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RESEARCH ARTICLE

Molecular profiling of blast resistance genes and evaluation of leaf and neck blast disease reaction in rice

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Abstract. In the present investigation, phenotypic evaluation of blast disease reaction was conducted at Ponnampet and Mandya districts of Karnataka, India, which indicated that the rice varieties such as IR64, Jaya, KMP153, IR30864, Mandya Sona-1, Mandya Sona-2, KCP-1, Dodda Byra, and Malgudi Sanna were susceptible to both leaf and neck blasts. Further, the rice varieties that were resistant to leaf blast such as KMP200, DHMAS70Q164-1b, Karibatta, Coimbatore Sanna and others showed susceptible reaction to neck blast only. In contrast, the varieties such as JyothixBR2655, Punkutt Kodi, Sirsi, 222 and Gangadale which were resistant to neck blast were found to be susceptible to leaf blast also. Only one variety, BR2655 showed resistance to both leaf and neck blast diseases. The genotypic studies using simple sequence repeat markers showed that the analysis of the distribution of resistance genes and genotyping of the selected rice varieties, and traditional rice varieties from different ecological regions with allele specific markers helped to identify 20 major blast resistance genes. The individual gene frequencies of the 20 major rice blast resistance genes varied from 10.34 to 100%. Less and more frequency of resistance gene distribution occurred in *Pi9* and *Pizt* gene, respectively. The result of this study would help to create strategies for improving rice blast resistance through genetic studies and plant–pathogen interaction.

Keywords. traditional rice varieties; leaf blast; neck blast; gene specific markers.

Introduction

Rice is the most valuable and primary food crop for more than 50% of the world's population (Khush 2005; Latif *et al.* 2011). The rice consumption is increasing day by day and the demand for it is also raising due to the increases in population. To meet the increasing demand for rice various studies have suggested that production has to be increased by more than 40% by the year 2030 (Khush 2005). This challenge could be met by development of high yielding rice varieties with tolerance to biotic and abiotic stresses (Selvaraj *et al.* 2011). Although, the potential yield of rice is 6500 kg/ha, the yield at farmer's level in Karnataka is 5017 kg/ha (Nirmala *et al.* 2009). This difference in yield levels could be mainly attributed to the major pests and diseases of rice. The major pests that attack rice in Karnataka include brown plant hoppers and yellow stem borers. Further, blast, sheath blight, sheath rot and bacterial leaf blight are the major diseases affecting the production of rice. Among biotic stresses, blast disease is the most harmful threat to high productivity of rice mainly due to its wide distribution and ability to survive in wide range of environmental conditions (Kwon and Lee 2002; Li *et al.* 2007).

Chikkaballi A. Deepak, Kodihally M. Harinikumar and M. P. Rajanna contributed equally to this work.

HBM: performed all the molecular work such as molecular analysis using trait specific markers and molecular diversity estimation. CAD: planned out the experimental design and assisted to draft manuscript preparation. KMH: associated with the survey of polymorphism among the rice varieties and traditional rice varieties. MPR: participated and planned the plot design at Ponnampet location. BSC: helped in all pathological related work performed in this work. All authors have read and approved the final manuscript.

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The blast disease in rice is caused by the fungus *Magnaporthe oryzae* which is geographically widespread and whose various host-limited forms collectively parasitize more than 50 different grass species (Ou 1985). The fungus colonizes almost all the parts of rice plant including leaves (leaf blast), node (nodal blast), panicle (neck blast), culm, glume and leaf sheath. Further, *Magnaporthe oryzae* infects rice plant at almost all stages of growth ranging from seedling stage to maturity. Breakdown of blast resistance is the major cause of yield instability in several rice growing areas. There is a need to develop strategies providing long lasting disease resistance against a broad spectrum of pathogens, giving protection for a long time over a broad geographic area, promising sustainable rice production in the future. So far, more than 125 blast resistance genes from Japonica (45%), Indica (51%) and other (4%) genotypes have been identified (Shikari *et al.* 2014) and 22 blast resistance genes have been isolated through map-based cloning (*Pib*, *Pita*, *Pi54(Pikh)*, *Pi9*, *Pid2*, *Pi2*, *Piz-t*, *Pi36*, *Pi37*, *Pik-m*, *Pi5*, *Pid3*, *pi21*, *Pit*, *Pb1*, *Pish*, *Pik*, *Pik-p*, *Pia*, *Pi1* and *Pi54rh*) (Ma *et al.* 2015). These qualitative and major *R* genes have been extensively used in blast resistance breeding programmes worldwide. We have undertaken this study to expand the existing knowledge in this area by identifying the major resistance (*R*) genes to *M. grisea* from released varieties of rice in Karnataka and traditional rice varieties (TRV) using molecular markers.

Material and methods

A total of 84 genotypes including 55 TRVs, 20 released varieties (RVs) and nine advanced breeding materials (ABMs) were selected for this study, provided in table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/. Their seeds were obtained from the germplasm collection of Rice Breeding Division, AICRP (Rice), Zonal Agricultural Research Station (ZARS), Mandya, India.

Phenotyping for blast disease resistance

TRVs, RVs and ABMs were evaluated for their reaction to leaf and neck blast at following two locations during Kharif 2018. Location-1: natural screening in a blast endemic area at Agricultural Research Station (ARS), Ponnampet, Karnataka (station situated at 12°19′45″ N 75° 53′44″ E); and location-2: artificial screening using blast disease inoculums and spreader row technique at Zonal Agricultural Research Station (ZARS), VC Farm, Mandya, Karnataka (station situated at 12° 31.45″ N 76° 53.74″ E). Layout planning is provided in figures 1 and 2 in electronic supplementary material. The disease reaction on each line was recorded after 15 days of inoculation, following standard 0–9 scale according to standard evaluation system developed by IRRI. HR-12 and Tetep were used as susceptible and resistant check, respectively.

Molecular analysis using gene specific markers

Isolation of genomic DNA: DNA was extracted from the frozen leaf samples (–80°C) using CTAB protocol of Chen *et al*. (2006). The DNA purity was analysed using advanced automated DNA quantifier, Biospec-nano (Spectrophotometer for life science).

PCR analysis

Twenty gene specific microsatellite markers (table 1) were amplified by PCR using unique flanking sequences as forward and reverse primers. The primer sequences synthesized from Sigma Inc and obtained from www.gramene.org and other previously published research work on blast resistance genes and associated markers.

Results

Leaf and neck blast reaction

The results of the leaf and neck blast evaluation of Ponnampet and Mandya regions are provided in table 2 in electronic supplementary material.

Leaf and neck blast disease reaction in Ponnampet

Thirteen varieties had disease scores of 2 to 3 and showed moderate resistance reaction (table 2a in electronic supplementary material). However, Mandya Sona-2, an advanced breeding line with fine grains, showed susceptible reaction and had a disease score of 7. Only one traditional rice variety by name 222 had a disease score of 0 and was found to be highly resistant. This may be due to the involvement of combination of multiple genes and interaction between them. Further, two more TRVs, namely Hasundi and Sarjana also showed resistant response but had a disease score of 1 (table 2b in electronic supplementary material). However, TRVs, such as Joopavadlu, Khuri Adhikshan, and more which were a part of the same experiment were found to be highly susceptible to neck blast and the variety KMP200 which was moderately resistant to leaf blast disease showed high resistance to neck blast. This result suggested that, some varieties that are moderately resistant against leaf blast disease may perform better with respect to neck blast.

Leaf and neck blast disease reaction in Mandya

None of the varieties showed high resistance reaction against *M. oryzae*. KMP200, BR2655 and DHMAS70Q 164-1b showed moderate resistant reaction and 10 varieties showed moderately susceptible reaction (table 2a in electronic

Table 1. List of SSR markers used in molecular validation of blast resistance genes.

	Marker	Gene	Forward primer	Reverse primer	Chr.	Product size (bp)		References
						'R' allele	'S' allele	
1	Pi54 MAS	*Pi54*	CAATCTCCAAAGTTTTCAGG	GCTTCAATCACTGCTAGACC	11	216	310	Ramkumar *et al.* (2011)
2	RM 224	*Pi1*	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	11	157	170	Hittalmani *et al.* (2000)
3	AP56595	*Pi2*	CTCCTTCAGCTGCTCCTC	TGATGACTTCCAAACGGTAG	6	288	310	Fjellstrom *et al.* (2004)
4	NMSM Pi-9-1	*Pi9*	CGAGAAGGACATCTGGTACG	GAGATGCTTGGATTTAGAAGAC	6	168	250	Qu *et al.* (2006)
5	K-2167	*Pik*	CGTGCTGTCGCCTGAATCTG	CACGAACAAGAGTGTGTCGG	11	300	619	Hayashi *et al.* (2006)
6	CKM 1	*Pikm*	TGAGCTCAAGGCAAGAGTTGAGGA	TGTTCCAGCAACTCGATGAG	11	174	213	Costanzo and Jia (2010)
7	RM 246	*Pitp*	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT	1	116	100	Barman *et al.* (2004)
8	RM 21	*Pi38*	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	11	157	170	–
9	Z 56592	*Pizt*	GGACCCGCGTTTTCCACGTGTAA	AGGAATCTATTGCTAAGCATGAC	6	292	250	Hayashi *et al.* (2006)
10	RM 229	*Pi7t*	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	11	116	150	–
11	JJ 803	*Pi5*	AAGTGAGCATCCAGTGCCTAATGA	AGCCGGTGCTCATAACACGTATTA	9	300	450	Lee *et al.* (2009)
12	MSM 6	*Pi40*	TGCTGAGATAGCCGAGAAATC	GCACCCTTTTCGCTAGAGG	6	256	270	Ramadevi *et al.* (2015)
13	RM 1337	*Pi20*	GCTGAGGAGTATCCTTTCTC	ACCATAGGAAGATCATCACA	12	210	190	Li *et al.* (2007)
14	RM 7102	*Pita*	TAGGAGTGTTTAGAGTGCCA	TCGGTTTGCTTATACATCAG	12	168	150	Conaway-Bormans *et al.* (2003)
15	RM 22585	*Pi33*	CACCGATTATTGTCGTATGG	AGTGAGGAAGGGAAGAATACG	8	245	280	–
16	RM 208	*Pib*	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	2	173	185	Fjellstrom *et al.* (2004)
17	RM 144	*Piks*	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	11	237	210	–
18	RM 19818	*Piz*	AACCCTAGACTACTCCCGGTCTCC	ACCTTGGCGAGCTCTGTTTCG	6	275	250	–
19	RM 527	*Piz5*	GGCTCGATCTAGAAAATCCG	GGCTCGATCTAGAAAATCCG	6	233	250	Fjellstrom *et al.* (2006)
20	OSR-3	*Pish*	AGCTAAGGTCTGGGAGAAACC	AAGTAGGATGGGGCACAAGCTC	1	150	130	–

Chr., chromosome number; R allele, resistance allele; S allele, susceptible allele.

supplementary material). The disease reaction of BR2655 and Raksha (moderately resistant) was in accordance with our expectation based on their performance in the farmer's fields. Further, the disease reaction manifested by KMP200 was encouraging as this is a variety in the pipeline yet to be released for the cultivation by farmers. The results of the 55 traditional varieties are summarized in table 2b in electronic material. On the other hand, varieties BR2655, Mandya Vijaya and Jyothi x BR2655 showed moderately resistant reaction to neck blast with a score of 3. The phenotypic studies for both leaf blast and neck blast resistance at Mandya identified BR2655 as moderately resistant. Although Mandya Vijaya and Jyothi x BR2655 were susceptible reaction for leaf blast but displayed moderate resistance to neck blast at Mandya (table 3a). Therefore, there is a big challenge for incorporation of neck blast specific genes to these advanced materials that they perform better against both leaf blast and neck blast diseases. Among the TRVs, the Punkutt Kodi, Putta Batta, Sirsi, 222, Coimbatore Sanna and Ratnachoodi showed resistance reaction for neck blast disease with the least score of 1. In contrast, most of the varieties were found to vary with their disease scores for leaf blast at two different locations. This discrepancy could be mainly due to the difference in the evaluation method particularly natural and or artificial inoculation.

Table 2. List of varieties and advanced breeding materials with similar phenotypic reaction for leaf blast and neck blast at both locations.

Reaction	Leaf blast reaction	Score	Total
HR	–	0	00
R	–	1	00
MR	KMP200, BR2655 and DH MAS 70Q 164-1b	2 to 3	03
MS	Thanu, MTU1001, KMP175 and KCP-1	4 to 5	04
S	–	6 to 7	00
HS	–	8 to 9	00
Reaction	Neck blast reaction	Score	Total
HR	–	0	00
R	–	1	00
MR	BR 2655	3	01
MS	IR-64, Jaya and Raksha	5	03
S	Mandya Sona-2 and JayaxASD-16.	7	02
HS	–	9	00

HR, highly resistant; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible; HS, highly susceptible.

Table 3. List of traditional rice varieties with similar phenotypic reaction for leaf blast at both locations.

Reaction	Name of traditional rice variety	Score	Total
HR	–	0	00
R	–	1	00
MR	Putta Batta, Tonnaru, Selam Sanna, Coimbatore Sanna, Kundi Pullan, Sada Holga and Hola Batta	2 to 3	07
MS	Dodda Byra, Malgudi Sanna, Padmarekha, Sahabag, Natibatta, Kaggali Kecrona and Jawahar	4 to 5	07
S	Kalanamak (Brown)	6 to 7	01
HS	Joopavadlu and Bebbana	8 to 9	02
Reaction	Traditional rice varieties	Score	Total
HR	–	0	00
R	–	1	00
MR	–	3	00
MS	Dodda Byra Bili Akki, Natibatta, Hasundi and Mysore Sanna	5	05
S	Navalisale, Karidaddi Budda, Moradda, Sahabag, GK1, Sada Holga, Theerthalli Local and PB Local	7	08
HS	Joopavadlu and Chinna Ponni	9	02

Comparison of leaf and neck blast disease reaction in two locations

Only three varieties, namely KMP200, BR2655 and DH-MAS70Q 164-1b were moderately resistant in both the locations (table 2). Similarly, phenotypic studies of traditional rice varieties for leaf blast disease revealed that there were several genotypes that had recorded similar disease reaction for leaf blast at both the locations (table 3). BR2655, the only variety showed similar reaction across locations (table 2). Following evaluation of TRVs at both the locations, we did not find any TRV that showed commonly high resistance to neck blast. This could be mainly because of the difference in race combinations that are prevalent at Mandya and Ponnampet. Further, specific isolates may cause neck blast disease, therefore most of the genotypes which were resistance to leaf blast like Sadaholga might be susceptible to neck blast.

Genetic diversity of blast resistant genes

Of the 20 R genes evaluated, genes 16, 8, 13 and 11 harboured in Tetep, HR12, BR2655 and Rajamudi, respectively. Popular varieties like IR 64, Jaya and Ratnachoodi possessed seven (*Pi2*, *Pik*, *Pikm*, *Pizt*, *Pi7t*, *Pita* and *Piz*), 11 (*Pi54*, *Pi1*, *Pi2*, *Pik*, *Pitp*, *Pi38*, *Pizt*, *Pi7t*, *Pi40*, *Piks* and *Piz*) and 12 (*Pi54*, *Pi2*, *Pikm*, *Pitp*, *Pizt*, *Pi7t*, *Pi5*, *Pi20*, *Pita*, *Pi33*, *Piks* and *Piz*) blast resistance genes, respectively.

Among the released varieties and advanced breeding materials, 10 were found to harbour *Pi54* blast resistance gene (table 3 in electroinc supplementary material) and of these traditional rice varieties, 13 TRVs were found positive for Pi54MAS marker with the fragment size of 216 bp for resistant allele (figure 3a in electronic supplementary material). It is clear from our study that the presence of *Pi54* gene in varieties and TRVs are 34.48% and 23.63%, respectively. Subsequently, nine varieties were positive for *Pi1* gene and among TRVs, eight were found positive for *Pi1* gene using RM224 marker (table 4 in electronic supplementary material). For *Pi2* gene, 86.20% of varieties were positive and 69.09% of TRVs were found to harbour *Pi2* blast resistance gene. NMSM Pi9-1 marker was used for *Pi9* gene evaluation and resistance allele size observed in 168 bp (figure 3d in electronic supplementary material), only three varieties were found to harbour *Pi9* blast resistance gene and 54.54% TRVs were clearly positive for marker (table 4 in electronic supplementary material). Varieties 82.75% had an amplicon of 300 bp indicating the presence of *Pik* gene (figure 3e in electronic supplementary material) and 52.72% of TRVs harboured *Pik* gene. Twenty varieties (68.96%) were found to harbour *Pikm* gene by using simple sequence repeat (SSR) marker called CKM-1 with 174 bp for resistant allele and 213 bp for susceptible allele (figure 4a in electronic supplementary material). Among TRVs, 46 (83.63%) clearly showed positive (table 4 in electronic supplementary material) and based on the amplification pattern of marker RM246 (figure 4b in electronic supplementary material) 13 varieties were positive for *Pitp* gene and 40 TRVs (72.72%) of the 55 clearly exhibited positive banding pattern from RM246. Varieties 31.03% and 56.36% traditional rice varieties harboured *Pi38* blast resistance gene. Surprisingly *Pizt* and *Pi7t* gene profiles reveals that all released varieties and advanced breeding materials and traditional varieties were found to harbour both the genes (figure 4, d&e in electronic supplementary material).

Pi5 positive varieties consisted of BR2655, KMP128, KMP153 and KCP-1 (figure 5a in electronic supplementary material) and similarly only 50.90% of TRVs were found to harbour *Pi5* resistance gene. Varieties 24.13%, and 90.90% of TRVs harboured *Pi40* (figure 5b in electronic supplementary material). RM1337 SSR marker indicated that only eight (27.58%) varieties harboured *Pi20* gene (figure 5c in electronic supplementary material) and 19 (34.54%) TRVs were found to harbour *Pi20* resistance gene. Genotypic evaluation is explained with RM7102 for *Pita/Pita2* gene 15 varieties and 28 TRVs were found to be positive for *Pita*/*Pita2*. Expected product sizes of the resistant and susceptible alleles of *Pi33* were 245 bp and 280 bp, respectively (figure 5e in electronic supplementary material). RM22585 SSR marker indicated that 15 varieties (51.72%) harboured *Pi33* gene. Similarly, 15 (27.27%) were found to harbour *Pi33* resistance gene.

RM208 marker elucidate about eight released varieties and advanced breeding materials (27.58%) and 14 TRVs (25.45%) were found to harbour *Pib* (figure 6a in electronic supplementary material). The presence of DNA band corresponding to 240 bp from the agarose gel images obtained after running the PCR products with genomic DNA from 29 released varieties and advanced breeding lines and primers of RM144 SSR marker indicated that only three genotypes harboured *Piks* gene. Similarly, among 55 TRVs, only 16 (29.09%) were found to harbour *Piks* resistance gene (table 4 in electronic supplementary material). SSR marker reveled that 27 varieties (93.10%) were found to harbour *Piz* blast resistance gene. The resistant and susceptible alleles were represented by a PCR product, with the primers of RM19818, 275 bp and 250 bp, respectively (figure 6c in electronic supplementary material). In a similar way, 33 TRV's (60.00%) were identified to be positive *Piz*. RM527 SSR marker indicated that only three genotypes harboured *Piz5* gene and eight genotypes (27.58 %) were identified to carry *Pish* gene.

Cluster analysis performed against varieties and TRVs

Clustering analysis was executed based on unweighted pair group method using arithmetic averages (UPGMA) using Jaccard's coefficient against 29 released varieties and advanced breeding materials with two checks grouped into two major clusters. Cluster I had highest number of varieties

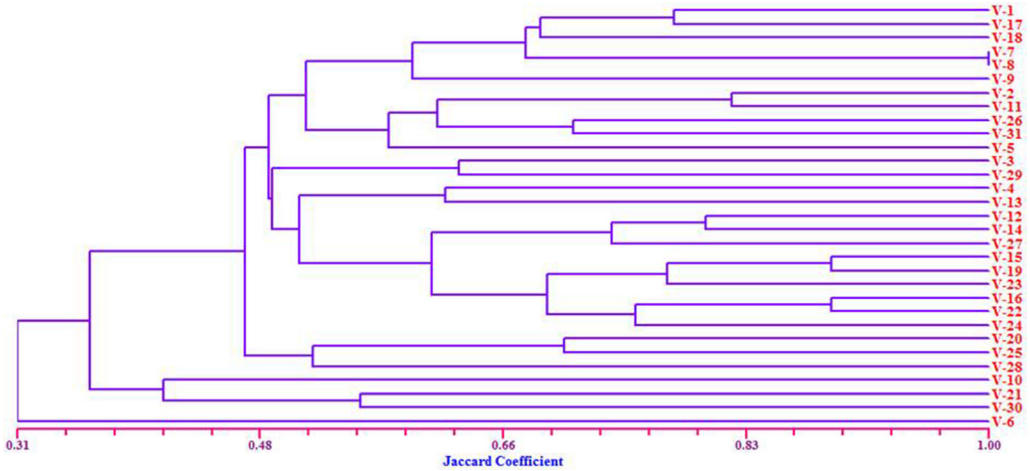

Figure. 1. UPGMA-based dendrogram of released rice varieties and advanced breeding materials using SSR markers data. V1, IR 64; V2, Jyothi; V3, BR 2655; V4, Jaya; V5, Thanu; V6, MTU 1001; V7, KMP 201; V8, KMP 200; V9, KMP 128; V10, KMP 153; V11, KMP 175; V12, Raksha; V13, Rashi; V14, Basamathi 370; V15, Mandya Vijaya; V16, JGL1798; V17, MTU 1010; V18, IR 30864; V19, BPT 5204; V20, CTH-1; V21, CTH-3; V22, Mandya Sona-1; V23, Mandya Sona-2; V24, Gangavathi Sona; V25, Jyothi X BR2655; V26, Jyothi X ASD-16; V27, KRH-4; V28, DHMAS 70Q 164-2a; V29, KCP-1; V30, HR-12; V31, Tetep.

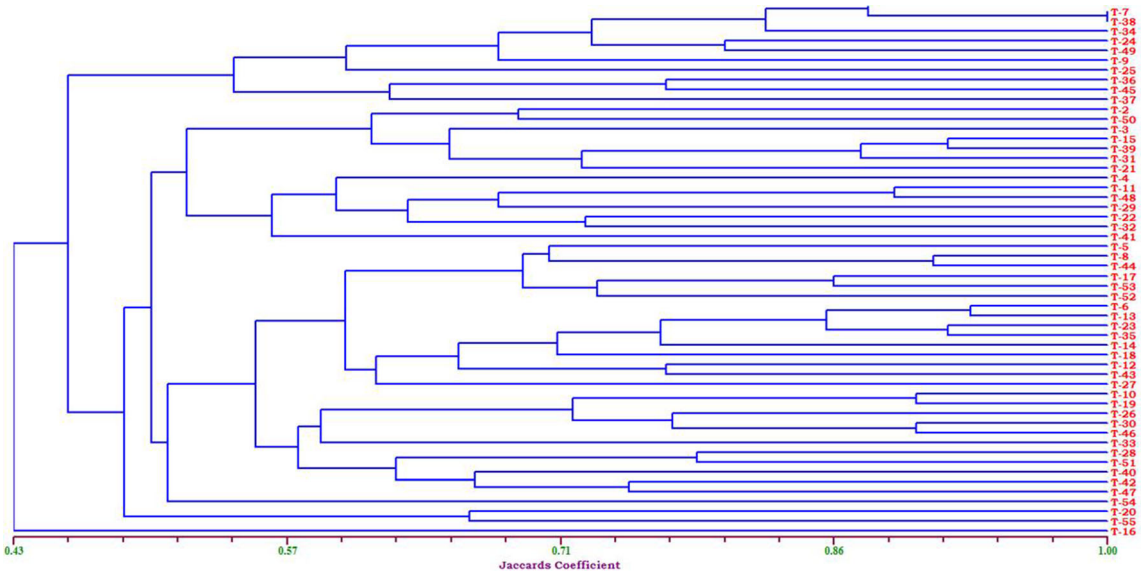

Figure. 2. UPGMA-based dendrogram of traditional rice varieties using SSR markers data. T1, Honne Kattu T2, Onamardini Nellu; T3, Dodda Byra; T4, Punkutt kodi; T5, Malgudi Sanna; T6, Bili Akki; T7, Karibatta; T8, Putta Batta; T9, Padmarekha; T10, Sirsi; T11, Sarjana; T12, 222; T13, Tonnaru; T14, Navalisale; T15, Karidaddi Budda; T16, Rajmudi; T17, Gangadale; T18, Moradda; T19, Tulasiya; T20, Kari Kandaka; T21, Laalya; T22, Sahabag; T23, Selamsanna; T24, Coimbatore Sanna; T25, GK1; T26, Jopavadlu; T27, Kaduvelpe; T28, Ugibatta; T29, Natibatta; T30, Kaggali Kecrona; T31, Khuri Adikshan; T32, Dunda; T33, Pushpa; T34, Kundi Pullan; T35, Black Sticky; T36, Kalanamak; T37, Kalanamak (Brown); T38, Sada Holga; T39, Bebbana; T40, Hasundi; T41, Hola Batta; T42, Theerthalli Local; T43, Anandi; T44, Nagaland Paddy; T45, Jawahar; T46, Kana Kunja; T47, Adikane Batta; T48, Nawara White; T49, Ubar Munda; T50, Rajakime; T51, PB Local; T52, Mysore Sanna; T53, Chinna Ponni; T54, Ratna Choodi; T55, Talehamsa.

(30) whereas cluster II had only one variety (figure 1). Cluster I was divided into two subclusters IA and IB. Further subcluster IA subdivided into subcluster IA-1 and IA-2. Maximum numbers of varieties which have shown leaf blast resistance in both the location are grouped into sub cluster IA-1 and susceptible universal variety HR-12 occupies in sub cluster IB in which KMP-153 and CTH variety susceptible varieties are grouped. Similarly, for TRVs also genetic similarity coefficient were calculated and dendrogram constructed (figure 2). The maximum similarity index of 0.60 was obtained between Karibatta and Sadaholga, while least similarity index was obtained among different pairs of traditional rice varieties. The average similarity coefficient of 0.27 was obtained by using SSR markers. The

dendrogram based on SSR markers revealed the presence of three major clusters that include cluster-I, cluster-II and cluster-III. Interestingly, most of the resistant and moderately resistant TRVs were grouped in different subclusters of major cluster II, and cluster I contain 38% of susceptible TRVs and only one popular TRV Rajamudi which was resistant reaction in Ponnampet location and moderate leaf susceptible in Mandya occupied in cluster III. This result gives a clear picture that the varieties which are specific and similar combination of blast resistance genes forms a group together whereas the varieties originated from same location did not show any grouping among them.

Discussion

Due to continuous exposure to severe disease pressure and proper maintenance of optimum relative humidity and associated microclimate that favours the disease incidence was more in Mandya compared to Ponnampet. Therefore, those TRVs that were found to be moderately resistant at Ponnampet were moderately susceptible at Mandya. Development of high yielding varieties has narrowed down the genetic base of plant breeding material of food crops, which limit their future improvements (Warschefsky *et al*. 2014). Accordingly, a protection measure necessitates constant progress to keep pace with the evolving pathogen (Vasudevan *et al*. 2016). Hence it is required to identify new resistance genes and alleles from advanced breeding materials or landraces. However, the genotypic diversity of most of the accessions has not been fully explored and understood. Hence we have examined the genetic diversity of the selected rice varieties which is being recorded and this allelic diversity data gives an idea for incorporation of best combination genes into elite or released variety.

Chethana *et al*. (2016) reported that during 2013 and 2014, the leaf blast disease was severe (>50%) in Kharif season in variety MTU 1001 in Cauvery command area where as in summer season Jaya variety was more affected. Similar to the reported incidents, our results also categorized MTU 1001 as moderately susceptible to leaf blast across locations for leaf blast. Further, the high yielding and long duration variety Jaya showed moderately susceptible and susceptible reaction to leaf blast at Ponnampet and Mandya locations, respectively. Changing climate and the emergence of new virulent races imposed a continuous threat to the rice production and global food security.

Moderate resistance to neck blast Gangadale was susceptible to leaf blast. Remaining TRVs, namely Sirsi, Honne Kattu, Punkutt Kodi and Rajamudi were moderately resistant to both leaf and neck blast reaction. Traditional rice variety Sarjana that had a leaf blast score of 1, was found to exhibit moderate resistance to neck blast. Similarly, 222 that had a very high resistance for leaf blast with the score of 0 showed moderate resistance reaction to neck blast at Ponnampet. This is because of relationships between leaf and neck blast has been partly documented and many questions remain unanswered. In (1992), Bonman thought quantitative resistance against leaf blast is positively correlated with quantitative resistance to neck blast; some cultivars may be relatively resistant to the disease on one organ type. Few varieties like KMP200, 222 and more possessed few R genes which exhibited resistant reaction this might be due to presence of novel R gene(s) or the combination of major R gene and major quantitative trait loci or minor gene interactions. On the other hand, Jaya Thanu, Mandya sona and many more popular varieties found to be carrying more R genes intriguingly showed susceptible reaction. Despite having maximum number of resistance R genes presence in KCP-1, Mandya sona, IR64 and others resistant capacity down due to mutations occurred in the R genes or evolution of new pathogen races. Yadav *et al.* (2019) reported landraces such as Erava pandy, Basmati(s), Maichakca, Gujuri, Kajal champa, Maichakca and Red binni found to be carrying 14 or more R genes from 24 R genes analysed showed susceptible reaction (score 7), this is because of advancement of new pathogen or less efficiency of major genes against harbouring pathogens.

Marker-assisted selection (MAS) is a classical tool in breeding for improved resistance to rice blast. For MAS, the selection is made based on DNA markers closely linked to a blast *R* gene that confers resistance to a particular race of the pathogen (Roy-Chowdhury *et al*. 2012). In the present study, genetic frequencies of the 20 major rice blast resistance genes varied from 10.34 to 100%. Similarly, the gene frequency of the nine major rice blast resistance genes varied from 6 to 97% in the in north east and eastern germplasm and the genetic frequencies of the 10 major rice blast resistance genes ranged from 19.79 to 54.69% (Imam *et al.* 2014). To our surprise *Pizt* and *Pi7t* genes present in all released varieties and advanced breeding materials and traditional varieties, similar results were obtained in evaluation of national rice varieties (NRVs) reveals that *Pib* gene appeared to be ubiquitous were detected in the all the 80 NRVs (Yadav *et al.* 2017).

Shikari *et al.* (2014) observed *Pi1* monogenic differentials with RM224 marker and specific isolates. It can be concluded that entries like Tetep amplified RM224 resistance specific allele and are likely to carry *Pi1* as per the response against specific isolates and susceptible allele was found in IR64 variety. In our study, *Pi1* gene analysis was done by correlating the genotypic results with Tetep (resistant allele) and IR64 (susceptible allele). AP56595 marker has been found to share 288 bp resistance allele sizes for the germplasm carrying *Pi2* allele (Liu *et al.* 2002) and similarly in our study *Pi2* gene with resistant allele size 288 bp was distributed with the frequencies of 86.20% in released varieties and advanced breeding materials and 69.09% in selected TRVs. Genotyping using gene based/linked markers were evaluated by Shikari *et al.* in (2014), reported only 26 from 100 germplasm harbours with *Pi9* gene and remaining were possessed with susceptible allele, within that IR-64 and

Tetep cultivars considered with susceptible allele and correspondingly, our study reported that IR-64 and Tetep cultivars were showing susceptible banding pattern for *Pi9* gene. Hayashi *et al*. (2006) found cosegregation of markers k-6816 and k-2167 with the gene *Pik* in F_2 population derived from Kanto 51 (*Pik*+) x OISL 235 (*Pik*). Presently, in our study we used K-2167 SSR marker to identify the *Pik* resistant allele which reveals that 82.75% and 52.72% of gene frequencies were observed in varieties and traditional rice varieties, respectively. Shikari *et al.* (2014) screened 100 germplasm for *Pikm* gene account with CKM-1 marker and found a total of 34 and 67 germplasm lines possessed resistance alleles for two genes, *Pikm* and *Pik*, respectively and their study accomplished that both *Pikm* and *Pik* were present in IR64 but only *Pik* was present in Tetep. We found the similar result for IR64 and Tetep cultivar with respect to *Pikm* and *Pik* gene in our testing. Singh *et al.* (2015) evaluated 192 germplasm lines identification for positive fragment of *Pitp* located on chromosome 11 with tightly linked SSR markers RM 246. Result indicates the absence of resistant fragment in IR64. In our study we have observed IR64 variety exhibited negative banding pattern from RM246 marker and varieties like Mandya Sona-1, Raksha, Basamathi and some other varieties showed positive allele size. *Pi38* was not much well studied gene, only limited foundation research was discovered to evaluate and understanding about this gene. Our current study was undertaken to expand upon the existing knowledge in this area by identifying and documentation of *Pi38* gene. Lee *et al.* (2009) suggested that SCAR marker JJ803 (derived from dominant marker JJ80-T3) cosegregated with *Pi5* mediated resistance at 0 cM and the marker is a part of 90 kb sequence which spans *Pi5-1* and *Pi5-3* subsequences in Nipponbare which however, lacks *Pi5-2* present in resistant RIL260. Since, *Pi5-1* and *Pi5-2* complement each other and condition *Pi5* mediated resistance; the absence of any of these will make germplasm susceptible even in case if it is positive for the marker JJ803.

However, till today only two genes (*Pi9* and *Pi40*) have been identified from wild species (Jiang *et al.* 2015). They have some durable effect in combined form (Joshi *et al.* 2009). So that it is chief to evaluation of *Pi40* gene in our study and combination of *Pi9* and *Pi40* found in few resistant varieties only and *Pi40* present in almost all TRVs since absence of *Pi9* results in susceptibility of maximum TRVs. In 2011, Wen *et al.* (2006) suggested that of the three SSR markers, RM1337, RM5364 and RM7102, cosegregated with *Pi20(t)*. RM1337 and RM5364 were found to be reliable markers of resistance conditioned by *Pi20 (t)* in a wide range of elite rice germplasm in China. Therefore, we evaluated *Pi20* blast resistance gene using gene linked marker RM1337 in our genotypic evaluation of varieties (27.58% gene distribution) and traditional rice varieties (34.54% gene distribution). *Pita* gene in US varieties has originated from Tetep and Tadukan, which happens to be the donor for K1 and most of the Japanese cultivars (Rybka *et al.* 1997). Identi-fication and validation of *Pita* genes reveals that the Indian rice germplasm are diverse and potential source of blast resistant lines which can be exploited in rice blast breeding programmes. In our study, ~50% of *Pita* gene frequency was observed in the evaluated varieties. Shikari *et al.* (2014) screened 100 germplasm in both phenotypic and genotypic analyses. They have determined the *Piz* gene distribution within the germplasm and results reveals that IR64 variety has *Piz* gene. Similar to this, same result was found in our experiment and suggested marker used in identification of *Piz* gene was appropriate. With the chosen 20 gene specific markers, we for the first time report the presence and absence these genes in the selected varieties and traditional rice varieties. More than 60% of the blast resistant germplasm with specific combination of resistance genes accessions were clustered in solitary group. Our study indicates, selecting resistant parents from the particular group and susceptible parent with better yielding traits from the other group has a genetic potential in the improvement of rice blast resistant variety.

In conclusion, phenotyping of BR2655, KMP 200, DHMAS 70Q-164-1b, Karibatta, Putta Batta, Tonnaru, Selam Sanna, Coimbatore Sanna, and a few more have been identified as leaf blast resistant varieties with the scores ranging between 0 and 3 at both the locations. Similarly, BR2655, Jyothi x BR2655, Punkutt Kodi, Sirsi, 222 and Gangadale showed resistance to neck blast disease at both the locations. These results provide clues about the specific resistance genes for leaf and neck blast and these varieties could be efficiently deployed in breeding programme to develop blast resistant cultivars. Cluster analysis was purely based on genotypic evaluation and specific combination of genes depicts the clear relationship of these genes between released varieties, advanced breeding lines and traditional rice varieties. Blast resistance genes such as *Pi1*, *Pi2*, *Pi54*, *Pi9*, *Pi40*, *Pi20* and *Pita* genes showed a broad resistance to blast which indicate that they could be effectively used as the main source of resistance in future resistance rice breeding programmes. The present study provided an overview of the genetic diversity of the selected rice varieties and TRVs for leaf and neck blast resistance. Besides, the accurate evaluation of blast resistance genes in rice varieties and the marker loci obtained are highly helpful and resourceful in the selection of resistant parents for blast disease and development of new breeding populations in future breeding programme. The information obtained from the phenotypic evaluation and genetic variability of the rice varieties and TRVs will be much helpful for appropriate selection of rice varieties in different blast prone areas and could also be utilized in gene deployment and gene pyramiding on the basis of prevalence of *M. oryzae* races and genotyping of blast resistance genes in this study will be useful in the marker-aided selection.

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