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Detection of QTLs controlling field blast resistance in rice (*Oryza sative* L.)

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Abstract

In order to detect quantitative trait loci (QTLs) controlling blast resistance, mapping population of 192 $F_{2:3}$ families derived from the cross of two Iranian rice varieties Tarom Mahalli (TAM) an blast-susceptible cultivar and the blast resistance cultivar, Khazar (KHZ) were developed. A SSR linkage map covering 1231.50cM of rice was constructed using 74 polymorphic SSR markers. In total, seven independent QTLs were detected through composite interval mapping to be associated with resistance against field blast on chromosomes 1, 3, 4, 5 and 11. Theses QTLs explained relatively high phenotypic variance of blast resistance and could be considered to use in marker assisted selection (MAS) programs for improving aromatic and susceptible varietyies.

Key words: Field Blast Resistance, Iran, Rice, SSR.
Abbreviations: FBR: Field blast resistance, KHZ: Khazar, TAM: Tarom Mahalli, QTL: Quantitative trait loci, MAS: Marker assisted selection.

Introduction

Rice (*Oryza sative* L.) is the stable food for almost half of the world's population and Major problems in rice production around the world are due to biotic and abiotic stresses against rice plants. Rice blast caused by the fungus *Magnaporthe grisea* is a one of the major rice disease in rice growing areas all over the world (Ou, 1985). Blast resistance in rice varieties is generally classified into two types. One is true and complete resistance controlled by race specific major gene. The other is partial or FBR associated with several genes having major and minor effects (Kiyosawa et al., 1967) and it observed as durable resistance in many cases (Bonman, 1992) that defined as the resistance which allows effective control of a parasite under natural field condition. After observing the rapid break down of true resistance genes to blast such as *Pik* and therefore improving of the level of FBR and developing lines with FBR has been a major goal in rice breeding. Molecular marker technology that widely used nowadays has been applied for the identification and mapping of genes conferring both complete and partial resistance, in particular (Kumar et al., 2009). Qualitative and quantitative blast resistances have been reported in various germplasm (Ou, 1985) and several qualitative resistance loci (major genes) have been studied and mapped in the rice genome (Mackill and Bonman, 1992; Yu et al., 1991). In addition four genes, *Pi-14(t)*, *Pi-16(t)*, *Pi-d(t)* and *Pi-25(t)*, have been mapped on chromosome 2 (Miyamoto et al., 1996; Pen et al., 1998; Pan et al., 1999; Li et al., 2000; Sallaud et al., 2003). In other hand Zhuang et al., (2002) with genetic analysis using of F_8 recombinant inbred population (Zhong 156 × Gumei 2) indicated that the resistance to leaf blast was controlled by two genes and the presence of resistant alleles at any loci would result in resistance. Two genes tentatively assigned as *Pi24(t)* and *Pi25(t)*, were mapped on to chromosome 12 and 6, respectively. Also ten QTLs for blast resistance have been mapped on the rice genome. Most of them are linked to qualitative genes previously reported (Wang et al., 1994). Effective loci were identified on chromosome 4 several QTL analysis (Higashi, 1995; Miyamoto et al., 1996; Fukuoka et al., 2001; Tabien et al., 2002). Fukuoka and Okuno (2001) detected five QTLs for FBR on chromosomes 2, 4, 9 and 12 which linked QTL to an RFLP marker, G271, at the middle of chromosome 4 held 47.8% of the phenotypic variance for FBR and another QTL on chromosome 4 held 31.0% of the variance for resistance. They designated *pi21* to the resistance gene that was mapped on chromosome 4 as a single recessive gene between RFLP marker loci *G271* and *G317* at a distance of 5.0 cM and 8.5 cM, respectively. A review of bibliographic genome (Yu et al., 1991) and recently 1,000 resistance gene analogs, 88 mapped resistance genes, 341 QTLs and 165 metaQTLs were reviewed by Ballini et al. (2009) that provide a useful update on blast resistance genes as well as new insights to help formulate hypotheses about the molecular function of blast QTL So that in the past few years, many major resistance genes for rice blast have been mapped through molecular marker technology. Although there is a good diversity for QTL analysis of FBR in Iranian rice varieties, there is not much information on QTL identification and their association with blast resistance within Iranian rice germplasm. Therefore in this study two local rice varieties, Khazar and Tarom Mahalli were selected and has been developed $F_{2:4}$ mapping population. Khazar (KHZ) is a dwarf, heavy tillering, high yielding and resistance to blast variety. In other hand Tarom Mahalli (TAM) is relatively taller, long grained and sensitive to blast. The objective of present study was to identify QTLs governing FBR in Iranian rice population. These QTLs could be further fine mapped and used to transfer into high yielding genotypes of rice.

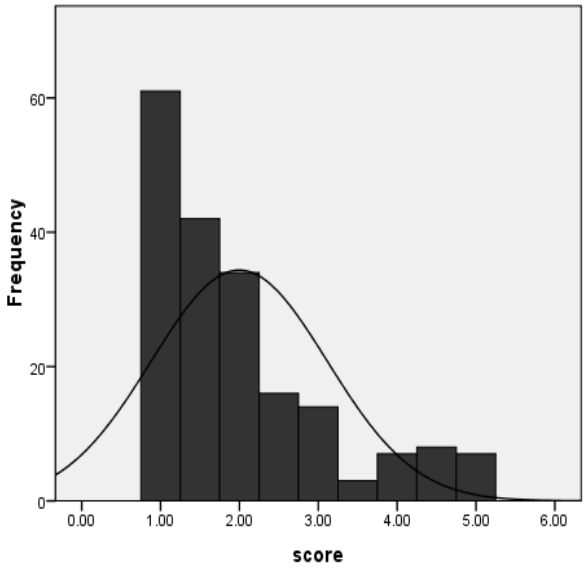

Fig 1. Frequency distribution blast resistance in F_3 families of derived from cross between KHZ and TAM.

Material and methods

Plant material

In order to provide of genetic material this experiment, F_2 population consisting of 192 individuals developed from a cross between Iranian *indicia* rice varieties TAM (sensitive to blast) and KHZ (resistance to blast). The parental plants and their F_2 192 individuals were grown in the paddy field of Rice Research Institute of Iran. Genomic DNA was extracted from the fresh leaves following the procedure described by Saghai Maroof et al., (1994). The matured seeds (F_3 generation) were collected from each F_2 plant for further blast resistance test.

SSR analysis and Linkage map construction

A total of 365 SSR primer pairs distributed evenly on 12 rice chromosomes were chosen according to McCouch et al., (2002) and then were tested for polymorphic survey. The primers exhibiting polymorphism were ussed to construct a linkage map of 12 chromosomes. Polymerase chain reaction (PCR) was carried out in a total volume of 10 µL per reaction containing 2 µL of template DNA, 0.39 µL of forward and reverse primers each of 10 pmol concentration, 0.6 µL dNTPs (2mM), 0.19 µL *Taq polymerase* (5 U/µL), 0.48 µL of $MgCl_2$ (50 mM), 1 µL of 10× PCR buffer and 5 µL steril nanopure H_2O. PCR amplification was performed on a thermal cycler (*Biometra Uno II,* Gottingen, Germany) in biotechnology laboratory of Rice Research Institute of Iran. The amplification products were separated on 6% polyacrylamide gels and detected by silver staining as described by Creste et al., (2001). A SSR linkage map was constructed using the program *Mapmanager QTbX17* (Manly and Olson, 1999) and genomic distances (cM) were calculated from recombination values using Kosambi mapping function (Kosambi, 1944)

Evaluation of field blast resistance

The F_3 seeds from 192 F_2 plant and those of two parents were sown into the field of the experimental farm Rice Research Institute of Iran in April 2007. All seeds were placed in three row plot 25 cm long with 25 cm on row. For basal fertilization, 15 g of N/m^2, 15 g of K_2O/m^2 and 32 g of P_2O_5/m^2 were applied. Twenty five F_3 plant from each F_2 individual were tested for degree of field blast resistance in paddy field in three replications. The degree of resistance of each F_3 family was evaluated using the criteria as described by Okutsu et al., (1984) via visual assessment of disease severity in five categories from 0 (no lesion area or a few brown spots) to five (necrosis of all leaves and sheaths).

QTL analysis

QTL identification related to blast resistance was sought using the software *QTL cartographer v. 2.5* (Basten et al., 2001). A minimum LOD score of 2.0 was used for the identification of putative QTLs, and the percentage of total phenotypic variation and additive effects explained by each QTL for field blast resistance were estimated. To identify additional QTLs that may have been masked by the major QTLs, composite interval mapping (CIM) method was employed. An automatic cofactor selection using a forward/backward regression was also performed with *QTL Cartographer v. 2.5* (Basten et al., 2001).

Result and discussion

Linkage map

Out of 365 SSR markers tested 85 produced polymorphic bands between the two parents and 74 primers amplified clear and scorable bands and were used for genotyping of F_2 population. The SSR linkage map constructed by 74 markers was covered a total of 1231.50 cM and encompassed the 12 rice chromosomes with a average two locus interval 19.83 cM. There were a few polymorphic markers were detected on chromosome 6, 7, 8 and 12. This homomorphism may reflect the genetic similarity between TAM and KHZ, because both varieties belong to *indica* subspecies. However two parental varieties TAM and KHZ are *indica*, SSR analysis showed high genetic variability between the two varieties on chromosomes 1, 2, 3, 4, 5, 9, 11 and 10.

Phenotypic evaluation and QTL analysis for field blast resistance

The frequency distribution in the F_3 families for segregating phenotypic classes of blast score is shown in Fig. 1. This distribution doesn't showed continuous variation with normal distribution. Therefore we used logarithmic transformation on data and maximum likelihood method for QTL mapping analysis. Seven QTLs controlling FBR were identified on chromosomes 1, 3 (two QTL), 4, 5 and 11 (two QTL). qBFR-1, qBFR-5 and qBFR-11b with LOD scores of 3.37, 2.64 and 2.66 showed the largest effects on FBR and explained 18.74, 17.33 and 22.63% of the total phenotypic variance, respectively (Table 1). The additive effect of a single QTL ranged from -0.209 to 0.489. The alleles of three QTLs qBFR-1, qBFR-5 and qBFR-11a, for increased FBR were from KHZ. In addition KHZ contributed the resistance allele for all loci except for the qBFR-3a, qBFR3b and qBFR11b. Also three QTLs qBFR-1, qBFR-5 and qBFR-11a, which played a major role in the expression of field resistance to blast in KHZ, were mapped on chromosome 1, 5 and 11 (Fig. 1). The QTLs of qBFR-1, qBFR-4 and qBFR-11a exhibited overdominance for decreased FBR whereas other QTLs exhibited partial dominance. QTL analysis using various genetic backgrounds and populations lead to identify several

Table 1. Putative QTLs for field blast resistance in the $F_{2:3}$ population derived from TAM × KHZ cross.

QTL	Chr.	Flanking Markers	Position	LOD	Additive effect	Dominance effect	Dominance ratio	Explained variance
qBFR-1	1	RM8068-RM8231	261.6	3.37	-0.128	-0.487	-3.80	18.74
qBFR-3a	3	RM5626-RM7389	78.2	2.34	0.414	-0.070	-0.17	15.45
qBFR-3b	3	RM7389-RM7000	88.8	2.55	0.489	-0.167	-0.34	17.61
qBFR-4	4	RM119-RM225	113.5	2.00	0.170	-0.458	-2.69	18.77
qBFR-5	5	RM421-RM480	10	2.64	-0.209	-0.318	-1.52	17.33
qBFR-11a	11	RM5474-RM144	6	2.35	-0.096	-0.384	-4.00	18.11
qBFR-11b	11	RM144-RM1341	36.6	2.66	0.379	-0.617	-1.63	22.63

QTLs that some of them share the same locus on the chromosome suggesting the existence of common locus for differentiation among rice varieties that could be considered as valuable QTLs because their specific stable. Several qualitative resistance loci have been studied and mapped in the rice genome. In addition the pyramiding of field resistance genes has been suggested as an effective way to improve blast resistance. It was also suggested that the combination of many genes in a resistance gene pyramid would enhance durability and effectiveness to the wider spectrum of races (Bonman, 1992). Therefore using of result of various mapping population could be valuable in breeding program. Noenplaba et al., (2006) using recombinant inbred lines (RILs) population derived from a cross between Khao Dawk Mali 105 (susceptible) and Jao Hom Nin (resistant) cultivars mapped QTLs related to blast resistance on chromosome 1. The peaks of QTL in their study were found near the RM212 marker, which was close to the RZ19–RG331 flanking markers reported by Prashanth et al., (2002) and Wang et al., (1994) who also identified QTL associated with blast lesion number, blast disease leaf area and blast lesion size. In this study, one QTL was detected between RM8068-RM8231on chromosome 1 that increased field blast resistance phenotype. Wu et al., (2004) using an advanced backcross population consisting of 80 BC_3F_3 lines derived from rice varieties Vandanal and Moroberekan have identified two QTLs for field blast resistance in the short-arm region of chromosome 3. For Judging about the positions of QTLs, it is likely that qBFR-3 did not coincide with the two QTLs reported by Wu et al., (2004) but Tabien et al., (2002) identified nine QTLs, one each on chromosome 1, 2, 3, 4, 6, 7 and 9, with two loci on chromosome 12 for field blast resistance in recombinant inbreed lines (RILs) derived from Lemont/Teqing and one (qBLSTads-3) was located at almost the same marker interval as that of our identified QTL. In other word the genetic position of qBFR-3a and qBFR-3b was very similar to that of qBLSTads-3. In report of Noenplaba et al., (2006) three QTL, qBL-3, qLB11-3 and qBL12-3, were detected with LOD scores of 12.18, 42.63 and 7.72 for leaf blast resistance, respectively. They were located on chromosomes 1, 11 and 12. Miyamoto et al., (1996) performed a linkage mapping of QTLs for field blast resistance using $F_{2:3}$ derived from a cross between the upland variety Kahei (high level of resistance) and the lowland variety Koshihikari (susceptible) and mapped two putative QTLs on chromosome 4 So that qBFR4-1 was mapped in the vicinity of RFLP marker G264 and explained about 62% of the total phenotypic variation in F_3 lines. Another QTL, qBFR4-2, was also found near the RFLP marker G271. These two QTLs on chromosome 4 explained about 71% of the phenotypic variation based on the analysis of a multiple QTL model. In population of this study one QTL was mapped on chromosome 4 was in the adjacent region of *Pi21* gene reported by Fukuoka and Okuno (2001) for FBR. Higashi (1995) suggested that the genetic factor controlling field blast resistance of upland varieties were located on chromosome 4. In addition, Fuluoka and Okuno (2001) detected two QTLs on chromosome 4, based on the genetic analysis using the upland cultivar Owarihatamochi as a resistance parent. In other hand Wang et al., (1994) suggested that QTLs for blast partial resistance were not located on chromosome 4 using recombinant inbreed lines derived from a cross between resistance cultivar Moroberekan (African variety, *japonica*) and CO39 as a susceptible parent. These results indicate that QTLs responsible for field blast resistance on chromosome 4 despite of high similarity in several varieties might be not specific to some varieties. Regarding to importance of other chromosomes in controlling of FBR Sirithunya et al., (2002) was showed that QTL for broad resistance spectrum to leaf and neck blast resistance located on chromosome 7 and 9. In particular, the QTL on chromosome 9 is near the *Pi5(t)* locus. In this research, one QTL was detected on chromosome 7. Zhuang et al., (2002) indicated that the leaf blast resistance was controlled by two genes *Pi24(t)* and *Pi25(t)* on chromosome 12 and 6, respectively and the presence of resistant alleles at any loci would result in resistance. In this study, the genomic segments RM8068-RM8231, RM421-R480 and RM5474-RM144 on chromosomes 1, 5 and 11 harboring qBFR-1, qBFR-5 and qBFR-11a were consistently detected for FBR with relatively high effect on resistance phenotypes. In the future, clarification of the relationship between the QTL found and major blast resistance genes should be carried out. Furthermore, since resistance genes chromosomes 1, 5 and 11 with alleles being contributed by KHZ are highly likely to confer durable resistance, introgression of these genes into sensitive variety through marker assisted selection should fulfill the objective of breeding for resistance to field blast.

Conclusion

In this study a SSR linkage map covering 1231.50cM of rice was used for QTL analysis of FBR. In total, seven independent QTLs were detected through composite interval mapping to be associated with resistance against field blast on chromosomes 1, 3, 4, 5 and 11. QTLs related to other studies and QTLs related to this study, could be combined in multiple crosses. Combination of these genes is likely to improve the resistance level to blast. It will be rather difficult, however, to combine these alleles related to blast resistance using conventional breeding procedure, because of difficulty in recognizing the resistance phenotypes in segregation populations. Use of DNA marker-assisted selection would make it easier to combine these two genes with large effects. Furthermore, marker-assisted selection now allows not only to combine genes at different QTLs but also to combine them with complete resistance genes. To make this strategy more feasible, identification of tightly linked DNA markers to the

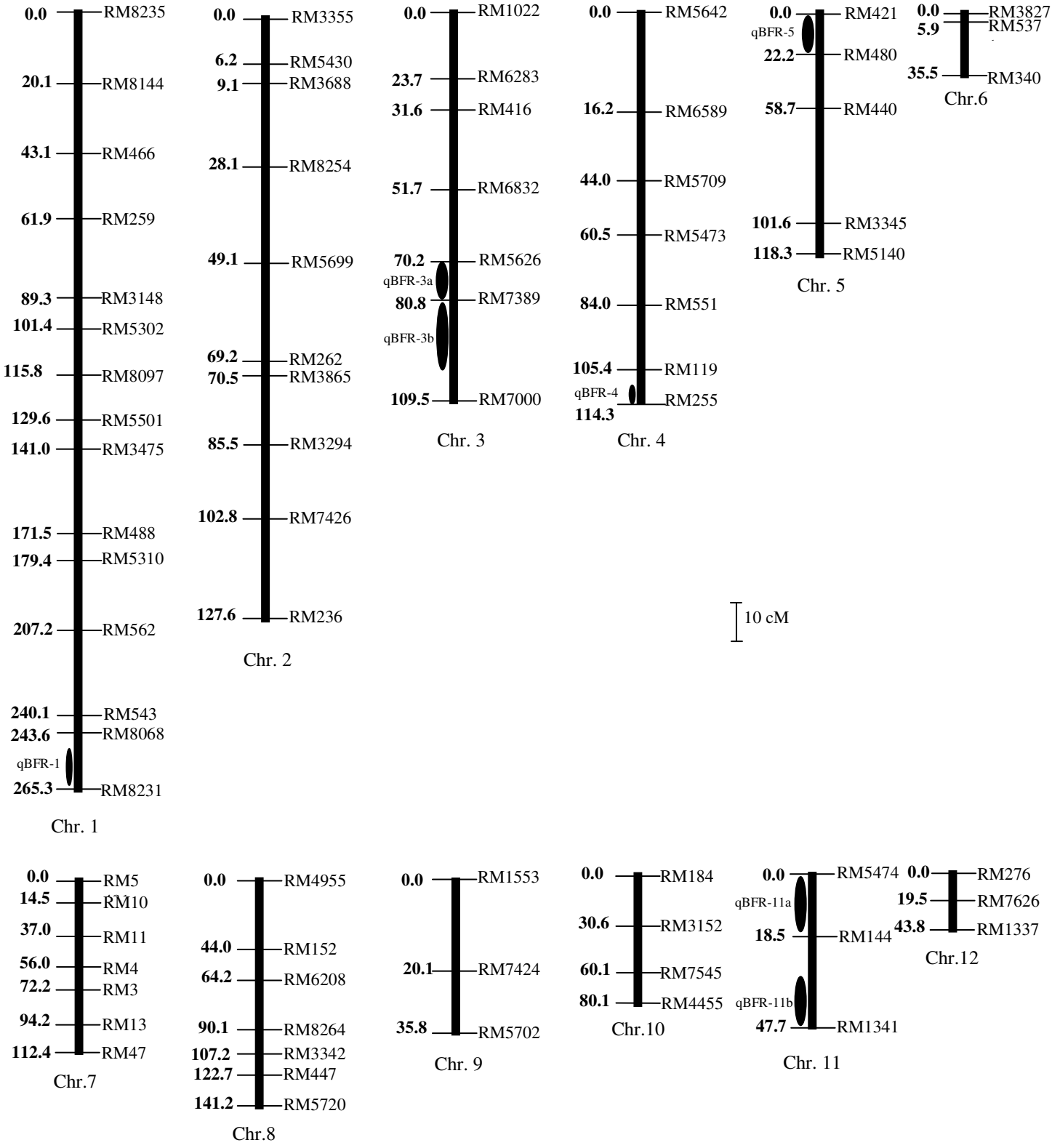

Fig 2. The position of QTLs for field blast resistance on $F_{2:3}$ population derived from cross between TAM and KHZ. Significance threshold for composite interval mapping determined at LOD=2 and distances in cM were indicated

QTLs for field blast resistance will be required based on the fine mapping of QTLs.

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