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Mapping resistance to cereal aphids in barley

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Abstract A set of 150 doubled-haploid (DH) barley (*Hordeum vulgare* L.) lines derived from the cross of Harrington/TR306 was used to determine the number and chromosomal location of quantitative trait loci (QTLs) controlling resistance to cereal aphids. The experiments were conducted under natural infestation in the field during two growing seasons: 1994 and 1995. Aphid resistance was measured by counting the number of aphids per plot. Counts were made on a weekly basis. Each year at the time of maximum aphid density there was an obvious difference in reaction between the parental genotypes. The DH lines showed continuous variation for aphid density. Simple interval mapping and simplified composite interval mapping revealed that the principal QTL determining cereal aphid resistance is on the distal region of the short arm of chromosome 1. This aphid-resistance QTL is linked with a heading-date QTL. At the time of highest aphid infestation, this QTL accounted for 31% and 22% of the total variance of aphid density in 1994 and 1995, respectively. A QTL on chromosome 5 was also detected but only by simplified composite interval mapping. However, the largest consistent effect was due to the QTL on the short arm of chromosome 1. This QTL may be a useful target for marker-assisted selection for adult plant cereal aphid resistance in barley.

Key words Barley · Cereal aphids · Quantitative trait loci · Gene mapping · Insect resistance

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Introduction

Cereal aphids, especially the corn leaf aphid (*Rhopalosiphum maidis* Fitch) and the bird cherry-oat aphid (*R. padi* L.), are the most important insect pests of barley in western Japan. They cause considerable damage to grain production through their sap withdrawal, injection of toxic saliva, excretion of honeydew, and transmission of virus diseases, especially barley yellow dwarf virus (BYDV).

Plant resistance to insect predation offers considerable advantages in agricultural practice. Increases in barley yield following the use of cultivars resistant to *R. maidis* were reported in the U.S.A. (Schalk and Ratcliffe 1977), as was a reduction in the use of insecticides (Auclair 1989). Painter (1951) defined resistance of plants to insect attack as the relative amount of heritable qualities possessed by the plant that influence the ultimate degree of damage done by the insect. Gill and Metcalfe (1977) reported that inheritance of resistance to *R. maidis* was dominant in barley seedlings. However, a genetic analysis of aphid resistance for adult barley plants is difficult due to uncontrollable environmental errors.

Regarding sources of resistance, wild barley (*Hordeum vulgare* subsp. *spontaneum*) can maintain the population of aphids at a low level by a number of mechanisms (Tsumuki et al. 1987, 1989; Kawada and Lohar 1989; Kanehisa et al. 1990; Moharramipour et al. 1997). Under natural conditions in the field, the resistance to infestation of cereal aphids showed quantitative inheritance (Moharramipour et al. 1996). To accumulate these quantitative resistance factors into one genotype, and in the interest of improving the level of resistance in our germ plasm, quantitative trait locus (QTL) analysis was used. The aim of the present study was to identify the location and effects of QTLs for adult plant field resistance to cereal aphid infestation.

Materials and methods

Plant materials

A set of 150 doubled-haploid (DH) lines of barley (*Hordeum vulgare* L.) derived from the cross of Harrington/TR306 was developed and supplied by the North American Barley Genome Mapping Project (NABGMP, Hayes 1996). Harrington is a two-rowed malting-quality standard in Canada and the U.S.A., and TR306 is a two-rowed high-yielding non-malting line (Kasha et al. 1994). Seed from a single head of each parent was used to generate the F_1. A population of DH lines was developed from the F_1 by the *Hordeum bulbosum* technique, as described by Chen and Hayes (1989).

Seeds of DH lines and parents were sown in the field at Kurashiki, Japan, in mid November and harvested in early June during the growing seasons of 1993–1994 and 1994–1995. Each plot consisted of approximately 20 plants in 100-cm-length and 90-cm-width rows.

Aphid density

The total number of cereal aphids belonging to four species – *R. maidis*, *R. padi*, *Schizaphis graminum* Rondani and *Sitobion akebiae* Shinji – were counted weekly on each of the 150 DH and parental lines in the field plots from the beginning of April until harvesting time for each of two growing seasons in 1994 and 1995. The number of all aphids was counted in each plot and the number of aphids per stem was calculated and determined as aphid density (aphid resistance), which was used for QTL analysis. The field plots were treated according to common agricultural practice. No pesticides were used. Plots were staked to prevent lodging.

Statistical analysis

Due to non-normal frequency distributions of the phenotypic data, $\log_{10}$ transformations were performed for the number of aphids per stem. For QTL analysis, a 127 marker subset of the skeletal molecular marker map was generated by selecting loci to distribute markers evenly across the seven barley chromosomes (Mather 1995). The phenotype data sets were analyzed by the Simple Interval Mapping (SIM) and simplified Composite Interval Mapping (sCIM) procedures of the software package MQTL (Tinker and Mather 1995), which can detect both QTL main effects and QTL × environment (QTL × E) interaction. Linkage groups were scanned at 5-cM intervals, and the probability of a QTL for each interval was expressed as a test statistic. Fifty three even-spaced background markers were specified for sCIM. Except for the multiple-environment sCIM analysis, where precise test statistics are not computable, type-I 5% significance thresholds were established with 1000 permutations.

Results

The density of natural aphid infestation was recorded in each of 150 DH lines and parents from April 13 to May 30 at weekly intervals in 1994, and from April 10 to May 8 at weekly intervals in 1995 (Fig. 1). *R. maidis and R. padi* predominated, and *S. graminum* and *S. akebiae* were observed intermittently in small colonies. The aphid density increased from the 2nd week of April and reached a maximum on May 1 in 1994 and on April 24 in 1995 (Fig. 1). *R. maidis* was the predominant species (about 80% of the total population) at the peak

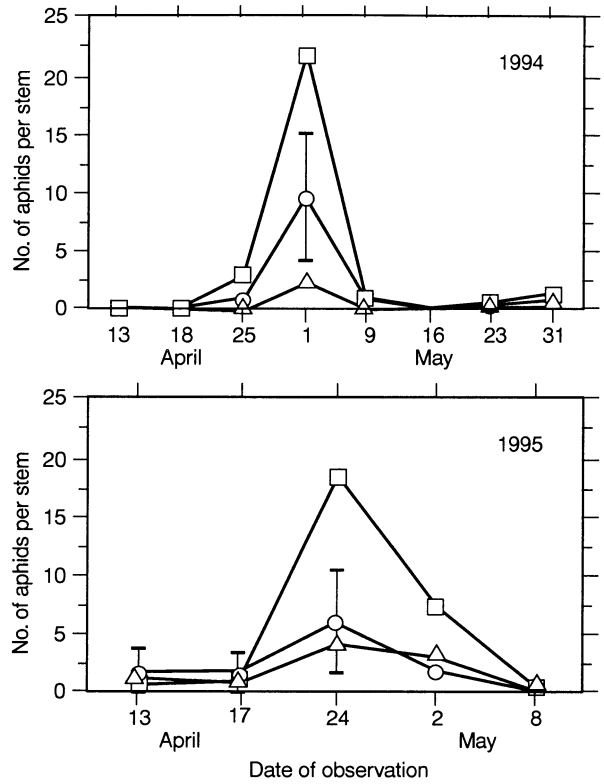

Fig. 1 Population density of cereal aphids in 150 DH lines of Harrington/TR306 in the field in 1994 and 1995. Values for DH lines are expressed as Means ± SD. □ = Harrington, △ = TR306 and ○ = DH lines

time in both years. The maximum differences of aphid density on Harrington and TR306 were also observed at these peaks (Fig. 1). The number of aphids per stem were 21.8 (1994) and 18.2 (1995) in Harrington, and 2.1 (1994) and 4.1 (1995) in TR306 (Fig. 1). Comparing the parental aphid resistance to other genotypes with known resistance planted in the same field, the aphid density in Harrington was close to the susceptible check OUL117 (*H. vulgare* subsp. *vulgare*), and TR306 was close to the resistant check lines OUH603 and OUH689 (both *H. vulgare* subsp. *spontaneum*). The frequency distribution of aphids in the DH lines was continuous and skewed to low density at each sampling, because a greater number of lines exhibited lower aphid densities. At the time of maximum density, the density varied from 2.5 to 27.5 in 1994 and from 0 to 25.0 in 1995 (Fig. 2).

Using SIM, we detected a highly significant QTL on the distal region of the short arm of chromosome 1 (Fig. 3). This favorable allele (low aphid density) was contributed by TR306. SCIM also identified this QTL within the same significant marker interval detected by SIM. As sCIM can estimate QTL position more precisely and accurately than SIM (Tinker and Mather 1995), the marker interval dRpg1/iPgd1A detected by sCIM most likely represents the best-estimate position

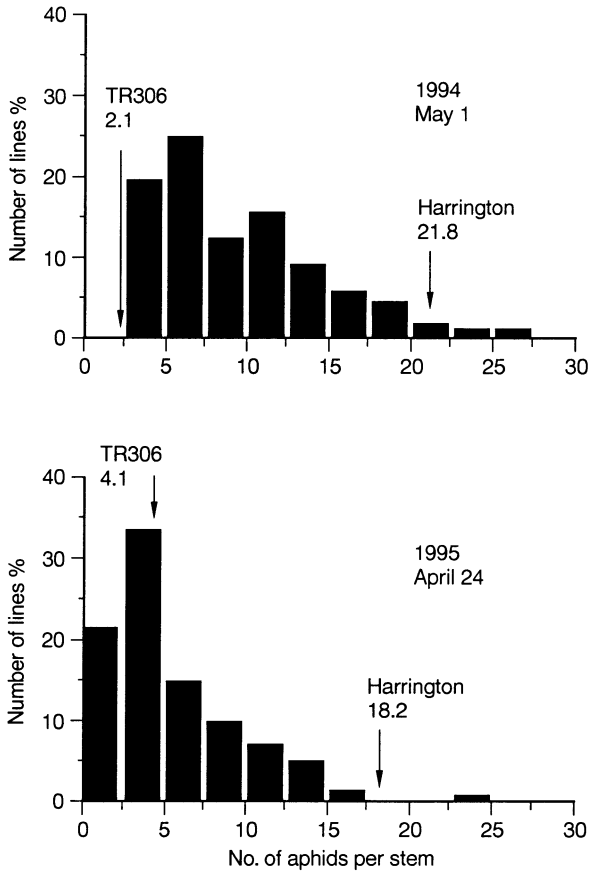

Fig. 2 Frequency distribution for the number of aphids per stem at the time of maximum cereal aphid densities on 150 DH lines from Harrington/TR306 in 1994 and 1995

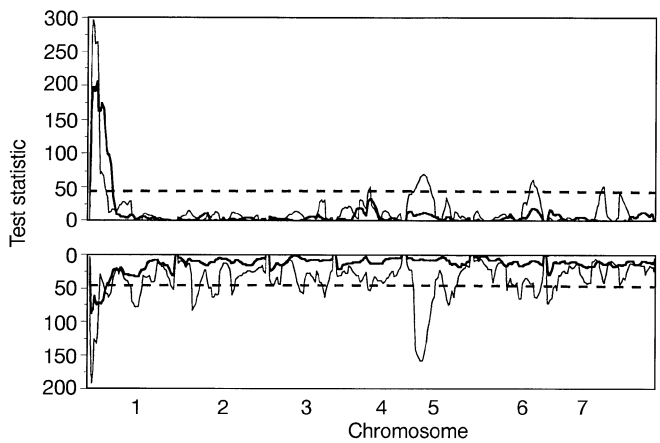

Fig. 3 Effect and position of QTLs for aphid resistance in barley. Both the QTL main effect (upper) and the QTL × environment interaction (lower) were calculated by simple interval mapping (*bold line*) and simplified composite interval mapping (*normal line*). The *Dotted line* indicates the 5% significant threshold level for simple interval mapping

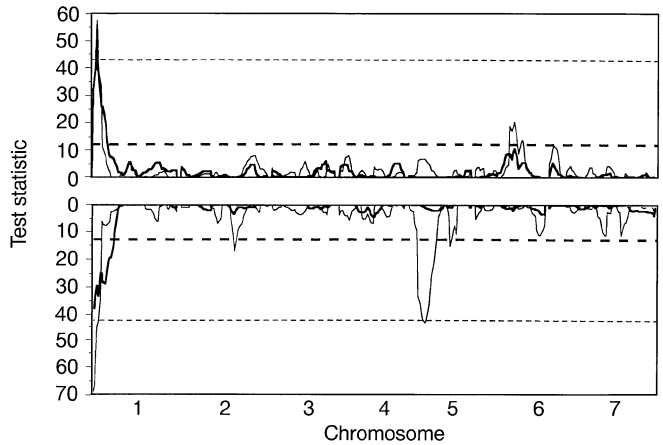

Fig. 4 Effect and position of QTLs for aphid resistance at maximum aphid density of May 1, 1994 (upper) and April 24, 1995 (lower). *Lines* indicate test statistics calculated by simple interval mapping (*bold line*) and simplified composite interval mapping (*normal line*). *Dotted lines* indicate 5% significant threshold levels

of this QTL. Both SIM and sCIM detected QTL × E interaction within the same significant marker interval of this QTL main effect (Fig. 3). SIM analysis of data from each sampling revealed that a QTL was at the marker interval of dRpg1/iPgd1A in every five observation in 1995. However, this QTL was not significant at five of eight sampling dates in 1994. Thus, the QTL × E interaction was due to change in magnitude of response rather than a change in the favorable allele. QTL analyses of data from the sampling dates having maximum aphid density in each year are shown in Fig. 4. Both SIM and sCIM revealed a significant QTL within the same interval of chromosome 1 in both years, as was found by the combined analysis shown in Fig. 3. These QTLs with the favorable allele contributed by TR306 accounted for 31% and 22% of the total variance in 1994 and 1995, respectively. A QTL was also detected at the aHor2/MWG943 interval on chromosome 5 though only by sCIM. This QTL was detected only at the time of maximum density (April 24), and a week later (May 2), in 1995 with a low level of significance (Fig. 4).

Discussion

Several genetic studies have reported resistance to aphids in species other than *Rhopalosiphum* spp. Using tertiary trisomic analysis, Gardenhire et al. (1973) mapped resistance to greenbug (*S. graminum* Rondani) on the centromere segment of barley chromosome 1, which does not include the distal region of the short arm (Sogaard and Wettstein-Knowles 1987). More recently, Nieto-Lopez and Blake (1994) found two QTLs

for Russian wheat aphid (*Diuraphis noxia* Mordvilko) resistance: one on chromosome 2 and one on chromosome 5. The symptoms caused by these aphid species were severe and mainly scored by the degree of chlorosis on barley leaves. Although the dominant aphid population was occupied by *R. maidis* and *R. padi* the present study also included small colonies of other species. However, the resistance factor found on chromosome 1 is mainly effective for these two *Rhopalosiphum* species and may not be identical to the genes found in the reports above, because of the different reactions and chromosomal locations among these resistance factors. On the other hand, the QTL found on chromosome 5 in this report may be identical to the resistance factor reported by Nieto-Lopez and Blake (1994) because of their similar chromosome location. However, it is difficult to estimate if the QTL is only effective for the Russian wheat aphid or is also effective for the other aphid species, since we did not evaluate aphid species separately.

It is probable that other aspects of plant growth and morphology affect aphid resistance. Especially, growth stage, as measured by heading date, may alter the feeding value of the barley plant and thus affect the density of aphids. According to the QTL analysis for the same DH population, a QTL for heading date was detected in 1995 (at aHis3A/ABG380) within an interval on chromosome 1 where a test statistic of aphid resistance was highly significant (Sato, unpublished). This QTL was not significant in 1994. This heading-date QTL was also identified at the same marker interval by Tinker and Mather (1994). The range of heading-date in the DH lines was from April 23 to 29 in 1994, and from April 11 to 22 in 1995. As the maximum aphid density (May 1 in 1994 and April 24 in 1995) was recorded just after all the lines headed in both years, heading might have had significant effects on aphid density. Also, the wider range of heading date in 1995 could affect aphid density. The correlation coefficients between heading date and aphid density ranged from 0.34 at the first sampling date to 0.04 at the last sampling date in 1995. No significant correlation was found between these characters in 1994. The distribution of test statistics at the maximum aphid density on chromosome 1 shows only one peak in 1994, but multiple peaks in 1995 (Fig. 4). As one of the peaks in 1995 is identified at the aHis3A/ABG380 interval, where the QTL for heading date is located, the QTL for heading date may have a coupling effect on the QTL for aphid resistance.

Other than heading date, QTLs for other important agronomic characters are also located near the aphid-resistance QTL on chromosome 1. Using the same population, the stem rust resistance gene *Rpg1* was mapped on the extreme subtelomeric region of chromosome 1 (Kilian et al. 1994). Also, a QTL for kernel weight is located in the same region (Tinker and Mather 1994). It is difficult to estimate if the QTL for aphid resistance has a biological relationship with these genes, such as being expressed by the same biochemical factor produced by Rpg1. However, due to linkage, these characters may be expressed by the same genotype. TR306 has a positive allele for all these characters, making it an excellent donor of aphid resistance, stem rust resistance and kernel weight, in a coupling configuration.

The aphid-resistance QTL detected on chromosome 1 in 1994 and 1995 is most likely an effect of the same locus. Aphid resistance is not a pleiotropic effect of the heading-date locus and is independent from heading date. Other QTLs had small effects or were detected only at periods of low aphid infestation. These may be due to experimental error, and are of less practical importance. Although we could not calculate true broad-sense heritabilities because of the unreplicable nature of the experiment, the approximate heritability was estimated using observations at the time of maximum and second-maximum aphid density in 2 years: April 25 vs May 1 in 1994 and April 24 vs May 2 in 1995. These approximate heritabilities were 36% in 1994 and 63% in 1995. The higher approximate heritability in 1995 might come from the effect of the heading-date QTL on chromosome 1 and the effect of the aphid-resistance QTL on chromosome 5 which was especially significant at April 24 vs May 2 in 1995. As the genotypic variances which accounted for the QTL on chromosome 1 were 31% in 1994 and 22% in 1995, this QTL might contribute most of the genetic variance in 1994, and one-third of the genetic variance in 1995. Therefore, marker-assisted selection for aphid resistance would involve markers on chromosome 1 and should allow for the marker iPgd1A to be used as a flanking marker for selection. Abundant markers are available for this region of the genome (Kleinhofs et al. 1993).

Phenotyping aphid resistance on adult barley plants is laborious and prone to uncontrolled error. Aphid density can be affected by environmental conditions, such as heavy rainfall or strong wind. This complicates selection, especially when a large number of genotypes are scored in an experiment. Tightly linked markers provide useful information for the selection of adult plant resistance, even when there is no aphid infestation. The QTL found on the short arm of chromosome 1 may be useful resistance factor for marker-assisted selection. However, the DH population used in this study was not developed especially for the analysis of aphid resistance. Since Tsumuki et al. (1987) reported a number of mechanisms in resistant lines which can maintain the population of aphids at a low level, there might be other resistance factors present in other barley germ plasms. These resistance factors should be mapped to establish their relationship with that mapped in TR306. Ultimately, durable and high levels of resistance may be achieved by pyramiding multiple resistance loci into single genotypes.

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