RESEARCH NOTE

Inheritance of blast resistance and identification of SSR marker associated with it in rice cultivar RDN 98-2

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Introduction

An F_2 population was developed from a cross between rice (*Oryza sativa* L.) genotypes, EK 70 (highly susceptible to blast) and RDN 98-2-3-5-14 (resistant to blast), to study the inheritance of blast resistance and to identify the marker associated with resistance. The F_2 population segregated in 3:1 ratio for resistance: susceptible under hot spot conditions for blast suggesting monogenic control of resistance in this population. Bulk segregant analysis conducted using a total of 25 SSR markers identified two SSRs to be polymorphic between the parents and the corresponding bulks. One of these SSR markers RM204 which has been reported to be mapped on the short arm of chromosome 6 and in close proximity of blast resistance gene/QTLs in other studies showed expected segregation ratio (1:2:1) for single gene model in the F_2 population. This marker was found significantly associated with blast resistance on regression analysis.

Rice is the world's largest food crop, providing caloric needs to millions of people daily. Rice suffers attack by a large number of pests and pathogens, which under epidemic conditions, cause serious yield losses. Among these, rice blast caused by *Magnaporthe grisea* (Hebert) Barr. (Barr 1977), synonym *M. oryzae* is one of the most devastating and destructive diseases of rice worldwide. Under heavy dew, arial plant parts are affected, leaf surface becomes speckled with oval lesions and plants are liable to lodging if nodes are infected. If the panicles are infected, it also results in severe yield losses (Ou 1985).

Resistance to the *M. oryzae* is a classic gene-for-gene system, where a major resistance gene is effective against pathogen strains containing the corresponding avirulence gene (Silue *et al.* 1992). Rice containing a *Pi* gene confers resistance to a *M. oryzae* race in a gene-for-gene manner. DNA markers have been used effectively to identify resistance genes (for details see Ballini *et al.* 2008) and marker-assisted selection (MAS) has been applied for integrating different resistance genes into rice cultivars lacking them. In order to identify the new sources of resistance against blast, there is need for identification of resistance genes in genetically diversified rice material. Therefore, the present investigation was undertaken with the objectives to first study the inheritance of resistance to blast disease in an F_2 mapping population specifically developed for this purpose and secondly to identify genes/QTLs associated with it. The ultimate objective of our exercise is to create a genetic mapping resource in the form of recombinant inbred lines (RILs) for future genetic studies from the population used in the present study.

Materials and methods

Plant material and phenotypic evaluation

The F_2 mapping population was derived from a cross made between a blast susceptible genotype EK 70 and a resistant variety RDN 98-2-3-5-14 (henceforth called RDN 98-2). EK 70, the local selection from Nasik district of Maharashtra state, India, having early maturity, tall plant stature and a long-slender grain is popular among farmers. However, it is highly susceptible to blast suffering a heavy losses. RDN 98-2, which was derived from a cross between a local selection Halvi Sal 17 and TN1 has mid-late maturity, tall plant stature with medium-slender grain type and is resistant to blast (figure 1 in electronic supplementary material

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at http://www.ias.ac.in/jgenet/). The genotype was tested at Agricultural Research Station, Lonavala near Pune, India, which is a 'hot spot' for rice blast during the years 2007 and 2008 following Uniform Blast Nursery (UBN) pattern and was found resistant to blast (score < 2) during both the years.

The F_2 population and the parents were evaluated for reaction to blast at ARS, Lonavala, during the rainy season of 2011 under natural blast epidemic conditions. Due care was taken for uniform and high inoculum pressure by planting two rows of susceptible check (EK 70) after every five test rows of F_2 population and the plot was surrounded from all the sides by five rows of susceptible check. The vulnerability of the host was increased by additional doses of N and avoiding use of P_2O_5 and K_2O. Moreover, the seedlings were inoculated three times at two days interval with the suspension of *M. oryzae* spores. The observations on infection type for blast were recorded on parents and 150 randomly selected F_2 plants on a scale of 0 through 9 as per standard evaluation system scale given by International Rice Research Institute, Manila, Philippines (table 1 in electronic supplementary material). Briefly, observations were recorded on each genotype at eight days interval starting from 15th day after planting and continued up to 45 days. The maximum score was considered for judging the reaction and further analysis. The genotypes with score of ≤3 were considered resistant.

DNA extraction, amplification and bulk segregant analysis

Genomic DNA was extracted from the leaves of both the parents and 150 individual F_2 plants following CTAB method as described by Doyle and Doyle (1990). The quality and quantity of DNA were estimated spectrophotometrically using a NanoDrop (ND-1000, Wilmington, USA). Bulk segregant analysis (BSA) method as suggested by Michelmore *et al.* (1991) was used for quick identification of SSR markers associated with blast resistance. Based on phenotypic observations, two bulks viz., susceptible bulk (B1) comprising of five susceptible F_2s and resistant bulk (B2) comprising of five resistant F_2s were made. These 10 F_2s were found homozygous when screened with the SSR markers used in the study. A pooled DNA sample was prepared for each bulk by mixing in equal quantity the DNA of five respective component F_2s.

The parents and the bulks were screened with 25 SSR primers (table 2 in electronic supplementary material) distributed over nine chromosomes of rice genome to determine polymorphism and possible association with blast resistance. These markers were selected based on previous studies on blast resistance in rice and from the panel of 50 standard SSR markers reported on the website www.gramene.org. Most of the major blast resistant genes were reported on chromosomes 2, 6, 9, 11 and 12 of rice genome and the markers associated/linked to these genes reported in the literature were used. In addition, some markers reported on www.gramene.org available with us were used. The PCR protocol involved a total volume of 20 μL containing 20 ng genomic DNA, 0.1 μM of each primer, 2.0 μL of 10× *Taq* DNA polymerase buffer (100 mM Tris pH 9.0, 500 mM KCl), 200 μM of each dNTPs and 1 U of *Taq* DNA polymerase. The reaction profile was 5 min at 95°C, 40 cycles of 30 s at 94°C, 30 s at 55°C/60°C annealing, 1 min at 72°C and 10 min at 72°C for final extension. The PCR products were electrophoresed on Metaphore agarose gel (2.5%) and visualized on Gel Documentation System (Flour Chem^{TM} Alpha Innotech Corporation, San Leandro, USA). The SSR markers found polymorphic among the parents and the bulks were used for F_2 progeny analysis. DNA of 150 F_2 progenies and parents were analysed to study cosegregation of these markers.

Data analysis

The clearly resolved amplicons of SSR were scored manually as homozygote for the allele for susceptible parent (0), homozygote for the allele for resistant parent (1) and heterozygote carrying the alleles from both parents (2) in the data sheet. Chi-square (χ^2) test was performed to test the goodness of fit of the F_2 population for the phenotypic and marker data by comparing an observed frequency distribution with an expected one. Marker-trait association was analysed by simple linear regression method to know the association between the markers and the blast score in a spreadsheet.

Results

Inheritance of blast resistance

The susceptible parent EK 70 showed highly susceptible reaction with lesion type of 9, whereas the parent RDN 98-2 was found to be resistant producing lesion type of 1 when exposed to natural inoculum pressure in the field. Among the randomly selected 150 F_2 plants, 111 plants showed resistant reaction and 39 plants showed susceptible reaction. None of the genotype showed the score of 0. The observed frequencies when tested for goodness of fit with chi-square (χ^2) test for single gene model showed goodness of fit ($P = 0.78$) to the expected segregation ratio (3:1).

SSR markers linked to blast resistance

Among the 25 SSR markers used, 14 markers reported polymorphism between two parents EK 70 and RDN 98-2 and two SSR markers, RM204 (figure 1) and RM215 reported polymorphism between susceptible and resistant parents and corresponding bulks indicating their possible association with blast resistance in the mapping population. The F_2 mapping population was genotyped with these two primers to study their possible association with blast resistance.

Segregation study with marker RM204 recorded a resistant allele of ~174 bp amplified in 33 plants, whereas a

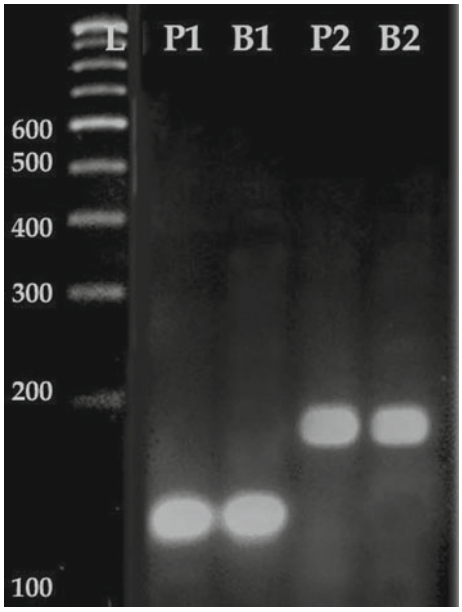

Figure 1. Results of bulk segregant analysis using the susceptible parent EK 70 (P1) and the resistant parent RDN 98-2 (P2), and their respective bulks (B1 and B2) with SSR marker RM204; L, 100-bp StepUp^{TM} DNA ladder (Genei, Bangalore, India).

susceptible allele of ~110 bp was amplified in 41 plants (table 1; figure 2 in electronic supplementary material). Seventy-three F_2 plants exhibited both the alleles (heterozygous). Genetic analysis with chi-square test indicated goodness of fit to the expected ratio of 1:2:1 for single gene model indicating the association of RM204 with blast resistant gene in the present population. This confirmed that the resistance for blast in present investigation was governed by single dominant gene. The simple regression analysis between phenotypic data of blast and the genotypic data of SSR marker RM204 indicated that the marker was significantly linked with blast resistance (table 2).

Another SSR marker RM215 which was polymorphic in BSA did not show a good fit to the expected segregation ratio for a single gene model. Further, on regression analysis this marker was not found to be associated with blast resistance (data not shown).

Table 1. Evaluation of the F_2 population with SSR marker RM204.

Category	Observed genotype	Expected genotype (1:2:1)	χ^2	*P*
Resistant	33	36.75	0.88	0.65
Heterozygote	73	73.5		
Susceptible	41	36.75		
Total	147*			

*Three genotypes did not amplify.

Table 2. Simple linear regression analysis of SSR marker RM204 with phenotypic data of blast incidence in rice.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	*F*	*P*
Regression	1	6.50	6.50	8.61	0.0038
Residual	148	111.79	0.76		
Total	149	118.29			

Discussion

Variation for blast score

Wide variation was observed for the blast score not only in the parental genotypes, but also in the F_2 population, which was seen from the entire range of score of blast incidence. This indicated that the parental genotypes used in the present study for developing the population was an appropriate choice and a permanent genetic stock in the form of RILs can be developed from the present F_2 population. Also, the natural blast inoculum pressure was high enough during field screening to allow evaluation of resistance and susceptibility in the population developed in the present study.

Inheritance of blast resistance in the F_2 population

The phenotypic data on the blast incidence showed that the F_2 population segregated in 3:1 ratio (resistance to susceptible). This indicated that the resistance in RDN 98-2 is governed by single dominant gene. Moreover, the SSR marker RM204 which was found associated with blast incidence through BSA in the present study segregated in 3:1 (1:2:1) ratio confirming the single gene control. The results obtained in this study are in accordance with several earlier reports including that of Chen *et al.* (1999), Sharma *et al.* (2007) and Ashkani *et al.* (2011). However, it should be noted that the material used in the present study was totally different from the one used in the above studies.

Comparison with the earlier results

During the last few years, genetics of blast resistance in rice has been extensively studied and many dominant R genes conferring complete resistance to *M. oryzae* have been identified. To date, approximately 73 major resistance genes and 350 QTLs have been mapped on almost all the chromosomes of rice, except perhaps chromosome 10 (Ballini *et al.* 2008; Ghaley *et al.* 2012).

Chromosome 6 of rice has been reported to contain many important genes and QTLs for blast resistance, many of which are closely mapped to each other. In the present study, SSR marker RM204 found associated with blast resistance has been mapped earlier by Chen *et al.* (1997) on the short arm of chromosome 6 at 17.2 cM, 31.1 cM by Temnykh *et al.* (2000) and 25.1 cM by Temnykh *et al.* (2001) in their respective genetic maps. Eizenga *et al.* (2006) reported marker RM225 to be close to the blast resistant genes *Pi22* and *Pi27*,

whereas Temnykh *et al.* (2001) mapped the marker RM225 on short arm of chromosome 6 at 1.1 cM away from the marker RM204. It suggests that the marker RM204 identified in present study is in close vicinity to the gene reported by Eizenga *et al.* (2006).

Further, Wang *et al.* (1994) reported QTLs for blast lesion number and blast diseased leaf area on the short arm of chromosome 6 with marker RZ398 as one closely linked to these QTLs. Chen *et al.* (1997) mapped the markers RZ398, RM204 and RM225 in the same genomic location on chromosome 6, whereas markers RZ398 and RM225 were colocalized in the map of Temnykh *et al.* (2001). It seems that the QTLs reported by Wang *et al.* (1994) for blast lesion number and blast diseased leaf area and the one identified for blast resistance in present investigation might be the same, as blast lesion number and blast diseased leaf area are highly correlated to blast resistance in the field condition.

Biradar *et al.* (2007) identified a QTL for silicon (Si) content on chromosome 6. The accumulation of silicon in plants helps in disease resistance and the relationship between Si content and blast resistance was confirmed by many scientists in rice (Fawe *et al.* 1998). The QTL for silicon content identified by Biradar *et al.* (2007) was at similar location to the QTLs reported for blast lesion number and blast diseased leaf area by Wang *et al.* (1994). Interestingly, the comparative analysis of the genetic maps of chromosome 6 reported by Wang *et al.* (1994), Chen *et al.* (1997), Temnykh *et al.* (2001) and Biradar *et al.* (2007) suggests that the QTL reported by Biradar *et al.* (2007) for silicon content and the one identified for blast resistance in the present investigation might be in close proximity, although they might not be the same.

The present study indicated that the SSR marker RM204 is associated with blast resistance gene/locus in the rice cultivar RDN 98-2 and also highlight the importance of BSA in establishing marker-trait association in a rapid way. The findings of this study could be directly useful in molecular analysis of segregating generations, breeding lines and varieties having RDN 98-2 as a parent.

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