Theor Appl Genet (2001) 102:916–920

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QTL analysis of resistance to the rice blast pathogen in barley (*Hordeum vulgare*)

Received: 7 January 2000 / Accepted: 22 September 2000

Abstract Barley is compatible with the rice blast pathogen (*Pyricularia oryzae* Cav.). Fiftyfour barley cultivars of diverse geographic origin and pedigree were inoculated with three isolates of the rice blast pathogen. All barley genotypes showed blast disease symptoms when inoculated at the seedling stage with each of the three isolates. However, one genotype showed quantitative resistance to all three isolates and three genotypes showed quantitative resistance to one or two of the isolates. By inoculating a set of doubled-haploid lines derived from the cross 'Harrington' (susceptible) and 'TR306' (resistant) with isolate Ken 54–20, we mapped quantitative trait loci (QTLs) determining seedling stage blast resistance. At all QTLs, TR306 contributed the resistance alleles. The four QTLs, when considered jointly, explained 43.6% of the phenotypic variation in blast symptom expression. A comparison of the blast resistance QTLs with other disease resistance QTLs reported in this population revealed a region on chromosome 4 (4H) with multiple disease resistance loci. It will be useful to capitalize on the syntenic relationship of rice and barley and to integrate information on species-specific resistance genes with information on the reaction of the two species to the same pathogen.

Keywords Barley · Blast · Disease resistance · Genome mapping · Quantitative trait locus

Communicated by M.A. Saghai Maroof

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Introduction

Rice blast (incited by *Pyricularia oryzae* Cav.) is one of the most important diseases of rice. The host range of the rice blast pathogen is wide and includes many genera of the Gramineae (Narita et al. 1956). Barley is reported to be compatible with the rice blast pathogen (Thomas 1940; Narita et al. 1956) and infection of barley fields in Japan by rice blast was reported by Kawai et al. (1979) and Matsumoto and Mogi (1979). A barley blast pathogen was reported to be pathogenic on rice over 80 years ago (Hara 1916). Recently, epidemics of blast disease on barley have occurred in northern Thailand during the months of November and December. Since these barley fields are located near rice fields and the two crops are produced in the same growing season, the same pathogen may cause blast disease in the two crops.

Yaegashi (1988) identified a single dominant gene, designated as *PHR-1*, which conditioned resistance in barley to an isolate of the barley blast fungus. The analysis was based on the F_2 progenies of the resistant genotypes 'Daisen Gold' and 'Miho Golden' crossed with susceptible varieties. *PHR-1* was characterized as conferring complete and race-specific resistance. However, no resistance genes to rice blast isolates that show the same phenotype as *PHR-1* have been identified in barley since this initial report. There are two papers reporting differences between barley varieties with regard to the incidence of blast disease under field conditions (Kawai et al. 1979; Matsumoto and Mogi 1979).

In this study, we report that a limited number of barley genotypes, including a mapping population parent from Canada, have partial, but relatively high, resistance to rice blast isolates at the seedling stage. As a first step towards characterizing the genetics of this quantitative resistance, we mapped rice blast resistance QTLs using a single rice blast isolate and a doubled-haploid (DH) barley mapping population derived from a cross between the susceptible variety 'Harrington' and the partially resistant variety 'TR306'.

Materials and methods

Fifty barley cultivars selected from the world collection of the Barley Germplasm Center at Okayama University, Japan, were used for characterizing diversity in host reaction to rice blast in barley. Harrington, TR306, Morex and Steptoe, the parental genotypes of mapping populations developed by the North American Barley Genome Mapping Project (http://www.css.orst.edu/barley/nabgmp/nabgmp.htm), were included in the germplasm survey. A set of 150 DH lines derived from the cross Harrington/TR306 was used for QTL analysis. Isolates Ken 54–20 (003.0), Ken 53–33 (137.0) and Ai 79–142 (037.3) of *P. oryzae* Cav. were obtained from the collection maintained at the Paddy Crop Disease Laboratory at the National Agriculture Research Center, Japan.

Ten seeds of each cultivar, or line, were sown in each of 12 rows in plastic trays (37 × 26 × 11 cm) and grown in a glasshouse. Seedlings were inoculated at the one-and-a half to the two-leaf stage by spraying a conidial suspension containing 5×10^4 spores/ml onto the leaves. The plants were incubated in a moist chamber at 25°C for 20 h, and then transferred to the glasshouse. The glasshouse was maintained at a temperature of 25°C day and 15°C night under natural daylight. Disease severity was scored 5 days after inoculation. The second leaf of each seedling was examined and rated on a 0–5 scale, where 0 = no symptom; 1 = percentage of diseased leaf area (DLA) of less than 1%; 2 = 1% < DLA < 5%; 3 = 5% < DLA < 10%; 4 = 10% < DLA < 50%; 5 = DLA is more than 50%.

For QTL analysis, a 127-marker subset of the skeletal marker map generated by Mather (1995) was used. The phenotype and genotype data sets were integrated and QTL analysis was conducted using the composite interval mapping procedures of PLABQTL (Utz and Melchinger 1996). Linkage groups were scanned at 1-cM intervals, and the probability of a QTL for each interval was expressed as a LOD score. Type-I 5% thresholds for LOD scores were estimated and used to declare QTL significance.

Results

All 54 barley genotypes were susceptible to the three rice blast isolates. Most of the barley genotypes developed large, susceptible lesions on entire leaves, and the leaves often died. The susceptible reaction of 'Harrington' to isolate Ken 54–20 (score = 4–5) is shown in Fig. 1. Four genotypes, C651, I656, I685 and TR306, formed only brown pinpoint lesions or small susceptible lesions and occasional large susceptible lesions. Lesion size in the partially resistant genotypes was isolate-dependent. A Canadian genotype, TR306, showed a partially resistant reaction (score = 1–2) to all three isolates (Fig. 1 and Table 1). An Indian genotype, I685, showed partial resistance similar to that of TR306 to the isolates Ken 54–20 and Ken 53–33 (scores = 2),

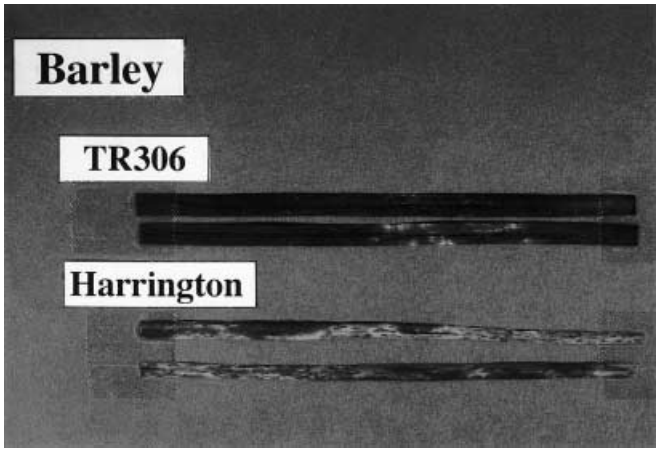

Fig. 1 Reactions of the barley genotypes TR306 and Harrington to the rice blast isolate Ken 54–20

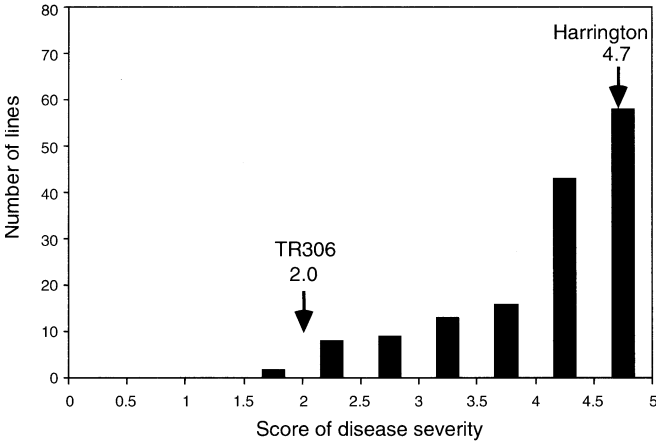

Fig. 2 Frequency distribution for the reaction to rice blast isolate Ken 54–20 on 150 doubled-haploid lines derived from the cross Harrington/TR306

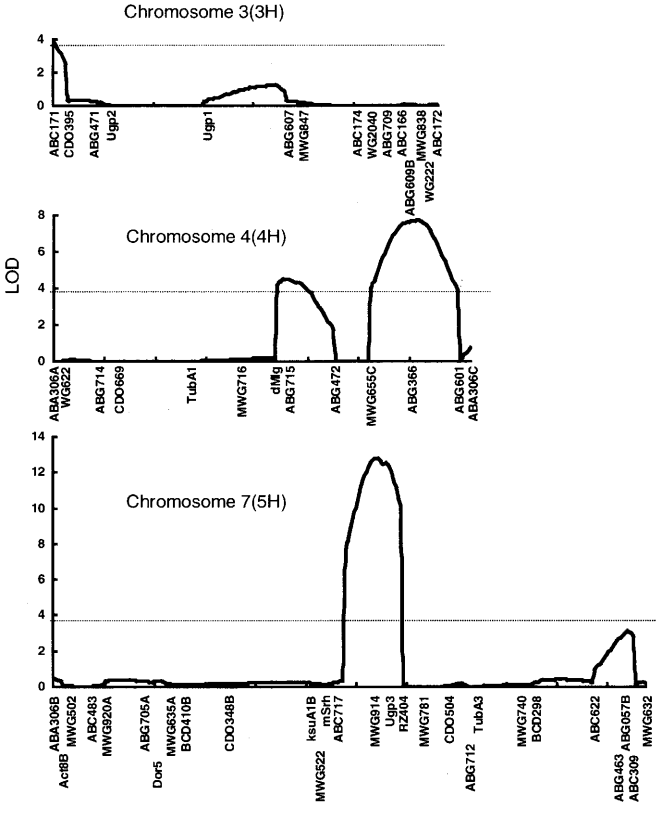

Fig. 3 Positions on QTLs for blast resistance detected by composite interval mapping on the map of Harrington/TR306. The *dotted line* indicates the 5% significant threshold LOD score

Table 1 Reactions of 50 barley accessions selected from the world collection and four parental genotypes of mapping populations to the three rice blast isolates

Accession no.	Name	Country of origin	Reaction to		
			Ken54–20	Ken53–33	Ai79–142
A009	Arivat	USA	5[a]	5	5
A024	Black Gatami	USA	5	5	5
A325	Rufflyn	USA	5	5	5
A607	Peatland	USA	5	5	5
A622	Gem	USA	5	5	5
B319	3368–1	Algeria	5	5	5
B341	4122	Egypt	5	5	5
B361	3683	Egypt	5	5	5
B635	3240	Egypt	5	5	5
B655	3608	Egypt	5	5	5
C603	Pinchiang Chaotung	China	5	5	5
C607	Hulan	China	5	5	3
C614	Lutai	China	5	5	5
C651	Himalayense Type 5	China	5	5	2
C659	Tibet Purple 1	China	5	3	5
E643	Ethiopia 129	Ethiopia	5	5	5
E688	Ethiopia 266	Ethiopia	5	5	5
E797	Ethiopia 593	Ethiopia	5	5	5
E847	Addis Ababa 48	Ethiopia	5	5	No data
E890	Dembi 5	Ethiopia	5	5	5
I603	N.P. 21	India	5	3	3
I614	B.E. 11	Pakistan	5	5	5
I636	Ghazvin 2	Iran	5	5	5
I656	Shirot	Pakistan	5	5	2
I685	Partek 2	India	2	1	3
J679	Kitagawa Chobo	Japan	5	5	5
J700	Akanmugi	Japan	5	5	5
J736	Kenyoshi 1	Japan	5	5	3
J793	Kinai 6	Japan	5	5	5
J808	Tochigi Goldenmelon	Japan	5	5	3
K604	Boseong Covered 5	Korea	5	5	5
K640	Sacheon Jecheon 5	Korea	5	5	5
K666	Jeomchon Naked 2	Korea	5	3	5
K696	Yeoju Rokujo Omugi	Korea	5	5	5
K714	Suncheon Native	Korea	5	5	3
N378	Spiche 5	Nepal	5	5	5
N380	Spiche 11	Nepal	5	5	5
N619	Bimtakothi 9	Nepal	5	5	3
N624	Sama 9	Nepal	3	3	5
N671	Ulleri 21	Nepal	5	5	3
T660	Turkey 180	Turkey	5	5	5
T694	Turkey 282	Turkey	5	5	5
T727	Turkey 381	Turkey	5	5	5
T760	Turkey 480	Turkey	5	5	5
T790	Turkey 570	Turkey	5	5	5
U302	3526	Spain	5	5	5
U366	Russia 16	Russia	5	5	5
U405	Sultan	Netherlands	5	5	5
U635	Germany 22	Germany	5	5	3
U703	Archer	UK	5	5	5
	Harrington	Canada	5	5	5
	TR306	Canada	2	2	2
	Steptoe	USA	5	5	5
	Morex	USA	5	5	5

[a] Disease severity, see Materials and methods

but the reaction of I685 to the isolate Ai 79–142 was susceptible (score = 3). A Chinese genotype (C651) and a Pakistani genotype (I656) were highly susceptible to the isolates Ken 54–20 and Ken 53–33 (scores = 5), but these genotypes were partially resistant to the isolate Ai 79–142 (score =2).

The disease-reaction phenotypes of the doubled-haploid (DH) population derived from Harrington/TR306 in response to inoculations with the Ken 54–20 isolate did not fall into discrete classes allowing for Mendelian analysis (Fig. 2). Using composite interval mapping, four chromosome regions were identified as contributing to blast resistance (Fig. 3). In all cases, resistance alleles were contributed by TR306. The most-significant and largest-effect QTL mapped to the ABC717 – MWG914 interval on the long arm of chromosome 7(5H) (LOD = 12.8, R^2 = 33.4%). Two QTLs mapped on the long arm of chromosome 4(4H). One QTL mapped to the

ABG366 – ABG601 interval (LOD = 7.7, R^2 = 21.8%); the other QTL mapped to the *dMlg* – ABG715 interval (LOD = 4.5, R^2 = 13.4%). The fourth QTL mapped to the distal region of chromosome 3(3H) and coincided with ABC171 (LOD = 3.9, R^2 = 11.8%). The multiple (4) QTL model explained 43.6% of the total phenotypic variation, with a LOD score of 18.0.

Discussion

We have demonstrated that some barley genotypes have partial resistance to the rice blast pathogen. Fiftyfour barley cultivars originating from different geographic regions were tested for resistance to the three rice isolates, and four genotypes showed partial, quantitative resistance to them. This resistance is quantitative with all three isolates tested, and is clearly different from the resistance to *Pyricularia grisea* (syn = *P. oryzae*) reported by Yaegashi (1988). A Canadian genotype, TR306, showed a similar resistance reaction to all three isolates, while the other three partially resistant genotypes (C651, I656 and I685) showed isolate-specific resistance. This isolate specificity suggests that a differentiation of the races of rice blast which corresponds to the genotypes of barley has occurred. Qi et al. (1998) recently demonstrated that in the barley: leaf rust (incited by *Puccinia hordei*) pathosystem some partial resistance QTLs show race specificity. It is interesting that TR306, a Canadian barley, shows partial resistance. There are no reports of rice blast in Canada and, as a result, the host and pathogen have not co-evolved. No barley germplasm from regions where rice blast is endemic exists in the pedigree of TR306.

Based on a QTL analysis of the DH mapping population derived from the cross between Harrington and TR306, there are at least four loci determining partial resistance. A number of QTLs conferring resistance to different diseases were also mapped in the Harrington/TR306 population by Spaner et al. (1998), which allows us to compare the genome location of blast resistance QTLs with QTLs determining resistance to more-common pathogens of barley. The blast resistance data were re-analyzed using MQTL (Tinker and Mather 1995), which was the software employed by Spaner et al. (1998), and the relationship of the blast resistance QTLs with the other disease resistance QTLs was confirmed. Two of the four blast resistance QTLs we detected in this study map to the same regions as QTLs conferring resistance to other diseases, as reported by Spaner et al. (1998). One of the blast resistance QTLs on chromosome 4(4H) mapped close to the vertical mildew resistance gene *dMlg* (Fig. 3). However, the blast resistance allele was contributed by TR306, whereas Harrington contributed the mildew resistance allele. The second blast resistance QTL on chromosome 4(4H) maps to the same region as the QTLs associated with resistance to stem rust (*Puccinia graminis* Persoon), scald (*Rhynchosporium secalis* Davis) and net blotch (*Phyrenophora teres* Drechs.). TR306 contributed resistance alleles at all of these QTLs. Multilocus clusters of resistance genes have been reported in a range of plant species (Hammond-Kosack and Jones 1997) including barley (Jørgensen 1992). QTL coincidence can be due to linkage or pleiotropy, and the two alternatives cannot be distinguished at the level of resolution afforded by this mapping population. The blast resistance QTL on chromosome 7(5H) was 14 and 19 cM, respectively, from QTLs conferring resistance to leaf rust and net blotch. The leaf rust resistance was conferred by the TR306 allele whereas the net blotch resistance was conferred by the Harrington allele.

As Narita et al. (1956) indicated, the rice blast pathogen can attack many genera of Gramineae. Accordingly, the data presented in this paper are interesting from the standpoints of host/pathogen specificity, the evolution of disease resistance genes, and quantitative resistance. Morgan et al. (1998) reported that resistance to pyricularia leaf spot in pearl millet was also caused by *P. oryzae*. These authors found a RAPD marker linked to blast resistance. Comparative mapping was forestalled by a lack of polymorphism of rice RFLP probes linked to rice blast resistance in pearl millet. If the genes determining resistance to the same pathogen in different species could be isolated, this would facilitate a study of the origin and divergence of plant disease resistance genes. By comparative RFLP linkage mapping, syntenic chromosome segments have been identified between barley and rice (Paterson et al. 1995; Saghai-Maroof et al. 1996). The order of RFLP and morphological markers is conserved in these regions. Accordingly, it should be possible, via comparative mapping, to align rice blast resistance QTLs in barley with the extensive catalogues of quantitative and qualitative blast resistance genes mapped in rice (McCouch et al. 1994; Kinoshita 1995). It may then be justifiable to expand the search for broad-spectrum resistance to rice blast in order to include alternative resistance alleles in the two genera.

There is evidence that plant disease resistance genes may be conserved across a range of plant species. Considering genes conferring resistance to obligate biotrophs, Yu et al. (1996) found a high degree of colinearity among RFLP markers common to small chromosome fragments in some species of Gramineae. Using resistance to *Cochliobolus carbonum* in maize as a model, Multani et al. (1998) demonstrated the loss of resistance by transposon insertion and a deletion in two resistance genes. Extending the idea to sorghum and rice, they found commonalities of the resistance genes in these species with those in maize. Resistance gene analogs (RGAs) may also be useful in finding and characterizing resistance genes (Mago et al. 1999). Leister et al. (1999) demonstrated relationships between mapped resistance gene homologues in rice (blast and bacterial blight) and barley (powdery mildew and stem rust). Toojinda et al. (2000) reported associations of RGA polymorphisms and QTLs associated with resistance to stripe rust (*Puccinia striiformis*, fsp. *hordei*) and barley yellow dwarf virus

(BYDV) in barley. Accordingly, there may be common resistance factors fundamental to multiple host/pathogen relationships which are modified by minor structural and regulatory changes. In this regard, it would be desirable to use the same pathogen to study resistance in multiple hosts. Blast resistance may be a useful model for such studies in the Gramineae. This preliminary report describes the resistance of barley to a rice blast isolate at the seedling stage. Additional research will be necessary to determine the specificity and agronomic utility of these rice blast resistance QTLs, as well as their relationships to other plant disease resistance genes.

Acknowledgements The authors thank Dr. H. Yaegashi (Saga University, Japan) for his valuable technical advice, and Drs. S. Surapong (IRRI Cooperative Project with Ministry of Agriculture and Cooperatives, Thailand) and T. Toojinda (Kasetsart University, Thailand) for their information and valuable discussion. The experiments comply with the current laws of the country.

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