Validation of rice blast resistance genes in barley using a QTL mapping population and near-isolines

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There are prior reports of *Pyricularia grisea*—the causal agent of blast of rice—causing disease in barley. In order to determine the specificity of this resistance in barley, we extended our previous mapping efforts to include blast isolates from barley and rice grown in Thailand and we assessed two resistance phenotypes: leaf blast (LB) and neck blast (NB). The largest-effect resistance QTL, on chromosome 1H, was associated with NB and LB and is located in a region rich in resistance genes, including QTL conferring resistance to stripe rust (incited by *Puccinia striiformis* f. sp. *hordei*) and the mildew (*Blumeria graminis* f. sp. *hordei*) resistance gene *Mla*. The LB, NB and mildew resistance alleles trace to one parent (Baronesse) whereas the stripe rust resistance allele traces to the other parent (BCD47) of the mapping population. Baronesse is the susceptible recurrent parent of a set of near-isogenic lines (NILs) for three stripe rust resistance QTL, including one on 1H. Unigene (EST) derived single nucleotide polymorphism haplotypes of these NILs were aligned with the blast mapping population QTL using *Mla* as an anchor. Baronesse and all NILs without the 1H introgression were resistant to LB and NB. However, two NILs with the 1H introgression were resistant to LB and NB. Both are resistant to stripe rust. Therefore, the QTL conferring resistance to stripe rust is separable by recombination from the blast resistance QTL.

Key Words: rice blast, barley, resistance gene, QTL mapping, near-isolines.

Introduction

Rice blast, caused by the fungus *Pyricularia grisea* (Cooke) Sacc. (teleomorph: *Magnaporthe grisea* (T.T. Hebert) Yaegashi & Udagawa, is the most destructive and cosmopolitan disease of rice (Puri *et al*. 2009). Blast in rice has two commonly recognized phases: leaf blast and neck blast. Leaf blast occurs most often during the plant's vegetative stage. Spindle-shaped lesions appear on the leaf blade and necrotic lesions are found at the leaf collar. Although leaf infection is sometimes found at the reproductive and ripening stages, the more serious symptom during these stages is neck blast (Bonman *et al*. 1989). Neck blast infections occur below the panicle, usually at the neck node, causing neck rot. If neck rot occurs early, the entire panicle dries prematurely and is sterile. Later infections may cause incomplete grain filling and poor quality. In farmer's fields, neck blast is often more destructive than leaf blast (Bonman *et al*. 1989) as it can lead to losses of up to 70% (Puri *et al*. 2009). Although neck and leaf blast are caused by the same pathogen, different genes are reported to determine the reaction to the two phases of disease (Bonman 1992, Ou 1985, Puri *et al*. 2009).

Blast disease on rice is a major threat to world food security. Of equal concern is the fact that the fungus can also cause disease on other agriculturally important crops, including barley (Chen *et al*. 2003, Inukai *et al*. 2006, Sato *et al*. 2001), perennial ryegrass (Williams *et al*. 2001), Italian ryegrass (Miura *et al*. 2005), and wheat (Urashima *et al*. 2004). In the case of wheat, blast disease emerged as a significant disease in Brazil in 1985 and rapidly spread to other wheat growing regions. This broad host range is of particular concern because cultural strategies, such as crop rotation, are simple and effective techniques for blast disease control in rice. If the crop that is grown in rotation with rice proves

to be compatible with *P. grisea*, then disease incidence will increase and a new alternative crop must be found. If two blast-susceptible crops are grown in rotation, there will be challenges to effectively deploying resistance genes in both crops. Likewise, in the face of continuous disease, dependence on fungicides will become increasingly costly and risky. A potentially positive aspect to broad host range is that the catalog of resistance genes available to plant breeders may be expanded and "non-host" resistance genes may prove to be more durable than qualitative resistance genes within a crop's gene pool. In the case of blast, it is increasingly difficult to determine which plant species are appropriately termed hosts and which are non-host. The most studied of the "novel" hosts for blast is barley, where a range of responses to the pathogen are reported (Chen *et al*. 2003, Inukai *et al*. 2006, Sato *et al*. 2001).

A gene (*PHR-1*) conferring qualitative resistance to rice blast in barley was reported by Yaegashi (1988). The dominant resistance allele at this locus was identified in two Japanese accessions: "Daisen Gold" and "Miho Golden". This gene has not been mapped. The more recent search for blast resistance genes in barley led to mapping QTL (Chen *et al*. 2003, Sato *et al*. 2001). The most recent and comprehensive report on the genetics of resistance of barley to blast is that of Inukai *et al*. (2006). They used a doubled haploid population of barley derived from the cross of BCD47 × Baronesse and two Japanese rice blast isolates (Ken 54-20 and Ken 53-33) to map a qualitative resistance gene (*Rmo1*) on chromosome 1H and QTL on chromosomes 1H, 3H, 4H and 7H. *Rmo1* and the QTL map to resistance gene-rich regions in barley and show some coincidence with syntenic resistance gene-rich regions in rice (Chen *et al*. 2003, Inukai *et al*. 2006). Because barley has been grown in parts of Asia for thousands of years—and often in rotation with rice in areas where rice blast is endemic—some host: parasite co-evolution could be expected. This could account for resistance in Daisen Gold and Miho Golden. Sato *et al*. (2001), Chen *et al*. (2003), and Inukai *et al*. (2006), however, found quantitative and qualitative resistance alleles in North American ("TR306" and "BCD47") and European ("Baronesse") germplasm. Rice blast is not endemic in the barley growing regions of Europe and North America where rice and barley are rarely grown in rotation. It is therefore surprising that barley germplasm from these areas would show differential reactions to a fungus with which it did not co-evolve. These reactions range from complete resistance to complete susceptibility.

The principal objective of this research was to determine the number, location, and interaction of genes determining blast resistance in barley using *P. grisea* isolates obtained from rice and barley grown in Thailand. We used the same doubled haploid population (BCD47 × Baronesse) that was used by Inukai *et al.* (2006) to map resistance to Japanese isolates in order to assess if the same or different resistance genes/QTL determined resistance to the different samples of isolates from the two different rice-growing areas. We then used a set of near-isogenic lines derived from Baronesse,

BCD47 and BCD12 to validate QTL effects. The near-isolines were developed for an ongoing assessment for quantitative resistance to stripe rust (incited by *Puccinia striiformis* f. sp. *hordei*). They were useful in the current experiments for validating blast resistance QTL on chromosome 1H because the stripe rust and rice blast resistance QTL occupy coincident linkage map positions and the donor of the stripe rust resistance QTL allele (BCD12) is susceptible to rice blast (Inukai *et al*. 2006 and this report). We reasoned that introgression of the 1H QTL allele from BCD12 into Baronesse would lead to susceptibility to rice blast.

Materials and Methods

Plant materials

The ORO (Oregon Resistance Opportunity) mapping population consists of 94 doubled-haploid (DH) lines developed from the F_1 of the cross between BCD47 × Baronesse. BCD47 is a two-rowed, spring growth habit DH line, developed via marker-assisted selection for alleles conferring resistance to barley stripe rust (incited by *Puccinia striiformis* f. sp. *hordei*) at QTL on chromosome 4H and 5H (Castro *et al*. 2003). Baronesse is a two-rowed, spring growth habit cultivar developed by Nordsaat Saatzucht GmbH and released in 1989 in Germany. The cultivar is grown extensively in the Pacific Northwest of the USA.

The BISON (Barley stripe rust ISOgeNic) lines are a set of QTL near-isogenic lines (NILs) representing all possible one-way, two-way, and three-way introgressions of alleles at barley stripe rust (BSR) resistance QTL on chromosomes 1H, 4H and 5H. The set also includes a NIL for a qualitative resistance gene on chromosome 7H. The BISON lines were developed by one cycle of marker assisted selection (MAS) and backcrossing of the iBISON (intermediate BISON) lines to the recurrent parent Baronesse. The development of the iBISON was described in detail by Richardson *et al*. (2006). Briefly, the BSR resistance QTL alleles on chromosomes 4H and 5H trace to BCD47 and the QTL allele on chromosome 1H comes from BCD12 (a sister line of BCD47). The MAS derivation of BCD12 and BCD 47 is described in detail by Castro *et al*. (2003). A "0 QTL" BISON (with susceptible alleles at all resistance loci) was developed from the same germplasm base as the other BISON lines using MAS. D3-6/B23 was the source of the qualitative resistance gene on 7H (Castro *et al*. 2003). Low density forward and background MAS in the iBISON and BISON were performed using an array of marker types, principally SSRs. Higher density markers were not available at the time this germplasm was developed. Detailed genotyping protocols are available at http://barleyworld.org/osubreeding/striperustmapping.php. Although the BISON lines were developed to validate BSR resistance QTL alleles, they are also useful for validating blast resistance QTL. Because Baronesse is resistant to *P. grisea* isolates from Japan (Inukai *et al*. 2006) and Thailand (this report), we hypothesized that introgression of a chromosome region containing a blast susceptibility allele

(or alleles) into Baronesse would lead to susceptibility in the resulting BISON NIL(s). For example, we would expect to see susceptibility to rice blast in the four NILs involving 1H (1H, 1H+4H, 1H+5H and 1H+4H+5H).

Pyricularia grisea isolates and culture

Four fungal isolates—TH16 (from Khon Kaen, Thailand), THL80 (from Khon Kaen, Thailand), THL142 (from Chiang Mai, Thailand) and THL222 (from Chiang Mai, Thailand)—were used. TH16 and THL80 were isolated from barley and THL142 and THL222 were isolated from rice. The virulence spectrum of the four isolates is described by Kongprakhon *et al*. (2009). The isolates were grown on rice polished agar medium (20 g rice polish, 2 g yeast extract and 20 g agar/l distilled water) at 24–25°C using a 12 h photoperiod for 7–8 days. Spores were harvested by flooding the culture plates with 8 ml of sterile water.

For phenotyping leaf blast (LB) in the ORO mapping population, barley plants were grown under greenhouse conditions (25–30°C continuous temperature with natural light) in 72-well plastic trays that measured 25.4 cm × 50.8 cm. In each well, three seeds of each DH line were sown. Two trays were needed to test the entire set of 94 DH lines, 50 in one tray and 44 in the other. Each tray also included three replicates each of the two parents (BCD47 and Baronesse) and also the two rice checks (IR64 and Azucena). Four experiments were performed to test the response to the four isolates (TH16, THL80, THL142 and THL222). For BISON lines, barley plants were grown under the same greenhouse conditions in 18-well plastic trays that measured 12.5 cm × 25.4 cm. Three seeds of each BISON line, BCD 12, D3-61B-23, CI10587, Baronesse, BCD47, IR64 and Azucena were sown per well. Each experiment was replicated four times. A commercial liquid fertilizer (16 : 16 : 16 N-P_2O_5-K_2O) was applied at a rate of 0.6 g/tray on a weekly basis. Additional nitrogen in the form of urea was applied at a rate of 10 g/tray for the mapping population and 2.5 g/tray for the BISON lines at 10, 3 and 1 day prior to inoculation in order to increase host susceptibility to blast. Seedlings were inoculated at the 1.5–2 leaf stage with each of the four isolates using 50 ml of 50,000 spore ml^{-1} suspensions, with 0.5 percent gelatin. Each tray of inoculated plants was then kept for one night in a controlled environment chamber at 24°C with near-saturated conditions of relative humidity. Trays were then returned to the greenhouse, which was kept at a continuous temperature of 25–30°C with natural light. Three, five, and seven days post-inoculation, lesion types were scored using the seven lesion type categories described by Roumen *et al*. (1992). According to this scale, 0 = resistant, 1–3 = intermediate and 4–6 = susceptible. For neck blast phenotyping, barley plants were sown in the field in early November at the Kamphaeng Saen Campus, Kasetsart University, Nakorn Pathom, Thailand. For each individual DH line and the parents, 50 seeds were sown per 150 × 50 cm plot. A three-replicate randomized complete block design was used. Inoculum (at the same concentration used for leaf blast

resistance phenotyping) was injected into the leaf sheath at heading. Six heads per DH line were inoculated with each of the four isolates. Neck blast was scored at seven and 14 days post-inoculation using a 0 to 6 scale based on the standard evaluation system for rice (IRRI 1996). According to this scale, 0 = resistant, 1–3 = intermediate and 4–6 = susceptible. Reaction types in the greenhouse for each DH line were scored on a per-well basis and in the field were scored on a per-plot basis (the score of the individual plant in each well/ plot with the highest score). Reaction types were highest at seven days post inoculation for both diseases. Therefore, the average scores across replicates from this time point were used as the phenotypic values for subsequent analyses.

Powdery mildew disease phenotyping

In order to determine the position of the *Mla* locus in the BISON, we used a comparative mapping strategy. *Mla* was mapped in the ORO population (Rossi *et al.* 2006) but the unigene-derived Single Nucleotide Polymorphisms (SNPs) used to genotype the BISON (see next section) have not been mapped in the ORO population. In order to assign a linkage map position to *Mla* in a mapping population that included the SNPs*,* we chose the Oregon Wolfe Barley (OWB) population (Costa *et al*. 2001, Szucs *et al*. 2009). The 2,383-locus OWB map is based primarily on the same Illumina Oigonucleotide Polymorphism Array (OPA) unigene-SNPs used to genotype the BISON (see next section). We mapped mildew resistance in the OWB based on the phenotypes of seedlings and adult plants. For the seedling test, a single pustule isolate of *B. g.* f. sp. *hordei* collected from the greenhouse was used. The avirulence/virulence formula of this isolate is *Mla1*, *Mla6*+*Mla14*, *Mla7*, *Mla9*, *Mla12*, *Mla13*+ *Mla-(Ru3)*, *Mlk*, *Mlg* + *Ml-(CP)*, *Mla7 + Ab*, *mlo*/*Mla3*, *Ml-*(La) on the Pallas differential genotypes described by Kølster *et al*. (1986). Conidia of this isolate were initially increased on susceptible barley cultivar 'Larker.' Inoculations were made on seven-day-old plants (first leaf fully expanded) by shaking conidia from the infected Larker plants onto the test entries. These plants were incubated at ambient greenhouse conditions (22–25°C) and assessed for their infection types 10–12 days later using the 0 to 4 scale of Mains and Dietz (1930). Infection types 0, 1 and 2 were considered indicative of resistance, whereas infection types 3 and 4 were considered indicative of host susceptibility.

For the confirmatory adult plant test, the 94 OWB DH lines and parents were grown under field conditions at the research facilities of Okayama University located at Kurashiki, Japan (34°35′N and 133°46′E). A natural epidemic of powdery mildew developed and due to the qualitative nature of disease symptoms, the phenotype was scored as resistant (no disease symptoms) or susceptible (disease symptoms present) at the mid-dough maturing stage of growth.

Genotyping and linkage map construction

For QTL mapping we used the ORO linkage map reported by Rossi *et al*. (2006). This map was constructed using

JoinMap 3.0 (Van Ooijen and Voorrips 2001) and the Kosambi mapping function (Kosambi 1944). The linkage map has 71 markers, mostly Simple Sequence Repeats (SSRs) comprising eleven linkage groups at LOD threshold grouping value of 4.0. The map covers 611.8 cM, corresponding to an average density of 8.7 cM per marker. Gaps in the map, due to a lack of polymorphic SSRs in some regions, led to multiple linkage groups per chromosome. In these cases, linkage groups were assigned to chromosomes based on the known locations of SSRs in denser maps and a letter suffix was used to designate each group (e.g. 7Ha, 7Hb).

The BISON lines were genotyped for 4,608 unigene-SNPs under the auspices of the Barley Coordinated Agricultural Project (CAP) (http://www.barleycap.org/). The development of the unigene-SNP markers is described by Szucs *et al.* (2009). Briefly, unigene-SNPs were used to design three Illumina 1,536-plex pilot Oligonucleotide Pool Assays (pilot OPAs; POPA1, POPA2, POPA3). The three POPAs were used to genotype the "CAP Core" germplasm array, which included the BISON, BCD12 and BCD47 using the Illumina GoldenGate BeadArray SNP detection platform. Genotyping assays were conducted at the Southern California Genotyping Consortium at the University of California, Los Angeles using DNA samples provided by Dr. Tim Close (University of California−Riverside). Complete genotype data for the CAP Core germplasm array is available at (http://www.barleycap.org/). SNPs are designated by their POPA numbers (e.g. 1_1311), where 1 = the POPA number (POPA1 in this case) and the subsequent four digits correspond to the unigene-SNP order in the corresponding POPA. The locus designations can be directly referenced to assembly #35 unigene numbers by referring to the barley SNP consensus map at HarvEST (http://harvest.ucr.edu/). For example, 1_1311 maps to the short arm of chromosome 1H and corresponds to assembly #35 unigene 5,087.

Statistical and QTL analyses

We calculated the Pearson correlation coefficients between traits and the significance of these correlations using the CORR procedure of SAS V9.1 (SAS Institute, Cary, NC, USA). The variance components were estimated using the VARCOMP procedure of SAS V9.1 with the restricted maximum likelihood method according to the following model:

$$Y_{ijk} = \mu + E_i + R_{j(i)} + G_k + GE_{ik} + \varepsilon_{ijk}$$

where Y_{ijk} was the infection score of the *k*th genotype (G) in the *j*th replicate (R) within the *i*th experiment (E), μ is the overall mean and ε_{ijk} was the residual error. Experiments were considered as fixed effects, and genotype and replicates as random effects. Heritabilities (h^2) were calculated with the following formula:

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2/e + \sigma_\varepsilon^2/re}$$

where σ_G^2 represents the genotypic variance, σ_{GE}^2 the genotype-by-experiment variance, σ_ε^2 the error variance, *e* the

number of experiments and *r* the number of replicates. Broad sense heritability on an entry mean basis was calculated with e = 4 and r = 4 for leaf blast and e = 4 and r = 3 for neck blast.

QTL analysis was performed using composite interval mapping (CIM) (Zeng 1994) implemented in Windows QTL Cartographer 2.5 (Wang *et al.* 2005). Cofactors for CIM were chosen using a forward-selection backward-elimination stepwise regression procedure with a significance threshold of 0.10. The maximum number of cofactors used was six. The scan window was set in 10 cM. Experiment-wise significance (alpha = 0.05) likelihood ratio (LR) thresholds for QTL identification were determined with 1000 permutations and expressed as LOD (LOD = 0.217 LR). For every significant QTL, we calculated individual R^2 (proportion of phenotypic variance explained by the individual QTL) and additive effect (expressed as one half of the difference between the two allelic classes). Negative values indicate that the parent line contributing the resistance allele was Baronesse. Epistatic interactions between QTL were tested by means of Multiple Interval Mapping (MIM, Zeng *et al.* 1999) using QTL Cartographer and the Bayesian Information Criteria (BIC-M0). In each experiment, we also calculated the R^2 of the multilocus model that included the QTL detected using CIM and their significant interactions detected using Multiple Interval Mapping (MIM). We also calculated the average TR^2, which is the proportion of variance explained by the QTL conditioned on the cofactors in CIM obtained with EQTL (QTL Cartographer v. 2.5).

Results

LB and NB QTL mapping

Baronesse and BCD47 showed contrasting LB and NB resistance phenotypes when inoculated with each of the four isolates. Overall, Baronesse showed high to intermediate levels of resistance, whereas BCD47 was susceptible (Fig. 1). The lack of discrete classes in the phenotypic frequency distributions is not due to errors in scoring: the h^2 of disease severity (DS) for LB and NB ranged 86–89%. Significant positive and negative phenotypic transgressive segregation was more prevalent for LB than for NB. For both LB and NB, inoculation with THL222 gave somewhat different results than inoculation with the other isolates. With this isolate, no DH lines were significantly more susceptible than BCD47. In order to determine, based on phenotype, if the same or different genes determined resistance to the four isolates, we calculated correlations between DS in response to inoculation with the four isolates (Table 1). The correlation coefficients for pairwise comparisons are significant and high with P-values averaging 0.1%. The highest correlations were observed among LB reactions and among NB reactions. The lowest correlations were observed between different isolates for the two different forms of blast (LB vs. NB).

The number, allele phase, and interaction of the LB and NB resistance QTL identified in response to inoculation with each of the four isolates are presented in Table 2 and
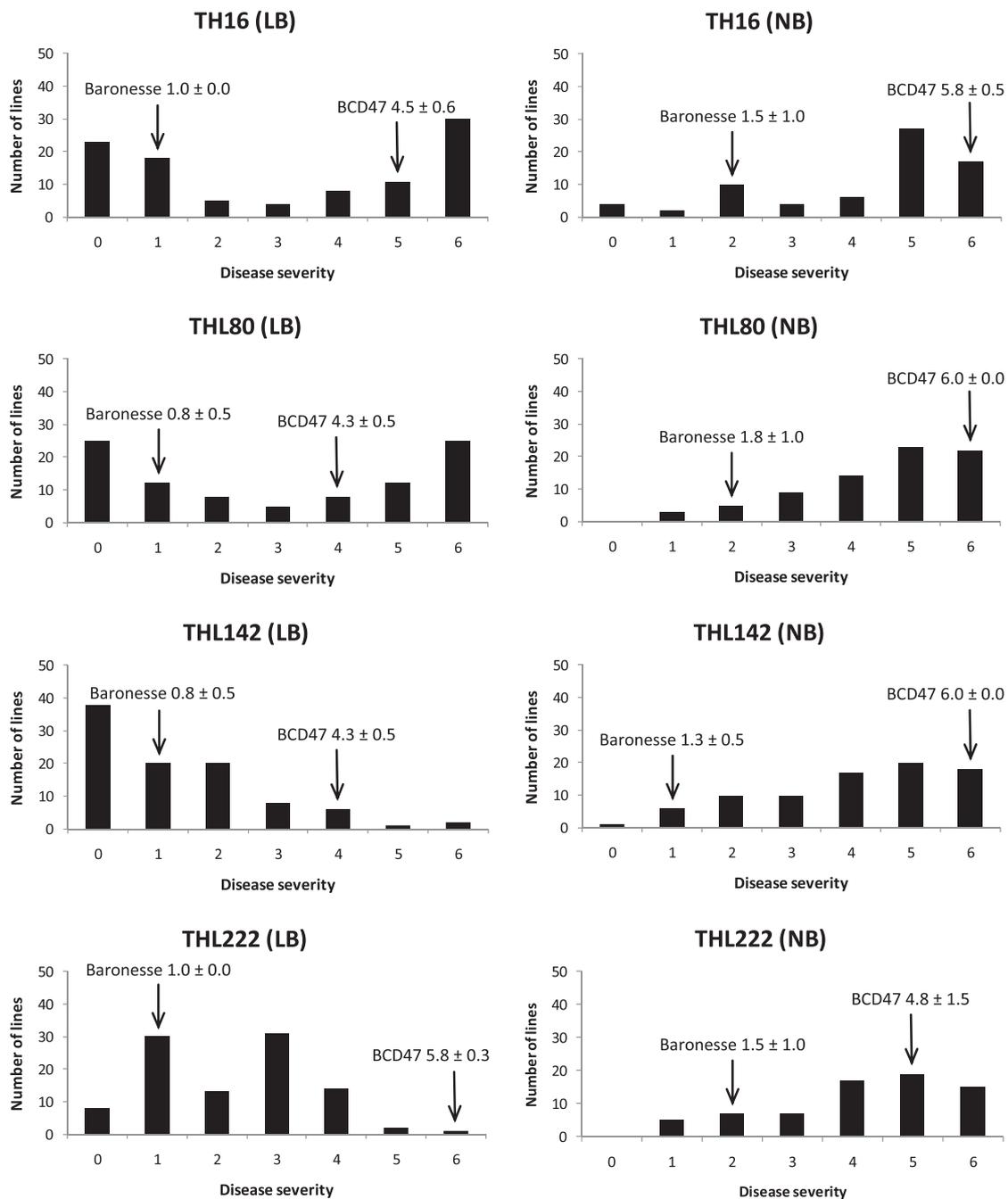
Fig. 1. Phenotypic frequency distributions of leaf blast (LB) and neck blast (NB) disease severity in the BCD47/Baronesse mapping population inoculated with each of four isolates (TH16, THL80, THL142 and THL222).

Fig. 2. A total of four QTL were detected. The QTL on chromosomes 1H, 4H and 7Hb had LOD scores ranging from 2.9 to 27.0 (Table 2). Baronesse contributed resistance alleles at two of the QTL (chromosomes 1H and 7Hb). BCD47 contributed the resistance allele at the QTL on chromosome 4H.

A QTL on chromosome 1H (k04435-Bmac399) was associated with LB and NB resistance to all four isolates. For LB, alleles at this QTL determined 65, 69, 51 and 71% of the phenotypic variance in resistance to TH16, THL80, THL142 and THL222, respectively. For NB, alleles at this

QTL determined 42, 37, 19 and 48% of the phenotypic variance in resistance to the same isolates. Two LB resistance QTL on chromosome 4H (HVMO3-Bmac030B and EBmac701-k07229) were identified with isolate THL222 (Table 2 and Fig. 2). Those QTL accounted for 6 and 3% of the phenotypic variance in DS. Baronesse contributed the resistance allele at both QTL. One NB QTL was detected on chromosome 7Hb and accounted for 13 and 10% of the phenotypic variance in DS in response to inoculation with isolates TH16 and THL80, respectively (Fig. 2). BCD47

Table 1. Pearson phenotypic correlation coefficients between disease scores observed in the ORO mapping population inoculated with four blast isolates (TH16, THL80, THL142 and THL222). LB and NB in brackets indicate leaf blast and neck blast, respectively

Variable	THL80 (LB)	THL142 (LB)	THL222 (LB)	TH16 (NB)	THL80 (NB)	THL142 (NB)	THL222 (NB)
TH16 (LB)	0.87***	0.64***	0.68***	0.70***	0.46***	0.46***	0.44***
THL80 (LB)		0.68***	0.73***	0.57***	0.40**	0.37**	0.42***
THL142 (LB)			0.73***	0.52***	0.28*	0.36**	0.33**
THL222 (LB)				0.52***	0.39**	0.37**	0.43***
TH16 (NB)					0.75***	0.49***	0.74***
THL80 (NB)						0.48***	0.83***
THL142 (NB)							0.53***

*, **, *** Significant at 5%, 1% and 0.1% level, respectively.

Table 2. Summary of leaf blast (LB) and neck blast (NB) resistance QTL detected in the BCD47/Baronesse DHL population in response to inoculation with each of four blast isolates (TH16, THL80, THL142 and THL222)

Isolate	QTL No.	Chrom.	Peak Position (2 LOD conf. interval)	Closet Marker	LOD	Additive effect	R^2	LOD Threshold
TH16 (LB)	1	1H	9.1 (0.2–48.8)	GMS21	22.4	1.7	0.65	2.5
TH16 (NB)	1	1H	9.1 (0.3–46.2)	GMS21	9.7	1.2	0.42	2.5
	2	7Hb	103.6 (93.5–104.0)	Bmac135	3.6	0.6	0.13	
THL80 (LB)	1	1H	9.1 (0.1–50.6)	GMS21	26.5	2.0	0.69	2.6
THL80 (NB)	1	1H	24.9 (6.4–46.9)	Bmac399	8.0	1.2	0.37	2.7
	2	7Hb	95.6 (78.8–101.3)	Bmac156	3.1	0.5	0.10	
THL142 (LB)	1	1H	9.1 (2.0–44.9)	GMS21	15.8	1.1	0.51	2.4
THL142 (NB)	1	1H	7.3 (1.6–23.4)	k08302	4.1	0.6	0.19	2.6
THL222 (LB)	1	1H	9.1 (1.4–50.6)	GMS21	27.0	1.2	0.71	2.5
	2	4H	44.6 (36.6–54.6)	Bmac030B	5.6	−0.4	0.06	
	3	4H	105.3 (89.6–116.9)	K03352	2.9	−0.2	0.03	
THL222(NB)	1	1H	34.9 (6.9–52.3)	Bmac399	6.1	1.1	0.48	2.5

contributed the resistance allele at this QTL, which was significant only in response to inoculation with blast isolates obtained from barley. We did not detect any significant QTL × QTL interaction.

Powdery mildew resistance phenotyping and mapping

The OWB seedling and adult plant response to powdery mildew infection data were identical and fit a 1 : 1 ratio, as expected for mono-factorial segregation in a doubled haploid mapping population. The resistance gene, designated as *RbgMD* (Resistance to *Blumeria graminis* from original parent source Multiple Dominant) maps to the short arm of chromosome 1H at the predicted position of *Mla* and cosegregates with locus 3-0952 on the unigene-SNP consensus map. The SNP consensus map is based on the OWB, Steptoe × Morex and Morex × arke populations. Therefore, locus 3-0952 defines the linkage map position of *RbgMD* on the SNP consensus map. Assuming *RbgMD* represents the effect of an allele at *Mla* (or a tightly linked locus), the ORO map and SNP consensus maps can be aligned with the BISON SNP haplotyes. This alignment is illustrated in bold font in Fig. 3.

QTL validation in the BISON

As shown in Fig. 3, Baronesse and all BISON without 1H introgressions tracing to BCD12 were resistant to LB. In contrast, BCD12, BCD47, BISON 1H and BISON 1H + 4H

+ 5H were highly susceptible to all isolates. The two LB-susceptible BISON have extensive introgressions from BCD12 that include locus 3-0952 (11.4 cM), which cosegregates with *RbgMD* in the Oregon Wolfe Barley (OWB) population based on seedling and adult plant response to infection. *Mla* lies at the peak of the LB QTL in the ORO population. Assuming that *RbgMD* and *Mla* are allelic or tightly linked, the most likely position of the LB QTL in the BISON is at locus 3-0952. There is no detectable introgression from BCD12 in BISON 1H + 5H until locus 2_0749 (17.3 cM), which is proximal to the LB QTL peak. BISON 1H + 4H is resistant to all isolates but has the same haplotype on the short arm of chromosome 1H as the LB-susceptible BISON 1H and BISON 1H + 4H + 5H.

Discussion

Our results agree with those of Inukai *et al*. (2006) in terms of location and effects of leaf blast resistance QTL in the ORO population on chromosomes 1H, 4H and 7H. We did not, however, detect the significant QTL on 3H reported by Inukai *et al.* (2006). These results show that the resistance QTL alleles are effective against the spectrum of virulence represented by two blast isolates from Japan and four blast isolates from Thailand. We extend these results to show that these QTL also confer resistance to blast isolates obtained from rice and barley in Thailand. We also show that many of
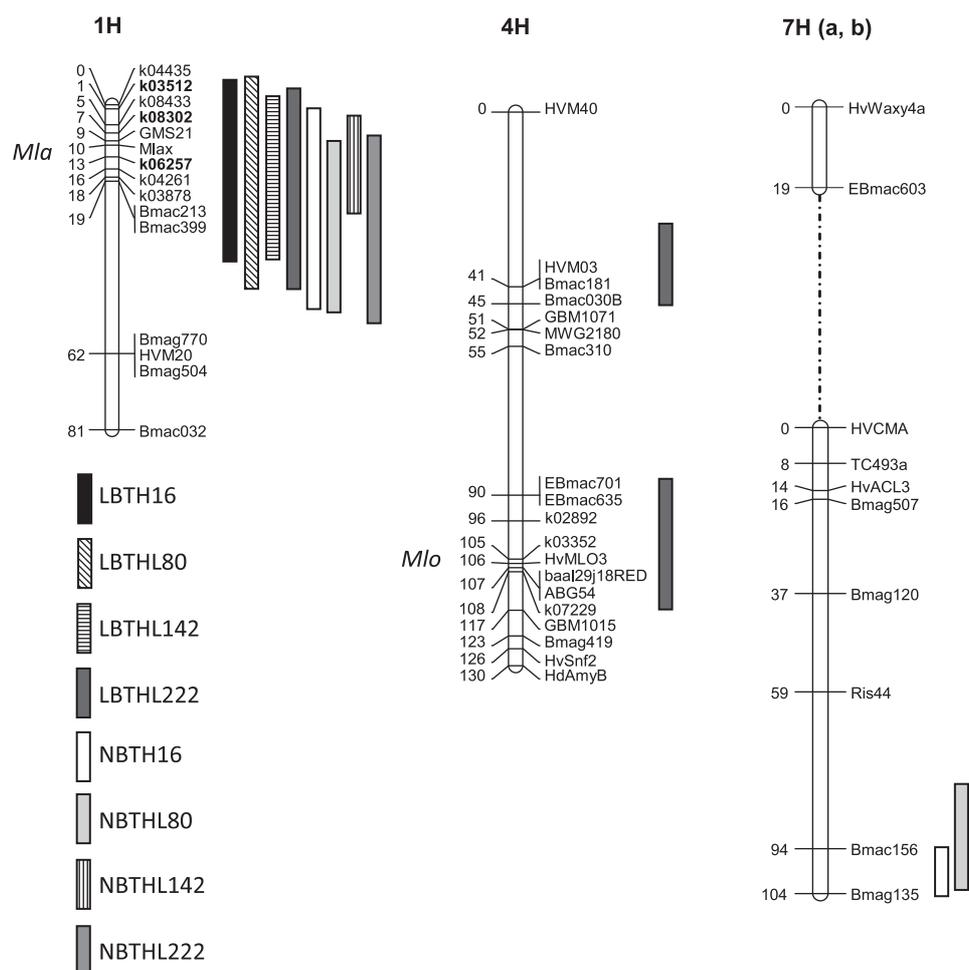
Fig. 2. Linkage maps of chromosomes 1H, 4H, and 7Hb in the BCD47/Baronesse mapping population. Distances are in Kosambi cM. The bars to the right of each linkage group indicate 2-LOD confidence intervals for leaf blast (LB) and neck blast (NB) resistance QTL detected in response to inoculation with each of four isolates (TH16, THL80, THL142 and THL222).

the same QTL confer resistance to leaf and neck forms of the blast disease, accounting for the positive correlations between the LB and NB phenotypes. The 1H QTL was detected for all isolates and for both leaf and neck blast. The 7H QTL was detected only for neck blast and the 4H only for leaf blast. These small and inconsistent effects may reflect isolate/disease specificity or they may be due to the relatively small size of the mapping population. Inukai *et al.* (2006) also detected smaller-effect QTL on 4H and 7H. It is not possible to state unequivocally that the large-effect 1H QTL is or is not allelic with the *RMo1* qualitative resistance gene. As observed in prior reports, at each of the genomic regions where we observed LB and/or NB QTL, there are prior reports of *R* genes and/or QTL forming resistance gene clusters (Chen *et al.* 2003, Inukai *et al.* 2006, Sato *et al.* 2001).

Although the BISON were developed to study the levels of resistance conferred by individual and multiple combinations of stripe rust resistance QTL, they were useful for validating a LB resistance QTL on chromosome 1H. In all cases, retention of Baronesse alleles on the short arm of chromosome 1H in the vicinity of *Mla/RbgMD* (e.g. Baronesse,

BISON 4H, BISON 5H, BISON 7H and BISON 4H+5H) resulted in LB resistance. Two of the 1H introgression BISON were LB-susceptible, and both have the BCD12 allele at 3_0952 (11.4 cM), which cosegregates with *Mla*. A likely explanation for the LB resistance of BISON 1H+5H is that it has the Baronesse LB and NB resistance gene (or genes): i.e. it has a Baronesse haplotype at all loci distal to 2_0749 (17.3 cM). A possible explanation for the LB resistance of BISON 1H+4H is that it retains an undetected segment from Baronesse in the vicinity of *Mla*. There is evidence for small introgressions and/or retention of small segments of recurrent parent genome. For example, BISON 1H+5H has only one unigene-SNP locus allele from BCD12 on the first 27 cM of the short arm of chromosome 1H, at locus 2_0749. In a preliminary test for barley stripe rust resistance under field conditions at Corvallis, Oregon in 2008 the following disease severities (on a plot basis) were observed: Baronesse (62%), BCD12 (12%), BCD47 (5%) BISON 1H (15%), BISON 1H+4H (10%), BISON 1H+5H (12%) and BISON 1H+4H+5H (<1%). These data suggest that the stripe rust resistance QTL is (or are) located in a very small
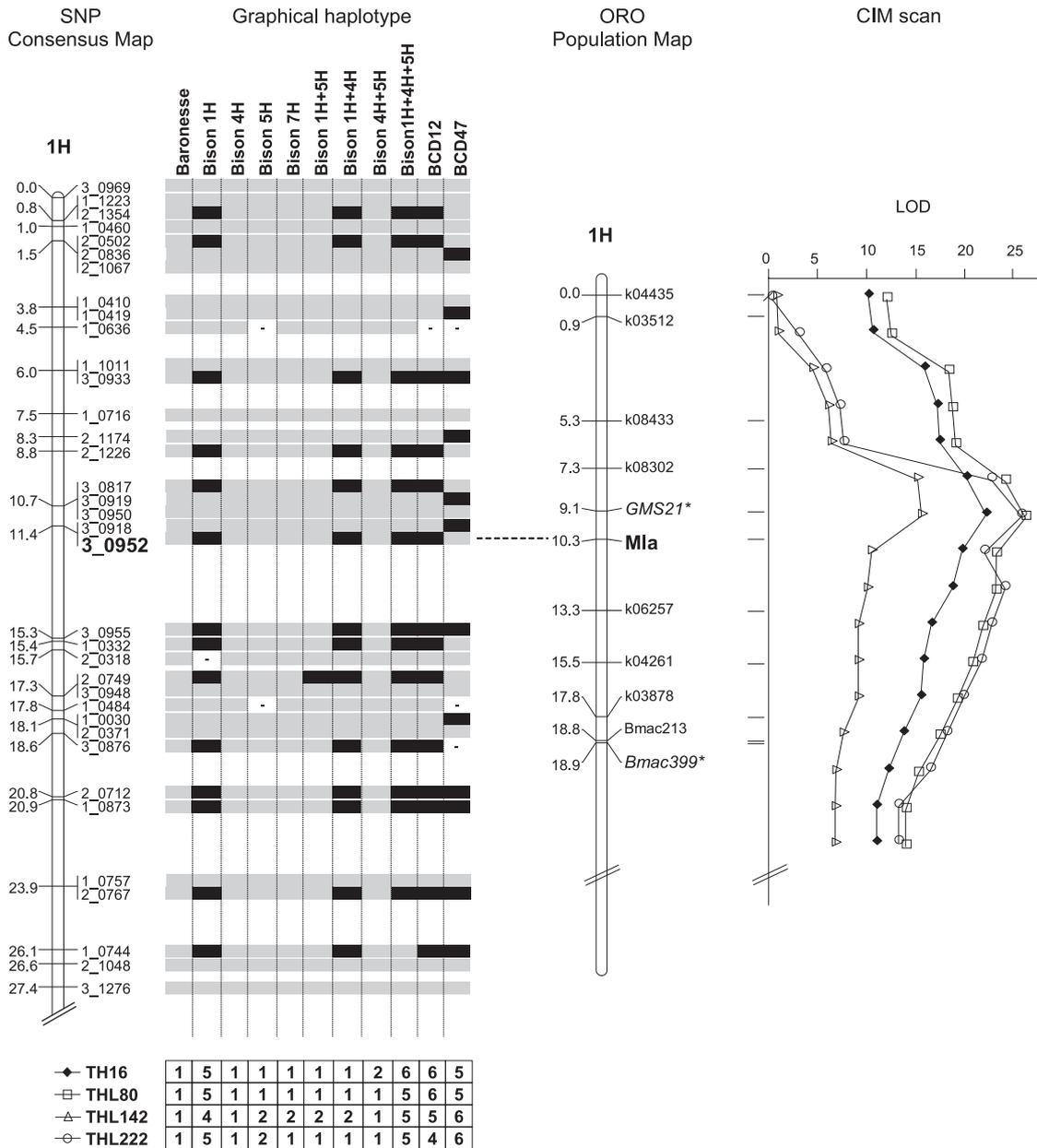
Fig. 3. The barley SNP consensus linkage map for the short arm of chromosome 1H (left panel) aligned with graphical SNP haplotypes for BISON lines (second panel from left to right), the ORO mapping population linkage map (third panel from left to right) and leaf blast resistance QTL scans for response to inoculation with each of four isolates of rice blast collected in Thailand (TH16, THL80, THL142 and THL222; fourth panel from left to right). Linkage map and BISON haplotype alignments are based on locus 3_0952 in the SNP consensus map and *Mla* in the ORO population map. The markers used for the MAS development of the BISON lines (GMS21 and Bmac399) are shown in italics and with an "*" on the ORO map. The leaf blast disease scores for each of four isolates (TH16, THL80, THL142 and THL222) are shown for the BISON lines, recurrent parent (Baronesse) and donor parents (BCD12 and BCD47) in the table below the graphical haplotypes.

introgression in BISON 1H + 5H. Following this line of reasoning, higher density mapping in BISON 1H + 4H might reveal retention of a small segment (in terms of map distance) of Baronesse chromosome 1 carrying the LB and NB resistance QTL. Small map (genetic) distances in barley can be very large physical distances (Künzel *et al*. 2000). Because BISON 1H + 5H is resistant to LB, NB and stripe rust, it could be of practically direct utility in breeding varieties with resistance to the three diseases. An alternative explana-

tion of the data is that the 1H + 4H BISON does not have the Baronesse resistance allele on 1H and that LB resistance is due to resistance alleles at QTL elsewhere in the genome (e.g. 4H and/or 7H). BISON 1H + 4H does have the BCD 47 resistance allele introgressed on 4H but this QTL allele alone would not be expected to lead to the high level of resistance (1–2) that was observed.

In conclusion, this research demonstrates the power of QTL mapping in a doubled haploid population, coupled with

validation in NILs, to detect genes conferring resistance to leaf and blast in barley. Because the BISON were developed when only a limited catalog of low-density makers was available for MAS, the extent of the introgression regions varies in each line and the introgressions were not strictly limited to the target QTL alleles. The high density SNP haplotypes do provide a useful retrospective view of the results of MAS in each isoline and do provide a basis for the future development of truly isogenic lines. BISON 1H + 4H may provide a useful starting point for isolating the LB resistance gene (or genes) on 1H and thus providing additional perspectives on the genetic basis of broad-based resistance. However, proving which gene, or genes, in the vicinity of *Mla* is responsible for LB resistance could be challenging: there are at fifteen genes associated with plant disease responses in a 261 kb nucleotide sequence that includes *Mla* in the cultivar Morex (Wei *et al*. 2002).

Acknowledgments

Literature Cited

Bonman, J.M., B.A. Estrada and J.M. Bandong (1989) Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Dis. 73: 388–390.

Bonman, J.M. (1992) Durable resistance to rice blast disease-environmental influences. Euphytica 63: 115–123.

Castro, A.J., F. Capettini, A.E. Corey, T. Filichkina, P.M. Hayes, A. Kleinhofs, D. Kudrna, K. Richardson, S. Sandoval-Islas, C. Rossi and H. Vivar (2003) Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. Theor. Appl. Genet. 107: 922–930.

Chen, H., S. Wang, Y. Xing, C. Xu, P.M. Hayes and Q. Zhang (2003) Comparative analyses of genomic locations and race specificities of loci for quantitative resistance to *Pyricularia grisea* in rice and barley. Proc. Natl. Acad. Sci. USA 100: 2544–2549.

Costa, J.M., A. Corey, P.M. Hayes, C. Jobet, A. Kleinhofs, A. Kopisch-Obusch, S.F. Kramer, D. Kudrna, M. Li, O. Riera-Lizarazu, K. Sato, P. Szucs, T. Toojinda, M.J. Vales and R.I. Wolfe (2001) Molecular mapping of the Oregon Wolfe Barleys: a phenotypically polymorphic doubled-haploid population. Theor. Appl. Genet. 103: 415–424.

International Rice Research Institute (1996) Standard evaluation system for rice, 4th ed. International Rice Research Institute, Manila, Philippines.

Inukai, T., M.I. Vales, K. Hori, K. Sato and P.M. Hayes (2006) *RMo1* confers blast resistance in barley and is located within the complex of resistance genes containing *Mla*, a powdery mildew resistance gene. Mol. Plant Microbe Interact. 19: 1034–1041.

Kølster, P., L. Munk, O. Stølen and J. Løhder (1986) Near-Isogenic barley lines with genes for resistance to powdery mildew. Crop Sci. 26: 903–907.

Kongprakhon, P., A. Cuesta-Marcos, P.M. Hayes, V. Hongtrakul, P. Sirithunya, T. Toojinda and N. Sangduen (2009) Four QTL in rice associated with broad spectrum resistance to blast isolates from rice and barley. J. Phytopathol. (in press).

Kosambi, D.D. (1944) The estimation of map distance from recombination values. Ann. Eugen. 12: 172–175.

Künzel, G., L. Korzun and A. Meister (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics 154: 397–412.

Mains, E.B. and S.M. Dietz (1930) Physiologic forms of barley mildew, *Erysiphe graminis hordei* Marchal. Phytopathol. 20: 209–239.

Miura, Y., C. Ding, R. Ozaki, M. Hirata, M. Fujimori, W. Takahashi, H. Cai and K. Mizuno (2005) Development of EST-derived CAPS and AFLP markers linked to a gene for resistance to ryegrass blast (*Pyricularia* sp.) in Italian ryegrass (*Lolium multiflorum* Lam.). Theor. Appl. Genet. 111: 811–818.

Ou, S.H. (1985) Rice Diseases, 2nd ed. Kew, Surrey, UK, Commonwealth Mycological Institute, Association of Applied Biology.

Puri, K.D., S.M. Shrestha, G.B. Khhatri Chhetri and K.D. Joshi (2009) Leaf and neck blast resistance reaction in tropical rice lines under green house condition. Euphytica 165: 523–532.

Richardson, K.L., M.I. Vales, J.G. Kling, C.C. Mundt and P.M. Hayes (2006) Pyramiding and dissecting disease resistance QTL to barley stripe rust. Theor. Appl. Genet. 113: 485–495.

Rossi, C., A. Cuesta-Marcos, I. Vales, L. Gomez-Pando, G. Orjeda, R. Wise, K. Sato, K. Hori, F. Capettini, H. Vivar, X. Chen and P. Hayes (2006) Mapping multiple disease resistance genes using a barley mapping population evaluated in Peru, Mexico, and the USA. Mol. Breed. 18: 355–366.

Roumen, E.C., J.M. Bonman and J.E. Parlevliet (1992) Leaf age related partial resistance to *Pyricularia oryzae* in tropical lowland rice cultivars as measure by the number of sporulating lesions. Phytopathology 82: 1414–1417.

Sato, K., T. Inukai and P.M. Hayes (2001) QTL analysis of resistance to the rice blast pathogen in barley (*Hordeum vulgare*). Theor. Appl. Genet. 102: 916–920.

Szücs, P., V.C. Blake, P.R. Bhat, S. Chao, T.J. Close, A. Cuesta-Marcos, G.J. Muehlbauer, L. Ramsay, R. Waugh and P.M. Hayes (2009) An integrated resource for barley linkage map and malting quality QTL alignment. The Plant Genome (in press).

Urashima, A.S., N.A. Lavorent, A.C.P. Goulart and Y.R. Mehta (2004) Resistance spectra of wheat cultivars and virulence diversity of *Magnaporthe grisea* isolates in Brazil. Fitopatol bras 29: 511–518.

Van Ooijen, J.W. and R.E. Voorrips (2001) JoinMap 3.0. Software for the calculation of linkage maps. Plant Research International, Wageningen, the Netherlands.

Wang, S., C.J. Basten and Z.B. Zeng (2007) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Wei, F., R.A. Wing and R.P. Wise (2002) Genome dynamics and evolution of the *Mla* (Powdery Mildew) resistance locus in barley. Plant Cell 14: 1903–1917.

Williams, D.W., P.B. Burrus and P. Vincelli (2001) Severity of gray leaf spot in perennial ryegrass as influenced by mowing height and nitrogen level. Crop Sci. 41: 1207–1211.

Yaegashi, H. (1988) Inheritance of blast resistance in two-rowed barley. Plant Dis. 72: 608–610.

Zeng, Z.B. (1994) Precision mapping of quantitative trait loci. Genetics 136: 1457–1468.

Zeng, Z.B., C.H. Kao and C.J. Basten (1999) Estimating the genetic architecture of quantitative traits. Genet. Res. 74: 279–289.