Establishing an association between molecular markers and sheath blight (*Rhizoctonia solani* Kuhn) resistance in rice

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Abstract

The use of multiple regression analysis to validate molecular markers associated with sheath blight resistance was carried out in 73 germplasm accessions of rice. Data on disease reaction was recorded as lesion size and used for calculation of AUDPC (Area Under Disease Progress Curve) and Percent Disease Index (PDI). A variation in AUDPC ranging from 217.78 to 793.33 with a mean value of 532.51 was recorded for the genotypes infected with sclerotia. PDI ranged from 28.89 to 84.44 with the mean values of 55.40 for infected lines. The AUDPC and PDI values for resistant check Tetep were found to be 432.70 and 45.22, respectively. Higher AUDPC and PDI values serve as an indication for susceptibility towards the disease reaction. The PIC value for the SSR loci varied from 0.225 to 0.743 with an average value of 0.47. Markers RM336, RM209, RM251 and RM224 were most informative on the basis of their high PIC values. UPGMA clustering based on molecular data and AUDPC were found to be in good agreement with each other. Twenty five germplasm accessions were grouped as common in cluster I of dendrogram generated by all alleles, cluster I of dendrogram constructed by Tetep specific alleles, and clusters I and II of the AUDPC dendrogram. Multiple regression analysis revealed that the two markers $RM251_{175}$ and $RM257_{150}$ may be considered as markers for association with low disease index (resistance to sheath blight) in rice. Amongst the germplasm accessions, IC383396 and IC426017 behaved as potentially resistant to sheath blight.

Keywords: germplasm accessions, multiple regression analysis (MRA), rice, sheath blight resistance, SSR markers.
Abbreviations: AUDPC_Area under disease progress curve, PDI_Percent disease index, MRA_Multiple regression analysis, PIC_Polymorphic information content, UPGMA_Unweighted pair group method with arithmetic mean.

Introduction

Sheath blight is one of the most devastating diseases causing huge damage to rice yield and quality globally every year. During favorable conditions this disease may cause yield reduction of up to 50% in major rice growing areas (Zheng et al., 2013). Losses depend on the growth stage of plant at which infection occurs, level of resistance in a particular genotype and prevailing environmental conditions. Sheath blight (SB) is caused by a semi-saprophytic soil-borne fungal pathogen, *Rhizoctonia solani* Kuhn having wide host range. The fungus survives either as sclerotia or mycelia in plant debris. The sclerotia float to the surface of flooded water in rice fields and germinate on plant sheaths forming appressoria for infection. Initial symptoms of sheath blight appear in the form of circular, oblong or ellipsoid, greenish grey, water soaked spots of about 1 cm long that occur on leaf sheaths near the water line. After the initial infection, the pathogen moves on the plant through surface hyphae and develops new infection structures over the entire plant,

causing significant necrotic damage (Ou, 1985). Sheath blight (SB) disease is prevalent in almost all major rice growing areas of the world *i.e.,* Bangladesh, China, Columbia, Cube, Germany, India, Indonesia, Iran, Japan, Korea, Malaysia, Moscow, Netherlands, Nigeria, Philippines, Senegal, Srilanka, Raiwan, Thailand, Trinidad, Tobago, U.K., U.S. and Vietnam (Dasgupta, 1992).

The immunities in rice germplasm have not been found so far, but moderate or partial resistance is available against the sheath blight pathogen (Liu et al., 2009). Some of the rice lines such as Tetep, Tadukan, Teqing, Jasmine 85, ZYQ8, Minghui 63, LSBR-5 and LSBR-33 have been found to show relatively high degree of quantitative resistance against this pathogen under field conditions (Li et al., 1995 and Pan et al., 1999). Rice sheath blight resistance has been reported to be a typical polygenic quantitative trait (Pinson et al., 2005 and Jia et al., 2009) and around 50 SB resistance quantitative trait loci (SBR QTLs) have been detected on all the 12 chromosomes of various rice species (Xu et al., 2011 and Wang et al., 2012). The spotting of molecular markers linked

with rice SB resistance can be used in breeding programs pertaining to incorporation of resistance toward this pathogen. Detection of molecular markers associated with various QTLs is the first step in marker assisted breeding (Misztal, 2006). Rice double haploid lines and RILs have been extensively used for QTL mapping for sheath blight resistance (Kunihiro et al., 2002; Han et al., 2002). However, the non-availability of mapping populations and substantial time needed to develop such populations are the major limitations in identification of molecular markers for specific traits. To overcome these limitations, the identification of molecular markers associated with trait of interest through multiple regression analysis, involving the use of diverse germplasm collections have so far been adopted in many crop species (Maureira-Butler et al., 2007; Dholakia et al., 2008; Ruan et al., 2009). These include RAPD markers associated with various polygenic traits in Asian rice (Virk et al., 1996) and protein content in wheat (Dholakia et al., 2001), SCAR markers associated with birch fiber length trait (Wang et al., 2008), ISSR markers associated with various yield traits (Vijayan et al., 2006) and protein content (Kar et al., 2008) in mulberry, and microsatellite markers associated with seed size in soybean (Hoeck et al., 2003) and mite resistance in coconut (Shalini et al., 2007). The present study involves an evaluation of genetic diversity and use of multiple regression analysis to validate the molecular markers associated with sheath blight resistance in rice.

Results

AUDPC and PDI of the lines infected with sclerotia

Data on disease reaction was recorded as lesion size and used for calculation of AUDPC and PDI (Table 1). A variation in AUDPC ranging from 217.78 to 793.33 with a mean value of 532.51 was recorded for lines infected with sclerotia. The genotype IC281785 exhibited highest area under disease progress curve, while the lowest mean value was exhibited by genotype IC346004. The lines with higher AUDPC were more susceptible to sheath blight than the lines with lower AUDPC values. The resistant check Tetep had an AUDPC value of 432.70. PDI values ranged from 28.89 to 84.44 with the mean values of 55.40 for infected lines. The genotype IC282815 exhibited highest (84.44) and IC346004 the lowest (28.89) PDI value. PDI for resistant check Tetep was found to be 45.22.

Genetic diversity based on AUDPC

The taxonomic distance matrix for 73 genotypes was employed for cluster analysis using UPGMA method and the dendrogram was constructed based on Jacquard's similarity coefficients of AUDPC (Fig 1). In the dendogram, the genotypes were grouped into four main clusters viz., Cluster I, II, III and IV consisting of 23, 27, 19 and 4 genotypes, respectively. Cluster I was subdivided into two main sub clusters Ia and IIb consisting of eleven and twelve genotypes, respectively. The major cluster, cluster II was subdivided into two sub clusters IIa (5 genotypes) and IIb (22 genotypes). Cluster III was subdivided into two main sub clusters namely IIIa (15 genotypes) and IIIb (4 genotypes). Cluster IV was not further subdivided.

SSR polymorphism and marker efficiency

Out of 16 primers used, 13 produced reproducible and polymorphic banding pattern, while three primers (sbq1,

RM5481 and RM338) were found to be monomorphic (Table 2). The thirteen polymorphic primers produced a total of 34 fragments, the size of whom varied from 100bp (marker K39512) to 220bp (marker RM536). Maximum number of fragments was produced by primers RM336 and RM210, yielding four fragments each. An average of 2.6 fragments per primer was produced by primers showing polymorphic amplification. Gel images showing SSR banding profile obtained by primer RM224 are presented in Fig 2. The PIC value of SSR markers in the present study ranged from 0.225 to 0.743 with an average PIC of 0.47. Markers RM336, RM209, RM251, and RM224 were most informative on the basis of high PIC value of 0.743, 0.694, 0.668 and 0.656, respectively. SSR marker K39512 showed least PIC value of 0.225.

Genetic diversity based on all the alleles amplified by SSR markers

A UPGMA cluster diagram obtained by all the 34 alleles (amplification products) generated by 13 polymorphic SSR markers grouped the 73 rice genotypes into eight major clusters (Fig 3). Cluster I, consisted of 38 genotypes which were again subdivided into two sub clusters, viz., Ia (14 genotypes) and Ib (24 genotypes). Tetep was allocated under sub cluster Ia. Cluster II includes only 3 genotypes. Cluster III contained twelve genotypes which were further divided into two sub clusters IIIa (6 genotypes) and IIIb (6 genotypes). Two genotypes were present in Cluster IV and six in Cluster V. Clusters VI and VII consisted of five and six genotypes, respectively. The cluster VIII was monogenic containing only one genotype.

Validation of SSR markers for Tetep specific alleles

Marker RM306, flanking the sheath blight resistant QTL *qSBR1-1*, amplified a fragment of 165 bp in Tetep and 47 other genotypes. RM251 linked to QTL *qSBR3-1* produced an amplification product of 175 bp in 20 genotypes including Tetep. SSR markers RM3691 and RM336 flanking the QTL *qSBR7-1*, produced the Tetep specific bands of 160 bp and 175 bp in 60 and 23 genotypes, respectively. Similarly, marker RM210 associated with QTL *qSBR8-1* produced a fragment of 210 bp in Tetep and other 29 genotypes. RM257 (*qSBR9-1*) amplified a band of 160 bp in 61 genotypes including Tetep. SSR markers sbq11 and RM224 flanking a major QTL *qSBR11-1*, amplified respective fragments of 160 and 140 bp in Tetep. Similarly, RM3428 and RM209 flanking the QTL *qSBR11-2* amplified 140 and 125 bp fragments in 62 and 30 genotypes, respectively. SSR markers RM536 and RM202 flanking the major QTL *qSBR11-3* produced the Tetep specific amplification products of 220 and 180 bp in 58 and 53 genotypes, respectively. Marker K39512 revealed the amplified fragment size of 100 pb in Tetep and 63 other genotypes.

Genetic diversity based on Tetep specific alleles

Based on the binary data generated by either presence or absence of an amplification fragment specific to resistant check Tetep, the cluster analysis was carried out for all the rice genotypes (Fig 4). In cluster analysis, the genotypes were grouped broadly into seven clusters, viz., Cluster I, II, III, IV, V, VI and VII. The major cluster i.e., cluster I consisted of 42 genotypes, which were further divided into five sub clusters, viz., Ia (10 genotypes), Ib (4 genotypes), Ic (14 genotypes), Id (5 genotypes) and Ie (9 genotypes). The check (Tetep) was

Table 1. Performance of rice germplasm accessions including check (Tetep) for sheath blight resistance.

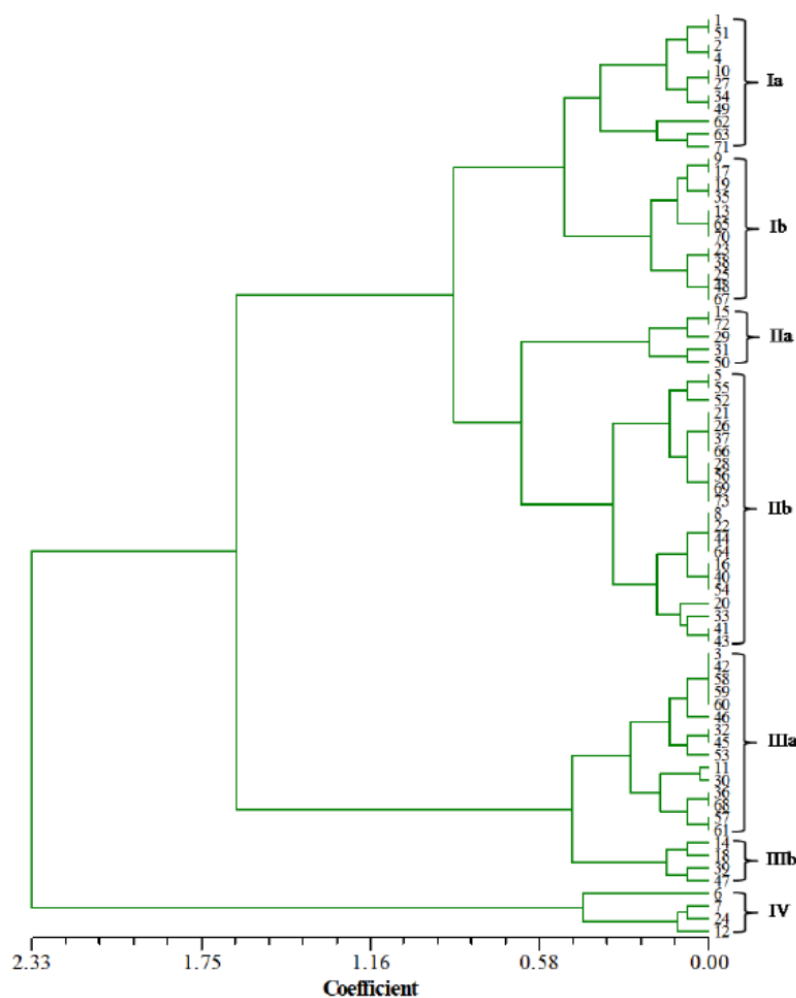| Parameter	Min.	Max.	Mean	SE (±)	Resistant check Tetep
AUDPC	217.78	793.33	530.35	13.38	432.70
PDI	28.89	84.44	55.32	1.40	45.22

Fig 1. Dendrogram based on Jaccard's similarity coefficient of AUDPC after inoculation of rice germplasm accessions with *Rhizoctonia solani*. Most of the moderately resistant genotypes have been grouped in clusters I and II of the dendrogram.

Table 2. Allele size (bp) and polymorphism information content (PIC) of the SSR primers used in present study.

| S. No.	Primer	PIC	No. of alleles amplified	Approx. size of amplified product (bp)	Allele size (as amplified in Tetep)
1.	Sbq1	0.00	1	210 (monomorphic)	210
2.	sbq11	0.421	2	150−160	150
3.	RM3691	0.260	2	130−160	160
4.	RM3428	0.236	2	140−170	140
5.	K39512	0.225	2	100−110	100
6.	RM536	0.335	2	210−220	220
7.	RM5481	0.00	1	100 (monomorphic)	100
8.	RM224	0.656	3	125−160	140
9.	RM306	0.516	3	150−210	165
10.	RM338	0.00	1	180 (monomorphic)	180
11.	RM257	0.315	2	110−150	150
12.	RM210	0.639	4	110−210	210
13.	RM202	0.362	2	160−180	180
14.	RM209	0.694	3	110−150	125
15.	RM336	0.743	4	140−210	175
16.	RM251	0.668	3	140−190	175
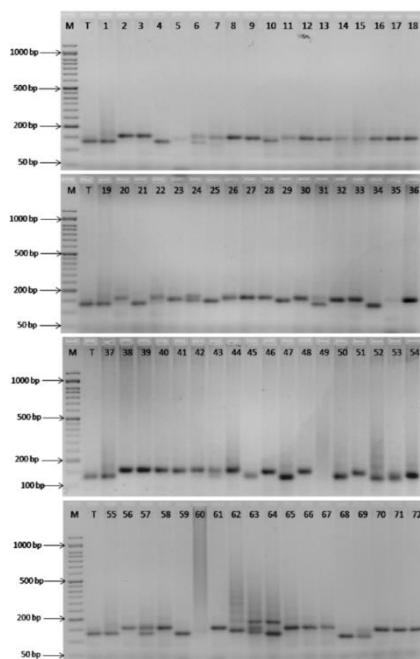
Fig 2. Gel images showing SSR banding profile obtained by primer RM224. Lane 1-72 represents the rice germplasm accessions; M= 100bp DNA size marker and T = Tetep (used as resistant check to rice sheath blight disease). RM224 was one of the most informative primers on the basis of its high PIC value of 0.656.

Table 3. Coefficient of dependent variable (AUDPC scores) in the stepwise multiple regression analysis for association with independent variables (SSR markers) towards sheath blight resistance in rice.

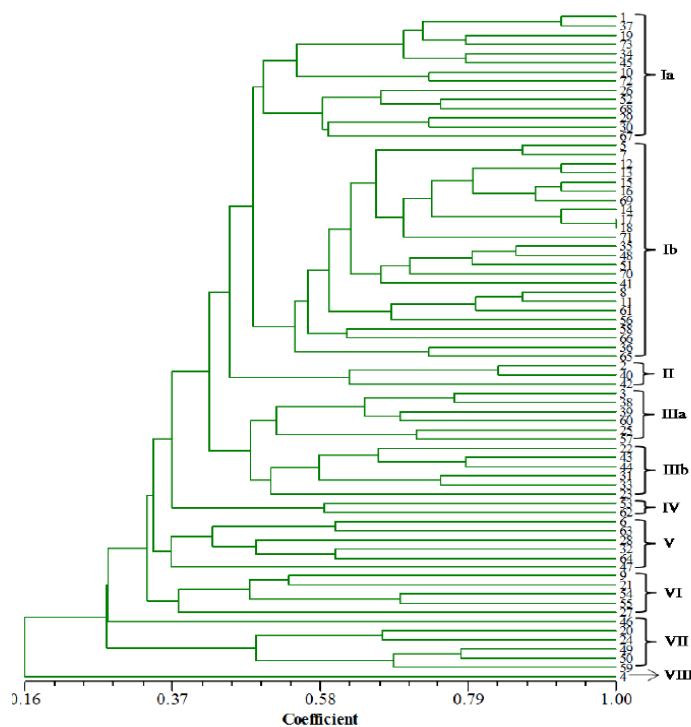| Marker	Un-standardized coefficient β	SE	Standardized coefficient β	T value	P value
$RM257_{150}$	0.451	0.134	0.359	3.377	0.001
$RM251_{175}$	0.315	0.118	0.284	2.667	0.010

Fig 3. Dendrogram of germplasm accessions based on Jaccard's similarity coefficients of all the alleles amplified by 13 polymorphic SSR markers associated with sheath blight resistance in rice.

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Table 4. SSR markers associated with AUDPC of sheath blight resistance in rice as revealed by stepwise multiple regression analysis.

Marker	R2	Adjusted R2	R2 Change	F Change	P value of F change
$RM257_{150}$	0.127	0.114	0.127	10.29	0.002
$+RM251_{175}$	0.207	0.184	0.081	7.11	0.010

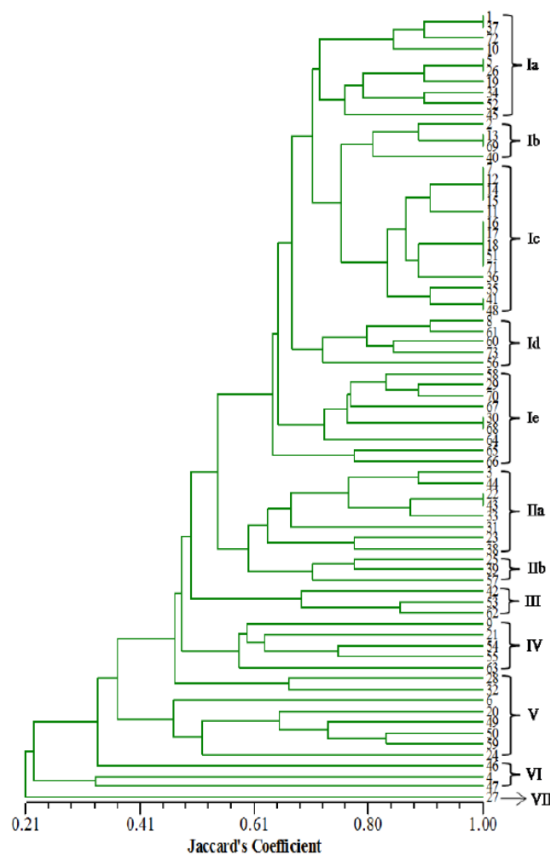+denotes the inclusion of marker in the proceeding step in stepwise MRA

Fig 4. Dendrogram of germplasm accessions based on Jaccard's similarity coefficients of Tetep specific alleles amplified by 13 polymorphic SSR markers associated with sheath blight resistance in rice.

under the sub cluster Id. Cluster II consisted of 11 genotypes which were again divided into two sub clusters, i.e., IIa (8 genotypes) and IIb (3 genotypes). The clusters III, IV, V, VI and VII were not subdivided and consisted of genotypes three, five, eight, three and one, respectively.

Multiple regression analysis

Stepwise MRA was attempted to delineate the correlation of SSR markers (independent variables) with AUDPC (dependant variable) for sheath blight resistance in the rice germplasm accessions. The results of multiple regression analysis have been presented in Tables 3 and 4.

Discussion

Absolute resistance to *R. solani* is not available in any of the rice germplasm accessions grown worldwide. A wide variation in susceptibility levels towards this pathogen is observed in different rice cultivars (Khush, 1977). The mechanism that provides quantitative resistance to *R. solani* in rice is still uncomprehended. However, some genotypes including the Indica rice line Tetep are well documented

sources of quantitative resistance to sheath blight. The genotypes with higher AUDPC and PDI values are more susceptible to sheath blight than those with lower values. AUDPC and PDI values in the present study are quite similar to that observed by Chand et al. (2006), Taheri et al. (2007) and Adhipathi et al. (2013).

In order to determine the utility of molecular markers associated with QTLs for sheath blight resistance, the genotypes were screened with 16 SSR markers. These markers were reported to be linked to different SB resistant QTLs by Channamallikarjuna et al. (2010). Thirteen SSR markers were found to be polymorphic and three revealed the monomorphic banding pattern. An average of 2.6 fragments per locus was produced by primers showing polymorphic amplification. This value is lower than the average of 5.89 per microsatellite locus reported by Lapitan et al. (2007) and 4.23 alleles per locus reported by Ghneim et al. (2008) for Venezuelan rice cultivars. However, Seetharam et al. (2009) obtained 2.48 average number of polymorphic fragments per primer which is lower than that of present study.

PIC value is a reflection of allele diversity and frequency among genotypes. The PIC value observed in the present investigation ranged from 0.225 to 0.743, which is

comparable to previous estimates of microsatellite analysis in rice viz., 0.20-0.90 with an average of 0.56 (Jain et al., 2003), 0.477 to 0.782 with an average of 0.634 (Matin et al., 2012) and 0.51 to 0.90 with an average of 0.79 (Singh et al., 2013). RM336 was found to be the most appropriate marker to discriminate the rice genotypes owing to its highest PIC value of 0.743.

The UPGMA dendrogram based on Jacquard's similarity coefficients of AUDPC grouped the genotypes into four main clusters, viz., Cluster I, II, III and IV. The cluster diagram obtained by all the 34 alleles, generated by 13 polymorphic SSR markers grouped the genotypes into eight major clusters. Based on the binary data generated by either presence or absence of an amplification fragment specific to resistant check Tetep, the genotypes were grouped broadly into seven clusters, viz., Cluster I, II, III, IV, V, VI and VII. In the previous studies, Tabkhkar et al. (2012) using SSR markers, grouped 48 rice genotypes in four main clusters and the dendrogram revealed that the landrace cultivars with good quality features were well separated from others. Kumar et al. (2014) grouped 134 rice genotypes into five clusters based on Euclidean cluster analysis.

The cophenetic correlation coefficient obtained in the present study was 0.84 for AUDPC. Its values for molecular data generated by all the alleles and Tetep specific alleles were found to be 0.79 and 0.81, respectively. The values can be interpreted as good fit. These values are quite similar to that observed by Ming et al. (2010). A high cophenetic correlation means that the dendrogram has utility in clustering of genotypes and it does not arise due to the distortion in data. In the present study, UPGMA clustering based on molecular data and AUDPC are in close agreement with each other. Twenty five genotypes have been grouped as common in cluster I of dendrogram generated by all alleles, cluster I of dendrogram constructed by Tetep specific alleles, and clusters I and II of the AUDPC dendrogram. Most of the moderately resistant genotypes are common to these clusters of the three UPGMA based dendrograms.

Based on AUDPC values, the genotypes were categorized into four classes viz., resistant (R; AUDPC below 316.94), moderately resistant (MR; AUDPC>316.94 to 436.22), moderately susceptible (MS; AUDPC>436.22 to 565.0) and susceptible (S; AUDPC>565.0). This classification is in agreement with that of Biswas (2011). AUDPC value of 432.70 was recorded by Tetep in the present investigation which categorized it into the group of moderately resistant genotypes. Tetep was also used as resistant parent in the studies carried out by Channamallikarjuna et al. (2010).

The association between SSR markers (independent variables) and sheath blight resistance in terms of AUDPC (dependent variables) was estimated through stepwise multiple regression analysis (Tables 3 and 4). The marker $RM257_{150}$ revealed maximum (0.356) and highly significant ($t = 3.377$; $P = 0.001$) correlation with AUDPC. The standardized beta coefficient was also very high (0.359) for this marker. Marker $RM251_{175}$ also showed a significantly high correlation (0.283) with AUDPC ($t = 2.667$; $P = 0.010$). Marker $RM257_{150}$ explains 12.7% of total phenotypic variation and together with marker $RM251_{175}$, it is able to explain 20.7% of the phenotypic variability. Thus, the two markers $RM251_{175}$ and $RM257_{150}$ can be taken as markers for association of low disease index (resistant to sheath blight) in rice. Sato et al., (2004) also reported two markers associated with sheath blight resistance in Tetep derived line (WSS2) of rice. Use of multiple regression analysis to identify molecular markers associated with resistance traits have been used in many plants species with great success (Virk et al., 1996 and

Kar et al., 2008). Ruan et al. (2009) estimated variability using ISSR markers in 52 accessions of sea buckthorn to find out markers associated with resistance to dried-shrink disease. Detection of markers associated with QTLs is the first step in marker assisted selection, whose success largely depends on the extent of genetic linkage between markers and concerned QTLs (Misztal, 2006 and Virk et al., 1996). The markers reported to be linked to rice SB resistance can be used in MAS breeding programs after validating them by testing their effectiveness.

Materials and Methods

Site of the study

The present study was carried out at the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (UP). The site of study is situated at 25^0 18´ N latitude and 83^0 03´ E longitude, at an elevation of 80.71 m above mean sea level.

Plant material

The research material consists of 73 morphologically diverse germplasm accessions of rice including one resistant check Tetep (Supplementary Table 1). These accessions were obtained from networking project involving National Research Centre on Plant Biotechnology (New Delhi), International Rice Research Institute (Philippines), Central Rice Research Institute (Cuttack, Odhisa) and Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU (Varanasi, India).

Method of inoculation

A virulent strain of *R. solani* 'MTCC-12227' was used for artificial inoculation of the test genotypes. The strain was grown on PDA medium at 28 ± 3^0C. Five plants from each of the two replications were randomly selected and tagged before inoculation. The selected plants were inoculated at boot stage of the crop with a bit (approx. 0.25 mg) of four-days-old immature sclerotium. For inoculation leaf sheath was opened carefully and inoculum was placed inside the sheath of tagged plants. Inoculation was done in the evening and inoculated plants were sprayed with few drops of sterilized water in the next morning.

Disease screening in field condition

The inoculated plants were regularly examined for appearance of symptoms starting from 48 hours of inoculation. Data on disease intensity was recorded after 20 days of inoculation (DAI) on three different dates at seven days interval (Kumar et al., 2008). The Area Under Disease Progress Curve (AUDPC) was calculated from disease severity (lesion length) by using the formula of Shaner and Finney (1977), Johnson and Wilcoxson (1982), and Campbell and Madden (1990).

$$AUDPC = \sum_{i=1}^{N} [\{(Y_i + Y_{(i+1)})/2\} \times (t_{(i+1)} - t_i)]$$

Where, Y_i = Disease level at the time t_i
$\{t_{(i+1)}-t_i\}$ = Time in days between two disease scores
Rating scale of Sharma et al. (1990) was followed to calculate the Percent Disease Index (PDI).
PDI = [Sum of all ratings/ (No. of observations x maximum rating scale)] x 100

Where 0 = No infection; 1 = Lesion limited to lower 20% of the height of the plant; 3 = Lesion limited to lower 21-30% of the height of the plant; 5 = Lesion limited to lower 31-45% of height of the plant; 7 = Lesion limited to lower 46- 65% of height of the plant; 9 = Lesion more than 65% of the height of the plant.

SSR analysis

For molecular studies, DNA extraction was carried out from leaves of 15 days old seedlings according to CTAB method with slight modifications (Doyle and Doyle, 1987). DNA quality was evaluated by electrophoresis in 0.8% agarose gel and quantification was accomplished using spectro-photometer. For marker analysis, 16 SSR markers reported to be linked to various rice sheath blight resistance QTLs (Channamallikarjuna et al., 2010) were employed (Table 2). DNA amplification was carried out in 10 μl reaction mixtures containing 2 μl template DNA (5 ng), 5 μl nano-pure water, 1 μl PCR buffer (10x), 0.48 μlMgCl$_2$ (50 mM), 0.6 μl dNTPs (2 mM), 0.4 μl of each primer (60 ng) and 0.12 μl of Taq DNA Polymerase (5 U/μl). Polymerase chain reaction (PCR) was carried out in a thermal cycler (Eppendorf, USA) to the temperature cycle profile: initial denaturation at 94°C for 4 min, 40 cycles each of 1 min denaturation at 94°C followed 30 sec annealing at 55°C to 65°C (depending on the primer employed) and 1 min extension at 72°C, and finally 4 min at 72°C for the final extension.

The amplified products were subjected to electrophoresis in 2.5 percent agarose gel prepared in 1x TAE buffer and stained with ethidium bromide. The electrophoresis was carried out in 1x TAE buffer at constant voltage of 65 V for 3 hours. The gels were visualized and photographs taken using gel documentation instrument (BioRad). Clearly resolved and unambiguous bands for each primer were scored in the form of matrix as 1 (presence) and 0 (absence) in each genotype. The binary data matrix was then utilized to generate genetic similarity data among the 73 rice genotypes.

Statistical analysis

The AUDPC estimates and binary data matrix generated by polymorphic SSR markers were subjected to further analysis using NTSYS-pc version 2.02 (Rohlf, 1998). The SIMQUAL program was used to calculate the Jaccard's similarity coefficients. The resulting similarity matrix was used for unweighted pair group method with arithmetic averages (UPGMA) based dendrogram using the sequential agglomerative hierarchical nested cluster analysis (SAHN) module of NTSYS-pc. In order to estimate congruence among dendrograms, cophenetic matrices were computed and compared using Mantel test. Polymorphic information content (PIC) was estimated using the formula suggested by Nei (1973).

PIC = $1 - \sum x^2 k$

Where, $x^2 k$ represents the frequency of the kth allele.

Association between SSR markers and rice SB resistance was estimated through stepwise multiple regression analysis (MRA) using the SPSS version 16.0. For stepwise multiple regression analysis, binary data generated by either presence or absence of an amplification fragment specific to resistant check Tetep in all the genotypes were used. The disease score in terms of AUDPC (quantitative trait) was treated as dependent variable and the thirteen polymorphic SSR markers were treated as independent variables. Selected markers were further tested with linear models for confirming the significance of β-statistics for each band identified by MRA.

Conclusions

Based on the cluster analysis of AUDPC and Tetep specific alleles, the genotypes IC383396 and IC426017 may be regarded as potentially resistant to sheath blight. Moreover, genotype IC281785 exhibited highest (793.33) and genotype IC346004 the lowest (217.78) area under disease progress curve, and thus may serve as prospective parents for developing mapping population for further genetic studies. It is to be worth mentioning that amplification of an allele specific to resistant check Tetep is not a sole criterion to confirm the resistance towards sheath blight. In order to use a particular molecular marker in MAS, association studies are essentially to be carried out with the trait of interest (sheath blight resistance in present case).

Acknowledgement

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