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MOLECULAR CHARACTERIZATION AND VALIDATION OF SSR MARKERS ASSOCIATED WITH SHEATH BLIGHT TOLERANCE IN MAGIC *INDICA* POPULATION OF RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Sheath blight, caused by *Rhizoctonia solani* Kühn, is one of the major constraints to rice production, and its quantitative inheritance limits the effectiveness of conventional breeding. The present study aimed to validate reported simple sequence repeat (SSR) markers associated with sheath blight resistance and assess the genetic diversity and marker–trait associations among selected MAGIC indica rice lines. Twenty relatively tolerant and 20 susceptible lines from Among 365 MAGIC *indica* lines phenotypically screened under field conditions, together with four check varieties, were selected for molecular characterization. A total of 136 SSR markers, including 55 sheath blight QTL linked and 81 genome wide markers, were screened, of which 9 SSR markers (6.62%) (RM336, RM224, RM209, RM216, RM205, RM202, RM12398, RM10255 and RM11229) were polymorphic. These markers detected 26 alleles with an average of 2.89 alleles per locus. The average major allele frequency, gene diversity, observed heterozygosity, and polymorphic information content (PIC) were 0.665, 0.457, 0.071, and 0.390, respectively. Marker RM336 was the most informative, exhibiting the highest gene diversity (0.63) and PIC (0.59). Allelic profiling using the resistant checks Tetep and Wazuhophek identified several MAGIC lines carrying multiple favorable alleles associated with sheath blight resistance, with genotypes 271, 291, 66, 313, 189, and 326 showing the highest recovery of resistant alleles. Single marker analysis revealed significant ($P < 0.01$) associations between all nine polymorphic markers and sheath blight related traits. RM205 showed the strongest association, explaining up to 71.2% of the phenotypic variation for relative lesion height and 63.4% for disease score. Neighbor joining cluster analysis grouped the 46 genotypes into three major clusters with six subclusters, indicating moderate genetic diversity and weak population structure. Tolerant and susceptible genotypes were distributed across the same clusters, reflecting the complex quantitative inheritance of sheath blight resistance.The validated SSR markers, particularly RM205, RM336, RM202, RM209, and RM224, together with the identified MAGIC lines carrying favorable alleles, represent valuable genetic resources for marker assisted breeding to develop sheath blight resistant rice cultivars.

Keywords: Sheath blight, MAGIC population, SSR markers, Genetic diversity, Single marker analysis, Marker validation, Polymorphic information content (PIC), Rice.

Sheath blight (SB), caused by the soil borne fungal pathogen *Rhizoctonia solani* Kühn, is one of the most destructive diseases of rice, resulting in significant yield and quality losses worldwide. Under favorable environmental conditions, yield losses may reach up to 50% in major rice growing regions (Zheng *et al*., 2013). Complete resistance to sheath blight has not been identified in cultivated rice germplasm; however, several genotypes, including Tetep, Tadukan, Teqing, Jasmine 85, ZYQ8, Minghui 63, LSBR-5, and LSBR-33, exhibit moderate to partial resistance and serve as valuable donors in breeding programs (Li *et al*., 1995; Pan *et al*., 1999; Liu *et al*., 2009). Resistance to sheath blight is a complex quantitative trait governed by multiple genes or quantitative trait loci (QTLs), each contributing a small phenotypic effect (Pinson *et al*., 2005; Jia *et al*., 2009). To date, more than 200 QTLs associated with sheath blight resistance have been identified using diverse mapping populations, including doubled haploid lines, recombinant inbred lines, near isogenic lines, and backcross populations (Zarbafi and Ham, 2019). However, the effects of many reported QTLs vary across genetic backgrounds and environments, limiting their consistent utilization in marker assisted breeding. Therefore, validation of molecular markers linked to these QTLs is essential before their deployment in breeding programs.

Multiparent Advanced Generation Intercross (MAGIC) populations provide a powerful resource for dissecting complex traits because they combine multiple founder genomes and accumulate extensive recombination through repeated intercrossing. Consequently, MAGIC populations possess high allelic diversity, enhanced mapping resolution, and

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greater statistical power for identifying marker trait associations compared with conventional biparental populations (Mackay and Powell, 2007; Cavanagh *et al*., 2008; Bandillo *et al*., 2013; Leung *et al*., 2015).The present study was undertaken to identify potential sources of sheath blight tolerance and validate SSR marker trait associations by evaluating 365 MAGIC indica rice lines for sheath blight resistance.

MATERIAL AND METHODS

Among the 365 MAGIC indica lines evaluated for sheath blight tolerance at ICAR–IIRR, Rajendranagar, 20 moderately tolerant and 20 susceptible lines, identified using the SES (IRRI, 1988) disease score (0–9 scale), were selected for molecular characterization using SSR markers. Molecular analysis was carried out in IBT, PJTSAU Rajendranagar with identified 20 sheath blight tolerant lines (103, 152, 188, 189, 205, 326, 350, 385, 271, 126, 291, 137, 263, 313, 66, 336, 151, 175 147 and 5) and 20 (40, 42, 267, 21, 382, 252, 27, 323, 285, 174, 38, 26, 279, 4, 172, 184, 245, 122, 390 and 363) susceptible lines of MAGIC *indica* population along with checks *viz.* Wazuhophek, Tetep and BPT-5204, Swarna respectively using SSR markers.

DNA extraction

Young leaf samples were collected from all selected genotypes and genomic DNA was extracted using the modified CTAB method (Murray and Thompson,1980). DNA quality and concentration were assessed by electrophoresis on 0.8% agarose gel, and DNA samples were diluted to a uniform concentration for PCR amplification. A total of 136 SSR markers, comprising 55 reported sheath blight QTL linked SSR markers and 81 genome wide SSR markers distributed across the 12 rice chromosomes, were used for genotyping. PCR amplification was carried out using standard reaction conditions, and the amplified products were separated by agarose gel electrophoresis. Bands were scored manually as polymorphic or monomorphic.

Genetic diversity analysis

Allelic data generated from polymorphic SSR markers were used to estimate the number of alleles, major allele frequency, gene diversity (expected heterozygosity), observed heterozygosity, and polymorphic information content (PIC) using PowerMarker version 3.25 (Liu and Muse, 2005). Genetic relationships among the selected genotypes were assessed by constructing a Neighbor-Joining (NJ) dendrogram based on simple matching coefficients using DARwin version 5.0.158 (Perrier and Jacquemoud-Collet, 2006).

Marker validation and Single Marker Analysis (SMA)

Allelic diversity of the selected MAGIC lines was assessed using the resistant checks Tetep and Wazuhophek as reference genotypes. The selected lines were screened with sheath blight resistance linked SSR markers, including qSBR7-1, qSB-9-2, qSBR11-1, qSBR11-2, qSBR11-3, RM216, RM10255, RM12398, and RM11229, to validate their association with sheath blight tolerance. Single marker analysis (SMA) was performed to determine the association between polymorphic SSR markers and sheath blight-related traits, namely plant height (PH), lesion height (LH), relative lesion height (RLH), and sheath blight disease score. Marker trait associations were estimated by regression analysis using Microsoft Excel, and the coefficient of determination (R^2), F value, and significance level were used to evaluate the strength of marker associations.

RESULTS AND DISCUSSION

Genotyping of the MAGIC population using SSR markers

Among the 365 MAGIC *indica* lines evaluated for sheath blight tolerance, none exhibited complete or moderate resistance to the disease. Therefore, the lines with a disease score of 5 (moderately susceptible) were considered relatively tolerant, as their reaction was comparable to that of the resistant checks Wazuhophek and Tetep. Based on the disease scores recorded under field conditions, 20 relatively tolerant (score 5) and 20 highly susceptible (score 9) MAGIC lines were selected for molecular characterization using SSR markers. A total of 136 SSR markers, comprising 55 reported sheath blight QTL linked markers and 81 genome wide SSR markers distributed across all 12 rice chromosomes, were screened for polymorphism among the selected genotypes (Table 1). Of the 55 QTL linked markers, six markers (RM336, RM205, RM216, RM202, RM224, and RM209) were polymorphic, while among the 81 genome-wide SSR markers, only three markers (RM11229, RM10255, and RM12398) showed polymorphism (Fig. 1). Overall, only nine

markers (6.62%) exhibited polymorphism and were subsequently used for genetic diversity analysis and marker trait association studies (Table 2).

The low level of SSR polymorphism observed in the present investigation is in agreement with earlier reports on sheath blight resistance in rice. Sharma *et al.* (2009) screened 808 SSR markers covering all 12 rice chromosomes and identified 149 polymorphic markers between the sheath blight susceptible cultivar Rosemont and the resistant cultivar Pecos. Similarly, Channamallikarjuna *et al.* (2010) evaluated 637 SSR markers between the parents HP2216 and Tetep and detected only 74 polymorphic markers for mapping sheath blight resistance QTLs. The limited polymorphism observed in these studies, as well as in the present investigation, suggests that polymorphism at sheath blight resistance-associated genomic regions is relatively low in rice. Therefore, identification and validation of informative SSR markers remain essential for marker-assisted breeding and the improvement of sheath blight tolerance.

Table 1. Summary of SSR markers screened for sheath blight tolerance in the MAGIC rice population

Marker category	No. of markers screened	Polymorphic	Monomorphic	Polymorphism (%)
Sheath blight QTL linked SSR markers	55	6	49	10.91
Genome-wide SSR markers	81	3	78	3.70
Total	**136**	**9**	**127**	**6.62**

Table 2. Polymorphic SSR markers identified among the selected MAGIC rice lines

S. No.	Marker	Chro.	Associated QTL/Region	Marker type
1	RM336	7	qSBR7-1	QTL linked
2	RM205	9	qSB9-2	QTL linked
3	RM216	9	Reported SB region	QTL linked
4	RM202	11	qSBR11-3	QTL linked
5	RM224	11	qSBR11-1	QTL linked
6	RM209	11	qSBR11-2	QTL linked
7	RM11229	1	Genome-wide	SSR
8	RM10255	1	Genome-wide	SSR
9	RM12398	2	Genome-wide	SSR

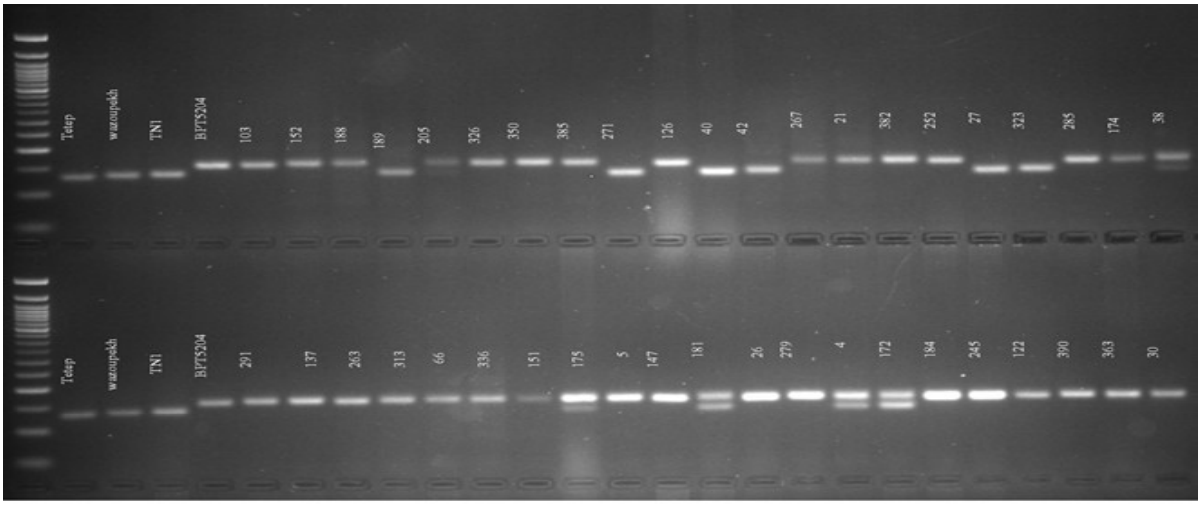

Fig. 1. PCR amplification profile of RM224 in sheath blight tolerant and susceptible MAGIC rice lines and check varieties

Genetic Diversity Analysis

Genetic diversity parameters, including the number of alleles, major allele frequency (MAF), gene diversity (expected heterozygosity; He), observed heterozygosity, and polymorphic information content (PIC), were estimated using PowerMarker version 3.25 (Liu and Muse, 2005). A total of 26 alleles were detected across the nine polymorphic SSR markers, with an average of 2.89 alleles per locus (Table 3). The number of alleles per marker ranged from 2 to 5. The highest number of alleles (5) was observed for RM336, followed by three alleles each for RM205, RM202, RM209, RM10255, and RM12398, whereas RM224, RM216, and RM11229 each amplified two alleles. The major allele frequency (MAF) ranged from 0.48 (RM205) to 0.79 (RM216), with an average of 0.66. Observed heterozygosity (Ho) ranged from 0.02 to 0.15, with the highest value recorded for RM336 (0.15), followed by RM224 (0.11) and RM216 (0.09), while the remaining markers showed lower heterozygosity (Table 3).

(expected heterozygosity) was 0.45, ranging from 0.21 (RM11229) to 0.63 (RM336). The relatively high gene diversity and PIC value exhibited by RM336 indicate its potential utility in future diversity studies and marker-assisted breeding for sheath blight tolerance. The mean PIC value obtained in the present study (0.45) is comparable with the values reported by Pal *et al.* (2004) (0.40) in Basmati and non-Basmati rice varieties and Salgotra *et al.* (2015) (0.40) in Basmati germplasm from the north western Himalayas. However, it was slightly lower than the average PIC value of 0.46 reported by Nachimuthu *et al.* (2015) in Indian rice landraces. Similarly, the average gene diversity (0.45) observed in the present study was marginally lower than the values of 0.52 and 0.54 reported by Nachimuthu *et al.* (2015) and Choudhary *et al.* (2013), respectively, in diverse rice germplasm collections. The average major allele frequency (0.66) observed in the present study is in agreement with the findings of Singh *et al.* (2016). Overall, these results indicate that the selected MAGIC population possesses moderate

Table 3. Genetic diversity parameters of the nine polymorphic SSR markers used for genotyping the selected MAGIC rice lines

Marker	Major allele frequency (MAF)	Sample size (N)	No. of observations	No. of alleles	Gene diversity (He)	Observed heterozygosity (Ho)	PIC
RM224	0.750	46	46	2	0.380	0.110	0.300
RM209	0.554	46	46	3	0.540	0.020	0.450
RM10255	0.750	46	46	3	0.410	0.070	0.370
RM12398	0.620	46	46	3	0.540	0.070	0.470
RM205	0.489	46	46	3	0.570	0.020	0.470
RM202	0.587	46	46	3	0.500	0.040	0.400
RM216	0.796	46	44	2	0.330	0.090	0.270
RM336	0.554	46	46	5	0.630	0.150	0.590
RM11229	0.880	46	46	2	0.210	0.070	0.190
Mean	0.665	46	46	2.89	0.457	0.071	0.390

Polymorphic information content (PIC), an indicator of the discriminatory power of a molecular marker (Kupper *et al*., 2011), ranged from 0.19 to 0.59. Among the markers evaluated, RM336 exhibited the highest PIC value (0.59), indicating that it was the most informative marker for assessing genetic diversity among the selected MAGIC lines. Moderate PIC values were observed for RM12398 (0.45), RM205 (0.45), and RM209 (0.45), suggesting that these markers also possess good discriminatory ability for diversity analysis. The average gene diversity

genetic diversity, with RM336 emerging as the most informative SSR marker among those evaluated.

Identification of Marker–Trait Associations for Sheath Blight Tolerance

Allelic Diversity Analysis

The 20 relatively tolerant and 20 susceptible MAGIC indica lines selected based on phenotypic screening were genotyped using the nine polymorphic SSR markers, comprising six reported sheath blight QTL linked markers (qSBR7-1, qSB9-2, qSBR11-1,

qSBR11-2, qSBR11-3, and RM216) and three genome wide SSR markers (RM10255, RM12398, and RM11229). The resistant cultivars Tetep and Wazuhophek were used as reference (differential) genotypes for scoring allelic diversity. The allelic profiles of the selected MAGIC lines were compared with those of the resistant checks, and the results are presented in Table 4. Among the selected genotypes, MAGIC line 271 exhibited the highest level of similarity with the resistant checks, sharing identical alleles at six sheath blight resistance linked markers (qSBR7-1, qSB9-2, qSBR11-1, qSBR11-2, qSBR11-3, and RM216) together with the genome-wide marker RM12398. Likewise, genotype 291 shared resistant alleles at six QTL linked markers (qSBR7-1, qSB9-2, qSB9-2, qSBR11-2, qSBR11-3, and RM216) and at RM12398. Genotype 66 possessed resistant alleles at six sheath blight associated markers along with the genome wide markers RM10255 and RM11229. Genotype 313 exhibited resistant alleles at four sheath blight QTL linked markers (qSBR7-1, qSB9-2, qSB9-2, and qSBR11-3) together with RM11229, whereas genotype 189 showed identical alleles at four QTL linked markers (qSBR7-1, qSB9-2, qSBR11-1, and qSBR11-2) and at RM12398. Similarly, genotype 326 shared resistant alleles at qSB9-2, qSB9-2, qSBR11-2, and qSBR11-3, in addition to RM10255. In contrast, genotypes 147 and 252 showed similarity with the resistant checks for only two markers each, indicating comparatively lower recovery of resistance-associated alleles.

Interestingly, a few phenotypically susceptible genotypes also possessed several resistance associated alleles. Genotype 172 showed similarity with the resistant checks for eight markers, genotype 184 for seven markers, and genotype 363 for six markers. The presence of resistance linked alleles in these susceptible lines suggests that sheath blight resistance is governed by multiple QTLs with small individual effects and is strongly influenced by genetic background and environmental factors. Therefore, the presence of favorable alleles at a few loci alone may not be sufficient to confer phenotypic resistance. The MAGIC indica lines exhibiting allelic profiles similar to those of the resistant checks Tetep and Wazuhophek are presented in Table 5. The present findings are in agreement with those of Singh et al. (2016), who screened 60 upland rice genotypes using SSR markers linked to eight major sheath blight resistance QTLs (qSBR7-1, qSBR9-1, qSBR8-1, qSBR1-1, qSBR11-2, qSBR11-1, qSBR11-3, and qSBR3-1) with Tetep as the differential genotype. They reported that RM336, linked to qSBR7-1, amplified a characteristic 158 bp allele in Tetep that was associated with sheath blight resistance. Similar marker based validation of resistance associated alleles has also been reported by Imam et al. (2014), who assessed the frequency of major blast resistance genes in rice germplasm using gene linked molecular markers.

QTL Analysis for Sheath Blight Tolerance Traits

Single-Marker Analysis

Single-marker analysis (SMA) was performed to identify and validate SSR markers associated with sheath blight tolerance related traits, namely plant height (PH), lesion height (LH), relative lesion height (RLH), and sheath blight disease score, using the phenotypic and genotypic data of the MAGIC *indica* population. The analysis was carried out using the simple linear regression approach described by Haley and Knott (1992). The results of the regression analysis revealed that all nine polymorphic SSR markers (RM224, RM209, RM10255, RM12398, RM205, RM202, RM216, RM336, and RM11229) showed significant associations with one or more sheath blight tolerance related traits (PH, LH, RLH, and disease score) (Table 6). These marker trait associations are in agreement with earlier reports, as the identified markers have previously been associated with sheath blight resistance in different rice mapping populations (Table 7).

The phenotypic variance explained (R^2) by the significant markers ranged from 0.14 to 0.90. The highest phenotypic variance ($R^2 = 0.90$) was recorded by RM10255 for plant height (PH), whereas the lowest ($R^2 = 0.14$) was observed for the same marker with sheath blight disease score. Similar results were reported by Boranayaka et al. (2018), who identified SSR markers RM518 and RM225 as significantly associated with water use efficiency (WUE) and nitrogen use efficiency (NUE), respectively, in the F_2 mapping populations of BPT 5204 × WAB 450 and BPT 5204 × Mysore Mallige using single marker analysis. The results of the present study indicate the

Table 4. Genotypic profiling of MAGIC indica rice lines using SSR markers linked to reported sheath blight resistance QTLs

S.No	qSBR7-1	qSBR11-1	qSBR11-3	qSBR11-2	Qsb9-2	qsbh9.2	no. of genes	Non gene specific			no. of genes	Total no. of genes
	RM336	RM224	RM202	RM209	RM216	RM205		RM12398	RM10255	RM11229		
Tetep	1	1	1	1	1	1	6	1	1	1	3	9
Wazuhophek	1	1	1	1	1	1	6	1	1	1	3	9
TN1	0	1	0	0	1	0	2	1	0	1	2	4
BPT	0	0	1	1	1	1	4	0	1	0	1	5
103	1	0	1	0	0	1	3	0	1	0	1	4
152	0	0	1	1	0	1	3	0	0	0	0	3
188	1	0	1	0	0	1	3	0	0	0	0	3
189	1	1	0	1	1	0	4	1	0	0	1	5
205	0	0	1	0	0	1	2	0	1	0	1	3
326	0	0	1	1	1	1	4	0	1	0	1	5
350	1	0	0	1	1	0	3	0	0	0	0	3
385	1	0	1	0	1	1	4	0	0	0	0	4
271	1	1	1	1	0	1	5	1	0	0	1	6
126	0	0	1	1	1	1	4	0	0	0	0	4
291	1	0	1	1	1	1	5	1	0	0	1	6
137	1	0	1	1	1	0	4	0	0	0	0	4
263	1	0	0	1	0	1	3	0	1	1	2	5
313	1	0	1	0	1	1	4	0	0	1	1	5
66	1	0	1	1	0	1	4	0	1	1	2	6
336	1	0	1	0	1	1	4	0	0	1	1	5
151	1	0	1	0	1	1	4	0	0	0	0	4
175	1	0	1	0	0	1	3	0	1	1	2	5
5	1	0	1	1	0	0	3	0	0	0	0	3
147	1	1	0	0	NA	0	2	0	0	0	0	2
40	1	1	0	1	1	0	4	1	1	0	2	6
42	1	1	1	1	0	0	4	0	0	0	0	4
267	1	0	1	0	NA	1	3	0	0	0	0	3

Cond.

S.No	qSBR7-1	qSBR11-1	qSBR11-3	qSBR11-2	Qsb9-2	qsbh9.2	no. of genes	Non gene specific			no. of genes	Total no. of genes
	RM336	RM224	RM202	RM209	RM216	RM205		RM12398	RM10255	RM11229		
21	1	0	1	1	0	1	4	0	1	1	2	6
382	1	0	1	0	1	0	3	0	0	1	1	4
252	1	0	0	0	0	0	1	1	0	0	1	2
27	1	1	0	1	1	0	4	1	0	0	1	5
323	1	1	1	1	NA	1	5	0	0	0	0	5
285	0	0	1	0	1	1	3	1	0	0	1	4
174	1	0	0	0	0	1	2	0	0	1	1	3
38	0	0	1	0	0	0	1	1	0	1	2	3
26	0	1	0	0	1	0	2	0	0	0	0	3
279	1	0	0	1	1	0	3	0	0	0	0	3
4	1	0	0	1	1	1	4	1	0	0	1	5
172	1	1	1	0	1	1	5	1	1	1	3	8
184	1	1	1	1	0	1	5	0	1	1	2	7
245	1	0	0	1	0	1	3	0	1	0	1	4
122	1	0	1	0	1	1	4	0	1	0	1	5
390	0	0	1	0	1	1	3	0	1	1	2	5
363	1	0	1	1	NA	1	4	0	1	1	2	6
Frequency%	80	25	70	52.5	50	67.5	-	25	35	32.5	--	-
Approx. size(bp)	200	130	180	150	91	188	-	253	119	339	-	-

Table 5. MAGIC *indica* lines sharing SSR alleles with resistant check varieties Wazuhophek and Tetep

S.No.	Chr. No	Marker	Allele Size(bp)	MAGIC lines showing equal banding pattern with Wazuhophek, and Tetep.
1	1	RM 11229	393	313, 66, 336, 175, 21, 382, 174, 38, 172, 184, 390, 363
2	1	RM10255	118	103, 205, 326, 66, 175, 40, 21, 172, 184, 245, 122, 390, 363
3	2	RM12398	252	189, 271, 291, 40, 252, 27, 285, 38, 4, 172
4	7	RM336 (qSBR 7-1)	218	103, 188, 189, 350, 385, 271, 291, 137, 263, 313, 66, 336, 151, 175, 5, 147, 40, 42, 267, 21, 382, 252, 27, 323, 174, 29, 4, 12, 184, 245, 122, 363
5	9	RM205 (qSBR 9-2)	122	103, 152, 188, 205, 326, 385, 271, 126, 291, 263, 33, 66, 336, 151, 175, 267, 21, 323, 285, 174, 4, 172, 184
6	10	RM216	146	263, 189, 151, 326, 350, 385, 271, 126, 291, 313, 66, 336, 151, 40, 21, 27, 285, 26, 279,4, 172, 122, 390
7	11	RM202 (qSBR 11-3)	194	103, 151, 205, 152, 188, 326, 385, 271, 126, 291, 137, 313, 66, 336, 15, 175, 5, 42, 267, 21, 382, 323, 285, 38, 172, 184, 122, 390, 363
8	11	RM224 (qSBR 11-1)	157	189, 205, 271, 147, 40, 42, 27, 232, 26, 172, 184
9	11	RM209 (qSBR 11-2)	139	152, 189, 236, 350, 271, 126, 291, 137, 263, 66, 5, 40, 42, 21, 27, 323, 279, 4, 184, 245

Table 6. Association of polymorphic SSR markers with sheath blight resistance-related traits based on single marker analysis (SMA)

Trait	PH		LH		RLH		Sheath blight score	
Marker	R Square	F value	R Square	F value	R square	F value	R square	F value
RM336	0.344	23.695**	0.323	21.498**	0.284	17.874**	0.3	19.362**
RM224	0.411	31.5**	0.5	45.042**	0.505	45.994**	0.467	39.506**
RM209	0.47	39.945**	0.469	39.852**	0.442	35.647**	0.46	38.441**
RM205	0.702	106.482**	0.702	106.406**	0.712	111.525**	0.634	78.042**
RM202	0.418	32.43**	0.385	28.3**	0.343	23.566**	0.374	26.929**
RM216	0.457	38.016**	0.457	38.016**	0.38	27.619**	0.378	27.369**
RM12398	0.474	40.595**	0.53	50.754**	0.507	46.413**	0.494	43.994**
RM10255	0.9	406.56**	0.38	27.633**	0.391	28.896**	0.14	31.29**
RM11229	0.469	39.845**	0.542	53.286**	0.515	52.391**	0.536	52.003**

Table 7. Previously reported SSR markers linked to sheath blight resistance QTLs used in the study

S.No	Chr.no	Marker	QTL	Scientist
1	7	RM336	qSBR 7-1	Channamallikarjuna *et al*. (2010)
2	9	RM205	qshb9.2	Tan *et al*. (2005)
3	11	RM202	qSBR 11-3	Channamallikarjuna *et al*. (2010)
4	11	RM224	qSBR 11-1	Channamallikarjuna *et al*. (2010)
5	11	RM209	qSBR 11-2	Channamallikarjuna *et al*. (2010)

possible presence of QTLs governing sheath blight tolerance, particularly on chromosome 11, where three reported QTL linked markers, RM209 (qSBR11-2), RM202 (qSBR11-3), and RM224 (qSBR11-1) showed significant associations with sheath blight tolerance related traits. These findings support the results reported by Channamallikarjuna et al. (2010), who identified major sheath blight resistance QTLs on chromosome 11 using the resistant donor Tetep.

While, single marker analysis serves as an effective preliminary approach for identifying marker trait associations, it lacks the resolution required to accurately define QTL intervals. Therefore, validation using high density markers, larger mapping populations, and advanced mapping approaches such as interval mapping (IM) or composite interval mapping (CIM) is essential for the precise localization of QTLs conferring sheath blight resistance.

Genetic relationships among Rice genotypes

Cluster analysis based on SSR marker data grouped the 46 rice genotypes into three major clusters (I, II, and III), with further subdivision into subclusters (Ia, Ib, IIa, IIb, IIIa, and IIIb). The neighbor-joining (NJ) radial dendrogram illustrating the genetic relationships among the 46 genotypes, comprising 21 sheath blight-tolerant genotypes, 21 susceptible genotypes, and four check varieties (two tolerant and two susceptible), is presented in Figure 3 and Table 8. Cluster I comprised 20 genotypes and was further divided into subclusters Ia and Ib. Subcluster Ia contained 18 genotypes, including nine tolerant genotypes (205, 152, 103, 188, 126, 350, 326, 189, and 271), five susceptible genotypes (21, 27, 323, 42, 271), and the four check varieties (Wazuhophek, Tetep, BPT-5204, and TN1), indicating a close genetic relationship among these genotypes. Subcluster Ib consisted of only two susceptible genotypes (38 and 174). Cluster II included 15 genotypes and was subdivided into IIa and IIb. Subcluster IIa comprised five tolerant and six susceptible genotypes, whereas subcluster IIb consisted of two tolerant genotypes (181 and 291) and two susceptible genotypes (30 and 363). Cluster III contained 11 genotypes and was divided into subclusters IIIa and IIIb. Subcluster IIIa included eight genotypes, comprising four tolerant genotypes (66, 263, 5, and 137) and four susceptible genotypes (172, 122, 245, and 390). Subcluster IIIb contained three susceptible genotypes (184, 274, and 26).

The clustering pattern revealed considerable genetic diversity among the evaluated rice genotypes. Interestingly, tolerant and susceptible genotypes were distributed across the same major clusters rather than forming completely distinct groups. This observation suggests that the SSR markers used in the present study captured overall genome-wide genetic diversity rather than exclusively differentiating sheath blight resistance. The close association of the resistant checks (Wazuhophek and Tetep) with several tolerant genotypes indicates the presence of shared genomic regions, while their clustering with susceptible genotypes reflects the complex and quantitative nature of sheath blight resistance. These findings are consistent with those reported by Lavanya and Betha (2019), who employed neighbor joining cluster analysis and observed three major genetic groups among rice genotypes. Their STRUCTURE analysis further revealed a weak population structure, with 66.7% of

Table 8. Distribution of MAGIC indica rice lines into different clusters based on neighbor joining cluster analysis

Clusters	Sub Clusters	Total No. Of Genotypes	Tolerant	Susceptible
I	Ia	14	9	5
	Ib	2	-	2
II	IIa	11	5	6
	IIb	4	2	2
III	IIIa	8	4	4
	IIIb	3	-	3

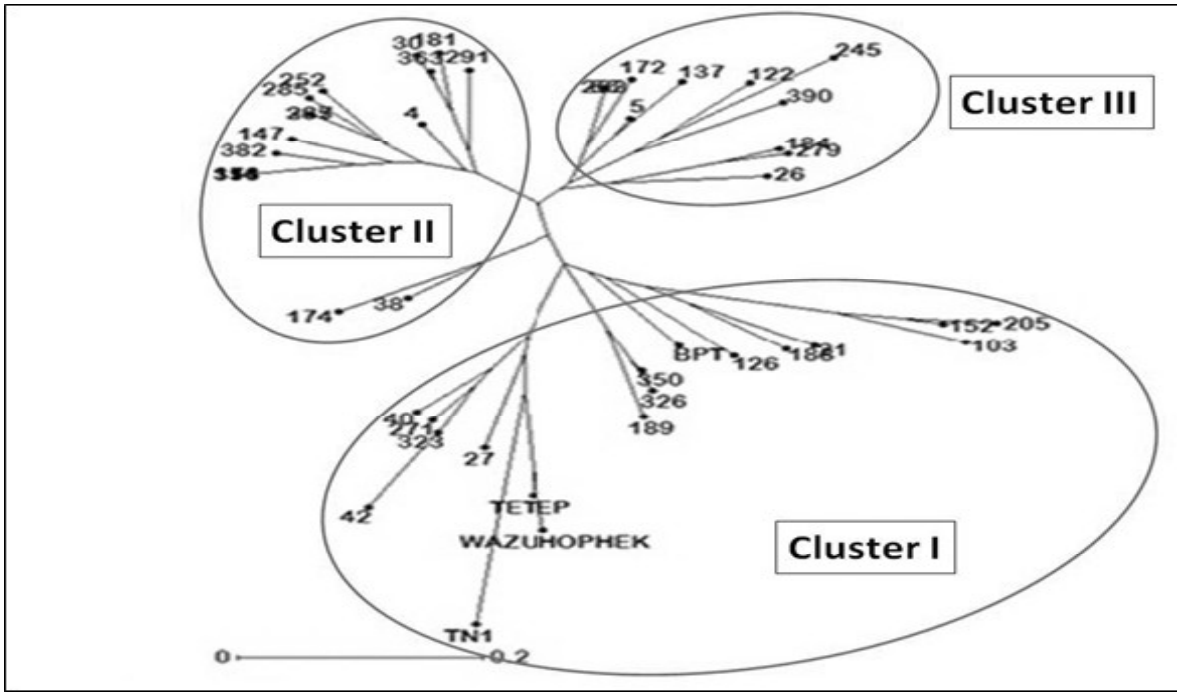

Fig 3. Neighbour joining tree showing genetic relationships among MAGIC *indica* lines and check varieties

the genotypes assigned to three subpopulations (K = 3) based on a membership coefficient (Q $\geq$ 0.75), while the remaining 33.3% were classified as admixed genotypes. The similarity in clustering patterns supports the existence of moderate genetic diversity and weak population stratification among rice genotypes evaluated for sheath blight resistance.

REFERENCES

Bandillo, N., Raghavan, C., Muyco, A., Sevilla, M.A.L., Lobina, I.T., Dilla-Ermita, C.J., Tung, C.W., McCouch, S., Thomson, M., Mauleon, R and Singh, R.K. 2013. Multi-parent advanced generation inter-cross (MAGIC) populations in rice: progress and potential for genetics research and breeding. *Rice*. 6(1): 11.

Boranayaka, M.B., Lokesha, R., Diwan, J.R and Patil, R. 2018. Marker validation in F2 population of rice (*Oryza sativa* L.) for water and Nitrogen use efficiency. *International Journal of Current Microbiology and Applied Science*. *7*(1):1275-1278.

Cavanagh, C., Morell, M., Mackay, I and Powell, W. 2008. From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. *Current opinion in Plant Biology*. 11(2): 215-221.

Channamallikarjuna, V., Sonah, H., Prasad, M., Rao, G.J.N., Chand, S., Upreti, H.C., Singh, N.K and Sharma, T.R. 2010. Identification of major quantitative trait loci qSBR11-1 for sheath blight resistance rice. *Molecular Breeding*. 25: 155-166.

Choudhary, G., Ranjitkumar, N., Surapaneni, M., Deborah, D.A., Vipparla, A., Anuradha, G., Siddiq, E.A and Vemireddy, L.R. 2013. Molecular genetic diversity of major Indian rice cultivars over decadal periods. *PloS one*. 8(6).

Haley, C.S and Knott, S.A. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*. *69*(4):315.

Imam, J., Alam, S., Mandal, N.P., Variar, M and Shukla, P. 2014. Molecular screening for identification of blast resistance genes in North East and Eastern Indian rice germplasm (*Oryza sativa* L.) with PCR based makers. *Euphytica*. 196(2): 199-211.

Jia, YL., Liu, G.J., Costanzo, S., Lee, S.H and Dai, Y.T. 2009. Current progress on genetic interactions of rice with rice blast and sheath blight fungi. *Front Agric China*. 3:231"239.

Kupper, C.P.J.M., Chitatto, A., Zucchi, M., Colombo, C., Carbonell, S., Cosata, M.J.M., Gazaffi, R.,

Franco, G.A.A., Decampos, T and De souza, A. 2011. Genetic diversity in cultivated carioca common bean based on molecular marker analysis. *Genetics and Molecular Biology*. 34: 88.

Lavanya, C and Betha, U.K. 2019. Molecular Diversity and Population Structure in Breeding Lines of Castor (*Ricinus communis* L.) Using SSR Markers. *International Journal of Current Microbiology and Applied Sciences.* 8(2):2319-7706 .

Leung, H., Raghavan, C., Zhou, B., Oliva, R., Choi, I.R., Lacorte, V., Jubay, M.L., Cruz, C.V., Gregorio, G., Singh, R.K and Ulat, V.J. 2015. Allele mining and enhanced genetic recombination for rice breeding. *Rice*. 8(1): 34.

Liu, G., Jia, Y., Correa-Victoria, F.J., Prado, G.A., Yeater, K.M., Mcclung, A and Correll, J.C. 2009. Mapping quantitative trait loci responsible for resistance to sheath blight in rice. *Phytopathology*. 99(9): 1078-1084.

Liu, K and Muse, S.V. 2005. Power Marker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*. 21: 2128–2129.

Mackay, I and Powell, W. 2007. Methods for linkage disequilibrium mapping in crops. *Trends in Plant Science*. 12:57–63.

Mullis, K.B., Faloona, F., Scharf, S., Saiki, R.K., Horn, G.T and Erlich, H. 1986. Specific enzymatic amplification of DNA invitro: the polymerase chain reaction. In Cold Spring Harbor Symposia on Quantitative Biology. *Cold Spring Harbor Laboratory Press.* 51: 263-273.

Murray, M.G. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research, 8(19): 4321–4325.https://doi.org/10.1093/nar/8.19.4321

Nachimuthu, V.V., Muthurajan, R., Duraialaguraja, S., Sivakami, R., Pandian, B.A., Ponniah, G., Gunasekaran, K., Swaminathan, M., Suji, K.K and Sabariappan, R. 2015. Analysis of population structure and genetic diversity in rice germplasm using SSR markers: an initiative towards association mapping of agronomic traits in *Oryza sativa. Rice*. 8(1): 30.

Pal, S., Jain, S., Saini, N and Jain, R.K. 2004. Identification of microsatellite markers for differentiating some Basmati and non-Basmati rice varieties. Indian Journal of Biotechnology.3:519-526.

Pan, X.B., Rush, M.C., Sha, X.Y., Linscombe, S.D., Stetina, S.R and Oard., J. 1999. Major gene, nonallelic sheath blight resistance from the rice varieties Jasmine 85 and Te Qing. *Crop Science*. 39:338"346.

Perrier, X and Jacquemoud-Collet, J.P. 2006. DARwin software http://darwin.cirad. fr/.

Pinson, S.R.M., Capdevielle, F.M and Oard, J.H. 2005. Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. *Crop Science*. 45: 503-510.

Salgotra, R.K., Gupta, B.B., Bhat, J.A and Sharma, S. 2015. Genetic diversity and population structure of Basmati rice (*Oryza sativa* L.) germplasm collected from North Western Himalayas using trait linked SSR markers. *Plos one*. 10: (7).

Scarcelli, N., Cheverud, J.M., Schaal, B.A and Kover, P.X. 2007. Antagonistic pleiotropic effects reduce the potential adaptive value of the FRIGIDA locus. *Proceedings of the National Academy of Sciences*. 104(43): 6986-16991.

Sharma, A., McClung, A.M., Pinson, S.R.M., Kepiro, J.L., Shank, A.R., Tabien, R.E and Fjellstrom, R. 2009. Genetic mapping of sheath blight resistance QTLs within tropical *japonica* rice varieties. *Crop Science.* 49: 256-264.

Singh, A.K., Singh, P.K., Khare, R., Singh, A., Singh, N.K and Singh, U.S. 2016. Identification of blast and sheath blight resistance genes in rice (Oryza sativa L.) through SSR markers. *Indian Journal of Biotechnology.* 15: 325-333

Zarbafi, S.S and Ham, J.H. 2019. An Overview of rice QTLs associated with disease resistance to three major rice diseases: Blast, sheath blight and bacterial panicle blight. *Agronomy*. 9(4): 177.

Zheng, A., Lin, R., Zhang, D., Qin, P., Xu, L., Ai, P., Ding, L., Wang, Y., Chen, Y., Liu, Y., Sun, Z., Feng, H., Liang, X., Fu, R., Tang, C., Li, Q., Zhang, J., Xie, Z., Deng, Q., Li, S., Wang, S., Zhu, J., Wang, L., Liu, H and Li, P. 2013. The evolution and pathogenic mechanisms of the rice sheath blight pathogen. *Nat Commun.* 4:14"24.