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Screening of Maintainer lines for Leaf Blast resistance through uniform blast nursery method in rice (*Oryza sativa* L.)

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Abstract
An experiment was conducted to evaluate the maintainer lines blast resistance and yield component traits in rice. Present investigation was carried out during *rabi* 2018-19 with 40 genotypes and TN 1 (susceptible check). The genotypes were screened against leaf blast by adopting Uniform Blast Nursery (UBN) method at Rice Research Centre, Rajendranagar and scored as per Standard Evaluation System (SES) scale (IRRI-2013). Accordingly, based on the disease scoring, the genotypes were categorized as resistant, moderately resistant, moderately susceptible and susceptible types. It was found that 13 lines were resistant to screening reaction, 12 lines were moderately resistant and 10 lines were found to be moderately susceptible and 3 lines were susceptible. Four genotypes *viz*., JMS 17B, JMS 18B, MTU 1216 and IR-BLZ-F4 were recorded higher mean for yield and its component traits along with blast resistance and hence these genotypes could be directly used as parents for development of heterotic hybrids with blast resistance and higher grain yield. Further the maintainer lines *viz*., MTU 1216 and IR-BLZ-F4 could be back crossed to any stable Cytoplasmic Male Sterile (CMS) lines for development of new blast resistant CMS lines which can be utilized as female parent in development of heterotic hybrids with leaf blast resistance.

Keywords: leaf blast, uniform blast nursery, host plant resistance, rice

1. Introduction

Rice is the most important food security crop and staple food of half of the world's population. Major increase in rice production occurred during the past four decades as a result of adoption of green revolution technology. Any decline in its acreage and production will have a perceivable impact on the state's economy and food security (Thippeswamy *et al*., 2016) [15]. Global rice demand is estimated to rise from 676 million tons in 2010 to 763 million tons in 2020 and to further increase to 852 million tons in 2035. This is an overall increase of 26% or 176 million tons in the next 15 years (Khush 2013) [6]. However, the rate of increase in rice production has slowed down. At the same time, yield loss caused by various diseases and insect pests need to be minimized as the rice crop is affected by about 36 fungal, 21 viral and 6 bacterial diseases (Ou, 1985) [8]. Among fungal diseases, the rice blast *Pyricularia oryzae* B. Couch 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 (Shafaullah *et al.*, 2011) [18]. 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.

Rice blast can infect the plant during nearly all growth stages. Repeated epidemics and frequent breakdown of rice blast resistance causing yield losses of 20–100% have been reported over the last decades in India and Japan (Khush and Jena 2009[5], Sharma *et al*., 2012) [23]. Effective management of such pathogens require constant breeding efforts for development of resistant cultivars. Host plant pathogens are fast evolving and can break down the resistance conferred by R genes, thus resulting in disease epidemics. These can be influenced by several factors, including weather conditions, disease pressure, as well as genome stability of the pathogen. The *M. oryzae* genome is rich in repetitive segments and retro-transposons (Dean *et al*., 2005) [2], which allow the fungus to frequently change pathogenicity or escape from host recognition by altering the effector molecules. This requires the continuous identification of new sources of host disease resistance against continuously evolving and geographically diverse pathogen races.

When predisposition factors (high mean temperature values, degree of relative humidity higher than 85-89%, presence of dew, drought stress and excessive nitrogen fertilization) favour epidemic development occurs (Piotti *et al*., 2005) [10]. Though many resistant varieties to *M. oryzae* have been developed, the resistance is not long lasting, because the high pathogen plasticity in the fields makes single resistance gene break down after three to five years of the cultivar release (Bonman *et al*, 1986; Lang *et al*, 2009) [1, 7]. Hence, development of broad spectrum and durable blast resistant varieties is essential for combating this disease, which requires continuous efforts of breeders and pathologists. Wild species of *Oryza* can be exploited to widen the gene pool of rice for biotic and abiotic stress (Ram *et al*, 2013) [12]. Host plant resistance proved to be the best strategy for blast disease management. Hence, development of blast resistant hybrids has gained importance. With the objective of identification of better parents for blast resistance, blast screening investigation was carried out.

2. Materials and Methods

The material for the present study includes 38 maintainer lines and 2 standard checks along with susceptible check (TN 1). These genotypes were sown during *rabi*, 2018-19 by adopting UBN (Uniform Blast Nursery) method.

The following method of isolation and maintenance of blast cultures described by Prasad *et al.* (2011) [11] was used throughout the study as mentioned here under.

2.1 Isolation and maintenance of blast cultures

Scraping method

The blast infected leaf bits are surface sterilized in 0.1% mercuric chloride and later washed in sterile distilled water for 3-4 times. Then the infected leaf bits are kept on leaf extract agar medium in a petriplate under aseptic condition and are incubated at 27°C for 3-4 days till mycelial growth is observed. The fungal mycelium is scraped from the infected leaf bit and transferred to a fresh petriplate containing sterile leaf extract agar. The petriplates are incubated at 27°C for further growth. The fungus is subsequently transferred to test tubes after the sufficient growth, containing sterile leaf extract agar for culture establishment.

Mass multiplication on oat meal agar

The blast fungus can also be multiplied on oat meal agar medium. Seven days old pre inoculated fungal agar block is aseptically transferred to sterile Oat meal agar containing petri plates and these plates are inubated at 28°C for 7 days till sporulation is observed. The rice blast fungus pathogen has been known as *Pyricularia grisea*. The teleomorph stage, *Magnaporthe grisea*, has not been found in nature, but it has been produced after crossing appropriate compatible isolates in the laboratory. The fungus produces simple, gray conidiophores that bear terminal, pear shaped, mostly two septate conidia.

2.2 Uniform blast nursery (UBN) method of screening

Uniform blast nursery (UBN) is a 10 × 1 m bed and the soil is enriched with FYM and recommended dose of fertilizers. Local susceptible variety such as TN 1 is sown as border rows on all sides of the bed. The susceptible check variety is sown after every twenty test entries. This helps to spread the inoculum. Test material is sown in 50 cm rows perpendicular to the border rows (Fig 1). Relative humidity is maintained with water sprinklers. The beds are covered with polythene sheets during night to maintain high humidity and to increase the disease pressure on the entries.

Inoculation

Spore suspension is prepared from 7-day-old blast culture grown on oat meal agar. The mycelium is scraped in 10 ml of distilled water and the solution is filtered through two-fold cheese cloth to remove the fungal debris. The spore concentration is adjusted to l × l05 spores per ml using heamocytometer. Using hand-held low volume (300 ml) capacity automizer, the spore suspension containing tween- 0.2% is sprayed uniformly over the 15-day-old seedlings. The inoculum is sprayed in the evening till the entire plant surface become wet with spore suspension and left overnight. Water is sprayed three to four times during day time to maintain high humidity. However, care was taken so that the water is not sprayed immediately after spraying inoculum. The minimum gap between spraying the inoculum and spraying water should be at least twelve hours. Scoring is done after 10-15 days of post infection depending on the severity of the infection on the each entry, resistant and susceptible check using Standard Evaluation System (SES, IRRI 2013) (Table 1). Based on leaf blast scores recorded, the genotypes are classified as highly resistant (0-1), resistant (1.1-3.0), moderately resistant (3.1-5.0), moderately susceptible (5.1-7.0) susceptible (7.1-8.9) and highly susceptible (9.0) (Fig 2).

Data on grain yield and its component traits *viz*., number of effective tillers per plant (cm), number of grains per panicle, grain yield per plant (g) recorded on these genotypes during *kharif*, 2018 were also mentioned for reference and identification of superior genotypes with blast resistance. The data was recorded on five random plants in each plot whereas a random sample in each plot was used to record 1000-grain weight (g) and mean values were calculated and subjected to analyses of variance.

3. Results and Discussion

Among the most devastating diseases that constrain rice production, rice blast ranks first because of its wide distribution and high incidence under favourable conditions. Although many resistant varieties have been developed, due to genetic plasticity in the pathogen genome, there is a continuous threat to the effectiveness of the developed cultivars (Patil *et al*. 2013) [9]. To breed rice varieties with more durable blast resistance, multiple resistance utilizing both qualitative and quantitative genes must be incorporated into individual varieties (Joshi *et al*. 2009) [4].

Identification of new donors is very important in development of resistant cultivars. Therefore, in the present study with the objective of identification of better parents for blast resistance, blast screening investigation was carried out with 40 genotypes with suitable check TN 1 (susceptible) by adopting UBN method and disease scoring is done using 0-9 Standard Evaluation System (SES) scale (IRRI 2013). Accordingly, based on the disease scoring, the genotypes are categorized as resistant, moderately susceptible and susceptible types (Table 2). The results indicate that among maintainer lines, 13 lines *viz*., CMS 11B, CMS 46B, JMS 13B, JMS 17B, JMS 18B, RNR 21280, RP 5950-24-6-2-1-1-B, SYE 160-7-19-7-23-16, MTU 1216, TP 30494, IR 10 F 388, RNR 26119, IR – BLZ-F4 were found to be resistant with less than 3.0 score, whereas 12 lines *viz.*, CMS 14B, CMS 23B, CMS 64B, JMS 11B, JMS 20B, WGL 44, RP 4993-183-9-2-1-1, JGL 1798. HMT Sona, OR 2573-11, CT – 18615-1-S-1-2-4 were found to be moderately resistant with disease score ranging from 3.1-5.0. While 10 lines were moderately susceptible (CMS 52B, CMS 59B, JMS 14B, JMS 21B, GNV 14-05, GNV 14-25, TULASI, RNR 26032, RNR 26075, Sharbati) and 3 lines were susceptible (TELLAHAMSA, R 1919-537-1-160-1, RNR 26992). The susceptible check, TN-1 was highly susceptible, however the checks, RNR 15048 (1.6) and MTU 1010 (3.6) were identified as resistant and moderately resistant, respectively.

Four genotypes *viz*., JMS 17B, JMS 18B, MTU 1216 and IR-BLZ-F4 were recorded higher mean for yield and its component traits with blast resistance (Table. 3), which could be directly used as parents for development of heterotic hybrids with blast resistance and higher grain yield. Similarly genotypes with higher mean for grain yield and its component traits with moderate resistance to leaf blast were CMS 14B, JMS 20B, WGL 44, RP 4993-183-9-2-1-1, CT– 18615-1-S-1-2-4 and OR 2573-11, could also be utilised as parents for developing of blast resistant, high yielding hybrids. While some genotypes *viz*., CMS 59B, JMS 14B GNV 14-25, GNV 14-05, RNR 26032, RNR 26075 and Sharbati were recorded moderate susceptibility but possess high mean yield and its component traits while some other genotypes were blast susceptible with high yield which were not suitable to use directly for development of blast resistant hybrids.

Similar field screening experiments were conducted for identification of location specific blast resistant lines by Srijan *et al*. (2015) [14], Hosagoudar and Jairam Amadabade (2017) [3] and Vinayak Turaidar *et al*. (2017) [17]

4. Conclusion

In present study none of the maintainer lines were shown highly resistant response against rice blast under nursery condition upon artificial inoculation. The genotypes which showed resistance to blast coupled with higher yield *viz*., JMS 17B, JMS 18B, MTU 1216 and IR-BLZ-F4 could be used as parents for development of blast resistant varieties and hybrids. could be back crossed to any stable CMS lines for development of new blast resistant CMS lines which can be utilized as female parent in development of heterotic hybrids with leaf blast resistance.

Table 1: Disease scoring is done using 0-9 SES scale (IRRI-SES, 2013)

Score	Description of symptom	Reaction
0	No lesions	Highly resistant
1	Small brown specks of pin-point size or larger brown specks without sporulating center	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Resistant
3	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves	Moderately Resistant
4	Typical susceptible blast lesions2-3 mm or longer, infecting less than 4% of the leaf area	Moderately Resistant
5	Typical blast lesions infecting 4-10% of the leaf area	Moderately Susceptible
6	Blast lesions infecting 11-25% of the leaf area	Moderately Susceptible
7	Blast lesions infecting 26-50% of the leaf area	Susceptible
8	Blast lesions infecting 51-75% of the leaf area	Susceptible
9	More than 75% leaf area affected	Highly Susceptible

Fig 1: Blast screening in Uniform Blast Nursery (UBN) Method.

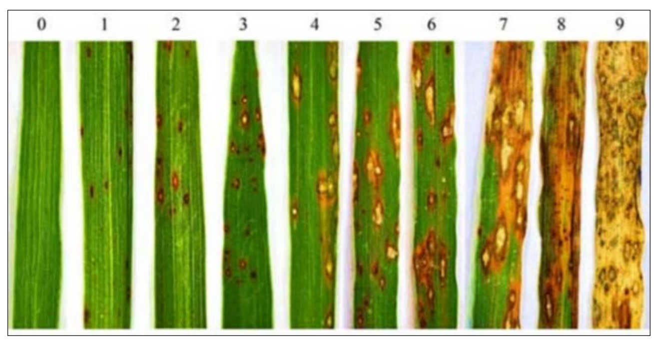

Fig 2: Scoring of leaf blast using 0-9 standard evaluation scale (SES IRRI, 2013)

Table 2: Blast reaction of various genotypes based on 0-9 scale (IRRI- SES, 2013) in rice.

S. No	Genotype	Reaction to leaf blast (Score 0-9 scale)	Disease reaction
1	CMS 11B	1.6	R
2	CMS 14B	3.6	MR
3	CMS 23B	3.6	MR
4	CMS 46B	2.3	R
5	CMS 52B	5.6	MS
6	CMS 59B	5.6	MS
7	CMS 64B	3.6	MR
8	JMS 11B	3.6	MR
9	JMS 13B	2.3	R
10	JMS 14B	5.6	MS
11	JMS 17B	2.3	R
12	JMS 18B	1.6	R
13	JMS 20B	3.6	MR
14	JMS 21B	5.6	MS
15	RNR 21280	2.9	R

16	WGL 44	4.3	MR
17	TELLAHAMSA	7.6	S
18	RP 5950-24-6-2-1-1-B	2.3	R
19	GNV 14-25	5.0	MS
20	GNV 14-05	5.6	MS
21	R 1919-537-1-160-1	8.3	S
22	RP 4993-183-9-2-1-1	3.6	MR
23	SYE 160-7-19-7-23-16	3.0	R
24	MTU 1216	1.6	R
25	TP 30494	1.0	R
26	JGL 1798	3.6	MR
27	HMT Sona	4.3	MR
28	IR 10 F 388	1.0	R
29	CT – 18615-1-S-1-2-4	3.6	MR
30	TULASI	6.3	MS
31	RNR 26119	1.6	R
32	RNR 26032	5.6	MS
33	RNR 26061	3.6	MR
34	RNR 26075	7.0	MS
35	OR 2573-11	4.3	MR
36	Sharbati	6.3	MS
37	IR – BLZ-F4	1.0	R
38	RNR 26992	7.6	S
Standard checks			
39	RNR 15048	1.6	R
40	MTU1010	3.6	MR
Susceptible check			
41	TN1	9	HS

R: Resistant, MR: Moderately Resistant, S: Susceptible, MS: Moderately Susceptible, HS: Highly Susceptible

Table 3: Grain yield and component traits of maintainer lines in rice.

S. No	Genotypes	No. of effective tillers per plant	No. of grains per panicle	1000-seed weight(g)	Grain yield per plant (g)
1	CMS 11B	24.2	133	19.31	27.88
2	CMS 14B	17.0	331	14.40	43.12
3	CMS 23B	17.8	143	22.99	24.52
4	CMS 46B	20.3	135	19.72	24.70
5	CMS 52B	20.8	152	21.78	27.07
6	CMS 59B	21.3	119	19.70	33.62
7	CMS 64B	16.7	215	18.37	26.45
8	JMS 11B	21.7	121	18.56	24.35
9	JMS 13B	16.4	274	15.44	26.57
10	JMS 14B	16.6	342	12.64	34.70
11	JMS 17B	16.0	328	11.23	30.97
12	JMS 18B	16.4	308	11.76	32.30
13	JMS 20B	16.0	268	12.98	29.70
14	JMS 21B	16.7	128	20.61	22.47
15	RNR 21280	16.2	236	13.03	24.46
16	WGL 44	14.6	263	12.92	30.84
17	TELLAHAMSA	16.0	130	21.24	26.10
18	RP 5950-24-6-2-1-1-B	13.2	258	13.05	28.62
19	GNV 14-25	14.6	334	14.52	32.66
20	GNV 14-05	14.0	254	14.26	29.53
21	R 1919-537-1-160-1	14.2	261	17.09	28.98
22	RP 4993-183-9-2-1-1	13.7	320	13.05	34.01
23	SYE 160-7-19-7-23-16	15.7	178	20.11	26.40
24	MTU 1216	16.9	160	23.04	30.85
25	TP 30494	16.5	167	21.53	29.01
26	JGL 1798	15.7	192	13.68	29.94
27	HMT Sona	16.6	215	14.10	23.00
28	IR 10 F 388	15.4	148	22.22	29.38
29	CT – 18615-1-S-1-2-4	13.2	215	23.45	34.05
30	TULASI	19.3	120	23.24	25.24
31	RNR 26119	15.5	365	12.28	32.17

32	RNR 26032	16.0	167	24.49	29.76
33	RNR 26061	16.6	183	19.32	15.85
34	RNR 26075	14.5	159	21.10	28.70
35	OR 2573-11	17.4	154	26.43	39.72
36	Sharbati	19.0	127	21.09	29.05
37	IR – BLZ-F4	14.2	257	21.55	29.72
38	RNR 26992	19.4	239	19.08	31.43
39	RNR 15048	14.7	310	11.81	35.54
40	MTU 1010	20.9	160	25.45	35.56

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