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Identification of Quantitative Trait Loci for Leaf Blast Resistance of Rice (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author LM designed the study, managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Author SKV performed the statistical analysis and editing of manuscript. Authors ASK and SBV provided major guidance and facilities for conducting experiment of the study. All authors read and approved the final manuscript.

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ABSTRACT

Blast is one of the economically important diseases of rice. Quantitative trait loci (QTL) analysis for partial resistance to leaf blast disease was carried out using 122 RIL population from the cross between Danteshwari (highly susceptible) and Dagad deshi (resistant) in blast endemic area of Ambikapur, north Chhattisgarh, India during wet season, 2013. Analysis indicated that approximately normal distribution of RILs for the trait leaf blast resistance. A linkage map was constructed from 122 RILs using 162 polymorphic SSR markers. Composite interval mapping was employed to identify QTLs. Five QTLs such as "*qLB12.1*", "*qLB12.2*", "*qLB12.3*", "*qLB12.4*" and "*qLB10.1*" were identified for leaf blast resistance using RIL population on chromosomes 12 and

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10, respectively. The QTLs, "*qLB12.2", "qLB12.3", "qLB12.4"* mapped both seedling and tillering stages of RIL population of rice. The LOD score and phenotypic variation explained by each QTL ranged from 6.898-3.021 and 15.86 - 56.24%, respectively. The *qLB12.2* was identified for both stage and found stable across the life of rice for leaf blast resistance. The resistance loci mapped to chromosome 10 specific to second replication. The QTLs identified could be used as a genetic resource in improvement of rice varieties for blast resistance. These findings laid the foundation for the development of a marker-assisted scheme for improving majority of rice varieties that are susceptible to blast and useful for fine mapping studies.

Keywords: Leaf blast; QTL mapping; RIL population; rice (Oryza sativa L.).

1. INTRODUCTION

Blast disease of rice caused by the filamentous fungus *Magnaporthe oryzae* has been one of the most damaging diseases of rice and remains one of the most difficult crop diseases to manage [1]. It is one of the limiting factors for rice production worldwide. Conservatively, each year enough rice is destroyed by rice blast disease to feed 60 million people [2]. The estimated annual yield losses upto 40-75% due to occurance of Blast [3]. Blast resistance in rice has been generally classified into two types: complete (qualitative) or true and partial (quantitative) or wild resistance [4]. Complete resistance is characterized by the prevention of infection in incompatible combinations of hosts and parasites and generally is controlled by a single major gene, under the control of the "gene-for-gene relationship" [5] with an avirulence gene in the blast pathogen [6]. On the other hand, partial resistance reduces the extent of pathogen reproduction in the compatible interaction [7,8]. Most of the partial resistance is non-race specific, quantitative and polygenic [9-10]. However, there are some exceptions such as *Pif* [11], *Pb1*(t) [12] and a partial gene in Chubu 32 [13], which are single genes conferring partial resistance to blast. True resistance is governed by qualitative gene also called major gene and field resistance by quantitative genes also called minor genes.

The deployment of resistant cultivar is the most effective and economical way of controlling blast disease, so breeding for resistant cultivars continue to be a priority in rice improvement [14]. Moreover, with the completion of the rice [15] and *M. oryzae* [16] genome sequences, rice blast disease has strength its position as a model for plant–pathogen interactions in monocotyledons [17-19]. Different strategies to breed durable resistance have been proposed to counter blast evolution. Some strategies, such as pyramiding [20], lineage exclusion [2], multilines [21] and mixtures [22] are based on the use of complete and specific resistance genes. Others are based on the accumulation of partial resistance [20], a strategy thought to be more durable because it is assumed to be more general. So far, 86 major *R* genes have been mapped on all of the rice chromosomes except for chromosome 3 [23]. To date, more than 70 genes and 347 quantitative trait loci (QTLs) have been detected over 12 chromosomes for blast resistance [24].

The quantitative resistance system that has been especially well characterized in rice is resistance to the blast fungus [25-27]. The first QTL analysis to blast resistance of rice by Wang [25]. Previously, the putative QTLs were identified for blast resistance on rice chromosomes using various population by different researchers [26, 28-42]. Once the tightly linked markers have been identified, the quantitative trait loci can be selected for breeding programs using marker-assisted selection strategy. So, the objective of present study was to identify QTLs related to rice leaf blast disease using an RIL population from the cross between cultivar Danteshwari (highly susceptible) and Dagad deshi (resistant).

2. MATERIALS AND METHODS

2.1 Plant Material and Phenotyping of Population

The evaluation of partial resistance to leaf blast in the Danteshwari × Dagad deshi crossed 122 F_{14} RIL population along with parents was conducted at RMD, College of Agriculture and Research Station, Ambikapur, C.G. India (23° 09'N and 83° 08'E at altitude of 611 meter above sea level) in wet season 2013. The characteristic features of parents given in Table 1. About 50 seeds of each line and the parents were sown in a 150 cm length row with 10 cm spacing on July, 2013. A complete randomized block design was used with two replications. The Swarna variety is highly susceptible to blast, used as check in border rows. The population was allowed to grow

for natural occurrence of the disease. The disease severity of leaf blast in each line of the population and parental cultivars were evaluated based on 0-9 score [43] of first date 13/08/2013 at seedling stage and second date 05/09/2013 at tillering stage. In Figs. 1 and 2, the disease scores ranged from 0 (no lesion) to 9 (more than 75% leaf area affected).

2.2 Phenotypic Analysis

The phenotypic data of each RIL given in Supplementary Table 1. The Mean and SD of the blast scores for each line and parents were calculated in Table 2. The disease scores of structural population RILs for leaf blast resistance in natural disease condition analysis by computer for graphical representation of continuous variation.

2.3 DNA Isolation and PCR Amplification

The genomic DNA isolated from leaf of single tagged plant using MiniPrep method [44]. The detail of DNA isolation method used as around 0.1 g of leaf sample was grinded in a 2 ml eppendorf tube contained 0.4 ml of extraction buffer with the help of MoBIO tissue lyzer. Then 0.4 ml of chioroform-isoamyl alcohol (24:1) mixture was added. Mixed well by vortexing. Centrifuged at 13000 rpm for 30 sec. Supernatant was collected and transferred to a new eppendorf tube. Then 0.8 ml of absolute ethanol was added and mixed properly by tube inversion. Centrifugation was done at 13000 rpm for 2 min. Supernatant was discarded and pellets were washed with 70% ethanol. Dried the pallets for 15-20 minutes. Pellets were dissolved in 50-100 µl (based on the size of pellet) TE buffer. The optimized PCR protocol was used for identify the informative SSR markers on the basis of parental polymorphism. Polymerase chain reaction (PCR) amplification for SSR was performed in a total volume of 20 µl and the reaction mixture contained 10 X Assay buffer, 1 mM dNTP mix, 5 pM forward and reverse primers, 40 ηg of template DNA and 1 unit Taq polymerase in 96 well veriti Applied Biosystems thermal cycler, USA. After an initial denaturation step of 95°C for 5 min, the amplification was carried out for 34 cycles comprising 1 min each of 94°C (denaturation), 55°C (annealing) and 72°C (extension). The final elongation step was extended to 7 min at 72°C followed by 4°C. After the PCR reaction was completed, 5 µl of 6 X loading dye was added to PCR amplicons and 7 µl (PCR product with dye) was loaded on 5% PAGE in a vertical electrophoresis system (CBS scientific, model MGV-202-33, USA) with 180V for 1.5 hours. DNA fragments were then stained with ethidium bromide and visualized with a UV transilluminator Bio-rad XR+ manufactured from USA.

Table 1. Characteristic features of parents

S. no.	Parent	Pedigree	Salient features
1.	Danteshwari	Shamridhi × IR 8608-298	High yielding, Dwarf, Early and high tillering, Resistant to gall midge, Early maturity 105 days, Long slender grain
2.	Dagad deshi	Land race	Strong culm, Tall, Shy tillering, Broad leaves, Bold seeded, Early maturity 100 days

Fig. 1. The natural occurrence of disease reaction with parents and RIL population

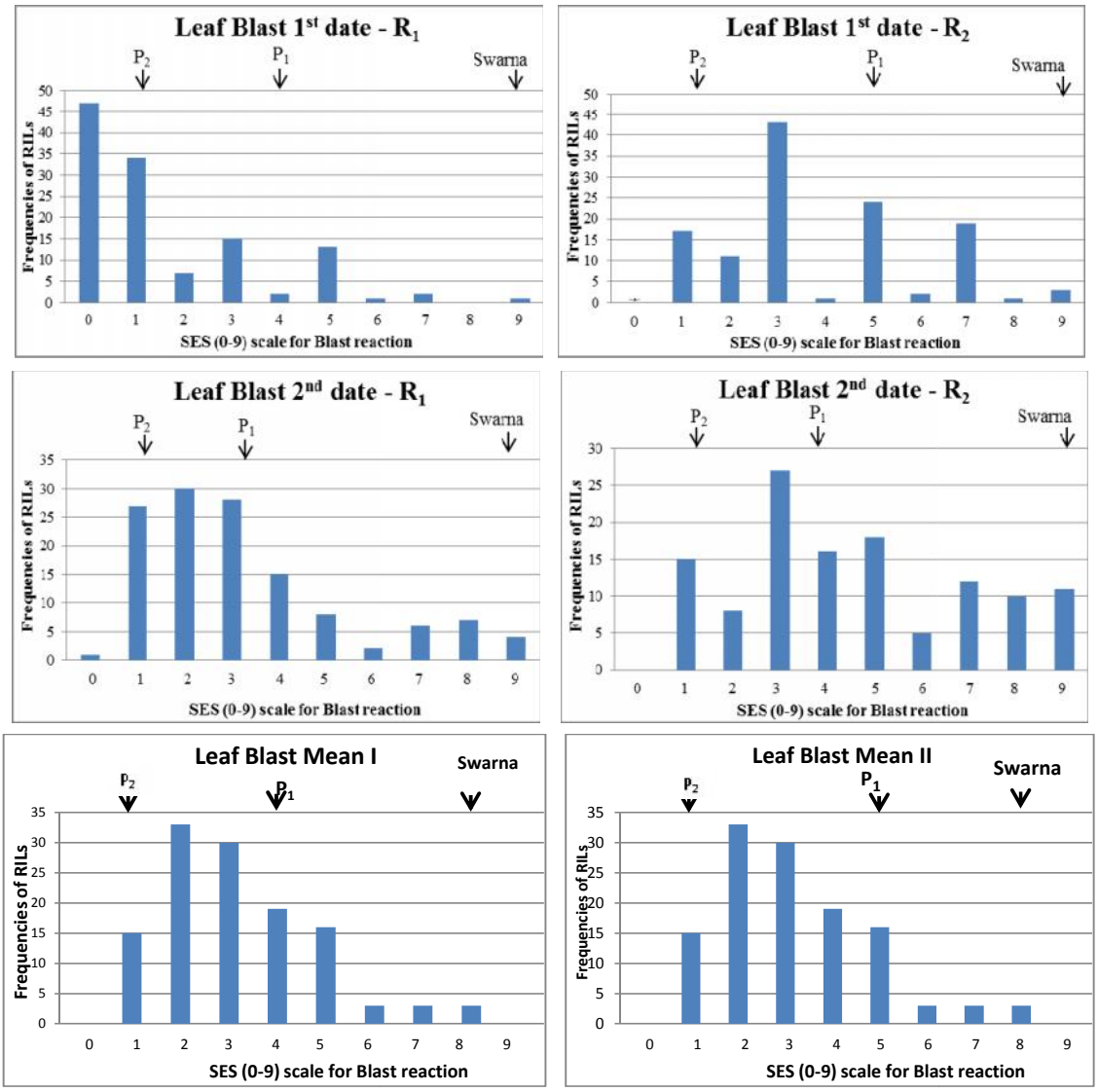

Fig. 2. The frequency distribution of RILs for leaf blast disease reaction of both stage and replications

Table 2. Statistics analysis of the blast resistance traits in parents and the RIL population

Traits	Parents				RIL population	
	Danteshwari		Dagad deshi			
	Mean	SD	Mean	SD	Mean	SD
Resistance at Seedling stage(rs)	3.5	4.9	1	1.4	2.7	1.5
Resistance at Tillering stage(rt)	6.5	3.5	2	0	3.8	1.9

rs=resistance at seedling stage, rt=resistance at tillering stage

2.4 Genotyping of RIL Population and Construction of Genetic Linkage Map

The polymorphism survey was conducted between the parents Danteshwari and Dagad deshi by using 830 SSR markers randomly distributed on all 12 rice chromosomes. Only 162 markers were found polymorphic. All 162 well distributed polymorphic SSR (RM and HvSSR) [45] markers were used to construct a linkage

map. The details of all 162 polymorphic markers are given in Supplementary Table 2. The genotypes data was prepared for each line based on the banding patterns. All of 162 clearly polymorphic markers were used in segregation analysis of the 122 RILs. The linkage map was constructed using MapMaker/exp ver. 3.0 program [46]. All pairs of linked markers were identified using the "group" command with an LOD value of 3.0. The marker order was determined using the "orders" and the "compare" commands and verified using the "ripple" command. The frequency of recombination between two markers was converted to genetic distance using Kosambi map function [47]. Assignment of linkage groups to the respective chromosomes was based on genetic maps developed by McCouch [45] and Gramene Annotated Nipponbare Sequence map [48]. The linkage map drawn by software QTL cartographer (version 2.5).

2.5 QTL Mapping

The data of two replications of two stages and their mean for each line was used as the raw value for QTL analysis. The composite interval mapping (CIM) was performed by QTL cartographer (version 2.5) [49]. The thresholds 0.05 significance level for CIM was determined using 1000 permutations for each trait [50]. The presence of putative QTLs declared if the LOD threshold was larger than 3 for the traits. The proportion of phenotypic variation explained by each QTL was calculated on the basis of R^2 value.

3. RESULTS

3.1 The Distribution of Resistance for Leaf Blast in the RIL Population

The phenotypically screened the structural population RILs derived from Danteshwari × Dagad deshi for the trait leaf blast resistance in natural disease load condition. The frequency distribution of score obtained in the experiment with natural disease occurrence was examined. Analysis indicated that approximately normal distribution was followed for the trait leaf blast resistance of RILs of different stages given in Fig. 2. The resistance segregation in the experiment varied dramatically. There were many lines showed resistance and many susceptible toward natural leaf blast disease. The parent Dagad deshi showed resistance toward leaf blast disease reaction and Danteshwari found highly susceptible. The incidence degrees of two parents Dagad deshi and Danteshwari to natural races were scores from 1 to 2 and 7 to 8 respectively. The maximum number of RILs shown blast resistance scores of 2, 4, 6 or 8 on SES (0-9) scale (IRRI, 2002) under natural infection. The check Swarna variety used in this experiment was highly susceptible to blast disease scored 9 on SES (0-9) scale (Fig. 1). The continuous variation of disease incidence in RILs indicates the existence of QTLs underlying the segregation of resistance. Interestingly, among lines few lines showed highly resistance to the disease occurrence, indicating that a combination of many genes was required to achieve different level of resistance.

3.2 Genetic Linkage Map

The molecular linkage map was constructed by multipoint analysis using the program MapMaker v.3.0 [46], with the LOD threshold fixed at 3.0 and based on the marker data of RIL population. The map distances between the microsatellite marker were presented in centiMorgan (cM), using the Kosambi function [47]. Out of 830 SSR markers screened for parental polymorphism, 162 (19.52%) were found polymorphic. A total of 162 well distributed polymorphic between Danteshwari and Dagad deshi SSR (RM and HvSSR) markers were used to construct linkage map. The map spanned approximately 3972.8 cM of the genome, with an average marker interval of 24.52 cM. The number of markers per chromosome ranged from 8 (chromosome 10) to 23 (chromosome 1), with an average of 13.5 markers per chromosome. The chromosomes 1 and 5 found the longest linkage groups, whereas chromosomes 10 and 7 were among the shortest. The order of microsatellites markers was followed after [45] and information of physical location available on Gramene [48] and Rice Genome Research Project [51]. There were 23, 12, 16, 11, 19, 9, 10, 12, 18, 8, 11 and 13 markers designated on linkage group 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12, respectively. The linkage group each of 12 rice chromosome given in Fig. 3.

3.3 QTL Mapping for Natural leaf Blast Races

The genotypic data and phenotypic data of field condition of natural blast disease infection were analyzed using QTL cartographer 2.5. There were five QTLs identified for leaf blast resistance using RIL population. The resistance loci

mapped to the chromosome number 10 and 12 of rice. The phenotypic variation explained by each QTL ranged from 15.86 to 56.24%. The QTL along with their LOD score and R^2 value worked out through composite interval mapping (Table 3 and Fig. 4).

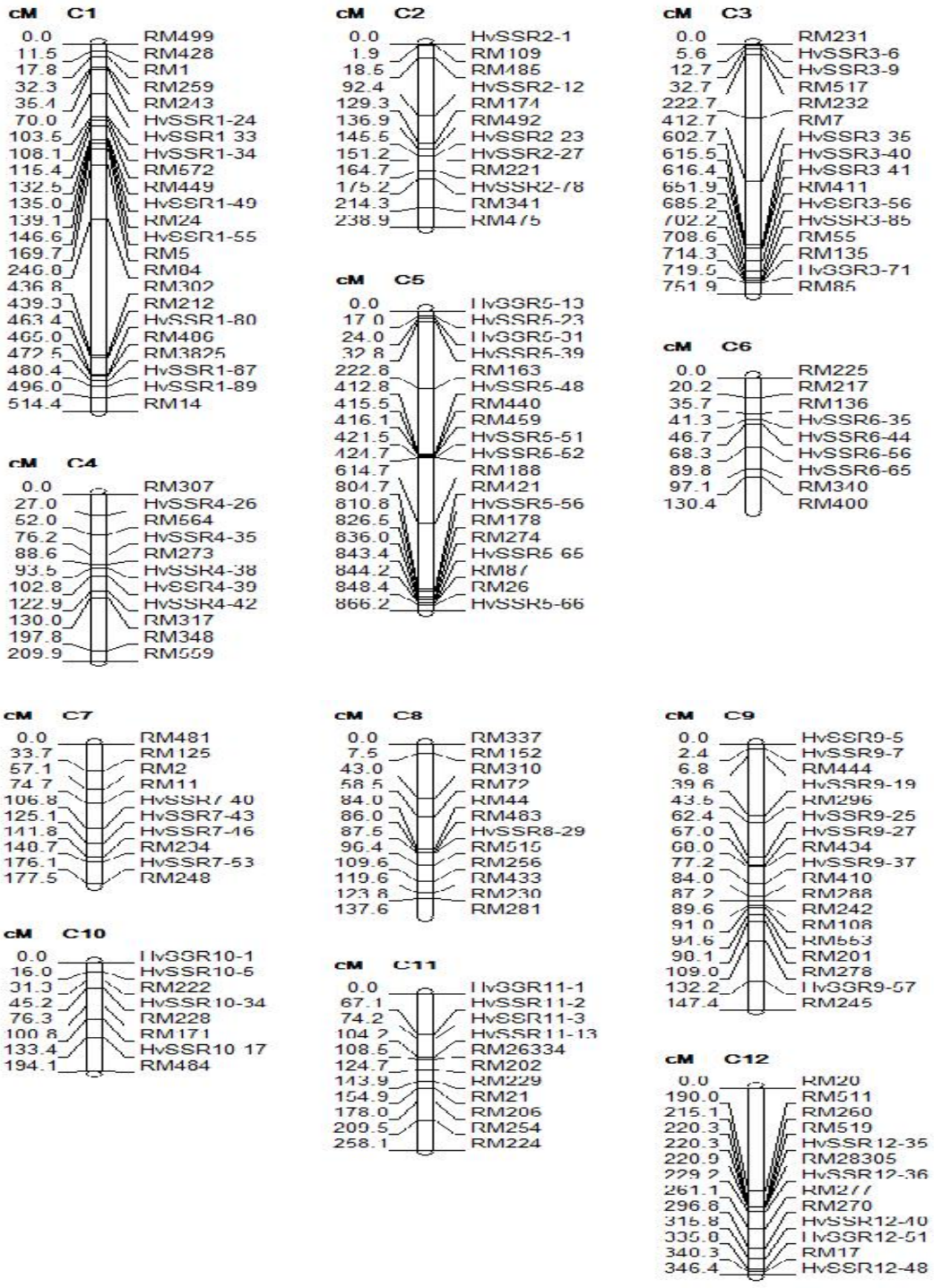

Fig. 3. The linkage group of 12 rice chromosomes with 162 well distributed SSR polymorphic marker

Table 3. QTLs underlying leaf blast resistance mapped by QTL cartographer 2.5

Trait	QTL	Chr.	Closely linked marker	Marker position (cM)	Marker interval		LOD	Additive effect	R^2
Blast R_1	*qLB12.1*	12	RM20	39	RM-20	RM-511	3.8627	-1.7729	0.2230
(Seedling	*qLB12.2*	12	RM20	179	RM-20	RM-511	6.5507	1.9454	0.5624
stage)	*qLB12.3*	12	RM511	195	RM-511	RM-260	3.0209	1.7696	0.5030
Blast R_2	*qLB10.1*	10	RM171	116.8	RM-171	HvSSR 10-17	6.5657	-1.5524	0.4641
(Seedling	*qLB12.4*	12	RM277	271.1	RM-277	RM-270	6.8985	1.7012	0.4605
stage)									
Mean I	*qLB12.4*	12	RM277	263.1	RM-277	RM-270	3.7701	1.086	0.2418
Blast R_1	*qLB12.2*	12	RM20	184	RM-20	RM-511	5.5785	1.8791	0.4271
	qLB12.3	12	RM511	194	RM-511	RM-260	3.9148	1.8794	0.3954
(Tillering	*qLB12.4*	12	RM277	267.1	RM277	RM-270	5.5569	1.9387	0.4806
stage)									
Blast R_2	*qLB10.1*	10	RM171	117.8	RM171	HvSSR 10-17	3.1492	-1.7886	0.4756
(Tillering	*qLB12.4*	12	RM277	268.1	RM277	RM-270	5.204	2.1427	0.4656
stage)									
Mean II	*qLB12.4*	12	RM277	261.1	RM277	RM-270	4.6023	0.9823	0.1586

(R_1= First replication, R_2= Second replication)

The significant QTL, *qLB12.1* was mapped on chromosome 12 for leaf blast resistance between markers RM20 and RM511 with LOD score 3.8627, which explained 22.3% of phenotypic variation for seedling stage of first replication. The QTL showed with a negative additive effect, which inherited from susceptible parent Danteshwari. Another QTL, on chromosome 12, *qLB12.2* also mapped linked to RM 20 marker with different position and LOD value 6.5507 and explained 56.24% of phenotypic variance. The same QTL also identified on tillering stage (second date) of first replication using QTL cartographer 2.5 with LOD value 5.5785 and explain 42.71% of phenotypic variance. This QTL identified for both stage and found stable across the life of rice for leaf blast resistance. The QTL with a positive additive effect showed that allele was inherited from resistance parent Dagad deshi acted to increase the measured trait leaf blast resistance.

Similarly, the QTL, *qLB12.3* was mapped between markers RM511 and RM260 on the chromosome 12 by QTL cartographer 2.5. This QTL was explained 50.3% of phenotypic variance with a LOD value of 3.0209. This QTL mapped for both stages and confined to first replication. The QTL also showed positive additive effect means allele from the resistance parent Dagad deshi acted to increase leaf blast resistance. One major effect QTL, *qLB12.4* was mapped between markers RM277 and RM270 in both seedling and tillering stage of both replications. The QTL with a LOD value 6.8985 and explained 46.05% of phenotypic variance at seedling stage. The QTL showed positive additive effect of value 1.7012. The allele from the resistance parent Dagad deshi acted to increase leaf blast resistance. The same QTL also workout at tillering stage with LOD value range 5.5569-5.204 and explained 48.06 - 46.56% of phenotypic variance with positive allele effect. The QTL, *qLB10.1* was identified on chromosome 10 between markers interval RM171 and HvSSR10-17. The LOD score of the QTL was 6.5657 and explained 46.41% of the phenotypic variation under heavy infection condition. The QTL showed negative additive effect of value -1.5524 and mapped in both stage and confined to second replication. The negative value of the additive effect showed that allele was inherited from susceptible parent Danteshwari.

4. DISCUSSION

The blast resistance in the cultivar Dagad deshi was found to have very complex inheritance. The Blast resistance in rice is generally classified into two types, complete and partial resistances [52]. The partial resistance reduces the extent of pathogen reproduction in the compatible interaction [7] and is non-race specific, quantitative and polygenic [9]. The present study was carried out to identify chromosomal regions for stable field resistance to natural blast races using RILs from the cross between Danteshwari and Dagad deshi. Among 122 RILs, the scores for disease severity in the leaf blast test ranged from 0 to 9 [43]. The normal distribution (Fig. 2) suggested that the resistance in these lines might be a partial resistance. The differences in the frequency distributions of resistance were

observed in RILs for blast disease. Some lines had a higher or nearly equal resistance to Dagad deshi and few lines were more susceptible than Danteshwari. This suggests multi-genic inheritance of QTLs for resistance to natural local races in the field.

In similar study, the frequency distribution of Broad resistance spectrum supported quantitative inheritance. A similar distribution of blast severity was observed in the field experiment at URRC [30]. The frequency distribution of disease severity of leaf and neck blast resistance in the 587 RILs screened by 3 selected isolates. The phenotypic distribution of blast reactions did not show discreate classes [34]. The distributions of the degree of incidence in four out of nine races screened by inoculation method in 190 RIL populations of two parents Suweon365 and Chucheong. The degree of incidence of race KI-197 showed normal distribution [50]. The RIL population exhibited transgressive segregations in both directions for all traits, and the population showed approximately normal distributions at all stages [37]. The frequency distribution of resistance levels of 112 $F_{2:3}$ segregating progenies exhibited continuous distribution, which indicated that blast resistance in IR71033-121-15 against two isolates (KI307 and KI209) was controlled by QTLs [38]. The compatible interaction between pathogen and resistant lines was occurred as marked by small lesions in resistant lines suggested that the resistance in these lines might be a partial resistance [53]. The frequency distribution for the phenotypic traits in 261 BC_2F_3 and 31 BC_2F_5 families shown normal curve for disease lesion against pathotype P7.2 and P5.0 [39]. The RIL population showed varying reactions to all 12 blast isolates with continuous frequency distributions and transgressive segregation in lesion score, indicating polygenic and quantitative resistance to blast in resistant landrace Bodao [40]. Blast resistance was evaluated in the F_3 lines of the cross Nekken 2 × Hokuriku 193 showed continuous distribution but the distribution was biased toward highly resistant plants, suggested that blast resistance in Hokuriku 193 was controlled by a small number of genetic factors with major effects [54].

In the other study, the segregation ratio of the F_3 lines was 3:1 for partial resistance to susceptibility; suggested that the partial resistance in Chubu 32 is controlled by a major gene [13]. The frequency distribution in the F_3 families for segregating phenotypic classes of blast score doesn't showed continuous variation with normal distribution [55]. The distributions of disease reactions of the two RIL populations was skewed toward resistance and this result suggests that there is a major resistance gene effect to IB54 [56].

4.1 Main-effect QTLs Identified in Both Stages

In the present study, five QTLs, *qLB12.1, qLB12.2*, *qLB12.3, qLB12.4* and *qLB10.1* were identified, against natural races in both seedling and tillering stage of RIL population of rice. The location of QTLs on chromosomes for leaf blast resistance showed in Fig. 4.

The *qLB12.1* found specific to replication (R_1) of seedling stage. The *qLB12.2* and *qLB12.3* identified for both stages with positive additive effect, with high phenotypic variance. The QTL, *qLB12.4* also identified for both seedling and tillering stages having positive additive effect means allele carried from resistance parent Dagad deshi. The QTLs also identified on these chromosomes by many other researchers showed their stability. Using a quantitative trait locus (QTL) mapping approach, 13 QTL on chromosomes 1, 2, 9, 11 and 12 were detected from Bodao. Among the 2 QTL on chromosome 12, *qtl12-2-3* was effective against 3 isolates, including 2009-12-3(ZC3), 2009-7(ZG1) and 2009-9(ZB15), yielding LOD scores of 7.90-13.30 and phenotypic variances of 11.68- 15.81%. The *qtl12-1-1* was effective against one isolate 2009-12- 3(ZC3) with an LOD score of 6.98 and phenotypic variance of 2.4 [40]. Five suggestive QTLs (*qBL11.2, qBL11.3, qBL12.1, qBL12.2, qBL12.3*) and one putative QTL (*qBL2.1*) were identified for pathotype P7.2 in advanced backcross BC_2F_5 families from *Oryza sativa* cv MR219/*O. rufipogon* IRGC105491 [39]. There were 22 quantitative trait loci (QTLs) conferring resistance to isolates identified and mapped onto rice chromosomes 1, 7, 9, 11 and 12. The single *qtl12-1-1* mapped on chromosome 12, effective against only one blast isolate and confers isolate-specific resistance [57]. The two QTLs, *qLB12.1* and *qLB12.2* were identified on chromosome 12, against the disease incidence for two and three years in Suwon between marker interval RM3455 - RM1377 and RM17 - RM1300 and explained to 4.8-5.2% and 20.6-25.2% of the total phenotypic variation, respectively. The *qLB12.2* was the major QTL explaining 11.8-37.7% of total phenotypic variation and was reduced to 0.69-1.44 of the degrees of disease incidence by the

allele effect of Suweon365 against all regions and years [50]. Furthermore, 9 QTLs have been mapped using RFLP markers on chromosome 1-4, 6, 7 and 9, with 2 loci on chromosome 12. The *qBLASTads-12-1*, also mapped similarly with major genes conferring resistance to the three major races of blast. The differential levels of susceptibility associated with *qBLASTads-2*, *qBLASTads-6* and *qBLASTads-12-1*, as detected in the presence of virulent races, suggests that these regions contain genetic factors conferring a hypersensitive response to some races and partial resistance to other races [31]. Five QTLs relating to leaf blast resistance have been detected on chromosomes 4, 6, 8, 11 and 12 from the BC_2F_2 population derived from the backcross of Koshihikari × *O. rufipogon* [33]. Two QTLs were detected on chromosome 4 and one each on chromosome 9 and 12 [29].Ten putative QTLs were identified for blast resistance on rice chromosomes 2, 3, 5, 6, 7, 8, 11 and 12 [28].

In contradiction, to most of partial resistance is non-race specific, quantitative and polygenic [9]. QTL analysis of the F_3 lines detected one QTL on chromosome 12 that contributed significantly to blast resistance. Notably, the QTL had a major effect and localized to the same region where *Pi20*(t), a broad-spectrum blast resistance gene, is positioned, suggesting strongly that the blast resistance of Hokuriku 193 was controlled by *Pi20*(t) [54]. Fourteen QTL were identified and mapped on three chromosomes: 1, 11 and 12 against leaf and neck blast. The QTLs on chromosome 12 showed a race-specificity for leaf and neck blast against THL318 and THL899. The two QTLs, *qLB12-2* and *qNB12-2* were detected on chromosome 12 with LOD scores of 24.64 and 7.45, respectively. These QTLs were coincidently located at the peak OSR32–RM309 interval for isolate THL318. The *qLB12-3* was mapped at the OSR32–RM309 marker interval and coincided with the *qLB12-2* and *qNB12-2* for isolate THL899 [34]. Totally 124 QTLs have been identified against 20 isolates using Cartographer software with a ZYQ8/JX17 DH population on 12 rice chromosomes. In comparison of their positions on chromosome, most QTLs are clustered together and distributed nearby the major genes especially the regions on chromosomes 1, 2, 8, 10 and 12 [58]. The majority of QTLs detected are race-specific and the partial resistance genes might be defeated major genes, with residual effectiveness and race specificity. Two main-effect quantitative trait loci (QTL) mapped on chromosome 12 to nearest marker R617 and R1933 to blast infection of recombinant inbred lines (RILs) inoculated with blast fungal isolate PH19 for disease severity. The QTL positioned to marker R1933 found common for both disease severity and lesion size of blast fungal isolate CM28 [32].

In this study, a QTL, *qLB10.1* was also identified on chromosome 10 in the marker interval RM171 and HvSSR10-17 through QTL Cartographer 2.5 with high phenotypic variance. The presence of this QTL was confirmed by presence of similar QTL, *qBL10.1* for pathotype P5.0 identified using the BC_2F_3 families derived from *Oryza sativa* cv MR219 × *O. Rufipogon* IRGC105491 on Chromosomes 10 [39]. A QTL, *qLS10* position near the marker RG241B was identified on chromosome 10 with 6.4% phenotypic variance, using 304 recombinant inbred lines of *indica* rice cross Zhong 156 × Gumei 2 [59]. Similarly, on chromosome 10, *Pi28*(t) a *R* gene in one of QTL identified through double haploid (DH) population derived from an IR64 by Azucena cross [26].

4.2 QTLs for Partial Resistance on Other Chromosomes of Rice

There were many other QTLs analysis on different chromosome for partial resistance (BRS) to leaf blast by different researchers [26, 27,30,35-38,53,55,56,60]. Two partial resistance QTLs, *qBR1.1* and *qBR6.1* and one major resistance QTL, *qBR11.1*, were identified in the B population. One partial resistance QTL, *qBR6.1* and one major resistance QTL, *qBR11.1* were confirmed with the S population [56]. The *qBR4-2* comprises three tightly linked QTLs that control blast resistance in a complex manner [60]. By using 148 Sequence Tagged Site (STS) and Single Sequence Repeat (SSR) markers, five QTLs on chromosomes 6, 7, 9 and 11 and seven epistatic QTLs were identified against two blast isolates (KI307 and KI209). Out of them two QTLs (*qKI307-2* and *qKI209-3*) shared a similar position on chromosome 11 [38]. In total, seven independent QTLs were detected through composite interval mapping to be associated with resistance against field blast on chromosomes 1, 3 (two QTL), 4, 5 and 11 (two QTL). Theses QTLs explained relatively high phenotypic variance of blast [55]. A QTL was identified near SSR marker of *RM 2136* at the end of long arm of chromosome 11 and explains 87% of phenotypic variation with 37% of additive effects [53]. Two major QTL (*r11a* and *r11b*) on chromosome 11 could be detected at all stages, whereas most QTLs were identified only at one

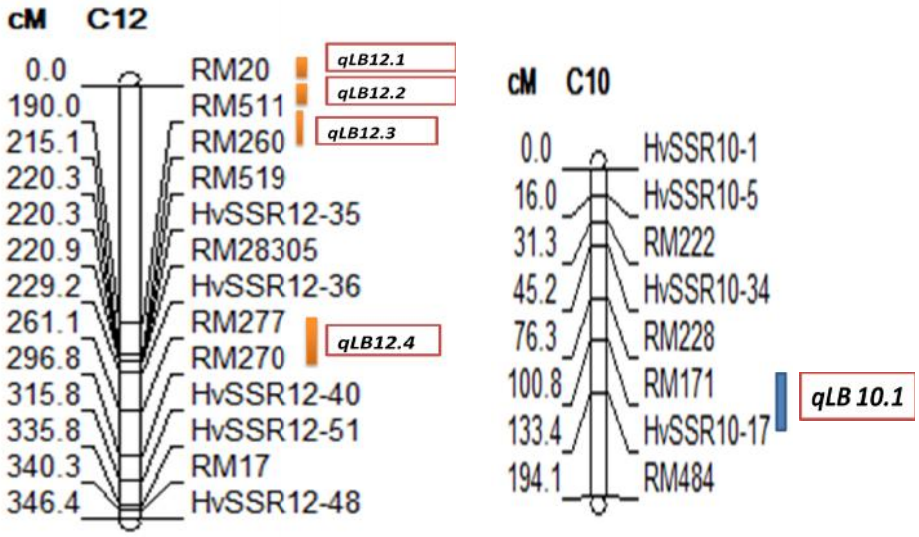

Fig. 4. The linkage map depicting location of QTLs for leaf blast on rice chromosome 10 and 12

or two stages in the RIL population for rice blast resistance under natural infection conditions [37]. A partial resistance QTL was identified on the long arm of chromosome 11 and explained 45.6% of the phenotypic variation [13]. Quantitative trait loci (QTLs) for broad resistance spectrum (BRS) to leaf blast were located on chromosomes 7 and 9. In particular, the *QTLch9* was mapped near the *Pi5(t)* locus. The *QTLch7* was located close to a previously mapped partial resistance QTL. Both loci showed significant allelic interaction. Genotypes having CT alleles at both *QTLch7* and *QTLch9* were the most resistant [30].

4.3 Potential Application Aspect of QTLs Identification

Resistance mediated by quantitative trait loci (QTLs), which usually have smaller individual effects than R-genes but confer broad-spectrum or non-race-specific resistance, is a promising alternative to less durable race-specific resistance for crop improvement, yet evidence that validates the impact of QTL combinations (pyramids) on the durability of plant disease resistance has been lacking. The five major effect QTLs "*qLB12.1",* "*qLB12.2",* "*qLB12.3",* "*qLB12.4"* and "*qLB10.1"* were identified in this study; on chromosome 10 and 12 for leaf blast could be use in pyramiding existing cultivars to the development of highly resistant rice cultivars, with durable broad spectrum resistance against the blast fungus. In other study, Pyramiding QTL alleles, each controlling a different response to *M. oryzae*, confers strong, non-race-specific, environmentally stable resistance to blast disease and results showed robust defence system provides durable resistance, thus avoiding an evolutionary ''arms race'' between a crop and its pathogen [61]. The pyramiding of four QTLs for blast resistance located on chromosomes 1, 2, 11 and 12, from two RD6 introgression lines. The results showed that the RD6 introgression lines carrying a high number of QTLs for blast resistance achieved from pyramiding have high levels of blast resistance and broad spectrum of resistance to the blast pathogens [62]. The QTLs identified in this study, will be valuable to further characterize and clone genes resistant to blast. In other study, the chromosomal segments associated with broad-spectrum quantitative disease resistance (BS-QDR) were identified in Rice. These segments contained numerous positional candidate genes identified on the basis of a range of criteria, and groups of genes belonging to two defense-associated biochemical pathways were found to underlie one BS-QDR region [63].

5. CONCLUSION

Blast disease is the most destructive disease worldwide. In this study, normal distribution suggested that the resistance in RILs might be a partial resistance. The SSR (RM and HvSSR) markers were used to construct linkage map covering approximately 3972.8 cM of the genome of rice. The linkage map was used for QTL analysis of leaf blast resistance. We

mapped five major effect QTLs namely "*qLB12.1",* "*qLB12.2",* "*qLB12.3",* "*qLB12.4"* and "*qLB10.1"* on chromosome 10 and 12 through composite interval mapping for leaf blast resistance using RILs population of rice from the cross of Danteshwari (highly susceptible) and Dagad deshi (resistant). Their position on respective chromosome showed as many similar QTLs mapped with different position with different population study. The QTLs "*qLB12.2",* "*qLB12.3",* "*qLB12.4"* mapped for both seedling and tillering stages of rice. This finding provides a new genetic resource for blast resistance improvement of rice varieties. These QTLs will be valuable to further characterize and clone genes resistant to blast. The identification of more tightly linked DNA markers to the QTLs for leaf blast resistance will be required by fine mapping. The markers linked to QTL identified in this study can be directly used in marker-aided selection (MAS), as explained of high phenotypic variance. Pyramiding of these QTLs into existing cultivars will lead to the development of highly resistant rice cultivars, with durable broad spectrum resistance against the natural leaf blast fungus.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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