IET 26274	3	R
Susceptible check			
49	TN -1	9	S

R-Resistant, MS- Moderately Susceptible, S- Susceptible

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