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Molecular marker analyses of powdery mildew resistance in barley

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Abstract Powdery mildew, caused by *Eryisphe graminis* f. sp. *hordei*, is one of the most important diseases of barley (*Hordeum vulgare*). A number of loci conditioning resistance to this disease have been reported previously. The objective of this study was to use molecular markers to identify chromosomal regions containing genes for powdery mildew resistance and to estimate the resistance effect of each locus. A set of 28 F_1 hybrids and eight parental lines from a barley diallel study was inoculated with each of five isolates of *E. graminis*.The parents were surveyed for restriction fragment length polymorphisms (RFLPs) at 84 marker loci that cover about 1100 cM of the barley genome. The RFLP genotypes of the F_1s were deduced from those of the parents. A total of 27 loci, distributed on six of the seven barley chromosomes, detected significant resistance effects to at least one of the five isolates. Almost all the chromosomal regions previously reported to carry genes for powdery mildew resistance were detected, plus the possible existence of 1 additional locus on chromosome 7. The analysis indicated that additive genetic effects are the most important component in conditioning powdery mildew resistance. However, there is also a considerable amount of dominance effects at most loci, and even overdominance is likely to be present at a number of loci. These results suggest that quantitative differences are likely to exist among alleles even at loci which are considered to carry major genes for resistance, and minor effects may be prevalent in cultivars that are not known to carry major genes for resistance.

Key words Restriction fragment length polymorphism (RFLP) · *Hordeum vulgare* · Quantitative resistance · *Erysiphe graminis* f. sp. *hordei* · Diallel

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Introduction

Powdery mildew, caused by *Erysiphe graminis* f. sp. *hordei,* is one of the most important diseases of barley (*Hordeum vulgare*). Controlling this pathogen using qualitative host resistance genes has been explored worldwide by plant breeders, but the frequent appearance of corresponding virulence genes within the pathogen population has allowed only limited success in combating the disease. An alternative approach would be to use quantitative resistance (QR), which would reduce the selection pressure resulting in resistance for a longer period of time. However, this type of host resistance is also limited by the problems associated with measuring quantitative differences in a breeding program and by the fact that the genes underlying QR are not well-described. Furthermore, there is uncertainty about the number of genes involved, and estimates of the number of resistance factors can only be regarded as the lower limit of the actual number of genes (Geiger and Heun 1989). Molecular markers such as restriction fragment length polymorphisms (RFLPs) provide an opportunity to analyze and tag quantitatively expressed genes and thereby help facilitate QR-oriented breeding efforts.

RFLP markers have been used to analyze quantitative traits such as yield obtained from diallel sets of maize lines (e.g., Lee et al. 1989; Smith et al. 1990; Dudley et al. 1991; Melchinger et al. 1992). However, similar RFLP analyses of quantitative traits from diallel sets have not been reported for self-pollinating species. This paper gives the results from molecular marker analysis of quantitative resistance to powdery mildew using a diallel set obtained by intercrossing eight barley lines.

Materials and methods

Cultivars and crosses

Eight cultivars of barley (*Hordeum vulgare* L.) that differ quantitatively in the level of resistance to powdery mildew were used in this

Table 1[a] Number of pustules per square centimeter leaf area and midparent heterosis (in parenthesis) for crosses and parents infected with five isolates of *Erysiphe graminis*

Crosses and Parents	Isolate				
	AR-4	BE-1	GI-1	OR-8	VA-4
FL×GP	2.96 (–0.30)	15.99 (–0.08)	8.36 (–0.06)	6.53 (0.01)	9.93 (–0.11)
FL×GR	0.64 (–0.73)	3.83 (–0.59)	1.78 (–0.66)	0.83 (–0.74)	2.08 (–0.66)
FL×HR	0.65 (–0.75)	5.47 (–0.50)	2.20 (–0.58)	1.25 (–0.60)	2.31 (–0.64)
FL×LN	1.26 (–0.51)	6.83 (–0.46)	2.35 (–0.59)	1.25 (–0.59)	2.82 (–0.58)
FL×ND	0.96 (–0.67)	6.90 (–0.36)	3.00 (–0.47)	1.72 (–0.45)	2.81 (–0.55)
PC×FL	2.53 (–0.18)	11.13 (–0.20)	5.38 (–0.15)	2.76 (–0.24)	5.89 (–0.25)
FL×TS	1.12 (–0.56)	7.10 (–0.38)	4.01 (–0.27)	1.89 (–0.44)	3.24 (–0.57)
GR×GP	3.65 (0.36)	14.04 (0.14)	7.92 (0.27)	4.72 (–0.04)	10.25 (0.42)
HR×GP	4.50 (0.55)	13.61 (–0.02)	7.23 (0.18)	5.62 (0.14)	7.97 (0.06)
LN×GP	4.21 (0.44)	15.19 (–0.02)	6.88 (0.03)	4.45 (–0.08)	9.82 (0.28)
ND×GP	3.73 (0.13)	15.21 (0.11)	7.78 (0.18)	5.08 (0.03)	8.64 (0.18)
PC×GP	3.79 (0.10)	17.76 (0.05)	7.74 (0.06)	4.37 (–0.20)	9.03 (0.02)
TS×GP	3.42 (0.18)	15.81 (0.10)	7.20 (0.12)	5.21 (0.01)	10.19 (0.18)
HR×GR	0.47 (–0.53)	3.12 (–0.47)	1.93 (–0.23)	0.89 (–0.44)	2.17 (–0.17)
LN×GR	0.67 (–0.34)	4.08 (–0.46)	1.84 (–0.39)	0.91 (–0.37)	1.74 (–0.37)
ND×GR	1.01 (–0.27)	7.35 (0.29)	2.21 (–0.25)	1.32 (–0.15)	3.19 (0.33)
PC×GR	1.43 (–0.07)	6.05 (–0.32)	2.55 (–0.30)	1.53 (–0.26)	2.14 (–0.46)
TS×GR	1.42 (0.43)	7.35 (0.16)	3.27 (0.17)	1.49 (–0.16)	4.80 (0.29)
HR×LN	0.53 (–0.58)	3.71 (–0.59)	2.43 (–0.17)	0.79 (–0.47)	1.67 (–0.46)
ND×HR	1.32 (–0.18)	6.66 (–0.09)	2.83 (–0.01)	3.32 (1.09)	3.39 (0.26)
PC×HR	1.40 (–0.20)	9.54 (–0.09)	3.59 (0.01)	1.81 (–0.14)	3.77 (–0.12)
TS×HR	1.80 (0.49)	7.90 (–0.01)	2.79 (0.03)	1.49 (–0.18)	5.12 (0.28)
ND×LN	1.14 (–0.30)	7.92 (–0.11)	2.24 (–0.34)	2.22 (0.52)	2.40 (–0.16)
PC×LN	1.32 (–0.26)	9.94 (–0.17)	3.60 (–0.12)	3.23 (0.64)	6.21 (0.40)
TS×LN	1.37 (0.11)	8.33 (–0.13)	3.01 (–0.07)	1.97 (0.18)	4.97 (0.19)
PC×ND	1.89 (–0.12)	11.33 (0.11)	4.50 (0.13)	2.42 (0.16)	4.60 (0.13)
TS×ND	1.75 (0.09)	9.83 (0.27)	4.26 (0.35)	2.13 (0.19)	4.57 (0.20)
PC×TS	2.53 (0.45)	12.92 (0.18)	5.69 (0.47)	3.30 (0.44)	7.30 (0.35)
FL	3.88	14.37	7.96	4.71	10.08
GP	4.60	20.40	9.89	8.28	12.13
GR	0.77	4.28	2.57	1.54	2.30
HR	1.22	7.48	2.40	1.61	2.89
LN	1.27	10.69	3.48	1.35	3.24
ND	2.00	7.09	3.29	1.57	2.50
PC	2.29	13.39	4.71	2.59	5.65
TS	1.20	8.45	3.02	2.00	5.13

FL, 'Flavina'; GP, 'Golden Promise'; GR, 'Grit'; HR, 'Hora'; LN, 'Luna'; ND, 'Nudinka'; PC, 'Proctor'; TS, 'Tosca'

[a] Disease data were provided by Dr. M. Heun. Heterosis values (in parenthesis) are based on our calculations

study: 'Flavina', 'Grit', 'Hora', 'Luna', 'Nudinka', 'Tosca', 'Golden Promise', and 'Proctor'. The first six cultivars all known carry *Mla*12, a major gene for mildew resistance, while the latter two cultivars do not have any known major gene for resistance (Heun 1987a).

All possible pairwise crosses were made among these eight parents, resulting in a diallel set of 28 crosses. At least 150 seeds were obtained for each cross, and these seeds were planted along with the eight parental lines to produce leaf tissues for powdery mildew resistance evaluation.

Powdery mildew resistance evaluation

Five isolates of *Erysiphe graminis* f. sp. *hordei*, AR-4, BE-1, GI-1, OR-8, and VA-4, were selected to represent the widest possible spectrum of virulence known for this pathogen (Heun 1987a). Leaf segments of the 28 F_1 hybrids and the eight parents, totalling 36 entries, were arranged in five complete Latin rectangles for mildew inoculation. Each of the 36 entries was replicated six times within a Latin rectangle, giving a total of 216 units. These 216 units were laid out in six vertical and six horizontal blocks with each entry represented once per block. Each of these 216 units consisted of five leaf segments (each 3.0 cm long and 0.7 cm wide) obtained from 5 different plants of the same entry. The leaf segments were plated on 0.5% agar containing 15 ppm benzimidazole. Each Latin rectangle was inoculated with one of the five aforementioned isolates using a settling tower at a density of 50±10 spores/cm^2. The leaf tissues were then incubated for 7 days, and the number of pustules/cm^2 leaf area was scored as a disease rating. The powdery mildew disease data (Table 1) reanalyzed here were originally collected by Dr. M. Heun, and these data were, in part, the basis of two earlier publications (Heun 1987a,b).

RFLP assays

DNA extraction, digestion, electrophoresis, and hybridization followed previously published procedures (Saghai Maroof et al. 1984; Zhang et al. 1993). DNA was extracted from bulked leaf tissue of about 10 seedlings for each of the eight parents. DNA was singly digested with six restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I) and probed for RFLPs with 73 cloned fragments. Over 400 probe enzyme (P/E) combinations were conducted which detected 84 Mendelian loci distributed along all seven barley chromosomes (Fig. 1). The clones were selected from two barley RFLP maps (Heun et al. 1991, Kleinhofs et al. 1993) to well represent each of the 14 chromosome arms. The number of clones varied from a minimum of 9 on chromosome 6 to a maximum of 16 on chromo-

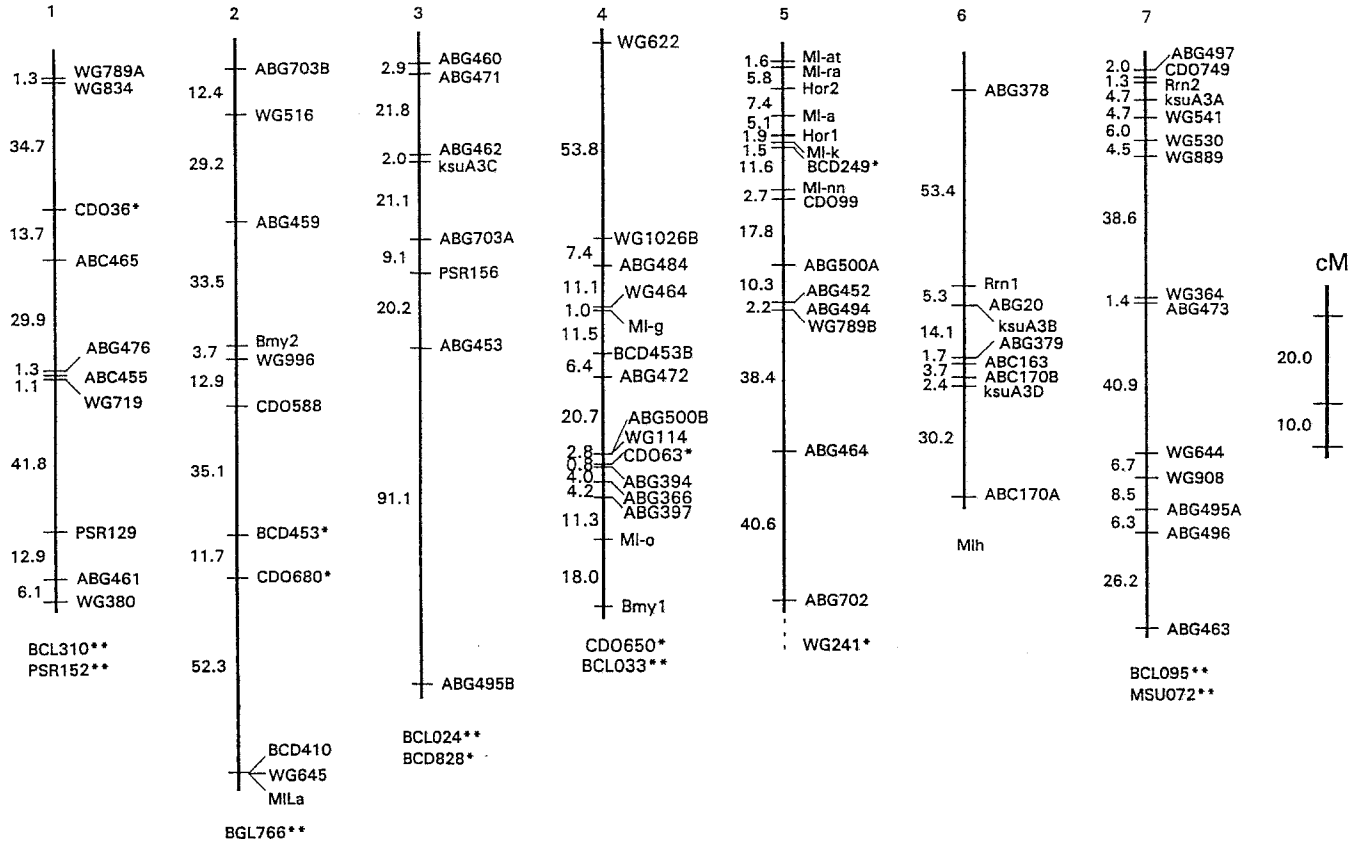

Fig. 1 A barley RFLP map was adapted from a 295-marker map (Kleinhofs et al. 1993) using the MAPMAKER computer program (Lander et al. 1987). Only the 84 RFLP markers of the present study are displayed (1 clone, BGL752, has not been mapped yet). *Numbers* to the *left* of the *horizontal lines* are map distances in centiMorgans. The 6 markers specified by* are from Heun et al. (1991) or Shin et al. (1990) maps. Exact chromosomal locations of the markers at the *bottom* of each *chromosome*, specified by**, are not known. Linkage relationships among the powdery mildew resistance genes and RFLPs are based on observations from several maps from separate studies. These approximate locations are *only* for explanation purposes and should be viewed with caution

some 7 and covered about 1100 cM of the barley genome (Fig 1). Molecular marker genotypes of the eight cultivars were used to deduce marker genotypes of the 28 F_1 hybrids to be used in statistical analysis.

Statistical analysis

The effect of a chromosomal region marked by an RFLP locus was assessed with a one-way analysis of variance using genotypes of the RFLP locus as the main effect and crosses and parents within genotypes as the error term.

Two genetic parameters, additive effect and degree of dominance, were estimated using a single-locus model for markers that detected significant resistance effects (positive markers). The additive effect (a), measuring half the difference between the two parents, is calculated as

$$a = \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} (N_{ij} \mid X_{ii} - X_{jj} \mid /2) / \sum N_{ij} \ ,$$

where X_{ij} is the average disease score of the individuals homozygous for the i^{th} allele of the RFLP locus, N_{ij} is the number of F_1s heterozygous for the i^{th} and j^{th} alleles and k is the total number of alleles at the locus. The dominance effect for each locus is calculated by

$$d = \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} [N_{ij} \mid X_{ij} - (X_{ii} + X_{jj})/2 \mid] / \sum N_{ij} \ .$$

It is necessary to take the absolute values for both estimates since the effects of different genotypes may cancel each other for loci with multiple alleles. The degree of dominance for a locus is given by d/a. A zero d/a indicates no dominance, d/a values between zero and 1 measure partial dominance and d/a values greater than 1.0 signify overdominance.

Results

Polymorphisms of the cultivars and probes

The level of polymorphism among the eight cultivars varied greatly from one probe to another. Twelve probes were monomorphic with all six enzymes, while the remaining 61 probes detected RFLPs among the eight cultivars. Of these 61 probes, 25 detected polymorphisms with all six enzymes, while the number of enzymes revealing polymorphisms for the other 36 probes varied from one to five, and the majority of the probes detected polymorphisms with two to four restriction enzymes.

Banding patterns revealed by 52 of the 61 polymorphic clones demonstrated typical single-locus variation, and each clone is mapped to a single chromosomal location (Heun et al. 1991; Kleinhofs et al. 1993). Each of the seven probes (ABG495, ABG703, BCD453, Hor, pTA71, WG380, and WG789) detected 2 distinct Mendelian loci,

thus, resolving a total of 14 loci. Restriction fragments detected by the probe KsuA3 were mapped to four locations on three different chromosomes, and those by replace βamy with βmy1&2 (β-amylase 1&2) were located on chromosomes 2 and 4. RFLPs for these 2 clones could not be resolved into their respective loci without a detailed inheritance study involving the eight parental lines used in this study. Therefore, data from these 2 clones will be excluded from further analysis.

There is obvious concurrence of restriction fragment length variants resolved by different enzymes with the same probe. Almost all of the polymorphic probes classified the eight parents exactly the same way by two or more enzymes. As an extreme example, 2 probes both revealed the same grouping of the parents with all six enzymes, indicating that the same insertion/deletion event was detected by different enzymes.

Relatedness of the parents

The RFLPs for enzymes that produced nonredundant information within each probe were used for assessing the relatedness of the eight parental lines. Consequently, the number of markers resulting from various probes were not necessarily equal. Both clones CDO749 and BGL766 as examples, detected polymorphisms among the eight parents with all six enzymes. Within CDO749, two enzymes (*Dra*I and *Xba*I) provided the same information regarding the similarity among the eight cultivars, and the information produced by the remaining four enzymes was different for each one and also from those of *Dra*I and *Xba*I. Thus, five pieces of information were obtained from this probe, and only two enzymes (*Dra*I and *Xba*I) produced redundant data concerning genetic differences among the eight parents. Within BGL766, however, all six enzymes revealed that one parent was different from the other seven cultivars, while the latter lines were not different from each other. Thus, only one piece of information was produced from this probe, and data from five enzymes were redundant. Therefore, RFLPs detected by CDO749 were treated as 5 markers for analyzing similarity among the parents, while data of BGL766 were used as only 1 marker.

Over all, nonredundant data consisted of 164 P/E combinations. The number of P/E combinations that detected differences between paired cultivars was used as the distance measure, and the relatedness of the parents was depicted with a cluster analysis using the Ward's minimum variance algorithm (Ward 1963). The cluster analysis placed the eight parents into three highly differentiated groups: 'Flavina', 'Nudinka', and 'Tosca' in the first group, 'Golden Promise', 'Hora', 'Proctor', and 'Grit' in the second group, and 'Luna' in the third group.

Comparison of this grouping with pedigree information showed that the similarity resolved by RFLPs did not always agree with parentage among these eight cultivars. Most of the discrepancies concerned the closeness of 'Grit' and 'Luna' with the other cultivars and also with each other. We suspect that the pedigree information may be incorrect or that very different selection pressures were applied in breeding these two cultivars.

Disease infection

The amount of disease as measured by the number of pustules/cm^2 leaf area differed greatly from one cultivar to another and also varied widely among the five isolates (Table 1). 'Grit' was the most resistant cultivar to all five isolates, and 'Golden Promise' was the most susceptible. The disease level of the other six cultivars varied more or less continuously between the two extremes. Among the five isolates, BE-1 appeared to be the most virulent, because it produced more disease than the other four isolates in all of the parents and F_1s. AR-4 was the least virulent isolate causing less disease than the other four isolates on all eight parents, as well as on 26 of the 28 F_1s.

Although the disease level in many F_1s was lower than the more resistant parents, a few F_1s had more disease than that in the more susceptible parents, while many F_1s were intermediate between the parents (Table 1).

Heterosis for disease reaction, measured as percent deviation from the midparent values, was common among the crosses for all five isolates of the pathogen. It varied from 74% less disease (negative heterosis) than the midparent of the cross between 'Flavina' and 'Hora' when inoculated with AR-4 to 109% more disease (positive heterosis) than the parental mean in the F_1 between 'Nudinka' and 'Hora' when inoculated with OR-8. Overall, there were more crosses with negative heterosis than positive heterosis for four of the five isolates (AR-4, BE-1, GI-1 and OR-8), while in the fifth isolate the numbers of crosses with negative heterosis were about equal to those with positive heterosis.

RFLP loci that detected significant effects on mildew resistance

A total of 27 marker loci (henceforth referred to as positive markers), or roughly 44% of the total polymorphic loci, detected significant effects of resistance to at least one isolate (Table 2). Most of the loci showed significant resistance effects to all five isolates.

All barley chromosomes except chromosome 3 have been reported to contain genes for powdery mildew resistance (Jensen et al. 1980; Hinze et al. 1991; Hilbers et al. 1992; Schuller et al. 1992; Heun 1993; Giese et al. 1993; see also Wettstein-Knowles 1993). In the present study, no effect was detected by markers on chromosome 3, and only minor effects were revealed by 3 markers on chromosome 1. Highly significant effects were revealed by markers on the remaining five chromosomes (Table 2).

The map positions for many of the marker loci that detected significant effects of resistance in this study corresponded well with the chromosomal locations of the known resistance genes from previous studies. For example, WG645, which is very closely linked to *Prx2* (peroxidase

2) on the tip of chromosome 2 (Kleinhofs et al. 1993), detected significant resistance effects to powdery mildew in our study. The peroxidase locus is closely linked to the *MlLa* locus (Giese et al. 1993), both of which reside within the region flanked by markers MWG660 and MWG97 (Hilbers et al. 1992). On the basis of published genetic maps (Wettstein-Knowles 1993), it can be deduced that the 4 positive markers on chromosome 4 (WG1026B, WG464, BCD453B, and CDO650) that detected highly significant effects are around the chromosomal region where the *Mlg* locus resides (Fig. 1). Of the other chromosome 4 positive markers, both ABG394 and ABG366 showed significant effects with one isolate (Table 2). These two loci are within 15 cM of the *Mlo* locus on chromosome 4 (Fig. 1, Hinze et al. 1991; Kleinhofs et al. 1993). A cluster of mildew resistance genes on chromosome 5 (Jensen et al. 1980; Wettstein-Knowles 1993) is located in the same block as Hor2, BCD249, and CDO99, which detected significant resistance effects in the present study. Marginal significance (at the 6% probability level) also was observed for at least one isolate with Hor1 (data not shown). The exact location of the *Mlh* locus which has been mapped to chromosome 6 (Hayashi and Heta 1985; Hilbers et al. 1992; see also Sogaard and Wettstein-Knowles 1987) is not known. Thus, correspondence between ABG20, a chromosome 6 marker with significant effects, and the *Mlh* locus could not be determined. Finally, CDO749, which showed significant effects with all five isolates, has been also reported to be linked to a gene of minor effect for powdery mildew resistance on chromosome 7 (Heun 1993). Therefore, it is highly likely that the effects detected by these markers reflect allelic differences at the respective loci with the reaction to the powdery mildew resistance in barley.

It is likely that the effects detected by a portion of the markers were results of sporadic correlations between unlinked markers (i.e., false positives) caused by the small number of parental lines included in the present study. To delineate the extent of false positive markers, product-moment correlations were calculated between RFLP patterns at all the positive marker loci (Table 2) by grouping the alleles of each locus into two classes. In preparing the RFLP data for correlation analysis, we assigned the most frequent allele (allele 1) a value of 1, all other alleles were grouped into a synthetic class (allele 2) and assigned a value of 0. For a diallelic locus, which was the case for a majority of the loci, such a grouping did not lose information. For a triallelic locus, which was the case for almost all the remaining loci, such dichotomization usually lumped alleles 2 and 3 into one group, and the latter allele was represented by one of the eight parents in most of the cases. Calculations showed that RFLP alleles of ABG476 and PSR129 on chromosome 1 were perfectly correlated with each other and with ABG20, a locus on chromosome 6, that detected highly significant resistance effects to all five isolates. This suggests that the minor effect detected by the two chromosome 1 markers may be due to an indirect effect from ABG20. Therefore, it is unlikely that chromosome 1 carries any locus with a major effect on powdery mildew resistance, although there has been evidence suggesting the existence of a possible locus with a minor effect (Heun 1993).

Table 2 RFLP loci that detected significant effects on resistance among the crosses and parents to five isolates of *Erysiphe graminis*

RFLP locus	Isolate					
	AR-4	BE-1	GI-1	OR-8	VA-4	Chromosome
ABG476	*	*	*	*	ns	1
ABC455	ns	*	ns	ns	ns	1
PSR129	*	*	*	*	ns	1
WG996	**	**	**	**	**	2
CDO680	ns	*	ns	ns	ns	2
WG645	*	**	*	**	*	2
WG1026	**	**	**	**	**	4
WG464	**	**	**	**	**	4
BCD453B	**	**	**	**	**	4
CDO650	**	**	**	**	**	4
ABG394	ns	*	ns	ns	ns	4
ABG366	ns	*	ns	ns	ns	4
BCL033	*	**	*	*	*	4
Hor2	**	**	**	**	*	5
BCD249	**	**	**	**	**	5
CDO99	**	**	**	**	**	5
WG789B	ns	ns	*	*	ns	5
ABG378	*	ns	*	**	*	6
ABG20	**	**	**	**	**	6
ABG497	**	**	**	**	**	7
CDO749	*	*	*	*	*	7
WG541	**	**	**	**	**	7
WG644	*	**	*	*	*	7
ABG495A	ns	**	ns	ns	ns	7
ABG496	**	**	**	**	**	7
ABG463	*	ns	*	**	*	7
BCL095	*	*	*	ns	ns	7

*, ** Effects significant at 0.05 and 0.01probability levels, respectively, as determined by AOV. In case different amounts of effects were detected with different enzymes for a given probe, only the result from the enzyme that detected the largest effect was entered into this table

Significant effects were also detected by KsuA3, but the RFLP bands could not be resolved into individual loci

The locus WG996 on chromosome 2, located on the opposite arm far away from WG645 (Fig. 1), was perfectly correlated with WG464 and nearly perfectly with BCL033, both of which are located on chromosome 4. WG996 also was significantly correlated with WG645. Thus, the effect detected by WG996 may be a false positive and caused by its sporadic correlation with other markers.

The minor effect revealed by WG789B on chromosome 5 was likely a result of its loose linkage to other markers on this chromosome. Chromosome 6 has been reported to carry a locus for mildew resistance (Hayashi and Heta 1985; Hilbers et al. 1992; see also Sogaard and Wettstein-Knowles 1987). The two positive marker loci on chromosome 6, ABG378 and ABG20, are about 55 cM apart; the effect detected by the latter marker was much larger than the former. Furthermore, ABG378 was perfectly correlated with ABG463 of chromosome 7 and also significantly correlated with 2 chromosome 5 markers, Hor2 and BCD249, while ABG20 was highly correlated only with BCL095, a chromosome 7 marker that detected a much smaller effect

Table 3 Additive effects and degree of dominance (in parenthesis) at the chromosomal region marked by each of the RFLP loci that detected significant effects of resistance to five isolates

RFLP locus	Isolate				
	AR-4	BE-1	GI-1	OR-8	VA-4
ABG476	0.73 (0.13)	2.50 (0.42)	1.25 (0.43)	1.03 (0.23)	
ABC455		3.23 (0.30)			
PSR129	0.73 (0.13)	2.50 (0.42)	1.25 (0.43)	1.03 (0.23)	
WG996	0.79 (2.62)	3.86 (0.55[a])	1.54 (2.00)	1.24 (0.40)	1.93 (1.11)
CDO680		3.23 (0.30)			
WG645	0.91 (0.10)	3.63 (0.19)	1.62 (0.01)	1.34 (0.11)	2.15 (0.03)
WG1026	1.15 (0.34)	5.05 (0.09)	2.23 (0.30)	1.68 (0.29)	2.72 (0.37)
WG464	0.80 (2.62)	3.86 (0.55[a])	1.54 (2.00)	1.24 (0.40)	1.93 (1.11)
BCD453B	0.66 (1.32)	2.57 (1.24)	1.34 (0.97[a])	1.14 (1.40)	1.75 (1.33)
CDO650	1.15 (0.34)	5.05 (0.09)	2.23 (0.30)	1.68 (0.29)	2.72 (0.37)
ABG394		3.23 (0.30)			
ABG366		3.23 (0.30)			
BCL033	0.78 (0.09)	3.71 (0.11)	1.44 (0.14)	1.10 (0.12)	1.95 (0.06)
Hor2	0.80 (0.01)	3.31 (0.12)	1.35 (0.17)	1.13 (0.19)	1.59 (0.41)
BCD249	0.73 (0.42)	3.22 (0.56)	2.38 (0.13)	1.94 (1.14)	1.82 (0.66)
CDO99	0.63 (0.31)	2.70 (0.70[a])	1.24 (2.68)	1.18 (1.88)	1.55 (2.04)
WG789B			1.57 (0.21)	1.34 (0.24)	
ABG378	0.95 (0.17)		1.47 (0.07)	1.57 (0.18)	1.75 (0.25)
ABG20	0.88 (0.12)	3.48 (0.11)	1.67 (0.29)	1.23 (0.02)	2.22 (0.09)
ABG497	0.90 (1.47)	3.44 (1.05)	1.73 (20.94)	1.87 (0.09)	2.39 (0.57)
CDO749	0.71 (0.25)	2.60 (0.19)	1.33 (0.15)	1.12 (0.18)	1.77 (0.28)
WG541	1.59 (0.47)	6.22 (0.19)	3.29 (0.30)	3.18 (0.01)	4.12 (0.34)
WG644	0.78 (0.09)	3.71 (0.11)	1.44 (0.14)	1.10 (0.12)	1.95 (0.06)
ABG495A		2.72 (1.84)			
ABG496	1.59 (0.47)	6.22 (0.19)	3.29 (0.30)	3.18 (0.01)	4.12 (0.34)
ABG463	0.95 (0.17)		1.47 (0.07)	1.57 (0.18)	1.75 (0.25)
BCL095	0.71 (0.44[a])	2.62 (1.03)	1.44 (0.64[a])		

[a] Over-dominance was detected in one or more crosses

than did ABG20. Thus, it is likely that the effect detected by ABG20 reflected an allelic difference at the *Mlh* locus, whereas that of ABG378 is a false positive.

Evidence has been presented for a mildew resistance locus near CDO749 of chromosome 7 (Heun 1993). In the present study, in addition to CDO749, significant resistance effects to at least one of the five isolates were detected by 7 additional markers distributed along chromosome 7, including: ABG497, WG451, BCL095, WG644, ABG495A, ABG496, and ABG463 (Fig. 1). The ABG497 locus is closely linked to CDO749 and revealed a consistently larger effect than did CDO749. Although ABG497 was significantly correlated with markers on chromosomes 2 and 4, it is unlikely that such a highly significant effect can be explained by such a sporadic correlation. It is more likely that ABG497 detected the same resistance effect as did CDO749, with the former being closer to the resistance locus than the latter.

Among the remaining chromosome 7 positive markers, 3 were correlated with positive markers either on chromosome 4 (WG644), 5 (ABG463) or 6 (BCL095). Consequently, the effects detected by these markers were almost certainly indirect effects of markers on other chromosomes. While not correlated with any other markers, RFLPs of WG541 and ABG496, both on chromosome 7, were perfectly correlated with each other, and the latter was linked to ABG495A. Thus, it is likely that the effect revealed by these three markers represents allelic differences of a second locus for resistance on chromosome 7.

Therefore, based on statistical inferences, our analysis detected the existence of genes for powdery mildew resistance on five chromosomes, i.e., chromosomes 2, 4, 5, 6, and 7, including a possible second locus on chromosome 7 that has not been reported in previous studies. Detailed studies are necessary to characterize the genes on the latter two chromosomes.

Additive and dominance effects of each locus

The estimates of additive effects varied greatly from one locus to another for a given isolate and also differed widely among the isolates for a given locus (Table 3). Differences were even larger among paired genotypes within loci, ranging from no effect to the maximum that can be obtained with this data set (details not shown).

The majority of the estimates for dominance were negative among the loci that demonstrated significant effects, indicating that dominance is in the resistance direction for most genes (data not shown). Also, most of the 114 locus-isolate combinations listed in Table 3 demonstrated partial dominance, and a few showed *d/a* values close to zero (≤ 0.01). A total of 24 cases had an average *d/a* of larger than 1.0, showing overdominance effects for resistance. In addition, there were 5 cases in which overdominance was

observed in certain crosses, although the overall average of *d/a* for those locus-isolate combinations was in the range of partial dominance. Again, all the estimates for overdominance were in the resistant direction, and values as large as 10–20 times the additive effects were obtained in certain instances (data not shown).

Heterozygosity and powdery mildew resistance

The product-moment correlation was calculated between the amount of disease and the level of heterozygosity of F_1s as measured by the number of RFLP loci showing different alleles between the parents. None of the correlations were significant; however, all correlation coefficients were negative, indicating that overall heterozygosity may confer a slight advantage for resistance. There was no significant correlation between heterozygosity and heterosis for any of the five isolates.

When only the markers detecting significant resistance effects were used for such calculations, the correlations were again not significant. Thus, the results indicate that the level of heterozygosity cannot be used as a predictor for disease resistance.

Discussion

The availability of RFLP linkage maps has caused renewed interest in diallel analyses as exemplified in a number of studies in maize. The purpose of the present study was to use RFLP markers to detect chromosomal regions associated with powdery mildew resistance in a set of barley diallel crosses. Remarkably, almost all of the known chromosomal regions previously reported to carry genes for powdery mildew resistance were detected with our analysis. The results also suggest the possible existence of at least 1 additional locus that has not been reported in previous studies.

As mentioned earlier, six of the eight cultivars used in this study are known to carry only *Mla12*, a major mildew resistance gene, while the other two cultivars do not have a known major gene. One prerequisite for being able to detect the presence of a locus in a study such as this is the existence of allelic differences at that locus among the parents. A large effect detected by a molecular marker signifies a large difference among alleles carried by the parents. Conversely, a small effect detected by a molecular marker indicates a small difference among alleles carried by the parents. The magnitude of the effects we have observed clearly indicates the existence of allelic differences in powdery mildew resistance among these eight parents at a number of chromosomal locations in addition to the *Mla* locus located on chromosome 5. Furthermore, we have found drastic differences in the amounts of additive and dominance effects within as well as among many marker locus/isolate combinations, indicating that the effects of alleles at the respective loci varied quantitatively rather than qualitatively. Thus, quantitative differences are likely to exist among alleles even at loci that are considered to carry major genes for resistance, and minor effects may be prevalent in cultivars that are not known to carry major genes for resistance. Such quantitative differences in allelic effects appear to be the basis for quantitative resistance.

A possible shortcoming associated with the analysis of the present study is the proportion of false positives that may be caused by sporadic correlations of RFLPs among unlinked markers due to the limited number of parental lines included in the diallel analysis. To reduce the extent of such problems, we evaluated positive markers according to their locations with respect to previously reported resistance loci. Markers were considered "true" positive whenever their location corresponded to the map position of a previously reported resistance locus. Markers were considered as "false" positives if their map locations did not correspond to the regions of known resistance genes and if they were highly correlated with those we had accepted as "truly" positive markers. Thus, the choice of "true" or "false" positive marker may be biased by prior knowledge, and it is possible that some markers from vicinities of previously reported resistance genes may still be false positive because of sporadic correlations. The extent of such false positives can be checked by inspecting the correlation of RFLP patterns between paired loci. For example, if we had not considered prior knowledge of gene location, the relatively small effect represented by WG645 of chromosome 2 and the minor effect detected by ABG394 and ABG366 on chromosome 4 would have been disregarded, since these markers were highly correlated with markers on other chromosomes that detected much larger effects. Conversely, the interpretations would still hold true for the remaining positive markers. Thus, prior knowledge of gene locations together with correlation analysis was helpful in reducing false positive problems in this diallel analysis, although the presence and effects of resistance genes among these eight parents may still need to be confirmed with segregation data.

We also have used the information from this data set to estimate some important genetic parameters concerning the effects of gene action based on a single-locus model. We found that, in general, additive genetic effects were the most important components conditioning resistance. However, dominance effects were considerable at most of the loci, and even overdominance was likely to be present at certain loci, as marked by some of the positive RFLP loci.

Two assumptions were made in the application of the single-locus model to estimate these genetic parameters. First, the gene effects of different loci were assumed to be independent of each other, i.e., epistasis was not present for powdery mildew resistance. Second, it was assumed that genes for resistance at loci other than the one being tested were randomly associated with each other and also with the genetic background.

As far as the first assumption is concerned, it is possible, at least in theory, to inspect the effect of epistasis with a diallel set by pulling out two-locus combinations and performing an analysis of variance to test the effects

of interaction components. For each combination to be repeated five times on the average, at least 45 data entries (F_1s and their parents) would be required to test the interaction between two unlinked diallelic loci. This number should then be multiplied by at least a factor of three for each additional locus. Obviously, such an analysis could not be accomplished in the present study because of the limited number of data entries (36 in all), which would form many empty cells upon cross-classification. Thus, our estimates of single-locus parameters may be biased in one way or another depending on the extent of epistasis.

The validity of the second assumption was checked using correlation at marker loci as an indication of the associatedness of the background genotypes. Our results suggest that this should not be a serious problem in this diallel set, especially for markers located in the vicinity of known genes.

In summary, we conclude that marker-based diallel analysis can be used to detect the effects of a specific gene as well as to determine the approximate map position of the locus, provided that the linkage map is adequately represented by the markers used in the survey. An advantage of such a diallel analysis, compared to those with segregating populations, is that a relatively large number of parents can be included in the analysis, thereby encompassing a broader spectrum for the genes or genotypes of interest. This may be particularly appealing for studying traits that are controlled by many genetic factors and for genes that are scattered among a large number of genotypes that cannot be accommodated in one or a few crosses, as is the case with resistance to many diseases.

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