Indian J. Genet., 70(1): 17-21 (2010)

Molecular analysis of QTLs associated with resistance to brown spot in rice (*Oryza sativa* L.)

J. L. Katara[1], H. Sonah, R. K. Deshmukh, Ravinder Chaurasia and A. S. Kotasthane

Department of Biotechnology, Indira Gandhi Krishi Vishwavidhyalaya., Raipur 492 012

(Received: July 2009; Revised: December 2009; Accepted: January 2010)

Abstract

Helminthosporiosis or brown spot disease is widely distributed and is known to cause heavy yield losses in rice. In present investigation, a set of 154 doubled haploid (DH) lines derived from a cross between CT 9993-5-10-1M and IR 62266-42-6-2 was used to identify quantitative trait loci (QTLs) for brown spot disease. DH lines along with parents were planted in two different soil environments with two replications each in RCBD and evaluated for the brown spot resistance. We have identified total 10 QTLs for brown spot resistance using MAPMAKER/QTL 1.1 distributed over the eight chromosome of rice. Out of 10 QTLs, four were consistently discovered in both the soil types. The QTL *BSq4.1* and *BSq11.1* located on the chromosome 4 and 11 were identified in both the environment with higher LOD score and phenotypic variance and will be good candidates for fine mapping and positional cloning studies.

Key words: Brown spot, Doubled haploid population, Rice, QTL analysis

Introduction

Helminthosporosis or brown spot disease of rice caused by *Helminthosporium oryzae* is widespread and occurs in all rice growing countries of the world. Most conspicuous symptoms of the disease occur on leaves and glumes of maturing plants. Symptoms also appear on young seedlings and the panicle branches in older plants. Leaf spots may be evident shortly after seedling emergence and continue to develop until maturity. Damage from brown spot is particularly noticeable when the crop is produced in nutritionally deficient or otherwise unfavorable soil conditions. Significant development of brown spot is often indicative of a soil fertility problem. It causes heavy losses, particularly in booting stage, when it reaches epidemic proportions. *Helminthosporium oryzae* contributed to Bengal famine of 1943, which arose concerns for the genetic vulnerability of our food plants and resulted in huge yield losses, extending as high as 90% in certain areas [1]. Yield losses in terms of weight of grains and poor germinability of infected seeds were attributed as much as 30 % and 60%, respectively [2-4].

Very few studies have been made for studying genotypic variability among rice cultivars for brown spot resistance [4-6]. No major gene confirming resistance to brown spot have been identified since racial picture of the pathogen is not deciphered [7]. With use of DNA markers and QTL mapping approaches, complex forms of disease resistance and their underlying genes are now far more accessible. Identification and mapping of QTLs is a valuable starting point for positional cloning of genes present in the QTL region. It can also help in the interpretation of the molecular and biochemical mechanism involved in host-pathogen interaction. The QTL studies conducted over several years and locations, provides information of the regions of genome consistently associated with the target traits. In present investigation we have identified stable QTLs for brown spot using a set of DH line population.

Materials and methods

The present study was conducted at the research farm of Indira Gandhi Agricultural University, Raipur, India (21.15^{o}N latitude, 81.86^{o}E longitude and 289.6 m above MSL). The plant material used in the study consisted of 154 DH line derived from a cross between CT 9993-5-10-1-M (*japonica* cultivar) resistant and IR 62266-42-6-2 (*indica* cultivar), susceptible for the brown spot, developed at Centro Internacional de Agricultura Tropical (CIAT), Columbia, and International Rice

[1]Corresponding author's e-mail: jlkatara@rediffmail.com

Research Institute (IRRI), Philippines. The 154 DH lines were planted along with parents in Randomized Block Design (RBD) in two different types of soils (vertisol and inceptisol) with two replications. The row to row and plant to plant spacing of 20 cm was maintained. Each line was planted in two rows of 2 m length. All normal packages of practices were followed to raise a good crop.

All the DH lines along with the parents were evaluated for field resistance to brown spot. Ten plants were selected randomly from each line and scored for brown spot resistance according to standard evaluation system for rice [8] (Table 1). Parental lines were replicated in each block every after 10 DH lines to confirm the uniformity of infection.

Table 1. Score* for the disease reaction of brown spot observed on doubled haploid rice lines

Score	Infected area (%)	Reaction
0	0-1	Highly Resistant (HR)
1	1-5	Resistant (R)
3	6-12	Moderately Resistant (MR)
5	13-25	Moderately Susceptible (MS)
7	26-50	Susceptible (S)
9	>75	Highly Susceptible (HS)

*According to standard evaluation system for rice (IRRI, 2002).

Previously constructed genetic linkage map consisting of 315 marker loci including 145 restriction fragment length polymorphisms (RFLPs), 158 amplified fragment length polymorphisms (AFLPs), and 12 simple sequence repeats (SSRs) were used for the QTLs analysis [9]. MAPMAKER/QTL1.1 software was used for interval mapping [10], and to estimate the percentage of the total phenotypic variance explained by each QTL. A threshold of LOD >2.0 is used per test to claim the presence of a QTL.

Results and discussion

Segregation of brown spot resistance

The frequency distribution of DH lines for brown spot resistance in both soils type and the phenotypic values of parents are shown in Fig. 1. Normal distribution was observed among genotypes for the brown spot resistance. The parental line CT 9993 was found to be resistant against brown spot while IR 62266 is moderately susceptible. Transgressive segregants were observed as some DH lines are highly susceptible than the IR 62266 and highly resistant than the CT 9993 parent.

QTL analysis for brown spot resistance

In this study, 10 QTLs for brown spot resistance was detected that were distributed across seven chromosomes of rice. The relative position and length of QTLs are presented in Figure 2. The marker interval, chromosomal location, additive effect and LOD score

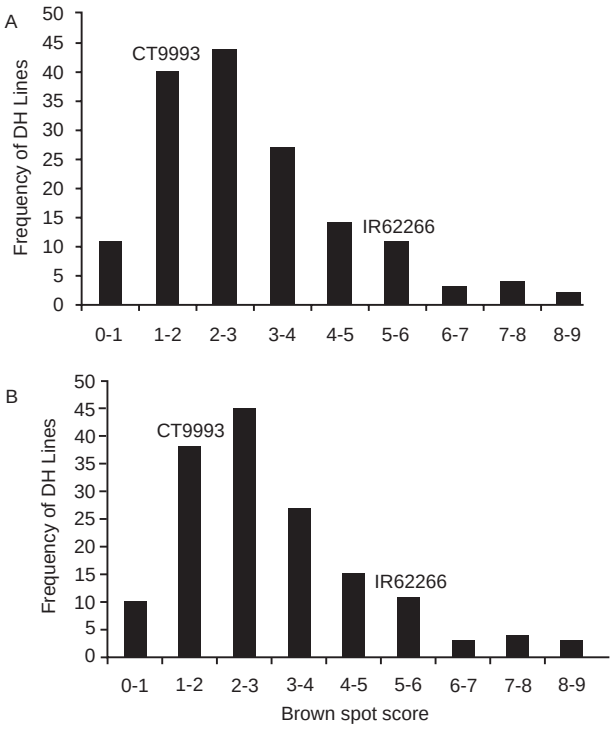

Fig. 1. Frequency distribution pattern of brown spot response of 156 DH lines derived from the cross CT 9993-5-10-1-M and IR 62266-42-6-2 in (A) Inceptisol and (B) Vertisol soils.

for the identified QTLs are presented in Table 2. In both soil types, QTLs identified with positive as well as negative additive effect, suggest the resistance alleles were contributed from both the parents and transgressive segregation pattern observed in both soil types.

QTL analysis for brown spot resistance in vertisol

Nine regions distributed over seven chromosomes were significantly associated with brown spot resistance observed in vertisol (Table 2). Out of the nine, three QTLs, *BSq4.1v*, *BSq6.1v* and *BSq8.2v* has highest phenotypic variation 18.5%, 11.8%, 17.6% and LOD

score 5.85,4.17 and 4.78, respectively.

QTL analysis for brown spot resistance in inseptisol

A total of six regions on five chromosomes were associated with brown spot score in inseptisol soil (Table 2). Four QTLs namely *BSq4.1i*, *BSq6.2i*, *BSq8.1i* and *BSq11.1i* were mainly contributing to the resistance against brown spot. Two QTLs *BSq2.1i* and *BSq2.2i* on chromosome 2 were detected with LOD score 2.86 and 2.26 and phenotypic variance 15% and 13.6%, respectively.

Several studies have shown a relationship between severity of brown leaf spot and soil status. From extensive investigations in Japan, it was concluded that the disease is associated only with abnormal or poor soil and that it serves as an index of such conditions. Studies have been conducted on 1000 ha area of irrigated rice at Caroni, over a period of three years and a significant correlation was observed among soil nutrient and disease index [11]. Vertisols is easily recognised because of their clayey textures, dark colours, and special physical attributes. This soils is very productive if well managed, but present constraints to low-input agriculture. Inceptisols are essentially intermediate between soils of any other order and soils that have no diagnostic subsurface horizon. These are mineral soils that have significant subsoil or surface alteration. Here we have conducted experiment in Inceptisols and Vertisols, because the difference in physical and nutritional status of these soils will helps to identify durable and consistent source of resistance.

In the present investigation QTL mapping was done with the moderate threshold LOD value of 2.0. Since the statistical threshold to be employed is one important point to detect putative QTL. Relatively low threshold are used to avoid false negatives, where as relatively high threshold levels are employed to avoid false positive. Therefore, putative QTLs detected may be under estimated and also may be biased towards genes with relatively large phenotypic effects. Some significant QTL may be false positives and QTLs responsible for significant variation within and between populations can be missed if the tested traits are fixed by chance for alleles with similar effect.

Table 2. QTLs detected with LOD score =2.00 by interval mapping using MAPMAKER/QTL 1.1, in a DH population of 154 rice lines derived from CT9993 and IR62266

S.No.	QTL	Marker interval	Chr. No.	Additive effect	Variation explained (%)	LOD score
		Vertisol				
1.	BSq2.1v	R1843-ME2_7	2	–0.54	8.4	2.01
2.	BSq2.2v	C1419-EM11_10	2	–0.49	6.2	2.11
3.	BSq4.1v	EMP2_2-ME10_11	4	0.91	18.5	5.85
4.	BSq6.1v	R2171-EMP3_5	6	0.57	11.8	4.17
5.	BSq8.2v	ME2_11-EM14_1	8	0.76	17.6	4.78
6.	BSq9.1v	K985-RM242	9	–0.48	7.2	2.49
7.	BSq11.1v	ME4_14-C477	11	0.71	13.1	3.81
8.	BSq11.2v	RG1109-G1465	11	–054	8.2	2.23
9.	BSq12.1v	G402-RZ76	12	0.58	9.4	3.25
		Inceptisol				
1.	BSq2.1i	R1843-ME2_7	2	–0.88	15.0	2.86
2.	BSq2.2i	C1419-EM11_10	2	–0.97	13.6	2.26
3.	BSq4.1i	EMP2_2-ME10_11	4	1.06	22.3	5.38
4.	BSq6.2i	RZ404-RG653	6	0.56	9.30	4.48
5.	BSq8.1i	RG1-G187	8	0.68	13.5	3.68
6.	BSq11.1i	ME4_14-C477	11	0.77	12.9	3.05

(Individual QTL are designated with the abbreviation of the brown spot (BS) and chromosome number. When more than one QTL affects a trait on the same chromosome, they are distinguished by decimal numbers, ‘v’ and ‘i’ for soil type)

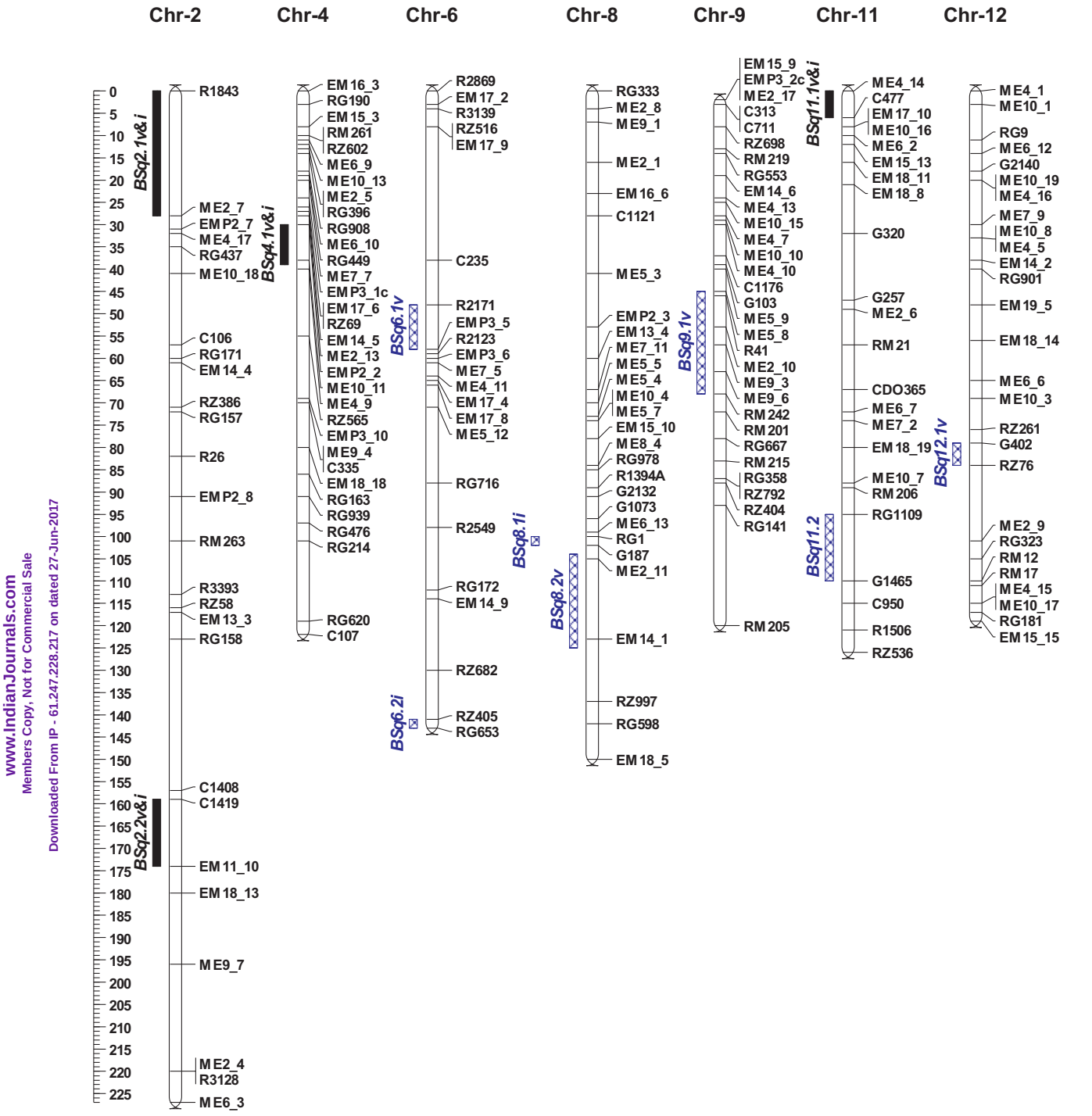

Fig. 2. Chromosomal location of QTLs for brown spot resistance identified in the CT 9993 x IR 62266 population under vertisol and inseptisol soil types. QTLs consistently identified in both soil types are denoted with solid bar and one of the soil types with cross lines bar

The availability of the complete rice genome sequence and rapid advances being made in the area of genomics will help to dissect and characterize QTLs [12]. QTL mapping will eventually provide an entry point for the most ambitious goal of map based cloning of resistance genes which have small and continuous effects on phenotype. In present study four QTLs for brown spot were consistently identified on three different

chromosomes of rice. The QTLs present on chromosome 2 have very less phenotypic variation but shows stable occurrence. The QTL *BSq4.1* and *BSq11.1* located on the chromosome 4 and 11 were identified in both the environment with higher LOD score and phenotypic variance (Table 2). The QTLs identified in present investigation may be good candidates for fine mapping and positional cloning studies. These QTLs might be useful for marker assisted resistance breeding in rice.

References

1. **Padmanabhan S. Y.** 1973. The Bengal Famine. Ann. Rev. Phytopath., **11**: 11-26.
2. **Bedi K. S. and Gill H. S.** 1960. Losses Caused by the Brown Spot Disease of Rice in Panjab. Indian Phytopath., **13**: 161-164.
3. **Herrera L. and Siedel D.** 1978. Influence of irrigation and soil leveling on infection of rice plants by *Cochlobolus miyabeanus* (Ito and Kuribayashi) Drechslera ex Dastur. Arch. Phytopoth, Pflanzenschutz., **14**: 263-265.
4. **Pannu P. P. S., Chahal S. S., Sharma V. K., Mandeep Kaur and Bagga P. S.** 2006. Occurrence of brown leaf spot of rice in Punjab, its effect on grain yield and its control. Indian Phytopath., **59**: 190-193.
5. **Ohata K. and Kubo C.** 1974. Studies on the mechanism of disease resistance of rice varieties to *Cochliobolus miyabeanus*. Bull. Shikoku. Agric. Exp. Stn., **28**: 17-57.
6. **Deren C. W., Datnoff L. E., Snyder G. H. and Martin F. G.** 1994. Silicon concentration, disease response and yield component of rice genotypes grown on flooded organic histosal. Crop Sci., **34**: 733-737.
7. **Sridharan A. and Menon M. R.** 1974. Studies on the isolation of *Helminthosporium oryzae*. Indian Phytopath., **27**: 131-133.
8. **IRRI (International Rice Research Institute).** 2002. Standard evaluation system for rice. SES (IRRI): 13-14**.**
9. **Kamoshita A., Zhang J., Siopongco J., Sarkarung S., Nguyen H. T., Wade L. J.** 2002. Effects of Phenotyping Environment on Identification of Quantitative Trait Loci for Rice Root Morphology under Anaerobic Conditions. Crop Science, **42:** 255-265.
10. **Lander E. S., Green P., Abrahamson J., Barlow A. and Daley M.** 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, **1**: 174-181.
11. **Phelps R. H. and Shand C. R.** 1995. Brown leaf spot disease and fertilizer interaction in irrigated rice growing on different soil types. Fertilizer Research, **42**:117-121.
12. **IRGSP (International Rice Genome Sequencing Project).** 2005. The map based sequence of the rice genome. Nature, **436**: 793-800.