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Phenotypic and Molecular Screening of Long Grain Rice (*Oryza sativa* L.) Genotypes Against Leaf Blast Disease

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ABSTRACT

The experiment was conducted during *rabi* (December, 2021 to March, 2022) at Agricultural Research Station (ARS), Kampasagar, Nalgonda district, Telangana, India to identify long grain rice genotypes comprising of released varieties and advanced breeding lines (ABLs) with blast resistance, superior yield, and good grain quality using Marker-Assisted Selection (MAS). Rice blast disease caused by *Magnaporthe oryzae* causes significant economic losses worldwide. Genetic resistance in cultivars was the most-effective strategy to manage this disease, offering an alternative to chemical control. Leaf blast resistance screening revealed that 5 and 14 genotypes were found resistant and moderately resistant to blast. Molecular profiling with 11 polymorphic markers linked to blast resistance genes (Pi1, Pi9, Pi20, Pi20, Pi2, Pi54, Pib, Pib, Pitp, Pizt and Pi38) identified the highest number of positive alleles in RNR 15435 (6) followed by KPS 13054 (5) and WGL-44 (5). Majority of the rice accessions had the R-genes Pitp and Pi20 but, genes Pizt, and Pi2 were present in all 30 rice accessions and none of these had Pi9. The most promising genotypes for blast resistance were RNR 15435, KPS 13054, KPS 12349, KNM 118, KPS 12774, KPS 3272 and R-RHZ-IB-80 which demonstrated strong marker-trait associations and had multiple resistance genes Pi1, Pi2, Pi20, Pi38, Pi54, Pib, Pitp, and Pizt. The associated R-genes might be utilized in rice breeding programmes through marker-assisted breeding, and the identified resistant rice accessions could be used as prospective donors for the production of new leaf blast resistant varieties in India.

KEYWORDS: Rice, leaf blast, R-gene, MAS, ABLs

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is the primary food for over half the world's population, providing crucial caloric and nutritional intake, and is a major driver of food security, especially in Asia. With the projected global population of 9.7 billion by 2050, rice production needs to increase by 42% from current levels to meet the rising demand (Pradhan et al., 2020). Globally, rice production is under severe threat due to fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*) causing blast disease (Pennisi, 2010; Fernandez and Orth, 2018). Pathogen affects the crop at all developmental stages and in favourable conditions, yields were drastically reduced up to 90% (Le et al., 2010, He et al., 2012; Singh et al., 2015). Blast disease epidemics were common in majority of the rice growing countries including India (Ariya-anandech et al., 2018; Karkute et al., 2023). Chemical control is expensive (Panda et al., 2017; Sahu et al., 2018) and ineffective under high disease infestation (Jeevan et al., 2020) resulting in pathogen resistance (Yamaguchi, 2004). Development of host resistance (R genes) is the most cost-effective and eco-friendly strategy to control rice blast disease. About 120 R genes were discovered so far for rice blast, of which 35 were successfully cloned and characterized for leaf blast resistance (Madhav, 2015; Wang and Valent, 2017; Kalia and Rathour, 2019). Majority of the cloned blast R genes share nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains in their protein sequences, except for a few (Pid2, Pi21, and Ptr) (Wang and Valent, 2017; Skamnioti and Gurr, 2009; Liu et al., 2010). According to gene-for-gene hypothesis, these R genes are race-specific and related to the hypersensitive response (HR) (Fukuoka et al., 2009). The *M. oryzae's* genome contains numerous repetitive DNA and retro-transposons (Dean et al., 2005), which might cause mutations in genes that mediate the pathogen's virulence and host range (Kang et al., 2001; Farman et al., 2002; Bohnert et al., 2004), allowing the fungus to develop new virulent races. The emergence of these races results in a change in pathogenicity, posing a threat to existing blast-resistant rice cultivars (Hittalmani et al., 2000). In rice breeding, marker assisted selection (MAS) has emerged as a potential method for blast disease resistance to incorporate desired gene(s) in early breeding generations (Wang et al., 2014) by gene pyramiding targeted R genes (Koide et al., 2010). Recently, molecular markers have been utilized to identify the gene of interest in rice germplasm (Zhang et al., 2014). Pyramiding of resistance genes using conventional breeding procedure is time consuming and less efficient because blast races carrying individual avirulence genes for the identification of the corresponding resistance genes are usually not present in nature. India's export potential for non-basmati long grain rice is strong, driven by global demand for its affordability and variety, particularly in Africa, the Middle East, and Asia, though climate risks and policy volatility can pose challenges. India is a leading exporter, supplying more than 11 mmt in 2023–24 (Anonymous, 2024). It is necessary to explore the germplasm for R genes to confer long term resistance to rice blast and to comprehend the resistant spectrum of relevant R genes against prevailing pathogen races to combat blast disease. The present study was undertaken to genotype 30 long grain non basmati rice accessions, including released varieties and advanced breeding lines (ABLs) using twelve different blast markers RM224, Nmsmpi9, RM1337, RM5364, AP5659-5, Pi54MAS, RM206, RM166, RM208, RM246, Zt56591, RM 21 targeting the genes Pi1, Pi9, Pi20, Pi20, Pi2, Pi54, Pi54, Pib, Pib, Pitp, Pizt, Pi38 respectively for blast resistance and to identify genotypes with blast resistance, superior yield, and good grain quality using Marker-Assisted Selection (MAS).

2. MATERIALS AND METHODS

2.1. Experimental site

The experiment was conducted during *rabi* (December, 2021 to March, 2022) at Agricultural Research Station (ARS), Kampasagar, Nalgonda district. The experimental farm was located in southern zone of Telangana at 16.85°N latitude and 79.56°E longitude and at an altitude of 152 m. The experimental material comprised of 30 long grain rice genotypes maintained at Agricultural Research Station (ARS), Kampasagar (Table 1). Of these, 13 were released varieties and 17 were advanced breeding lines. In addition to these, two genotypes, TN-1 and Tetep, were chosen as susceptible and resistant checks, respectively for leaf blast.

2.2. Phenotyping of rice germplasm lines for blast disease resistance

Phenotyping of rice germplasm lines for blast disease resistance was carried out at ARS, Kampasagar during *rabi* 2021–22. The Method adopted for screening of blast resistance was Uniform Blast Nursery (UBN) Method. Soil was pre treated with FYM and recommended dose of fertilizers. Each rice entry (30 plants test entry^{-1}) was raised in 50 cm long rows on nursery beds with a 10 cm row spacing in a uniform blast nursery for leaf blast (UBN). Local susceptible variety TN1 was sown after every 5 test entries and on border rows on all sides of the bed to ensure adequate inoculums load and disease transmission. Tetep was used as resistant check variety.

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 test entries. For primary screening in the Uniform Blast Nursery (UBN), Inoculation was done on border lines after 10 days from the date of sowing and

Table 1: List of long grain rice genotypes used in the study

Sl. No.	Genotypes	Grain length (mm)	Released variety/ Advanced breeding line	Source
1.	RNR11718	7.55	Released variety	RRC,ARI, Rajendranagar
2.	RNR18833	10.40	Released variety	RRC,ARI, Rajendranagar
3.	RNR15435	10.46	Released variety	RRC,ARI, Rajendranagar
4.	JGL3855	7.51	Released variety	RARS, Jagtial
5.	JGL28545	8.08	Released variety	RARS, Jagtial
6.	JGL24423	9.08	Released variety	RARS, Jagtial
7.	KNM 733	7.64	Released variety	RARS, Jagtial
8.	KNM1638	7.74	Released variety	ARS, Kunaram
9.	KNM 118	8.45	Released variety	ARS, Kunaram
10.	IR64	9.32	Released variety	IRRI, Phillippines
11.	WGL44	7.74	Released variety	RARS, Warangal
12.	KPS4329	8.47	Advanced breeding line	ARS, Kampasagar
13.	KPS13054	8.04	Advanced breeding line	ARS, Kampasagar
14.	KPS12982	8.37	Advanced breeding line	ARS, Kampasagar
15.	KPS13072	7.56	Advanced breeding line	ARS, Kampasagar
16.	KPS12349	7.60	Advanced breeding line	ARS, Kampasagar
17.	KPS12928	8.11	Advanced breeding line	ARS, Kampasagar
18.	KPS12288	7.01	Advanced breeding line	ARS, Kampasagar
19.	KPS12981	7.39	Advanced breeding line	ARS, Kampasagar
20.	KPS6343	9.16	Advanced breeding line	ARS, Kampasagar
21.	KPS2943	8.32	Advanced breeding line	ARS, Kampasagar
22.	KPS6226	7.70	Advanced breeding line	ARS, Kampasagar
23.	KPS3272	8.33	Advanced breeding line	ARS, Kampasagar
24.	KPS6218	8.26	Advanced breeding line	ARS, Kampasagar
25.	KPS12630	8.15	Advanced breeding line	ARS, Kampasagar
26.	KPS12774	8.15	Advanced breeding line	ARS, Kampasagar
27.	KPS12306	7.62	Advanced breeding line	ARS, Kampasagar
28.	DRRDhan45	9.84	Released variety	IIRR, Rajendranagar
29.	CRDhan311	7.66	Released variety	NRRI, Cuttak
30.	R-RHZ-IB-80	9.00	Advanced breeding line	IGKV, Raipur

disease was allowed to spread naturally via wind dispersal. The inoculum was sprayed in the evening till the entire plant surface became wet with spore suspension and left overnight. Water was sprayed three to four times during day time to maintain high humidity. However, care was taken so that the water was not sprayed immediately after spraying inoculum. The minimum gap between spraying the inoculum and spraying water should be at least 12 h.

From 25 days after sowing until the susceptibility check showed 85% of the blast disease symptom, the disease spectrum of all the test entries was recorded. Blast severity on leaf segments of each test entry was recorded visually using disease scale of 0–9 (Anonymous, 2013). Blast incidence was graded as 0=no visible symptoms were scored (Highly resistance–HR); 1=dark brown pin point lesions (Resistance–R); 2–3=expanding dark brown spots without sporulating lesions (Moderate resistance–MR); 4–5=small circular sporulating lesions (Susceptibility–S) and 6–9=large expanding lesions with sporulation areas (High susceptibility– HS).

2.3. DNA isolation and genotyping

Genotyping of the tested entries was conducted at

ICAR-Indian Institute of Rice Research (IIRR). Leaf samples of thirty genotypes and checks grown in uniform blast nursery (UBN) at Agricultural Research station, Kampasagar, Nalgonda district were collected after 21 days of planting. DNA extraction was done using Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980) with slight modifications. The quality and quantity of the extracted DNA was assessed using agarose gel electrophoresis. After that, the isolated DNA samples were diluted to a final concentration of 50 ng μl^{-1} using 1X TE buffer for PCR amplification.

Molecular profiling of 32 rice lines for the presence of major blast resistance genes was carried out using 12 linked molecular markers. The detailed information on blast resistance genes and their corresponding primer pairs used in this investigation was listed in Table 3. PCR was carried out in a total volume of 10 µl containing 30–50 ng of template, gene linked primers (0.5 µm each; sequences were provided in Table 3, 1×PCR buffer, 0.5 mm dNTP mix, and 5 unit of Taq DNA polymerase (Bangalore Genei, India). The PCR cycle settings for the primers RM224, Nmsmpi9, RM1337, RM53764, AP 5659, Pi54MAS, RM206, RM166, RM208, RM246, Zt56591, RM21 were 94˚C for 5 min, followed by 35 cycles of 94˚C for 30s, 58˚C for 30s and 72˚C for 1 min and a final extension at 72˚C for 7 min. PCR amplification was done twice for each marker to double check the results. The amplicons were then differentiated electrophoretically on the agarose gel of appropriate concentration and voltage was set at 5–6V cm^{-1}, intense bands were visualized under UV light. The size of the amplified fragments was calculated using 100 bp ladder or 100 bp ladder (Bangalore Genei, India) as size reference standards and the scoring was done for the PCR analysis as presence (1) or absence (0).

2.4. Allele scoring

The presence or absence of an allele was indicated as 1 and 0, respectively, in the amplified PCR products of 11 markers.

3. RESULTS AND DISCUSSION

3.1. Phenotyping of germplasm lines

Thirty long grain rice genotypes were assessed against rice blast and based on reaction, categorized into resistant, moderately resistant, moderately susceptible and susceptible (Table 2). Of these, three genotypes viz., KPS 2943, KPS 3272 and KPS 6218 were found resistant with a score of 1. Five genotypes, JGL 3855, KPS 12349, KPS 12774, CR Dhan 311 and R-RHZ-IB-80 were found moderately resistant with a blast score of 2. Another set comprising of nine genotypes, RNR 15435, JGL 28545, KNM 733, KNM 118, IR 64, KPS 13054, KPS 6343, KPS 6226 and KPS 12630 were also classified as moderately resistant with blast score 3. Genotypes like RNR 11718, RNR 18833, JGL 24423, KNM 1638, WGL 44, DRR Dhan 45 and five advanced breeding lines fell into the moderately susceptible category with a score of 4–5, while two genotypes i.e., KPS 12982 and KPS 13072 were classified as susceptible with a score of 6–7. None of the genotypes fell into highly susceptible category (scores 8–9). In rice, large scale

Table 2: Screening of long grain rice genotypes for blast resistance using 0–9 SES scale (Anonymous, 2013)

Sl. No.	Entry	Reaction to blast (Score 0–9 Scale)	Disease reaction
1.	RNR 11718	4	MS
2.	RNR 18833	4	MS
3.	RNR 15435	3	MR
4.	JGL 3855	2	MR
5.	JGL 28545	3	MR
6.	JGL 24423	4	MS
7.	KNM733	3	MR
8.	KNM1638	4	MS
9.	KNM118	3	MR
10.	IR64	3	MR
11.	WGL44	4	MS
12.	KPS4329	4	MS
13.	KPS13054	3	MR
14.	KPS12982	7	S
15.	KPS13072	6	S
16.	KPS12349	2	MR
17.	KPS12928	4	MS
18.	KPS12288	5	MS
19.	KPS12981	4	MS
20.	KPS6343	3	MR
21.	KPS2943	1	R
22.	KPS6226	3	MR
23.	KPS3272	1	R
24.	KPS6218	1	R
25.	KPS12630	3	MR
26.	KPS12774	2	MR
27.	KPS12306	4	MS
28.	DRRDhan45	4	MS
29.	CRDhan311	2	MR
30.	R-RHZ-IB-80	2	MR
31.	TN1 (Ch)	6	MS
32.	Tetep (Ch)	1	R

R: Resistant; MR: Moderately resistant; MS: Moderately susceptible; S: Susceptible

cultivation of high yielding rice varieties replaced land races and traditional varieties resulting reduced genetic diversity, thus limiting the chances of improving the varieties with the available genetic resources (Yadav et al., 2019; Tanksley and McCouch, 1997). With the widespread cultivation of narrow genetic base cultivars over a large area, blast pathogen population was subjected to selection pressure as a result, new virulent races were emerged. Globally, rice production has become a threat as a result of the emergence of these new virulent races. However, the problem could be overcome by identifying promising donors for unique functional genes or alleles that would help to combat the disease and ensure sustainable rice production (Yadav et al., 2019, Vasudevan et al., 2014). The present experiment investigated the genetic diversity of released varieties and advanced breeding materials for blast resistance genes using 11 molecular markers.

3.2. Genotyping of rice germplasm lines for blast resistance

Genotyping of 30 rice genotypes was done using eleven gene based molecular markers for nine major blast resistant genes along with field screening. Under uniform blast nursery (UBN), 30 rice accessions along with Tetep and TN-1 as resistant and susceptible checks, respectively were screened for leaf blast disease resistance. Of these, 3 (9%) and 14 (44%) genotypes were found resistant and moderately resistant to leaf blast disease, respectively. Surprisingly, 3 accessions that were found resistant to leaf blast resistance were advanced breeding lines and among the 14 moderately resistant genotypes seven each were released genotypes and advanced breeding materials.

The molecular profiling of 30 long grain rice accessions for blast resistance genes using eleven gene-specific markers revealed significant variation in the presence of resistance genes. Eleven linked blast markers RM224, Nmsmpi9, RM1337, RM5364, AP5659-5, Pi54MAS, RM 116, RM 208, RM 246, Zt56591, RM21 targetting blast resistance genes Pi1, Pi9, Pi20, Pi20, Pi2, Pi54, Pib, Pib, Pitp, Pizt and Pi38, respectively (Table 3) and the presence or absence of rice blast resistance genes was presented (Table 4). Allele scoring data of 30 genotypes revealed that the frequency of the positive alleles of R genes ranged from

Table 3: Details of markers used for molecular screening of blast resistant genes in 30 rice genotypes

Molecular markers	Linked gene	Primer sequence	Chr	'R' allele	'S' allele	Reference
RM224	Pi1	F:CTCGATCGATCTTCACGAGG	11	130	160	Hittalmani et al., 2000
		R:TGCTATAAAAGGCATTCGGG				
Nmsmpi9	Pi9	F:CGAGAAGGACATCTGGTACG	6	168	250	Qu et al., 2006
		R:GAGATGCTTGGTTTAGAAGAC				
RM1337	Pi20	F:GCTGAGGAGTATCCTTTCTC	12	210	190	Li et al., 2008
		R:ACCATAGGAAGATCATCACA				
RM5364	Pi20	F:GTATTACGCTCGATAGCGGC	12	148	300	Li et al., 2008
		R:GTATCCTTTCTCGCAATCGC				
AP5659-5	Pi2	F:CTCCTTCAGCTGCTCCTC	6	288	250	Fjellstrom et al., 2006
		R:TGATGACTTCCAAACGGTAG				
Pi54MAS	Pi54	F:CAATCTCCAAAGTTTTCAGG	11	216	359	Ramkumar et al., 2011
		R:GCTTCAATCACTGCTAGACC				
RM166	Pib	F:GGTCCTGGGTCAATAATTGGGTTACC	11	258	-	Fjellstrom et al., 2004
		R:TTGCTGCATGATCCTAAACCGG				
RM208	Pib	F:TCTGCAAGCCTTGTCTGATG	2	173	185	Fjellstrom et al., 2004
		R:TAAGTCGATCATTGTGTGGACC				
RM246	Pitp	F:GAGCTCCATCAGCCATTCAG	1	116	100	Ma et al., 2014
		R:CTGAGTGCTGCTGCGACT				
Zt56591	Pizt	F:TTGCTGAGCCATTGTTAAACA	6	257	-	Hayashi et al., 2006
		R:ATCTCTTCATATATATGAAGGCCAC				
RM 21	Pi38	F:ACAGTATTCCGTAGGCACGG	11	157	170	Gowda et al., 2006
		R:GCTCCATGAGGGTGGTAGAG				

Table 4: Heatmap depicting combinations of blast resistance genes in rice genotypes

Sl. No.	Genotypes	Genes-Markers											Gene combinations	Total genes
		RM 224	RM 166	RM 21	RM 246	RM 208	RM 13 37	RM 53 64	Pi54 MAS	Zt 565 91	AP 565 9-5	Nms-mpi9		
		Pi1	Pib	Pi38	Pitp	Pib	Pi20	Pi20	Pi54	Piz-t	Pi2	Pi9		
1.	KNM733	0	0	0	0	0	0	0	0	1	1	0	Piz-t, Pi2	2
2.	KPS6218	0	0	0	1	0	0	0	0	1	1	0	Pitp, Piz-t, Pi2	3
3.	KPS12306	0	0	0	0	0	0	0	0	1	1	0	Piz-t, Pi2	2
4.	KPS12928	0	0	0	0	0	0	0	0	1	1	0	Piz-t, Pi2	2
5.	JGL3855	0	0	0	0	0	0	0	0	1	1	0	Piz-t, Pi2	2
6.	KPS13072	0	0	0	0	0	0	0	0	1	1	0	Piz-t, Pi2	2
7.	JGL24423	0	0	0	0	0	1	0	0	1	1	0	Pi20, Piz-t, Pi2	3
8.	KPS12982	0	0	0	0	0	0	0	0	1	1	0	Piz-t, Pi2	2
9.	JGL28545	0	0	0	0	0	1	0	0	1	1	0	Pi20, Piz-t, Pi2	3
10.	KPS12349	1	0	0	0	0	1	0	0	1	1	0	Pi1, Pi20, Piz-t, Pi2	4
11.	KPS6343	0	0	0	0	0	1	0	0	1	1	0	Piz-t, Pi2	2
12.	RNR11718	0	0	0	0	0	0	0	0	1	1	0	Piz-t, Pi2	2
13.	CRRDhan311	0	0	0	0	0	1	0	0	1	1	0	Pi20, Piz-t, Pi2	3
14.	KNM1638	0	0	0	0	0	0	0	0	1	1	0	Piz-t, Pi2	2
15.	IR64	0	0	0	0	0	0	0	0	1	1	0	Piz-t, Pi2	2
16.	KNM118	0	0	0	1	0	1	0	0	1	1	0	Pitp, Pi20, Piz-t, Pi2	4
17.	RNR18833	0	0	0	1	0	0	0	0	1	1	0	Pitp, Piz-t, Pi2	3
18.	KPS12774	0	0	0	1	0	1	0	0	1	1	0	Pitp, Pi20, Piz-t, Pi2	4
19.	RNR15435	0	1	0	1	0	1	0	1	1	1	0	Pib, Pitp, Pi20, Pi54, Piz-t, Pi2	6
20.	KPS12288	0	0	0	1	0	1	0	0	1	1	0	Pitp, Pi20, Piz-t, Pi2	4
21.	KPS3272	0	0	0	1	0	1	0	0	1	1	0	Pitp, Pi20, Piz-t, Pi2	4
22.	KPS6226	0	0	0	1	0	0	0	0	1	1	0	Pitp, Piz-t, Pi2	3
23.	KPS2943	0	0	0	1	0	0	0	0	1	1	0	Pitp, Piz-t, Pi2	3
24.	R-RHZ-IB-80	0	0	0	1	0	1	0	0	1	1	0	Pitp, Pi20, Piz-t, Pi2	4
25.	DRRDhan45	0	0	0	1	0	1	0	0	1	1	0	Pitp, Pi20, Piz-t, Pi2	4
26.	KPS12630	0	0	0	1	0	0	0	0	1	1	0	Pitp, Piz-t, Pi2	3
27.	WGL44	1	0	0	1	0	1	0	0	1	1	0	Pi1, Pitp, Pi20, Piz-t, Pi2	5
28.	KPS13054	0	0	1	1	0	1	0	0	1	1	0	Pi38, Pitp, Pi20, Piz-t, Pi2	5
29.	KPS12981	0	0	0	1	0	0	0	0	1	1	0	Pitp, Piz-t, Pi2	3
30.	KPS4329	0	0	0	1	0	0	0	0	1	1	0	Pitp, Piz-t, Pi2	3
No. of positive alleles		2	1	1	16	0	14	0	1	30	30	0		

two (nine genotypes) to six (single genotypes) among the 30 genotypes. The highest number of resistance genes was detected in RNR 15435 with 6 genes followed by 5 genes in WGG 44 and KPS 13054. Among the resistance genes, Pizt and Pi2 were the most prevalent present in all genotypes, while Pi9 linked to Nmsmpi9 and Pi20 linked to RM5364 were absent in all genotypes.

Electrophoresis pattern of each SSR marker linked to blast resistant gene in rice genotypes was shown in Figure 1. Estimation of PCR results for eleven blast resistance genes viz., RM224 (Pi1), Nmsmpi9 (Pi9), RM1337 (Pi20), RM5364 (Pi20), AP5659-5 (Pi2), Pi54MAS (Pi54), RM166 (Pib), RM208 (Pib), RM246 (Pitp), Zt56591 (Pizt), RM21 (Pi38), were determined by visualization of amplicons on near 130 bp, 168 bp, 210 bp, 148 bp, 288 bp, 216 bp, 258 bp, 173 bp, 116 bp, 257 bp and 157 bp of positive fragments, respectively.

Using AP5659-5 marker to visualize a 288 bp amplicon, the rice blast R-gene Pi2 was dis covered on chromosome number 6 in all the genotypes. On the contrary, Pi9 rice blast resistance gene was determined by visualization of amplicons on 168 bp of positive fragment using SSR primer Nmsmpi9 on the chromosome number 6 and was not detected in all the genotypes. Pi20 resistant gene on chromosome 12 was amplified with markers RM1337 and RM5364. Amplicon sizes were 210 bp and 148 bp for markers RM1337 and RM5364, respectively. Pi20 was scored positive for RM1337 marker in resulted in 14 genotypes, while it was tested negative in all the 30 genotypes for marker RM5364.

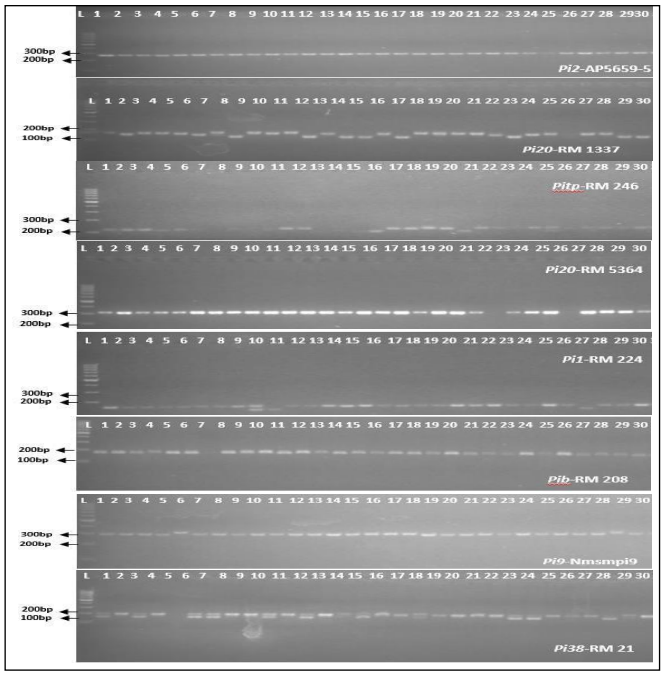

Figure 1: Screening of rice genotypes for blast resistance genes using agarose gel electrophoresis

Visualization of 116 bp amplicon using RM246 marker was found for Pitp rice blast resistance gene on the chromosome number 1 in 14 genotypes. Marker RM224 was found to amplify gene Pi1 with amplicon size of 130 bp on chromosome number 11 only in two accessions i.e., KPS 12349 and WGL 44. Pib rice blast resistance gene was determined by visualization of amplicons on 173 bp and 258 bp of positive fragments using SSR primers RM208, RM166 on the chromosome number 2 and 11, respectively. Existence of Pib gene was not detected with marker RM208, while it was scored on RNR15435 with marker RM166.

Using the linked marker RM21, the blast resistance gene Pi38 on the chromosome number 11 was tested positive on KPS13054. Blast resistance gene Pi54 was also scored in KPS13054 with marker Pi54MAS. Results from genotyping studies for blast resistance revealed that among eleven blast resistant genes studied, genes Pi2 and Pizt were found in all genotypes, gene Pi20 was present in 14 genotypes (JGL24423, JGL28545, KPS12349, KPS6343, CRR Dhan311, KNM118, KPS12774, KPS12288, RNR15435, KPS3272, R-RHZ-IB-80, DRR Dhan 45, WGL44, KPS13054), gene Pitp was detected in 14 genotypes (KNM118, RNR18833, KPS12774, RNR15435, KPS3272, KPS6226, KPS2943, R-RHZ-IB-118, DRR Dhan 45, KPS12630, WGL44, KPS13054, KPS12981, KPS4329), gene Pi1 was present in 2 genotypes (WGL44, KPS12349), genes Pi54 and Pib were found in RNR15435, gene Pi38 was scored in KPS13054 and gene Pi9 was absent in all genotypes. The genotypes which were found resistant to blast in phenotypic screening showed positive for three genes viz., Pitp, Pi2 and Pizt. Whereas genotypes which were found susceptible to blast in phenotypic screening had only two genes Pi2 and Pizt.

3.3. Marker trait association

The genotypes were categorized into four groups based on their response to blast disease and their marker genotype (Table 4). Genotypes that were phenotypically resistant and carried positive alleles of resistance genes (R/+) were considered to exhibit a strong marker-trait association. Some genotypes displayed phenotypic resistance without the presence of resistance genes (R/-), while others showed susceptibility but had positive resistance alleles (S/+). Moreover, some genotypes were both phenotypically susceptible and lacked resistance alleles (S/-). Phenotypic screening against blast incidence was highly challenging as it was heavily influenced by developmental stage and environmental factors. However, using a linked marker associated with the R genes was the easiest and most reliable way to identify individual/multiple gene(s) (Susan et al., 2019; Hayashi et al., 2006). The frequency of R-gene positive alleles ranged from 0% to 100%, with the genetic frequency of 11 major blast resistance genes ranging from

18 to 55%. Genotype RNR 15435 had the maximum 6 resistant genes followed by WGL 44 and KPS 13054 with 5 resistant genes (Jia et al., 2004; Ingole et al., 2014; Yan et al., 2017). Similar findings were reported by Yadav et al. (2017), Susan et al. (2019) and Jeevan et al. (2023).

The most promising genotypes for blast resistance were RNR 15435, KPS 13054, KPS 12349, KNM 118, KPS 12774, KPS 3272 and R-RHZ-IB-80 which demonstrated strong marker-trait associations and had multiple resistance genes such as Pi1, Pi2, Pi20, Pi38, Pi54, Pib, Pitp, and Pizt. These genotypes possessed high genetic potential for blast resistance with a blast score of 1 or 2 or 3. In particular, genotypes RNR 15435 and KPS 13054 possessed 6 and 5 resistant genes, respectively and were identified as promising candidates for breeding blast resistance in rice crop improvement.

In the present study, majority of the rice accessions had the R-genes Pitp and Pi20 but, genes Pizt, and Pi2 were present in all 30 rice accessions. The Pi2 gene was detected in the majority of the rice accessions with high resistance to leaf blast (Imam et al., 2014). Of the 52 rice accessions tested, 12 had Pizt gene in the study of Jeevan et al. (2023). Earlier research findings found that gene Pi2 expressed NBS-LRR type R proteins which were responsible to increase the resistant ability in rice against leaf blast disease across a broad spectrum of pathogenic races (Deng et al., 2006; Meng et al., 2020). Gene Pi1 was found in only two accessions KPS 12349 and WGL 44, Pib and Pi54 genes were detected in RNR 15435, Pi38 was found in KPS 13054 and none of the accessions had Pi9 gene. Conversely, Pib gene was found in all eighty rice accessions studied by Yadav et al. (2017) and Pi1 gene was found in 39 landraces in the study of Ingole et al. (2014). Similar study of rare prevalence of Pi9 gene was documented (Imam et al., 2014; Yadav et al., 2017). This could be due to the origin of Pi9 gene in the wild species *O. minuta* and its subsequent introgression into Indica rice (Imam et al., 2014). All the three resistant genotypes viz., KPS 2943, KPS 3272 and KPS 6218 had all the three resistant genes Pitp, Pizt and P2 indicating strong association between resistant genes and observed phenotypes. Contrary to this, majority of the moderately susceptible genotypes to blast also had these three resistant genes suggesting lack of correlation between genotype and phenotype.

4. CONCLUSION

The identified resistant germplasm for leaf blast through phenotyping and the molecular characterization of blast resistance genes could act as promising donors for development of leaf blast resistance cultivars. This led to sustainable rice production in blast-endemic areas promoting sustainable agricultural practices and enhanced food security.

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