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Abstract

Aphis gossypii and *Bemisia tabaci* are severe hemipteran pests of melon crops and breeding for resistance to both insects is required to reduce pesticide use. Resistance was evaluated for its effect on behaviour and biotic potential of both hemipterans in a population of RILs derived from the cross Védrantais X PI 161375. Insect variability was considered using two *A. gossypii* clones and two *B. tabaci* populations. Two additive QTLs affected the whiteflies. Four additive QTLs and two couples of epistatic QTLs affected the aphids. Among them, a major QTL affects both behaviour and biotic potential of *A. gossypii* and therefore a same R gene induces both antixenosis and antibiosis. This major QTL colocalizes with the *Vat* gene belonging to the NBS-LRR gene family. No loci affected both aphids and whiteflies contrary to what was observed for the *Mi1.2* gene, a NBS-LRR gene in tomato. Original populations with different allelic compositions at QTLs affecting *A. gossypii* were built by one inter-crossing of RILs used for the mapping process. The genetic background was shown homogeneous between these populations what allowed validating QTLs and investigating the effect of allelic combinations at QTLs. Effects of QTLs were stronger than expected and some QTLs had a wider spectrum than expected. This strategy of validation appeared rapid and low cost.

Introduction

Hemiptera contains major pests of cultivated plants especially in three superfamilies: whiteflies (mostly pantropical), aphids (mostly in the northern temperate regions) and leafhoppers (worldwide). These pests have piercing-sucking mouthparts to probe plant tissues intra and intercellularly. They are phloem feeders and drain plant nutrients what causes direct damages. Moreover, because of their diet rich in sugars, they produce sticky and sugary excreta covering the foliage and serving as substrate to sooty mold fungi. They also deliver viruses and bacteria. Plant responses to hemipteran insects have substantial overlap with responses mounted against microbial pathogens (Kaloshian and Walling, 2005); even if genetic control of plant resistance to insects has been poorly studied compared to resistance to pathogens (Yencho et al., 2000), the heredity of resistance to hemipterans has been described in various plant species, mainly in cereals. Major genes have been identified in most of cases for hemipterans control. Only two genes of resistance to insects have been cloned so far and both belong to the NBS-LRR family resistance genes. The *Mi-1.2* gene, which confers resistance to the nematode *Meloidogyne incognita* and other species of nematodes in tomato, was also shown to confer resistance to the aphid *Macrosiphum euphorbiae* (Rossi et al., 1998) and to the whitefly *Bemisia tabaci* (Nombela et al., 2003). The *Vat* gene isolated in melon confers resistance to *Aphis gossypii* (Pauquet et al., 2004).

Aphids and whiteflies cause direct-feeding damages on melon. *A. gossypii* is the only aphid species able to colonize melon plants. *B. tabaci* is the most damaging whiteflies species in melon crops because of its huge and extending distribution. To control both species, insecticides have been frequently applied and both insects developed insecticide resistances. Because their geographical distribution overlaps in the main production areas, breeding for resistance to both insects is required to reduce the pesticide use. Several sources of resistance to *A. gossypii* and to *B. tabaci* have been identified in melon. *A. gossypii* resistant accessions have been largely described since the 1970s, particularly the Indian and Korean accessions PI 414723 and PI 161375 (Kishaba et al., 1971; Bohn et al., 1972; Lecoq et al., 1979; Pitrat and Lecoq, 1980); in both accessions, the resistance is controlled by the *Vat* gene (Pitrat and Lecoq, 1982). Nevertheless, resistance might be variable according to aphid clones. As early as 1971, Kishaba et al. (1971) pointed out that the melon resistance to the US south-eastern aphids was inefficient against the south-western aphids. In the same way, Soria et al. (2000) observed low resistance levels to *A. gossypii* clones from Spain in accessions that exhibited a high level of resistance to French *A. gossypii* clones. The *Vat* gene effect on different clones of *A. gossypii* is unknown so far. Resistance to *B. tabaci* was investigated more recently during the

1990's. Even if resistance and tolerance were described in several melon accessions few studies dealt with resistance to a characterized biotype. Sauvion et al. (2005) identified several accessions resistant to *B. tabaci* biotype B, among them PI 161375 and PI 414723, also resistant to *A. gossypii*. To our knowledge, inheritance of resistance to *B. tabaci* in melon has not been investigated.

In the present study, we used molecular markers to decipher *A. gossypii* and *B. tabaci* biotype B resistance in melon in quantitative trait loci. Variability of *A. gossypii* was considered using two genetically distant clones and variability of *B. tabaci* biotype B was considered using two natural populations. This study was conducted in a population of recombinant inbred lines (RILs) derived from the cross Védrantais X PI 161375 (resistant to both pests) to identify possible QTLs effective against a large spectrum of hemipterans. Populations with different allelic combinations at QTLs were built in a homogeneous genetic background in order to validate the QTL effects.

Materials and Methods

Plant material and genetic map
Védrantais is a commercial French line of Charentais type (Vilmorin, France). PI 161375 is a Korean accession, resistant to *B. tabaci* and *A. gossypii*. A recombinant inbred line progeny (RILs, F7, F8) was issued from the cross Védrantais X PI 161375.

A genetic map was built using the map produced by Périn et al. (2002) enriched by SSR markers developed by Ritschel et al. (2004) and Gonzalo et al. (2005). Among more than 800 markers available for this progeny in the lab, 216 markers were selected (88 SSR, 98 AFLP, 17 ISSR, 5 phenotypic, 5 RFLP and 3 RAPD) to genotype190 RILs. The linkage groups were determined with the 'group' command of Mapmaker (Lander et al., 1987), using a minimum logarithm of the odds ratio (LOD) of 8 as threshold for linkage detection. The order of the markers on each linkage group was determined using the 'order' command (minimum LOD 6) and marker's position was confirmed using the 'ripple' command. The map was drawn using the MapChart software. The assignment of the linkage groups were as in Périn et al. (2002).

Insects
The seedlings for the mass rearing of aphids and whiteflies were grown in insect proof greenhouses. Two clones of *A. gossypii*, NM1-Lab and 4-104, collected on cucurbits in south-eastern France, were used for the resistance tests. They were genotyped using 8 microsatellite markers and were shown to have a NM1 genotype and a C9 genotype respectively, as described by Carletto et al. (2009). The mass rearing of aphids was conducted on melon (cultivar Védrantais) in a room maintained at 24:18°C, 18:6h photoperiod. Two days after inoculation of plants by apterous adults, adults were removed to obtain 7 days later 5-7 day-old aphids for inoculation. Natural populations of *B. tabaci* were used because clonal lines can not be obtained as *B. tabaci* reproduces by arrhenotokous parthenogenesis. Two populations were caught in Guadeloupe during the cropping seasons 2000-2001 (Bt 2001) and 2001-2002 (Bt 2002) and identified as B biotype accordingly to De Barro and Driver (1997). The mass rearing of whiteflies was conducted on cabbage (*Brassica oleracea*, cultivar Copenhague) in a room maintained at 25-27°C and a 12:12h photoperiod. The day before inoculation, adults were removed from the cages to obtain newly hatched females (unmated) for inoculation.

Resistance to *A. gossypii* assays
All experiments were conducted in a room maintained at 24:18°C, 18:6h photoperiod. We evaluated behaviour and biotic potential components as different factors of resistance.

The behaviour component was the acceptance by aphids 48h after infestation of a plant. Ten 5-7 day-old apterous aphids were deposited on the first or second leaf of 2 week-old seedlings. Two days later, the number of *A. gossypii* remaining on each plant was recorded. Each experiment comprised one plant of 90 to 100 RILs and of Védrantais, PI 161375 and the F1 as controls. One hundred thirty-four RILs were observed. After obtaining 8-10 data per RIL, a new set of experiments were conducted with the RILs exhibiting a coefficient of variation over 30% (observed more frequently with the clone 4-104).Twenty-one experiments were conducted to obtain 8-16 data per RIL with the NM1-lab clone and 40 experiments were conducted to obtain 8-34 data per RIL with the 4-104 clone. The six

populations (A, B, C, D, E and F) built to validate QTLs (see below) were evaluated for acceptance using 35-50 plants per population in four independent tests (A, B, C with each clone on one hand and D, E, F with each clone on the other hand).

Biotic potential was explored through two life history parameters of aphids: the pre-reproductive period and the fecundity of an adult during a period equivalent to the pre-reproductive period. Two adult aphids were caged for egg-laying onto the leaves of a 2-3 week-old plantlet. The day after (d_0), 2-3 nymphs were kept and the adults and other nymphs were removed. The nymphs were daily observed until they died or reached the adult stage. When they produced their first progenies, the day d_n was scored. The pre-reproductive period was estimated as $d_{Ag} = d_n - d_0$. The first adult obtained in each cage was transferred onto a new leaf. The progenies laid out by this adult during a period equivalent to d_{Ag}, P_{Ag}, were counted and removed every two days. Each experiment comprised one plant of 90 to 100 RILs and of Védrantais, PI 161375 and the F1 as controls. One hundred thirty-eight RILs were observed. After obtaining 5-8 data per RIL, a new set of experiments were conducted with the RILs exhibiting a coefficient of variation of the parameters over 30%. Five to thirteen data per line were obtained for both parameters. A simplified test was used to evaluate aphid fecundity on populations combining QTLs (see below) : 2 adult aphids were deposited for egg-laying onto the leaves of a 2-3 week-old plantlet; a glue ring was placed around the peduncle of each leaf to prevent aphid escaping. The day after, 1 nymph was kept and the adults and other nymphs were removed. The progenies produced (when nymphs reached the adult stage) were scored as long as the aphid is alive.

Resistance to *B. tabaci* assays

Experiments were conducted at INRA in Guadeloupe island, Petit-Bourg (French West Indies), from May to August in 2001 and 2002. The seedlings were bred in an insect proof greenhouse. The acceptance, which is a behaviour parameter, was not observed for *B. tabaci* as mobile apterous forms does not exist on this species and acceptance tests with alates are not adapted for genetic studies. One month-old plants were transferred in a screenhouse. One newly hatched female of *B. tabaci* was caged onto a leaf for oviposition. The number of progenies per female, P_{Bt}, was estimated by counting empty puparium 15 to 30 days after infestation. Altogether, progenies of 7 to 17 females were observed for 111 RILs with the *B. tabaci* population Bt 2001 and for 68 among the 111 RILs with the population Bt 2002. Védrantais, PI 161375 and the F1 were included as controls in each experiment.

Data and QTL analyses

The phenotypic values in parental lines were compared taking into account the interval of confidence of the mean estimated as IC = $t_{(0,05,\ n-1)}s$ / $\sqrt{n}$ with t the student value, n the number of data and s the standard error.

Narrow sense heritability of each trait (h^2) was calculated as follows: $h^2 = \sigma^2_g / (\sigma^2_g + (\sigma^2_e/n))$ where σ^2_g is the genetic variance, σ^2_e is the environmental variance and n is the mean number of replicate per genotype. We looked for transgressive RILs among the extreme resistant RILs exhibiting a [mean ± IC] not overlapping with [mean ± IC] of PI 161375 and among the extreme susceptible RILs exhibiting a [mean ± IC] not overlapping with [mean ± IC] of Védrantais. We selected the five most extreme RILs and we compared the data with the parent's data obtained in the same tests (unilateral Mann and Whitney test with exact p). The correlation between all traits observed in the RIL population was investigated using the r coefficient of Pearson.

The additive QTLs were detected using QTL Cartographer software (Basten et al., 1997) with the composite interval mapping procedure. Five markers, selected by stepwise regression analysis, were used as co-factors, with a window of 10 cM and a walking step of 2 cM. The thresholds of significant LOD scores were fixed after 1000 permutations. When several QTLs were detected within less 20 cM interval, only the marker with the highest LOD value was retained. When several markers were significantly associated with the resistance, we considered the overall region as a single QTL and indicated the linked marker exhibiting the highest R^2 value. The epistatic QTLs (digenic interactions) were detected using the two-way analysis of variance (ANOVA, procedure of S-Plus software) between the 216 markers. The p values were corrected for Bonferroni effect as $p_{cor.} = p (216 \times 215)/2$ and then, the threshold $p_{cor.} = 0.05$ was reached when $p = 2.15\ 10^{-6}$. For the detected QTLs, the homogeneity of the variances of the trait between the 4 genotype's classes was verified using a Levene

test. The QTLs were named as followed: the two first letters Ag for *A. gossypii* or Bt for *B. tabaci*, followed by A when the QTL controlled the acceptance or by B when the QTL controlled a biotic parameter, followed by two numbers, X.x, for the xth QTL described on the Xth linkage group (in roman numeral). As an example, *AgA-V.1* is the first QTL described located on the linkage group V that controls an *A. gossypii* traits, the acceptance. We calculated the adjusted global R^2 from an ANOVA, taking into account the markers with the highest LOD from an ANOVA for each QTL.

Breeding melon populations combining resistance QTLs to *A. gossypii* in a homogeneous genetic background

We built new families of plants carrying various allelic combinations at QTLs in a homogeneous genetic background. We chose to create a homogeneous background at a population level by obtaining a heterozygous background between Védrantais and PI 161375 at most loci. The first step was to select RILs derived from the cross Védrantais X PI 161375 on the basis of the allelic composition of the markers at the resistance loci to *A. gossypii*. Seventy RILs were selected and divided into 2 families. The family 1 comprises the RILs selected on the basis of 4 QTLs affecting the acceptance by the NM1-lab clone (2 additive QTLs and one couple of epistatic QTLs), the family 2 comprises the RILs selected on the basis of 2 additive QTLs affecting the acceptance by the 4-104 clone. Each family was composed of three groups: i) the RILs with all the resistant alleles (15 RILs in the family 1 and 11 RILs in the family 2); ii) the RILs with the resistant allele at the major QTL and the susceptible alleles at the others QTLs (11 RILs in the family 1 and 13 RILs in the family 2); iii) the RILs with the susceptible allele at the major QTL and the resistant alleles at the others QTLs (9 RILs in the family 1 and 11 RILs in the family 2). The RILs belonging to a same group were inter-crossed using the pollen mixture technique. Six populations named A, B, C, D, E and F were constructed by mixing an equal quantity of seeds collected on every RIL belonging to a same group. The populations A, B and C derived from the RILs of the family 1 and the RILs D, E and F derived from the RILs of the family 2 (see table 4).

To evaluate the homogeneity of the genetic background within the families, we calculated the expected heterozygosity at each marker in each population derived from the inter-crossing process. Within the group of RILs constitutive of a population, at the marker i, the allele PI 161375 has a frequency p_i, and the allele Védrantais has a frequency q_i such as $p_i+q_i = 1$. The expected heterozygosity in the derived population at the marker i is $H_i=2p_iq_i$. When $p_i=q_i=0.5$ within the group of RILs constitutive of a population, the expected heterozygosity in the derived population is maximum ($H_i =0.5$), i.e. 50% of the plants are heterozygous at the marker i, and 25% of the plants are homozygous for each allele. Then, the genetic background is homogeneous between the populations of a family when $H_i = 0.5$. To calculate the expected heterozygosity, we selected sets of markers without missing data in the studied population. The number of markers took into account was 101, 195 and 104 for the populations A, B and C and 285, 228 and 267 for the populations D, E and F.

The acceptance of each population was predicted according to the allelic combination of the homozygous QTLs in the population. For each QTL selected, the markers with the highest LOD from an ANOVA were included in a linear model to predict the phenotypic value of this population.

Results

Acceptance by *A. gossypii* and biotic potential of *A. gossypii* and *B. tabaci* in Védrantais and PI 161375

Resistance parameters were observed on PI 161375, Védrantais and the F1 with two clones of *A. gossypii* and two natural populations of *B. tabaci* biotype B.

The acceptance, which is a behaviour parameter, was only observed for *A. gossypii* for technical reasons. We observed the mean number of adults remaining on plants 48h after infestation by 10 aphids (Table 1). Acceptance was significantly reduced on PI 161375 compared to Védrantais, 70% with the NM1-lab clone and 30% with the 4-104 clone. The acceptance of the F_1 was intermediate between the parents for both clones of *A. gossypii*.

The number of progenies produced by one female, which is a biotic parameter, was observed for both pests (Table 1). The *A. gossypii* NM1-lab clone produced two fold less progenies on PI 161375 than on Védrantais (t test, p= 0.02). The number of progenies produced on the F1 was close to the

number of progenies produced on PI 161375. The population Bt 2001 of *B. tabaci* produced more progenies than the population Bt 2002 on Védrantais as well as on PI 161375. Both populations produced two fold less progenies on PI 161375 than on Védrantais (t test p<0.01 for both populations), The number of progenies on the F1 was close to the number of progenies produced on PI 161375 with Bt 2001 and close to the number of progenies produced on Védrantais with Bt 2002.

Resistance to *A. gossypii* and *B. tabaci* in a RIL population (Védrantais X PI 161375)
One hundred thirty-four RILs were assessed for the acceptance by two aphid clones, NM1-lab and 4-104. The heritability of the acceptance was 0.92 for the NM1-lab clone and 0.96 for the 4-104 clone. No significant transgressive line was observed for the acceptance by the NM1-lab clone. Acceptance by the 4-104 clone was reduced on the transgressive line, RIL181, compared to PI 161375 (p=0.02) with only 2.3 adults in average remaining on the RIL181 *vs* 4.2 adults on PI 161375. The acceptance by the NM1-lab clone was correlated to the acceptance by the 4-104 clone (Table 2), suggesting common genetic factors for the resistance control toward both clones.

One hundred and twenty seven RILs were assessed for two biotic parameters of the *A. gossypii* NM1-lab clone, the duration of the pre-reproductive period, d_{Ag}, and the number of progenies produced by one female, P_{Ag}. The heritabilities were 0.78 for d_{Ag} and 0.85 for P_{Ag}. We observed a transgressive RIL for P_{Ag}: aphids produced 64.2 progenies on the RIL208 and 46.0 on Védrantais (p=0.03). The two components assessed for the biotic potential of *A. gossypii,* d_{Ag}, and P_{Ag}, were negatively correlated (Table 2), the shortest d_{Ag} and the highest P_{Ag} inducing the highest biotic potential. This correlation suggested the involvement of common genetic factors for the control of these two traits. Moreover, d_{Ag} and P_{Ag} were correlated to the acceptance parameter (Table 2), suggesting that common genetic factors control the acceptance by *A. gossypii* and the biotic potential of *A. gossypii*.

One hundred and eleven RILs were assessed for a biotic parameter, the progenies produced by a whitefly, P_{Bt}, with two natural populations of *B. tabaci*. The heritabilities of P_{Bt} were 0.62 with Bt 2001 and 0.74 with Bt 2002. We observed a transgressive RIL (p=0.04): *B. tabaci* Bt 2002 produced 103 progenies on the RIL140 and only 40 progenies on Védrantais. The number of progenies produced by the whiteflies Bt 2001 was not correlated to the number of progenies produced by the whiteflies Bt 2002 (Table 2), suggesting an independent genetic control of these traits. The component assessed for resistance to *B. tabaci*, P_{Bt}, was not correlated to any components assessed for *A. gossypii*, suggesting an independent genetic control for resistance to *A. gossypii* and to *B. tabaci* (Table 2).

Mapping QTLs of resistance to *A. gossypii* and *B. tabaci*
Genetic map
The 216 markers designed a framework map consisting in 12 linkage groups (corresponding to the basic number of chromosomes in melon) and covering 1312 cM (Kosambi) (Figure 1). The median distance between two markers was 5.1 cM (3.3 cM for the first quartile and 7.8 cM for the third quartile). Therefore the melon genome was well covered by the marker set.

Resistance to A. gossypii
Several QTLs controlled the acceptance by *A. gossypii* (Table 3 and Figure 1). Three additive QTLs, *AgA-V.1*, *AgA-IX.1* and *AgA-IX.2*, affected the acceptance by the NM1-lab and the 4-104 clones. The resistant allele originated from PI 161375, the resistant line, for these three QTLs. The major QTL *AgA-V.1* colocalized with the *Vat* locus. *AgA-V.1* equally affected the NM1-lab and 4-104 clones (R²=71% and R²=66%). *AgA-IX.1* reduced the acceptance by the clone NM1-lab (R²=6.0%) whereas *AgA-IX.2* reduced the acceptance by the clone 4-104 (R²=4.2%). A couple of epistatic QTLs *AgA-VII.1-XI.1* reduced the acceptance by the NM1-lab clone with a R² value over 20%, nevertheless this R² value (issued from an ANOVA) can not be compared to the R² calculated for additive QTLs (issued from composite interval mapping analysis). The global R² was estimated at 82% for acceptance by *A. gossypii* NM1-lab clone, 74% for acceptance by *A. gossypii* 4-104 clone.

Several QTLs affected the biotic potential of *A. gossypii*. NM1-lab clone (Table 3 and Figure 1). One major and additive QTL, *AgB-V.1*, controlled d_{Ag}, the duration of the pre-reproductive period, and P_{Ag}, the number of progenies produced by one female. Its effect (R²) was 55% on d_{Ag}, and 67% on P_{Ag}. *AgB-V.1* peaked at 1.4 cM of the *Vat* locus for d_{Ag} and 8.0 cM of the *Vat* locus for P_{Ag}, its resistant

allele originated from PI 161375. One minor QTL *AgB-IV.1* had an additive effect (R^2=9.3%) on d_{Ag}, its resistant allele originated from the susceptible line Védrantais. A putative QTL (not shown in the table 3) affecting the acceptance by the NM1-lab clone (R^2=2.9%, p=0.066) colocalized with AgB-IV.1. A couple of epistatic QTLs, *AgB-VII.1-XII.1* affected over 20% d_{Ag} (value not comparable to the R^2 calculated for additive QTLs). The global R^2 of the QTLs was estimated at 68% for d_{Ag}, and at 62% for P_{Ag}.

Resistance to B. tabaci biotype B
Two additive QTLs affected the number of progenies produced by one female of *B. tabaci*, P_{Bt} (Table 3 and Figure 1). *BtB-VII.1* affected the Bt 2001 population (R^2=17.9%) and *BtB-IX.1* the Bt 2002 population (R^2=13.8%). The resistant alleles at both QTLs originated from PI 161375. No epistatic QTL affecting *B. tabaci* was detected. The poor QTL detection for resistance to *B. tabaci* is linked to a weak heritability of P_{Bt}. The inflated phenotypic variance in some RILs may be due to the fact that we used unexpected mated females that usually produce more progenies, instead of unmated females. Some RILs that were phenotyped for their *B. tabaci* resistance, were not fully genotyped and therefore, for some markers, the analysis was affected by a reduced effective size of the sample of RILs.

QTL validation in 6 populations combining QTLs of resistance to A. gossypii
Populations were built displaying different allelic combinations at the QTLs affecting either the aphid NM1-lab clone (populations A, B and C, family 1) or the aphid 4-104 clone (populations D, E and F, family 2). The genetic background is homogeneous within a family when the expected heterozygosity is H=0.5 at all the markers. In order to check the homogeneity of the genetic background within a family, the expected heterozygosity at each marker was estimated in each population (Figure 2). The heterozygosity was over 0.4 for about 70% of the markers in the populations A, B and C and for about 80% of the markers in the populations D, E and F. Less than 7% of the markers have a nil heterozygosity whatever the population, these markers are in the vicinity of the QTLs selected to build the populations and therefore are homozygous as expected. For the last 10-15% of the markers, the allelic composition was unbalanced (0.1<H<0.3). Thus, we considered that the populations A, B and C on one hand and the populations D, E and F on the other hand have a homogeneous genetic background.

The phenotypic value was predicted for each population according to its allelic composition at each QTL (Table 4). As expected, the population A which contains the resistant allele at all the QTLs affecting the NM1-lab clone (*AgA-V.1, AgA-IX.1, AgA-VII.1-XI.1*) was predicted to exhibit a reduced acceptance by the NM1-lab clone when compared with the population B which contains only the resistant allele at the major QTL (*AgA-V.1*). The population B was predicted to exhibit a reduced acceptance by the NM1-lab clone when compared with the population C which contains only the resistant allele at the minor QTLs (*AgA-IX.1*, *AgA-VII.-XI.1*). In the same way, the population D that contains the resistant allele at the two QTLs affecting the 4-104 clone (*AgA-V.1*, *AgA-IX.2*) was predicted to exhibit a reduced acceptance by the 4-104 clone when compared to the population E which contains only the resistant allele at the major QTL (*AgA-V.1*). The population E was predicted to exhibit a reduced acceptance by the 4-104 clone when compared to the population F which contains only the resistant allele at the minor QTL (*AgA-IX.2*).

The six populations were evaluated for acceptance by the NM1-lab and 4-104 *A. gossypii* clones (Table 4). In order to observe the effect of the major QTL *AgA-V.1,* we compared two couples of populations with the same allelic composition except at the *AgA-V.1* locus, i.e. A *vs* C and D *vs* F. The resistant allele at *AgA-V.1* reduced the acceptance from 54% (A *vs* C) to 61% (D *vs* F) of the NM1-lab clone. In the same way, the resistant allele at *AgA-V.1* reduced the acceptance by the 4-104 clone from 31% (A *vs* C) to 55% (D *vs* F). These results confirmed the strong effect of *AgA-V.1* on the acceptance by the NM1-lab clone as well as by the 4-104 clone. Its effect appeared slightly stronger on the NM1-lab clone than on the 4-104 clone.

In order to observe the effect of the minor QTLs *AgA-IX.1* and *AgA-VII.1-XI.1,* we compared a couple of populations with the same allelic composition except at *AgA-IX.1* and *AgA-VII.1-XI.1* loci, i.e. A *vs* B (Table 4). The resistant alleles at *AgA-IX.1* and *AgA-VII.1-XI.1* reduced the acceptance by the NM1-lab clone of 24% as expected and, surprisingly, reduced the acceptance by the 4-104 clone of

31%. This effect of the minor QTLs *AgA-IX.1* and *AgA-VII.1-XI.1* was not predicted on the 4-104 clone. In order to observe the effect of the minor QTL *AgA-IX.2*, we compared a couple of populations with the same allelic composition except at the *AgA-IX.2*, i.e. D *vs* E (Table 4). The resistant allele at *AgA-IX.2* reduced the acceptance by the 4-104 clone of 20% as predicted, but this difference was not significant (p=0.11). Surprisingly, the resistant allele at *AgA-IX.2* reduced the acceptance by the NM1-lab clone of 29%. Its effect was not predicted on the NM1-lab clone.

 AgB-V.1, the only QTL predicted to affect the number of progenies, mapped very close of *AgA-V.1*. We checked the allelic composition at the nearest marker of *AgB-V.1* in the 6 populations built to validate the acceptance. The populations D, E and F appeared appropriate for validating *AgB-V.1*, i.e their allelic composition is homogonous at AgB-V.1 (the same than at AgB-V.1). As expected the number of progenies produced was strongly reduced when the resistant allele at *AgB-V.1*is present*:* 2.4 progenies were produced on D and 35.6 on F (p<0.0001)*.* As expected the fecundity was not affected by the allelic composition at *AgA-IX.2* (D vs E)*:* 2.4 progenies produced on D and 3.1 on E (p=0.84). We used a simplified test to evaluate the populations instead of the test used for the RILs evaluation. Therefore, the magnitude of the predicted effect (from the QTL analysis) of the populations cannot be compared to the magnitude of the observed effects of the populations for the fecundity parameter.

Discussion

Plant resistance to insects is mostly quantitatively inherited and most of the QTLs identified control traits that are a response of the plant to the pest attack (usually scored as damages) (Yencho et al., 2000). Few QTLs that affect the insect biology have been described so far (Maliepaard et al., 1995; Yencho et al., 1996; Alam and Cohen, 1998; Yamasaki et al., 1999; Duan et al., 2007), very few affecting aphids (Castro et al., 2005). In our study, we focused on resistance parameters that reveal an effect on the behaviour and the biotic potential of the insect, because we consider that these effects are a more direct and reliable measure of the resistance than damages. Moreover these parameters may allow further modelling of the impact of plant genotypes on insect dynamics. We identified 10 genome locations on 5 linkage groups of the melon genome involved in resistance to hemipterans.

<u>The same locus affects both the behaviour and the biotic potential of aphids</u>
 Since Painter (1951), entomologists have distinguished two mechanisms of resistance to insects: antixenosis that affects the behaviour of insects and, antibiosis that affects their biotic potential. Insect behaviour is a complex trait that involves physical and chemical interactions with hosts, parasites and environment. The biotic potential of insects depends on different life traits as fecundity, mortality, duration of larval development etc… In our study, two major QTLs affecting either the behaviour or the biotic potential of *A. gossypii* colocalized with the *Vat* gene and should correspond to this gene. In the same way, the tomato *Mi-1.2* gene alters the feeding behaviour of the aphid *M. euphorbiae* and drastically decreases its fecundity and longevity (Kaloshian et al., 1997). The AKR gene in *Medicago truncatula* induces deterrence and low biotic potential of the aphid *Acyrthosiphon kondoi* (Klingler et al., 2005). The behaviour and the biotic potential of several aphid species appear affected by a same major R gene. Thus, antixenosis and antibiosis should be considered as two responses of aphids to R genes. The melon *Vat* gene and the tomato *Mi-1.2* gene belong to the NBS-LRR family of R genes (Milligan et al., 1998; Pauquet et al., 2004), and NBS-LRR genes are also candidates for the *M. truncatula* AKR locus (Klingler et al., 2005). NBS-LRR proteins have been shown to be involved in the recognition of pathogens (McHale et al., 2006) and are therefore probably involved in the recognition of aphids. This recognition induces a complex plant response which, interestingly, leads aphids to modify their behaviour (antixenosis effect). On a *Vat*-resistant melon plant, *A. gossypii* seldom reaches the phloem, stops feeding in phloem when reached (Chen et al., 1996; Klingler et al., 1998), and then the starvation affects its biotic potential.

 QTLs affecting the biology of several hemipterans have been described in tomato (Maliepaard et al., 1995), wheat (Castro et al., 2005) and rice (Alam and Cohen, 1998; Wang et al., 2004; Duan et al., 2007). These QTLs affect the behaviour, the biotic potential, or both of their targets. Here we showed that minor additive or epistatic QTLs affected either the behaviour or the biotic potential of *A. gossypii*. One of these minor QTLs, *AgB-IV.1,* affected the biotic potential of *A. gossypii* and was a putative QTL affecting the acceptance by the NM1-lab clone. Therefore, as for major genes,

antixenosis and antibiosis should be considered as two responses of hemipterans to QTLs of resistance.

Specificity of the resistance loci to hemipterans

Several major genes for resistance to aphids have been described. More often the resistance conferred by these genes is biotype-specific, such as the resistance to *Amphorophora idaei* in raspberry (Sargent et al., 2007), the resistance to *Schizaphis graminum* and *Diuraphis noxia* in wheat (Berzonsky et al., 2003) and, the resistance to *Dysaphis devecta* in apple trees (Alston and Briggs, 1977). In our study, we used two distantly related clones of the *A. gossypii* species, the 4-104 clone with a C9 genotype, and the NM1-lab clone with a NM1 genotype (Carletto et al., 2009). We identified a major QTL that reduces acceptance by both *A. gossypii* clones. This major QTL colocalizes with the *Vat* gene, which has been characterized so far using the NM1-lab clone (NM1 genotype). We showed here that the *Vat* gene also reduces acceptance by a C9 clone. Moreover, we used two hemipteran species, *A. gossypii* and *B. tabaci* to track QTLs with a broad effect on piercing sucking insects. No QTL affecting both *A. gossypii* and *B. tabaci* was detected in the RIL population we used. The *Vat* gene did not confer any resistance to *B. tabaci* biotype B as already suggested by Sauvion et al. (2005). These results contrast with the spectrum of the tomato *Mi-1.2* gene, which confers resistance to different pests such as nematodes, aphids, whiteflies and psyllids (Milligan et al., 1998; Nombela et al., 2003; Casteel et al., 2006) but confers resistance to a single clone of the aphid *M. euphorbiae* (Goggin, 2007). Specificity of resistance to hemipterans remains poorly studied, but knowledge from the *Mi-1.2* and *Vat* genes suggested that the NBS-LRR genes offer an unpredictable spectrum of resistance against hemipteran species.

Effect of allelic combinations at QTLs

To validate the QTLs, we opted to compare populations with a homogeneous genetic background (at the population level) and different allelic combinations at QTLs. The populations were derived from RILs used for the QTL mapping by inter-crossing set of RILs with the same allelic combinations at QTLs. This original strategy offers several advantages: i) the new populations are obtained in one generation, the expected homogeneity between populations corresponds to the expected homogeneity after 5 to 6 back-crosses between a line and a recurrent parent, ii) it is not necessary to carry on any new genotyping, iii) the effects of the QTLs (and of the combinations of QTLs) can be evaluated within a confidence interval and, iv) the effect of the detected QTLs on different clones can be investigated. This strategy requires the inter-crossing of enough RILs with the same allelic combinations to obtain a high heterozygosity level in the population. The expected heterozygosity can be checked before inter-crossing, especially if the number of RILs available is low. If needed, the heterozygosity can be inflated by inter-crossing each RIL used as a female by a pollen mixture excluding its own pollen. This strategy also requires the phenotyping of at least 30 plants per population because the genetic background is homogeneous at the population level (the genetic background of each plant is distinct to each other).

In this study, the validation procedure allowed confirming the strong effect of the major QTL *AgA-V.1* on acceptance by *A. gossypii*; its effect appeared even slightly stronger than expected. We showed that the combination of minor QTLs (additive and epistatic) have a significant effect on acceptance by *A. gossypii*; this combination effect appeared stronger than expected (acceptance reduced of 6% according to the predicted values and of 24% according to the observed values). Moreover, according to the clone, we observed a significant but unexpected effect of some combinations (acceptance reduced of 30% with the NM1-lab clone while no reduction was expected). New clones representative of all the *A. gossypii* variability will be used to investigate the spectrum of efficiency of these combinations of QTLs. The effect of associating a major QTL with minor QTLs on durability of the major QTL will be investigated.

Melon breeding perspectives

Aphids, as whiteflies, invade crops in low numbers early in the season and their population increase gradually over generations before reaching damaging levels. Kennedy et al. (1987) suggested that, for such pests, even low or moderate levels of all types of resistance could increase the time necessary to the population to reach a damaging level.

Although *B. tabaci* is considered as a devastating pest on several crops, loci affecting the biology of *B. tabaci* has been only characterized in tomato (Nombela et al., 2003). In our study, two minor QTLs, each of them specific to a population of *B. tabaci* biotype B, were detected. Most likely, the lack of control over the variability of *B. tabaci* biotype B impaired the detection of QTL. *B. tabaci* has been structured in 12 major clades according to the COI sequences (Boykin et al., 2007). Intra-clade or intra-biotype variation has been only investigated in whiteflies from Asia-Pacific region (de Barro, 2005). Thus, more efforts are needed to improve genetic studies i) to characterize the intra and inter biotype variability of *B. tabaci* populations infecting melon crops and ii) to control the breeding of *B. tabaci* in the mass rearings in the aim of inflating heritability of resistance in biological tests.

The *A. gossypii* genotypes that colonize cucurbits crops belongs either to the NM1 genotype, up to now only identified in France, or to a cluster of a dozen of related genotypes (Carletto et al., 2009). In this cluster, the C9 genotype is the most frequent and is worldwide distributed. In our study, we showed that the *Vat* gene affects *A. gossypii* NM1-lab clone, with a NM1 genotype, and 4-104 clone with a C9 genotype. For some clones having a C9 genotype (including the clone 4-104), Lombaert et al. (2009) did not observe any significant difference in residence time (a parameter comparable to acceptance) on *Vat*- and non *Vat*-melon. This lack of consistency with our results could be due to a lack of power to reveal difference (β risk) in the biological test used by Lombaert et al. (2009). Moreover, we showed that the accession PI 161375, the accession carrying the resistant allele at the *Vat* locus, was resistant to different clones belonging to the NM1 or C9 genotypes (Boissot et al., 2008). Altogether, these results suggest that the *Vat* gene affects different clones of NM1-lab and C9 genotypes of *A. gossypii* and it appears as a solid basis for breeding resistance for all production areas. Moreover, the resistance could be reinforced by minor additive and epistatic QTLs whose efficiency when combined to the *Vat* gene was proved in our study. We will investigate the effect of these minor QTLs on the durability of the *Vat* gene using the populations combining the *Vat* gene with different QTLs.

Acknowledgments

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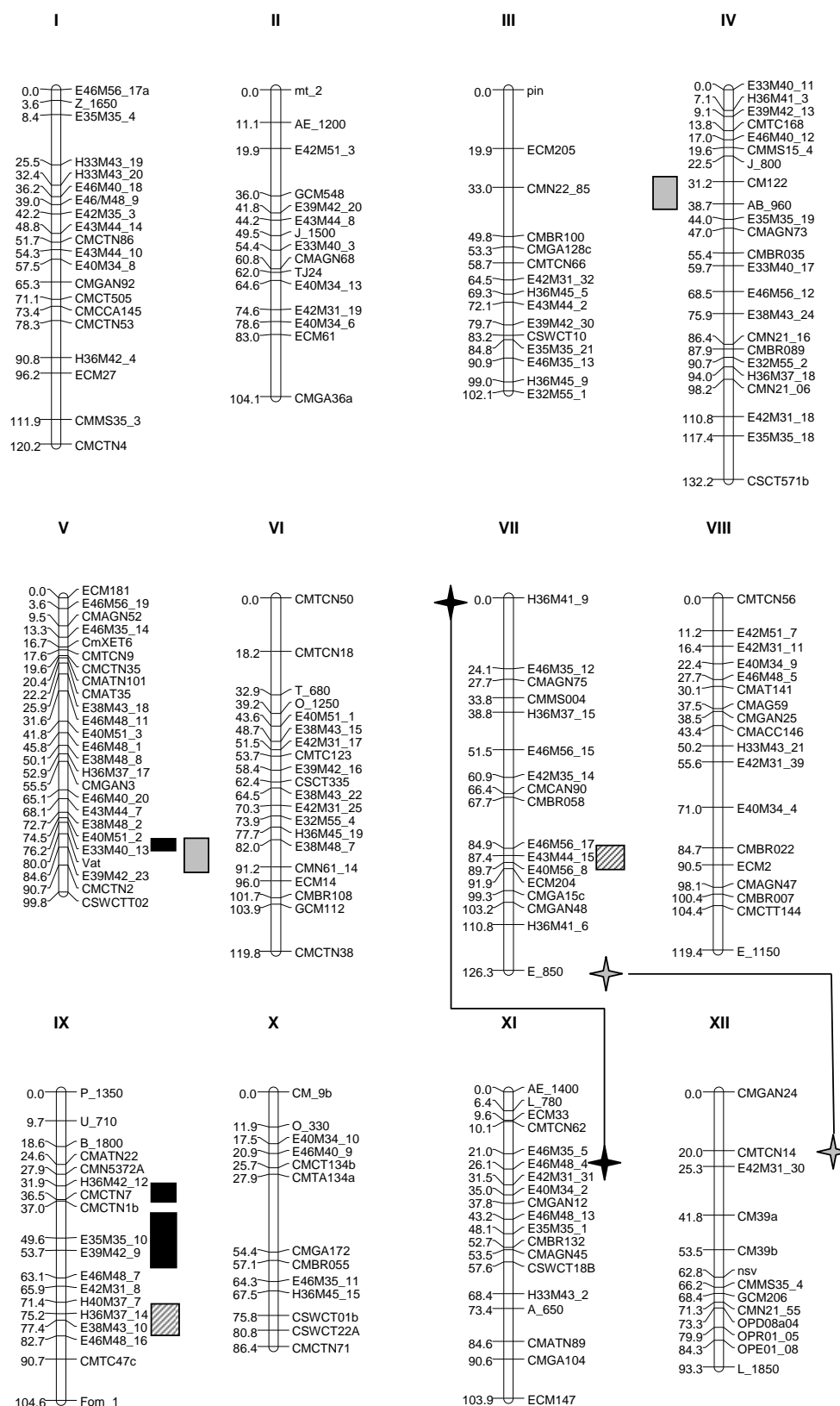
Fig. 1 Genetic map of melon (Védrantais X PI 161375) and QTLs affecting hemipterans: plain: *A. gossypii* and striped: *B. tabaci*, black: acceptance, grey: biotic potential, rectangle: additive QTL (right side: PI 161375 allele for resistance, left side: Védrantais allele for resistance) and star: epistatic QTL (same side of the two linkage groups: cis effect, opposite of the two linkage groups: trans effect).
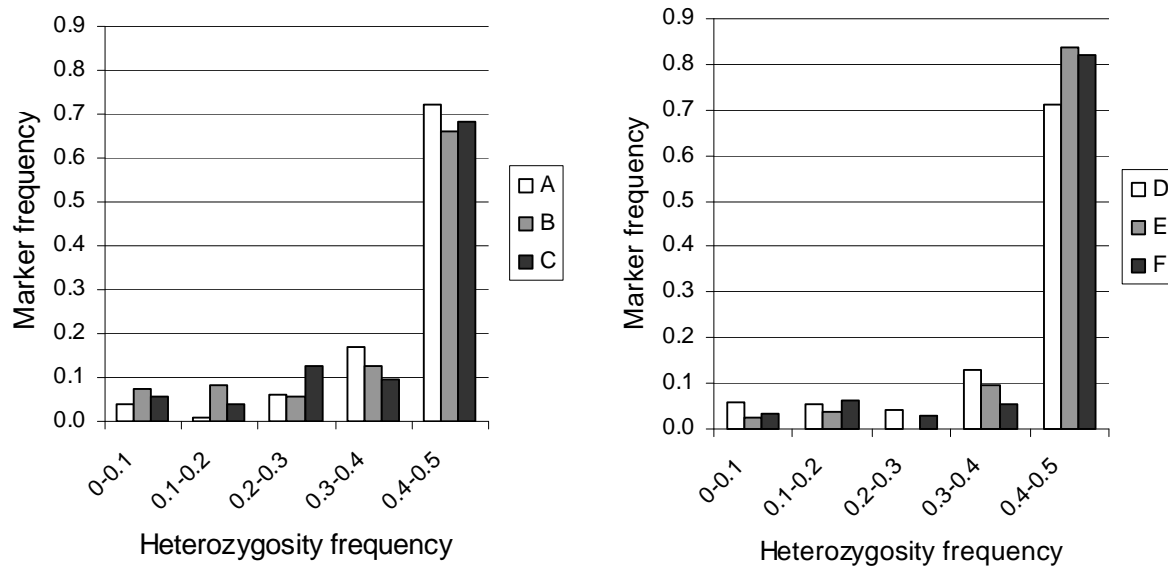
Fig. 2 Heterozygosity frequency in the populations of the family 1 combining the 4 QTLs, (populations A, B, C), and in the populations of the family 2 combining 2 QTLs, (populations D, E and F) for a set of markers (from 101 to 195 markers in the first family and 225 to 285 markers in the second family).

Table 1 Resistance parameter (mean ± CI 95%) to *A. gossypii* and *B. tabaci* observed on PI 161375, Védrantais and the F_1: acceptance (aphids remaining 48h after infestation by 10 aphids) and progenies produced by one female (during a time as long as the pre-reproductive period for *A. gossypii* and during all the life for *B. tabaci*)

| | Acceptance by | | Progenies produced by | | |
| | *Aphis gossypii* | | *Aphis gossypii* | *Bemisia tabaci* biotype B | |
	NM1-lab [a]	4-104 [a]	NM1-lab [a]	Bt 2001 [b]	Bt 2002 [b]
Védrantais	8.3 ± 1.0	7.1 ± 0.4	40.3 ± 12.7	112 ± 33	40 ± 12
PI 161375	2.6 ± 1.0	4.9 ± 0.8	19.7 ± 11.9	54 ± 27	17 ± 12
F_1	5.4 ± 1.7	5.8 ± 0.7	8.4 ± 7.6	53 ± 27	39 ± 18

[a] clone, [b] population

Table 2 Pearson correlation matrix between parameters of resistance to *A. gossypii* and *B. tabaci* calculated on a set of RILs derived from the cross Védrantais X PI 161375 (in bold the significant r at p<0.05)

| | *Aphis gossypii* | | | *Bemisia tabaci* | |
| | Acceptance | d_{Ag} [x] | P_{Ag} [y] | P_{Bt} [z] | P_{Bt} |
	4-104	NM1-lab	NM1-lab	Bt 2001	Bt 2002
A. gossypii					
Acceptance NM1 lab	**0.81**	**-0.83**	**0.78**	-0.01	0.01
Acceptance 4-104		**-0.77**	**0.68**	-0.03	0.04
d_{Ag} NM1-lab			**-0.80**	0.02	0.08
P_{Ag} NM1-lab				-0.05	0.00
B. tabaci					
P_{Bt} Bt 2001					0.08

[x] pre-reproductive period of *A. gossypii*
[y] progenies produced by one female *A. gossypii* during a period as long as d
[z] progenies produce by one female *B. tabaci* during all its life

Table 3 QTLs with an additive effect (Composite interval mapping) and an epistatic effect (ANOVA) on the acceptance by *A. gossypii* and the biotic potential of *A. gossypii* NM1-lab clone (NM1 genotype) and 4-104 clone (C9 genotype) and on biotic potential of *B. tabaci* biotype B.

Trait	QTL	LG[a]	Marker[b]	Nb[c] Ind	Position[d] cM	LOD[e] Value	P[f]	Resistant[g] allele
Aphis gossypii acceptance by NM1-lab clone								
	AgA-V.1	V	Vat	118	78.1-82.6	39.5***		PI 161375
	AgA-IX.1	IX	H36M42_12	120	31.1-37.2	5.9***		PI 161375
	AgA-VII.1-XI.1	VII	H36M41_9	106	0		0.03	Epistasis trans[h]
		XI	E46M48_4		26.1			
A. gossypii acceptance by 4-104 clone								
	AgA-V.1	V	Vat	120	78.2-81.6	35.7***		PI 161375
	AgA-IX.2	IX	E35M35_10	126	40.5-58.5	3.7**		PI 161375
A. gossypii biotic potential NM1-lab clone								
d_{Ag}	AgB-V.1	V	E33M40_13	112	78.1-82.0	27.2***		PI 161375
	AgB-IV.1	IV	CM122	79	31.2-42.6	5.8**		Védrantais
	AgB-VII.1-XII.1	VII	E_850	75	126.3		0.03	Epistasis cis[h]
		XII	CMTCN14		20.0			
P_{Ag}	AgB-V.1	V	E39M42_23	108	86.3-89.5	19.5***		PI 161375
Bemisia tabaci biotic potential population 2001								
P_{Bt}	BtB-VII.1	VII	E43M44_15	37	85.8-94.2	3.6*		PI 161375
B. tabaci biotic potential population 2002								
P_{Bt}	BtB-IX.1	IX	H36M37_14	59	72.0-82.0	4.0**		PI 161375

[a] Linkage group
[b] The nearest flanking marker to QTL
[c] The number of RILs genotyped for the identified QTL-linked marker
[d] Estimated position of the QTL within ± 1 LOD unit
[e] LOD value for the additive QTLs with significance at 5% (*),1% (**), 0.1% (***) after 1000 permutations
[f] P value corrected for Bonferroni effect for the epistatic QTLs (See materials and methods)
[g] Parental allele which contributed to the resistance
[h] Trans: Resistant alleles are Védrantais X PI 161375 or PI 161375 X Védrantais, Cis: Resistant alleles are Védrantais X Védrantais or PI 161375 X PI 161375

Table 4 Acceptance by NM1-lab and 4-104 *A. gossypii* clones (adults 48h after infestation by 10 adults) on 6 populations combining QTLs of resistance: predicted from the QTL analysis (ANOVA) and observed on the populations.

| | Allele at the resistance locus[y] | | | | Acceptance by two *A. gossypii* clones | | | |
| | | | | | Predicted values from QTL analysis | | Observed values on the population[z] | |
Population	*AgA-V.1*[l]	*AgA-IX.1*[m]	*AgA-VII.1-XI.1*[m]	*AgA-IX.2*[n]	NM1-Lab	4-104	NM1-lab	4-104
A	R	R	R	H	4.3	5.1	3.8[a]	4.6[a]
B	R	S	S	H	4.6	5.1	5.0[b]	5.8[b]
C	S	R	R	H	8	7.3	8.4[c]	6.7[c]
D	R	H	H	R	4.6	4.6	2.6[a]	3.1[a]
E	R	H	H	S	4.6	5.1	3.7[b]	3.9[a]
F	S	H	H	R	8.5	6.8	6.7[c]	6.9[b]

[l] QTL detected with both aphid clones

[m] QTL only detected with the NM1-lab aphid clone

[n] QTL only detected with the 4-104 aphid clone

[y] R: homozygous for the resistant allele, H: either homozygous (R or S alleles) either heterozygous, S homozygous for the susceptible allele

[z] means significantly different at 5% within column for each family (A, B and C family 1 or D, E and F, family 2).