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G. Backes · L. H. Madsen · H. Jaiser · J. Stougaard
M. Herz · V. Mohler · A. Jahoor

Localisation of genes for resistance against *Blumeria graminis* f.sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line

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Abstract The aims of this investigation have been to map new (quantitative) resistance genes against powdery mildew, caused by *Blumeria graminis* f.sp. *hordei* L., and leaf rust, caused by *Puccinia hordei* L., in a cross between the barley (*Hordeum vulgare* ssp. *vulgare*) cultivar "Vada" and the wild barley (*Hordeum vulgare* ssp. *spontaneum*) line "1B-87" originating from Israel. The population consisted of 121 recombinant inbred lines. Resistance against leaf rust and powdery mildew was tested on detached leaves. The leaf rust isolate "I-80" and the powdery mildew isolate "Va-4", respectively, were used for the infection in this experiment. Moreover, powdery mildew disease severity was observed in the field at two different epidemic stages. In addition to other DNA markers, the map included 13 RGA (resistance gene analog) loci. The structure of the data demanded a non-parametric QTL-analysis. For each of the four observations, two QTLs with very high significance were localised. QTLs for resistance against powdery mildew were detected on chromosome 1H, 2H, 3H, 4H and 7H. QTLs for resistance against leaf rust were localised on 2H and 6H. Only one QTL was common for two of the powdery mildew related traits. Three of the seven QTLs were localised at the positions of the RGA-loci. Three of the five powdery mildew related QTLs are sharing their chromosomal position with known qualitative resistance genes. All detected QTLs behaved additively. Possible sources of the distorted segregation observed, the differences between the results for the different powdery mildew related traits and the relation between qualitative and quantitative resistance are discussed.

Keywords QTL · *Hordeum* · *Blumeria graminis* · *Puccinia graminis* · Resistance gene analogs

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G. Backes (✉) · A. Jahoor
Plant Research Department, Risø National Laboratory,
P.O. box 49, DK-4000 Roskilde, Denmark
e-mail: gunter.backes@risoe.dk
Tel.: +45-4677-4115, Fax: +45-4677-4122

L.H. Madsen · J. Stougaard
Department of Molecular and Structural Biology,
Laboratory of Gene Expression, University of Aarhus,
Gustav Wieds vej 10, DK-8000 Aarhus C, Denmark

H. Jaiser
Pajbjergfonden, Gersdorffslundvej 1, DK-8300 Odder, Denmark

M. Herz · V. Mohler
Lehrstuhl für Pflanzenbau und Pflanzenzüchtung,
Department Pflanzenwissenschaften,
Wissenschaftszentrum Weihenstephan für Ernährung,
Landnutzung und Umwelt, Technische Universität München,
Alte Akademie 12, D-85350 Freising-Weihenstephan, Germany

Current address:
M. Herz, Bayerische Landesantalt für Bodenkultur und Pflanzenbau,
Am Gereuth 2, D-85354 Freising-Weihenstephan, Germany

Introduction

Powdery mildew caused by *Blumeria graminis* f.sp. *hordei* and leaf rust caused by *Puccinia hordei* are two of the economically most important foliar diseases on barley (*Hordeum vulgare*) in the temperate climate zone, as these diseases can reduce the kernel yield of the affected plants dramatically (Griffey et al. 1994; Conry and Dunne 2001). Genetic resistance is the most economic and sustainable way to control these diseases in barley. However, the restricted availability of new resistance genes in the gene pool of cultivated barley and a rapid development of the pathogen toward new virulences (Hovmøller et al. 2001) forces the breeders to look for effective resistance genes in the wild relatives of this crop. In the past, wild barley (*H. vulgare* ssp. *spontaneum*), the ancestor of the cultivated barley, has shown its ability to provide the barley breeders with numerous effective resistance genes (Jahoor and Fischbeck 1993; Schönfeld et al. 1996; Sato and Takeda 1997; Ivandic et al. 1998; Zeller 1998; Garvin et al. 2000).

By the availability of dense linkage maps of the barley genome (Graner et al. 1991; Kleinhofs et al. 1993) and progresses in the field of molecular genetics, two major

Table 1 Primer for *Pst*I/*Msp*I-AFLP reaction

*Pst*I–adaptor	5′-GAAGGAGCTCTAGAAGA-3′ 3′-CCTCGAGATCTTCTGC-5′
*Msp*I–adaptor	5′-ACACGAATTCGGATCCGTGCA-3′ 3′-GCTTAAGCCTAGGC-5′
*Pst*I + 0 – primer	5′-ATTRCGGATCCGTGCAG-3′
*Msp*I + 0 – primer	5′-GGAGCTCTAGAAGACGG-3′

advancements were made in the scientific area of the resistance of barley against diseases: individual gene loci (quantitative trait loci, QTLs) have been identified and mapped as the cause for quantitatively inherited resistance (Heun 1992; Backes et al. 1995) and genes for specific resistance reaction were cloned and their function characterized (Buschges et al. 1997; Zhou et al. 2001).

Quantitative resistance is characterized by a more or less continuous transition from susceptible to resistant genotypes in a segregating population, while clear groups of resistant and susceptible lines can be defined for qualitative genes. For quantitative resistance against powdery mildew and leaf rust in barley, QTLs were localized in different genetic backgrounds (Heun 1992; Chen et al. 1994; Thomas et al. 1995; Backes et al. 1996; Qi et al. 1998; Spaner et al. 1998; Falak et al. 1999; Kicherer et al. 2000; Toojinda et al. 2000).

To finally isolate QTLs and thereby to gain knowledge about the function of these genes, several approaches were proposed and applied. One of these approaches includes the marker-assisted generations of backcrosses to decrease the chromosomal fragment, where the respective QTL is localised (Young 1996). Alternatively, candidate genes (DNA markers with known function in plant resistance reactions, such as glucanases, chitinases or PR-proteins) and/or Resistance Gene Analogs (RGAs) can be included in the linkage maps for QTL analysis in order to draw conclusions from possible co-localisations of QTLs and candidate genes and/or RGAs (Pecchioni et al. 1999; Pflieger et al. 1999; Geoffrey et al. 2000). RGAs are DNA-markers representing sequences containing motifs typical for most (hyper sensitively acting) plant disease resistance genes like nucleotide binding sites (NBS), leucine rich repeats (LRR) or kinase domains (Staskawicz et al. 1995).

In the present investigation, quantitative resistance against powdery mildew and leaf rust has been examined in a population derived from a cross between a wild barley line (*H. vulgare* ssp. *spontaneum*) and a barley cultivar. RGAs have been included to test for eventual co-localisation between resistance-QTLs and these markers. The presence of such co-localisations is a first step toward the isolation of these QTLs and a deeper insight into the function of these genes. This is also a starting point for a discussion about the concept of "quantitative resistance".

Materials and methods

Plant material

The population used for mapping consisted of 121 recombinant inbred (RI) lines from a cross between the Israeli *H. vulgare* ssp. *spontaneum* line ''1B-87" and the two-row spring barley (*H. vulgare* ssp. *vulgare*) cultivar "Vada". This cultivar is known to possess *MlLa* powdery mildew resistance (Schwarzbach and Fischbeck 1981). Beside that, "Vada" is known to have a high level of quantitative resistance against leaf rust (Parleviet 1975). The wild barley line ''1B-87" was collected in Israel in 1975 (Fischbeck 1981) and showed a high level of resistance both against leaf rust and powdery mildew in the field trial.

Disease assessments

Powdery mildew infection was observed in a field experiment in 1998 on the site of the plant breeder Pajbjergfonden, Horsens, Denmark. The plants were sown 10-04-98 as two rows per line with a susceptible spreader in-between. Disease severity was screened at the beginning (11-06-98) and the maximum stage of disease development (06-07-98) in a scale from 1 (resistant) to 9 (susceptible). Furthermore, powdery mildew resistance was assessed in an experiment on detached leaf segments on a water-based agar as described in Backes et al. (1996). In this experiment, the isolate "Va-4", that is virulent against the *MlLa* resistance, was applied on ten leaf segments of each RI-line. Each leaf segment was scored individually from 1 (resistant) to 9 (susceptible) and the mean of the ten segments was assigned to the respective line.

For the exploration of leaf rust resistance, a modified leaf segment test according to Walther (1991) was performed. In this test, 4–3 leaf segments of each line were infected with the highly virulent leaf rust isolate I-80 that overwhelms all recently known major resistance genes except *Rph7*. After the appearance of symptoms, the leaf segments were screened on 3 subsequent days. The area under the disease progress curve (AUDPC) was computed and expressed as the percent of the AUDPC value of "Vada" from the same infection.

Marker assays

Two to three leaves were freeze-dried, ball-milled with two 3-mm steel-balls in a ball-mill (Retsch) and plant DNA was isolated in a 2 ml reaction tube according to the CTAB-protocol (Saghai-Maroof et al. 1984).

RFLP analyses were performed according to Kicherer et al. (2000). The primers used for microsatellite analysis and their PCR conditions are described in Ramsay et al. (2000). *Eco*RI/*Mse*I AFLPs were produced by applying the protocol for the Perkin-Elmer AFLP Plant Mapping Kit (Perkin-Elmer 1997) but by using custom-made labelled primer sets. Primers for *Pst*I/*Msp*I AFLPs are listed in Table 1. Promega *Taq*-polymerase was used for the PCR reactions with the microsatellite and AFLP primers. The detection of microsatellite- and AFLP-pattern was carried out on an ABI Prism 377 sequencer.

For the development of resistance gene analog primers, PCR fragments derived from barley resistance gene analogs were originally obtained using degenerate oligonucleotide primers corresponding to variants of the conserved P-loop Kin-1a GMGGV/IGKTT and GLPL motifs of the NBS-LRR type of disease resistance genes (Staskawicz et al. 1995) to amplify *H. vulgare* cv Regatta DNA (Madsen et al., in preparation). To map individual RGAs, primers detecting allelic differences between ''1B-87" and "Vada" were developed and used to determine the genotype of the individual RI-lines of the segregating population.

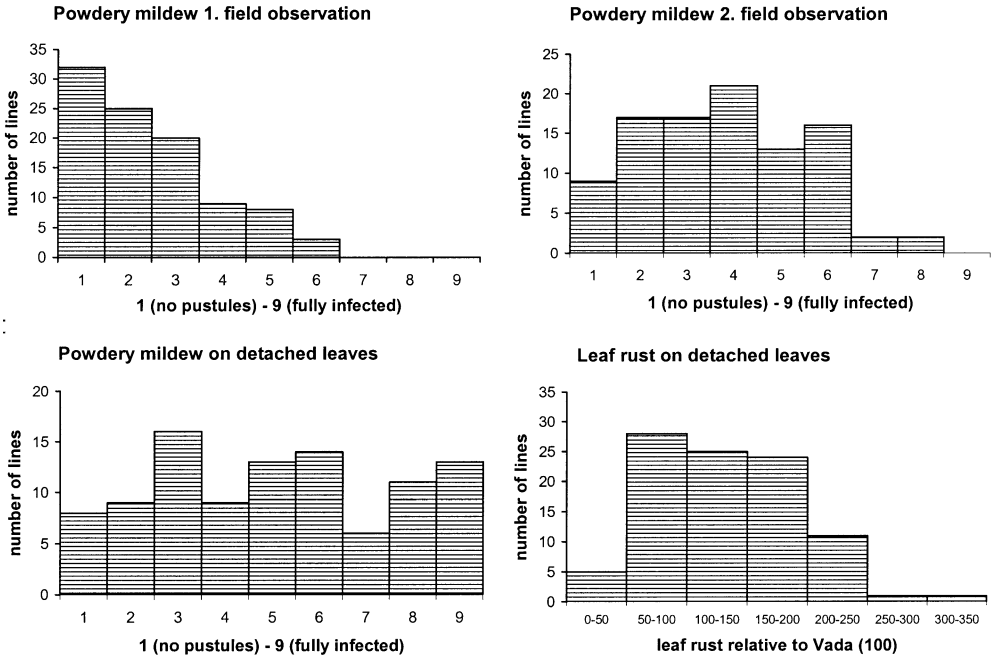

Fig. 1 Distributions over lines for the different traits observed

Mapping

The linkage groups were established by using LOD-data as calculated by JOINMAP 2.0 (Stam and Van Oijen 1995) to cluster the markers into linkage groups. Ordering of the markers was carried out in GMendel W32 v.8.0b (Holloway and Knapp 1993) using the 'kSAR' function. To avoid ordering to be influenced by the starting order of loci, a Monte Carlo simulation was performed with 200 replications with the 'monte' function of this programme. Markers showing a high variance in this programme were checked for genotyping errors and, consequently, some were excluded from the map. The resulting unambiguous map was checked by using the 'SAL' ordering function in GMendel. Distorted segregation of the markers was investigated by a χ^2-test in GMendel.

QTL analysis

Trait data were inspected for outliers and the distribution over the lines of the population. Deviation from normality was analysed by the Shapiro-Wilk test using STATISTICA software package (StatSoft Inc 2001). A non-parametric QTL analysis was performed using the Kruskal-Wallis rank sum test as a procedure of the MapQTL programme v. 3.0 (Van Oijen and Maliepaard 1996). The maximum individual error probability for accepting the position of a QTL in this experiment was chosen to be 0.0005. This corresponds to an overall error probability of 0.05, with 100 unlinked markers applying a standard Bonferroni correction. As this correction is exact only for unlinked experiments, this procedure is very conservative concerning the acceptance of QTLs (Lynch and Walsh 1998). Additivity of the QTL effects was tested by a goodness of fit-test of the expected values for groups of marker patterns under the assumption of additivity against the observed values.

Results

Trait data

The distribution of the different disease observations in the field and on detached primary leaves is shown in Fig. 1. None of the observations showed a normal (Gaussian) distribution. As expected, the second field observation for powdery mildew showed, on average, higher values than the first field observation.

Linkage map and marker allele segregation

For the map construction, marker data from 367 loci were available. Of these, 322 were assigned to linkage groups. Further loci were excluded during the mapping procedure as they showed only one or no recombination event with other loci and/or led to ambiguities in the ordering as revealed by the Monte Carlo simulation in GMendel, thus either indicating potential errors in genotyping (especially with dominant marker types) or erroneous attachment to a group. The largest reductions happened in linkage group 2Hb (from 50 to 26 loci) and 7H (from 62 to 30 loci). The final map consisted of 221 loci: 58 RFLP loci, 16 microsatellite loci, 13 RGA loci and 134 AFLP loci (Fig. 2). All chromosomes but 2H are represented by one coherent linkage group. For 2H, two groups had to be defined (2Ha and 2Hb). The complete map length is 1.264 cM; the average interval length is 5.8 cM.

Distorted segregation is the deviation of segregation rations from the expected Mendelian fractions (Lyttle 1991). There are 4 groups of linked markers with distorted segregation (Fig. 2). Two are localised on 1H, both distorted toward the allele from "1B-87" and two groups on 3H and 6H, respectively. In the latter group, the "Vada"-allele is predominant in the population. In all the four groups, a clear peak of distorted segregation, more or less in the middle of the respective group is observed. For the two groups on 1H, the markers with the highest

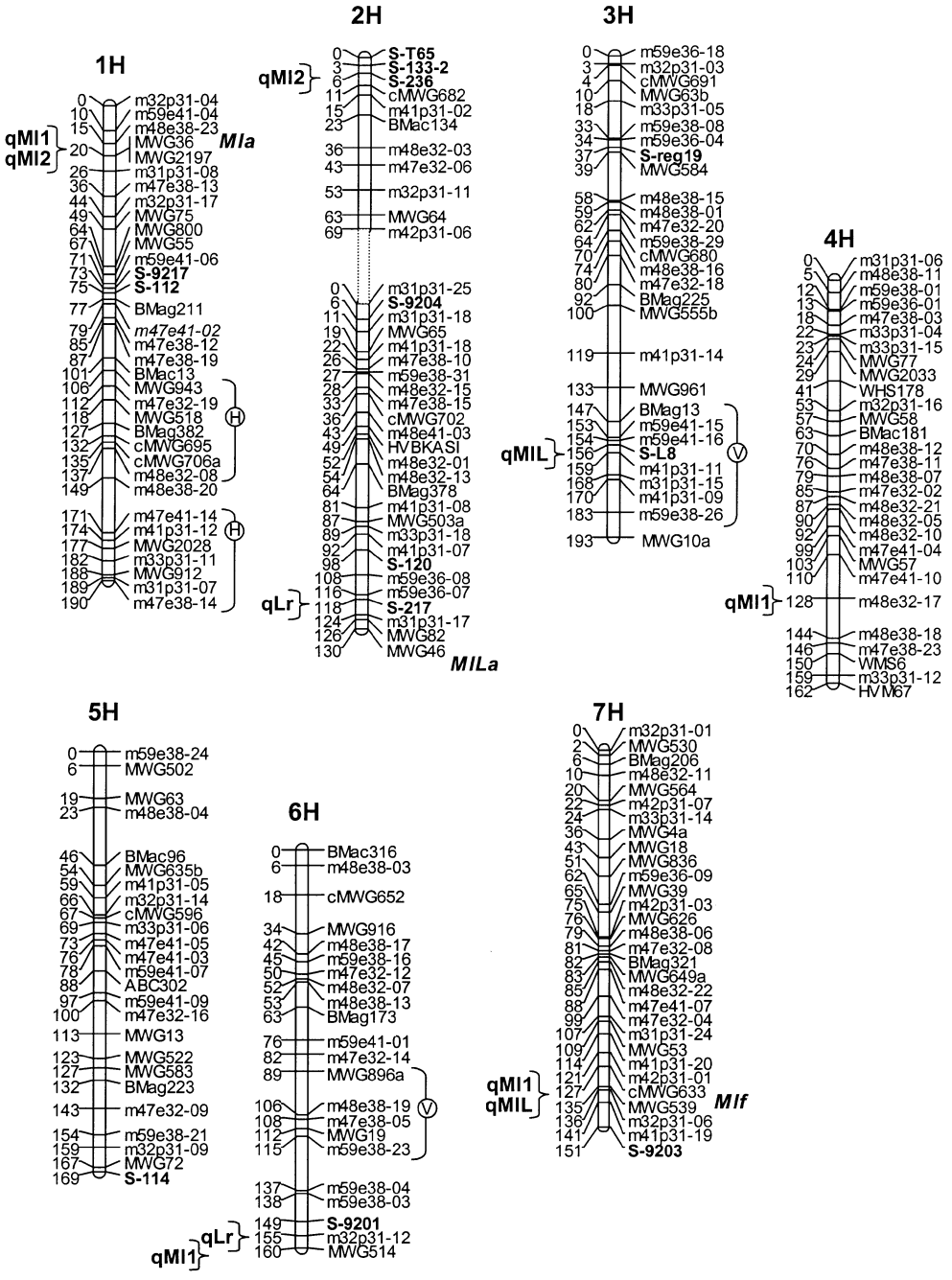

Fig. 2 Linkage map of the population "1B-87 × Vada" including QTL-positions {with curved half-brackets: qM11 = powd. mildew 1. field obs., qM12 = powd. mildew 2. field obs., qMIL = powdery mildew on detached leaves, qLr = leaf rust field obs.}, known qualitative powdery mildew resistance genes in the vicinity of the QTLs and assignment of markers with distorted segregation [with squared brackets], "V" in favour of the "Vada" allele and "H" for distortion in favour of the allele from *H. spontaneum* beside the marker with the highest distortion

distorted segregation were the RFLP-marker MWG518 with a ratio of lines with the "Vada"-allele: lines with the "1B-87"-allele of 1:3.1 and the AFLP-marker m47p31-12 with a ratio of 1:2.3. The maximum distorted segregation on 3H is shown by the RGA-marker S-L8 (ratio 3.5:1) and on 6H by the AFLP-marker m48e38-19 (1.8:1).

QTL analysis

As all traits deviated significantly from normality, as analysed by a Shapiro-Wilk test, non-parametric QTL analysis was performed. The markers with significant Kruskal-Wallis- (K-) values for at least one of the powdery mildew related observations, their position on the chromosomes and the K-values and significances for all three powdery mildew-related observations are shown in Table 2. The markers with a significant K-value for leaf rust are revealed in Table 3.

Table 4 displays the pattern group means for the significant markers together with their positions on the chromosomes for powdery mildew and leaf rust. Figure 2 shows the position of these significant QTLs on the linkage map.

For the first field observation, QTLs were found on chromosomes 4H with the highest K-value at the posi-

Table 2 QTLs localised for the different powdery mildew resistance observations

Chrom.	Pos.	Marker	1. Field observ.		2. Field observ.		Detached leaves	
			K	Error	K	Error	K	Error
1H	19.5	MWG2197	8.4	<0.005	20.2	<0.0001	3.7	>0.05
2Ha	5.8	S-236	7.5	<0.01	14.8	<0.0001	4.6	<0.05
3H	156.2	S-L8	3.5	>0.05	5.2	<0.05	13.6	<0.0005
4H	127.7	m48e32-17	12.8	<0.0005	4.1	<0.05	10.8	<0.005
7H	134.8	MWG539	12.4	<0.0005	6.1	<0.05	16.7	<0.0001

Legend: 'Chrom' = chromosome where the marker is located, 'Pos.' = position of the marker on this chromosome, 'Marker' = marker name, 'K' = K-value from Kruskal-Wallis test, 'Error' = error probability for dividing the population into two groups according to the respective marker label

Table 3 QTLs localised for leaf rust

Chrom.	Pos.	Marker	Leaf rust.	
			K	Sign.
2Hb	118,2	S-217	13.4	<0.0005
6H	155.2	m32p31-12	20.1	<0.0001

Legend see Table 2

Table 4 Means for groups of lines with the same marker pattern at highly significant marker positions

Chrom.	Pos.	Marker	'Vada'	'1B-87'
Powdery mildew, 1. observation				
4H	127.7	m48e32-17	2.92	1.81
7H	134.8	MWG539	2.85	1.59
Powdery mildew, 2. observation				
1H	19.5	MWG2197	4.85	2.69
2Ha	5.8	S-236	3.05	4.53
Powdery mildew on detached primary leaves				
3H	156.2	S-L8	5.96	2.82
7H	134.8	MWG539	6.51	3.25
Leaf rust				
2Hb	118.2	S-217	160%	108%
6H	155.2	m32p31-12	183%	112%

Legend: 'Chrom' = chromosome where the marker is located, 'Pos.' = position of the marker on this chromosome, 'Marker' = marker name, 'Vada' = mean of the group with the 'Vada'-pattern for the respective marker, '1B-87' = mean of the group with the '1B-87'-pattern for the respective marker

tion of the AFLP-marker m48e32-17 and on 7H with the highest K-value at the position of the RFLP-marker MWG 539. The difference between the means of groups with the marker alleles from either "Vada" or "1B-87" was 1.1 and 1.3 points respectively on the 1–9 scale. The data from the second field observations proposed QTLs on the chromosomes 1H and 2Ha near or at the positions of the RFLP-marker MWG2197 and the RGA-marker S-236. The differences between the means of marker-allele groups were 2.2 and 1.5 points respectively. QTLs

Table 5 Check for additivity of the marker effects

Powdery mildew, 1. observation

$\chi^2 = 0.207$ ($p = 0.976$)

4H	7H	Exp.	Obs.	n
V	V	3.61	3.43	21
V	H	2.51	2.20	15
H	V	2.35	2.13	16
H	H	1.25	1.67	24

Powdery mildew, 2. observation

$\chi^2 = 0.131$ ($p = 0.988$)

1H	2H	Exp.	Obs.	n
V	V	4.14	3.94	18
V	H	5.62	5.55	22
H	V	1.98	2.41	17
H	H	3.46	3.15	20

Powdery mildew, detached leaves

$\chi^2 = 1.424$ ($p = 0.700$)

3H	7H	Exp.	Obs.	n
V	V	8.32	6.92	26
V	H	5.06	4.62	22
H	V	5.18	5.51	11
H	H	1.92	3.40	15

Leaf rust

$\chi^2 = 10.18$ ($p = 0.017$)

2Hb	6H	Exp.	Obs.	n
V	V	203%	200%	15
V	H	131%	116%	19
H	V	149%	139%	9
H	H	77%	102%	11

Legend: the two first columns describe the allele pattern of the markers with highly significant Kruskal-Wallis-results, the column head is the chromosome, where the respective marker is located, 'V' is the "Vada"-pattern, 'H' is the pattern of "1B-87". 'Exp.' is the expected mean of the group under the assumption of additive QTL effects, 'Obs.' is the observed value for this group mean. 'n' is the number of observations in the respective groups. The sum of 'n' does not reflect the whole number of lines, as only lines with both respective flanking markers having the allele from the same parent were taken into account

for powdery mildew resistance revealed on detached leaves were localised on the chromosomes 3H (RGA-marker S-L8) and 7H (RFLP-marker MWG539) with a difference of the marker-allele groups of 3.1 and 3.3 points respectively. At the very conservative individual significance level of 0.0005, only the QTL on chromosome 7H was supported by two observations: the first field observation and the observation on detached leaves. Nevertheless, accepting QTLs at a lower significance level (<0.05), at all prior QTL positions, QTLs were detected for at least two powdery mildew resistance related observations (see Table 1). For all these QTLs, with the exception of the one for the second field observation on 2Ha, the resistant allele was contributed by ''1B-87" (Table 4).

For leaf rust, two QTLs were detected, one on chromosome 2Hb, near or at the RGAP-marker S-217, with a difference for the marker-allele groups of 52%, and one on 6H, near or at the AFLP-marker m32p31-12, with a marker-allele group difference of 71% (Table 3, Fig. 2). For both QTLs "1B-87" contributed the allele that resulted in the more resistant genotype.

Concerning the test for additivity (Table 5), for the powdery mildew related observations, the coincidence between the calculated and observed values was very close, thus proposing additive gene effects. For leaf rust, however, a significant deviation from the expected values was observed. Similar to the powdery mildew observations on detached leaves, the highest deviation occurred in the group, where the two resistant alleles are combined. The model including both resistant alleles resulted in a higher expected resistance level than observed from field data.

Discussion

Distorted segregation

Distorted segregation was often encountered in crosses between wild species and cultivars as well as in crosses between subspecies. This can be caused by different factors such as hybrid sterility, incompatibility and nuclear cytoplasmatic interaction (Cheng et al. 1998). In rice, where crosses between *indica*- and *japonica*-types are frequently performed, 14 gametophyte genes causing partial sterility and distorted segregation were localised as *ga-1* to *ga-14* (Lu et al. 2000). Furthermore, loci with similar effects were found in *Aegilops tauschii* (Faris et al. 1998) and barley (Konishi et al. 1990; Cheng et al. 1998). Such gametophyte genes could eventually explain the four groups of markers with distorted segregation. The *ga*-genes detected in barley were localised on 3H (*ga2*, Konishi et al. 1990), 4H (*L*, Cheng et al. 1998) and 7H (*ga*, von Wettstein-Knowles 1993). Of these, the *ga2* locus could be the reason for the distorted segregation on 3H on the linkage map presented here. The locus for brittle rachis (*btr*), which also could be a reason for distorted segregation, because of problems in harvesting the material, is also localised on 3H, but on the opposite arm of the chromosome (Franckowiak 1997).

Differences in powdery mildew related observations

Even though QTLs for one of the three powdery mildew related observations (first field observation at the beginning of the infection, second field observation at the climax of the infection and tests on detached leaves) always have correspondent peaks of the K-value with a lower significance (Table 2), the difference between the QTLs found for the different powdery mildew resistance-related observations is obvious. It is interesting to note, that the QTLs linked with the two RGA-loci cannot be detected in all three powdery mildew-related observations.

Differences between the field experiment and the experiment on detached leaves could be explained by the divergent isolate structures: on the one side the infection with the single defined isolate "Va-4", and on the other side the exposure to an unknown mixture of isolates. Isolate-specificity of quantitative resistance loci has been shown in many cases (Danesh and Young 1994; Leonards-Schippers et al. 1994; Backes et al. 1996; Caranta et al. 1997; Li et al. 1999; Qi et al. 1999). Beside that, it has been often discussed that results obtained on primary detached leaves might not be representative for the resistance of the adult plant, and that at least some of the factors effective in the field (adult-plant resistances) are not detectable at this stage (Backes et al. 1996; Kicherer et al. 2000; Qi et al. 2000).

The differences between the early and late powdery mildew field observation are more difficult to explain. The only common QTL is the one located on 1H and the first field observation shares more QTLs with the experiment on detached leaves than the second one. This could be due to the fact that different mechanisms of defence are involved in resistance. The first observation and the experiment on detached leaves are effected by resistance loci that prevent or delay the development of the pathogen until the appearance of the first colonies. The second observation is also influenced by other resistance loci reducing the propagation of the pathogen, e.g. by reducing the number of infective conidia produced by the first colonies. Besides that, the population structure of the pathogen in relation to virulence might have changed between the two observations.

Comparison with previously located resistance loci

The chromosomal positions of the QTLs localised in the present experiment were compared with the positions of known loci for qualitatively and quantitatively inherited resistance against leaf rust and powdery mildew in barley. For the comparison with the positions of powdery mildew resistance loci, a joined map including powdery mildew resistance loci by Mohler (1997) was used. The

map of Qi et al. (1998), together with the map of Becker et al. (1995), was applied to look for co-localisations with known qualitative and quantitative leaf-rust genes.

The closest coincidences were found on chromosome 6H and on 2H. On chromosome 6H, a QTL for quantitatively inherited resistance against powdery mildew was detected about 25 cM proximal from the position of the RFLP-marker MWG514 (Backes et al. 1995). On chromosome 2H, a locus for quantitatively inherited resistance against leaf rust (*Rphq2*) was localised about 20 cM distally from the position of the RGAP-marker S-217 by Qi et al. (1998). Taking into account the relatively large confidence interval for the localistation of QTLs and the element of uncertainty added by the indirect comparison of map positions, these QTLs detected in this experiment possibly confirm the ones localised before on 2H and 6H.

It is interesting to note that "Vada", which was the resistant parent in the population explored by Qi et al. (1998, 1999) and led to the localisation of ten QTLs for leaf rust resistance, all with the resistant allele contributed by this variety, did not show up with any QTL in this analysis. This may partially be due to the fact that an isolate used in this case (I-80) was chosen to show differences between the "1B-87" and "Vada". In the experiment mentioned above, Qi et al. (1999) also observed a certain amount of isolate specificity. Furthermore, if we accept that the QTL for leaf rust resistance on chromosome 2H is the same as the one detected by Qi et al. (1998, 1999), then we must conclude that the allele of "1B-87" at this QTL results in interaction with isolate "I-80", in a considerably higher quantitative resistance as the allele of "Vada" even though the same "Vada"-allele appeared in the experiment of Qi et al. (1999) as a major genetic component conferring resistance to this variety.

There might also be QTLs not detected in this experiment, because of the non-parametric method that had to be used and only has a restricted power to detect QTLs, the limited size of the population or because of chromosome regions not covered by the linkage map. The fact, that the leaf rust resistance of the RI-population ranged from 13% to 350%, with the AUDPC-value of "Vada" given as 100%, shows that there must be genetic factors in "Vada" conferring quantitative resistance.

In comparison with the position of loci for qualitatively inherited resistance, several co-localisations were observed. The strong QTL for powdery mildew resistance in the first and the second observation on chromosome 1H (near or at the position of the RFLP-loci MWG36 and MWG2197) is localised quite precisely at the position of the *Mla*-powdery mildew resistance locus. The same is true for the QTL for powdery mildew resistance in the first observation on chromosome 4H (near or at the position of the AFLP-marker m48-e38-18) and the position of the *mlo*-powdery mildew resistance locus (compared with Thomas et al. 1998). Finally, at the position of the *Mlf*-locus on 7H, a QTL for powdery mildew resistance in the first field observation and in the test on detached leaves (at or near the position of the RFLP-marker MWG539) was observed. The *MlLa*-locus is situated distal to the last marker on chromosome 2H, MWG46. Therefore, no QTL could be detected at this position in the field experiments. On detached leaves, the isolate used was virulent against the *MlLa* resistance to exclude an influence of the *MlLa* locus on the results of this experiment.

For the two QTLs for leaf rust resistance, these co-localisations of loci for qualitative and quantitative did not appear. Nevertheless, a gene for qualitative resistance against barley stripe disease, which first has been identified in "Vada" (Skou and Haahr 1987) on 2H, is localised in the same region as the QTL for resistance against leaf rust (near or at the position of the RGAP-locus S-217). This could be similar to the co-localisation of the resistance gene *Pm1* against *Blumeria graminis*, the resistance gene *Lr20* against *Puccinia triticina* (former *Puccinia recondita* f.sp. *tritici*) and the resistance gene *Sr15* against *Puccinia graminis* on chromosome 7A of wheat (Hart et al. 1993). The differences between the observations for powdery mildew and leaf rust concerning these co-localisations could simply be due to the lower number of QTLs for leaf rust (two compared to five for powdery mildew) detected in this investigation.

Quantitative and qualitative resistances

Two results of the present investigation show the tight relationship between so-called "qualitative" and "quantitative" resistances: the co-localisation of loci for quantitative and qualitative resistance and the co-localisation of QTLs and RGAs. The latter is true, as the conserved motifs, which were used to obtain the RGAs, were taken, as mentioned above, from NBS-LRR type disease resistance genes. These resistance genes are known to cause qualitative resistance against the respective pathogens. Caranta et al. (1997) detected QTLs for resistance against potyvirus Y and potyvirus E in potatoes in the vicinity of previously located qualitative resistances. Li et al. (1999) found a QTL for resistance against *Xanthomonas oryzae* in rice at the position of the qualitative resistance gene *Xa4*. Keller et al. (1999) localised a QTL for resistance of wheat against powdery mildew at or nearby the position of the qualitative *Pm5* resistance locus. Geffroy et al. (2000) found QTLs for partial resistance against *Colletotrichum lindemuthianum* in common bean to be partially localised at positions of RGAs and one QTL in a chromosomal position of a previously localised qualitative gene. This tight relation between "qualitative" and "quantitative" resistance is further supported by the fact that the effect of resistance-QTLs can differ significantly using different defined isolates (Danesh and Young 1994; Leonards-Schippers et al. 1994; Caranta et al. 1997; Li et al. 1999; Qi et al. 1999) and that often resistance is determined genetically by one or two major genes together with several minor genes (Pecchioni et al. 1996; van der Voort et al. 1998; Kicherer et al. 2000).

The co-localisation of qualitative and quantitative resistance can be caused by several factors:

(1) a gene at a QTL results in complete susceptibility against the isolates with virulence and complete resistance against the avirulent isolates, but the inoculum is a mixture of isolates and therefore, only a part of the pathogen population is affected. (Keller et al. 1999).

(2) Combinations of qualitative resistance genes can result in higher resistance levels than the individual genes, as demonstrated for leaf rust in wheat by Kloppers and Pretorius (1997). This could produce results like the ones for the leaf rust QTLs in this investigation, where the effect of the combination of resistant alleles is lower than expected by the assumption of an additive model.

(3) A "defeated" qualitative resistance gene acts as a quantitative gene. An example of this kind of behaviour has been found in the investigation of Li et al. (1999) where one locus was identified as qualitatively acting after infection with one isolate and as a QTL after infection with two other isolates.

(4) A qualitative resistance locus represents an "extreme" allele of a quantitative locus.

(5) As most resistance genes seem to have a clear tendency to cluster in multigene families (Yu et al. 1996; Wei et al. 1999), some QTLs could be "mild" members of these families.

(6) As regulatory elements and genes necessary for complementary factors in resistance reaction can be located in close linkage to the qualitative resistance genes (Kalavacharla et al. 2000), these genes can be localised as QTLs near the respective qualitative genes.

Nevertheless, it should be clearly stated, that QTLs can be of a completely different nature than qualitative resistance genes. Zhu et al. (1999) co-localised QTLs for *Fusarium* head blight resistance and QTLs for inflorescence traits and plant height. Taller plants with longer, more open inflorescences and insignificant lateral florets were more resistant than the opposite plant type. In this case, QTLs for plant architecture have probably pleiotropic effects on disease resistance.

Looking at all these facts and arguments, it might be concluded, that the terms "qualitative resistance" and "quantitative resistance" might not be used to define two different kinds of resistance as originally intended by Van der Plank (1968). Basically, "quantitative" and "qualitative" describe only the way one is treating the data: if we can not see clear Mendelian groups, we have to use quantitative methods. The additional benefit of these quantitative methods is that we can find additional loci, interactions between loci and interactions between loci and environmental factors as shown by Chantret et al. (2000) for the powdery mildew resistance gene *MlRE* in wheat.

Parleviet (1975) differentiated a "hypersensitive" and a "partial" type of resistance, where the latter is not based on hypersensitivity, is race-non specific and more durable. This definition conflicts partially with actual results, as significant interaction between the genotypes of the pathogen and the plant are described to occur also in partial resistance (Qi et al. 1999). Moreover, in the near future, the description of the resistance mechanisms as the absence of one type of mechanism (hypersensitivity) might no longer be satisfying. As we are moving toward to a better understanding of the mechanisms involved in plant disease resistance, a characterisation of resistance loci by function will be possible. Techniques like RGAs and candidate gene identification will contribute to this development.

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