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ORIGINAL PAPER

Genetic architecture of factors underlying partial resistance to *Alternaria* leaf blight in carrot

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Abstract In most production areas, *Alternaria* leaf blight (ALB) is recognized as the most common and destructive foliage disease in carrot. To assess the genetic architecture of carrot ALB resistance, two parental coupling maps were developed with similar number of dominant markers (around 70), sizes (around 650 cM), densities (around 9.5 cM), and marker composition. The $F_{2:3}$ progenies were evaluated in field and tunnel for two scoring dates. The continuous distribution of the disease severity value indicated that ALB resistance is under polygenic control. Three QTLs regions were found on three linkage groups. Two of them were tunnel or field specific and were detected only at the second screening date suggesting that the expression of these two QTLs regions involved in resistance to *Alternaria dauci* might depend on environment and delay after infection.

Introduction

Carrot (*Daucus carota* spp. *sativus*) is one of the most popular and commonly consumed vegetables worldwide (Farrar et al. 2004), with 21 million ton produced in 2003 on an area of one million ha. *Alternaria* leaf blight (ALB), which is the most common and destructive foliage disease of carrot, is due to *Alternaria dauci*. This fungus is responsible for severe infection that leads to loss of photosynthetic tissues, weakening or killing the leaves (Ben-Noon et al. 2003). During mechanical harvesting, carrots are lifted from the ground by their leaves (Farrar et al. 2004). Therefore, foliage disease can decrease the harvesting effectiveness and substantially reduce the root yield (Soylu et al. 2005). Losses of 40–60% have been observed (Ben-Noon et al. 2003) and could even reach 100% when weakened-leaves break during harvest, thus leaving the roots in the soil. The disease already reported in a great number of production areas, is currently considered to be an important world-wide disease problem (Pryor et al. 2002).

Different ways to protect carrots from ALB are available, but none of them is really efficient, particularly when infection is severe. Agricultural practices such as seed and foliage treatment, crop rotation, and foliage destruction after harvesting could help to control the disease. Fungicides are intensively sprayed on fields, but this approach is costly and not always effective (Ben-Noon et al. 2001). Indeed, the development of fungicide resistance, particularly to iprodione, has been documented (Gaube et al. 2004; Pryor et al. 2002). ALB management models are being developed to optimize the number of treatments and dates (Rogers and Stevenson 2006). Non-fungicidal treatments have also been tested using: only those using gibberellins had an effect on the disease, when the disease pressure was low and the gibberellin concentration was high (Farrar et al. 2004).

The development of genetic resistance offers a promising alternative. Breeders have developed ALB partially

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resistant carrot cultivars but, even though different levels of disease control were obtained, no complete resistance has been observed (Pryor et al. 2002). Very little is known about the genetic control of this trait. Simon and Strandberg (1998) reported partial resistance when they evaluated carrot inbred lines for their resistance to *A. dauci*. Boiteux et al. (1993) found a narrow-sense heritability rating of 0.4 for leaf blight disease in a half-sib population. These results suggest that resistance is under polygenic control and, even though environmental conditions affect the expression of this trait, that resistance could be increased by genetic improvement. However, nothing is known about the number of genetic factors involved and their contribution to resistance. Moreover, these two papers described resistance among imperator and Brasilia carrot varieties. In Europe, only Nantes carrots are marketed.

The aim of the present study is, firstly, to check if resistance in Nantes types is also under a polygenic control and, secondly, to investigate the genetic architecture of factors involved in such ALB resistance, using a QTL detection approach.

Materials and methods

Plant materials

The mapping population was derived from a cross between a susceptible line S269 (Vilmorin) obtained from Carentan (France) and a partially resistant line R268 obtained from the Vilmorin breeding program.

One hundred and seventy-one F_2 genotypes and 171 $F_{2:3}$ progenies were obtained by selfing the F_1 hybrid and F_2 genotypes, respectively. F_3 plants obtained from a single F_2 plant belonged to the same $F_{2:3}$ progeny.

Two cultivars, i.e., two hybrids widely grown in France, were also studied: Presto (Vilmorin) is a highly susceptible cultivar (Ben-Noon et al. 2001; Villeneuve et al. 2001) and Bolero (Vilmorin) is partially resistant to ALB (Corbaz and Perko 1995; Simon and Strandberg 1998).

Phenotypic evaluations

Three experiments were conducted: one with the parents of the mapping population, the F_1 hybrid and reference cultivars compared under tunnel conditions, and two on the $F_{2:3}$ progenies both in tunnel and in the field. In this paper, they are, respectively called the “parent experiment” and “$F_{2:3}$ experiments”. Only 129 $F_{2:3}$ progenies among the 171 of the mapping population were studied because there were not enough seeds obtained from the 42 other $F_{2:3}$ progenies to perform the experiments.

$F_{2:3}$ experiment and parent experiment in tunnel were performed from June to November at the National Institute of Horticulture (INH) in Angers (France) in 2002 and 2003, respectively, with the same experimental design and inoculation protocol. Plants were grown in a randomized complete block design (RCBD) with two blocks. Each plot contained 80 plants. Twenty lines of each reference genotype were randomly introduced in the trial. Moreover, Presto was sown around each block to ensure an uniform fungus development.

Plants were artificially infected with a single-conidial isolate of *A. dauci* (P2 strain) collected from naturally infected carrots grown in Cestas (Gironde, France) and reported as being aggressive by Gaube et al. (2004). The inoculum preparation procedure was described in Pawelec et al. (2006). In summary, the first inoculation was performed when plants showed three true leaves and the second one 15 days later. A conidial suspension of 4–5 $\times$ 10^3 mL^{-1} conidia was sprayed to runoff with an atomizer on leaves. A high relative humidity (95–100% RH) was maintained for 48 h after inoculation.

The $F_{2:3}$ experiment in the field was performed from June to November 2002 in Les Landes, France. Plants were grown in an RCBD with three blocks. Each plot contained 300 plants of a $F_{2:3}$ progeny. A row of cv Presto was sown between each $F_{2:3}$ row in order to check for contamination uniformity. When contamination was not uniform, each score was corrected according to the formula: $N_c = N_{F_{2:3}} - (N_P - N_{PM})$ where $N_{F_{2:3}}$ was the score of the $F_{2:3}$ progeny before correction, N_c was the score of the $F_{2:3}$ progeny after correction, N_P was the score of the plot of Presto near the $F_{2:3}$ plot and N_{PM} was the mean cv Presto score for the experiment.

As Les Landes is a region where deep attacks of *A. dauci* occur each year, the present experiment was naturally infected without any additional inoculation.

Disease severity values (DSV) were visually scored on a 0–9 scale (Pawelec et al. 2006), where 0 means that no visible disease damage was observed in a plot, and 9 means that the plants were totally blighted.

In the parent experiment, disease development was assessed as soon as the first symptoms appeared and reassessed every 15 days for two rounds.

In $F_{2:3}$ progenies, disease development was assessed only twice because there was no further disease development due to the environmental conditions.

Four scores were thus obtained for each $F_{2:3}$ progeny. The traits were named score1T, score2T, score1F, and score2F, respectively, for score one in tunnel, score two in tunnel, score one in the field, and score two in the field.

Most data were analyzed with SAS software (version 8.1) (SAS Institute Inc 1991). The Student-Newman–Keuls test was performed using the PROC GLM procedure to

compare the DSV of the two parents, the F_1 hybrid and the mean DSV between the two parents. The Qstats function of the QTL cartographer software package was used to test $F_{2:3}$ data normality (Basten et al. 2000). The PROC CORR procedure of SAS was performed on the $F_{2:3}$ progenies data to determine correlations between score1T, score2T, score1F, and score2F. The PROC GLM procedure was also used to calculate the broad-sense heritability according to the formula: $h^2 = \sigma_G^2/(\sigma_G^2 + \sigma_G^2/n)$, where σ_G^2 and σ_E^2 were the genetic and residual variances, respectively, estimated as $\sigma_G^2 = (MS_G - MS_E)/n$ and $\sigma_E^2 = MS_E$, with MS_G and MS_E being the genetic and residual mean squares and n being the population size (Gallais 1990).

Genetic map construction

Total genomic DNA was extracted from root tissues of 171 F_2 plants according to Briard et al. (2000). Three kinds of dominant markers were used: amplified fragment length polymorphism (AFLP) markers, inter-simple sequence repeat (ISSR) markers, and amplified polymorphic fragments of resistance gene analog (APF-RGA) markers. All amplifications were performed on a PTC100 cycler (MJ research).

The AFLP protocol was the same as that described by Briard et al. (2000). The ISSR protocol was as described by Le Clerc et al. (2005) and slightly modified. DNA was 1/10 diluted in distilled water. PCR reactions were performed in a total volume of 6.25 µL containing 1.25 µL of diluted DNA, 0.1 mM of each dNTP (Eurogentec), 1 mM $MgCl_2$ (Perkin Elmer), 0.25 µM of a single primer, and 0.375 units of Silverstar DNA polymerase (Eurogentec).

For the amplification of APF-RGA markers, approximately 30 ng of DNA (3 µL) were digested for 2 h at 37°C with 1.5 units of Mse (invitrogen) in a 3.5 µL reaction volume (0.65 µL 10× Mse buffer, 0.3 µL Mse enzyme, 2.55 µL water). Subsequently, 6.5 µl of the digested DNA solution was subjected to ligation with Mse restriction-site derived adapter for 16 h at 16°C in a 6.3 µL reaction volume (1.2 µL), Mse adapter (50 pmol), 0.25 µL T4 DNA ligase (Eurogentec), 1.28 µL 10× ligation buffer, 3.57 µL water).

An aliquot of this ligation mix was diluted 20× with TE buffer and preamplified with adapter-derived primers having one additional (+1) nucleotide (C) at the 3′ end. Each 10.2 µL preamplification volume contained 1 µL diluted ligated DNA, 4 pmol each primer specific to the adapter and Mse site, 1 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 units TAQ polymerase (Eurogentec), 1 µL 10× Taq buffer, 7.3 µL water. After 5 min denaturation at 94°C, this reaction was performed for 20 cycles with the following cycling profile: 30 s at 94°C, 1 min at 56°C, 1 min at 72°C, and 5 min at 72°C.

The preamplification products were 50-fold diluted and 1 µL was used as template for selective amplification. The amount of 10 µL reaction volume contained 17 pmol primer specific to an RGA locus (Ptokin 1, NBS2), 1.7 pmol Mse primer with a (+3) extension (three random nucleotides at the 3′ end), 0.75 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 units TAQ polymerase (Eurogentec), and PCR buffer 1×. The thermal cycling conditions were as follows: one cycle of 5 min at 94°C, 40 cycles of 1 min at 94°C, 1 min at 50°C, 30 s at 72°C, and one cycle of 5 min at 72°C. Ptokin 1 was described first by Chen et al. (1998).

The AFLP, ISSR, and APF-RGA primer sequences are shown in Table 1. The electrophoresis and silver staining procedures were previously described by Briard et al. (2000). Electrophoresis was carried out in a 6% denaturing polyacrylamide gel with 7.5 M urea at a constant power of 60 W using TBE buffer 1×. The procedure lasted 2–3 h for APF-RGA and 4.5 h for ISSR and AFLP.

The chi-square test was performed to test the segregation distortion of each marker. A ratio of 3:1 was expected with an F_2 population and dominant markers.

Genetic maps were constructed with mapmaker software (Lander et al. 1987) using the Kosambi mapping function (Kosambi 1944). Markers were grouped when LOD $\geq$ 4.

The dataset was split into two subsets, with each containing dominant markers only in the coupling phase. Two

Table 1 Sequences of the three selective nucleotides for AFLP and sequences of the ISSR primers and APF-RGA primers (three selective nucleotides of the second primer)

Marker type	Restriction enzymes	Primer sequence
AFLP	EcoRI/MseI	AAG/CAG
		AAG/CTA
		AGG/CTA
AFLP	EcoRI/TaqI	ATG/ACT
		ATG/ACA
AFLP	HindIII/MseI	AAG/CAG
		AAG/CAT
		AGC/CAG
		AGC/CAT
AFLP	HindIII/TaqI	AAG/AGC
		AAG/ATG
		AGC/AGC
ISSR[a]	–	VHVCTCTCTCTCTCTCT
		HVHTCTCTCTCTCTCTC
		HBHAGAGAGAGAGAGAG
APF-RGA	Mse	GCATTGGAACAAGGTGAA/CTA
		GCAACAGAAGGGTTGGGGTGG/CAG
		GCAACAGAAGGGTTGGGGTGG/CTA

[a] V = G, A, C; H = A, T, C; B = C, G, T

parental maps were thus built. When a marker was present for the S269 parent (respectively, R268) and F_2 progeny, it belonged to the marker subset used to construct the "S269 map" (respectively, "R268 map"). A parental map was built for each subset. The "Group" mapmaker function was performed on an all marker set (mixed-phase marker set) to determine which markers belonged to the same linkage group and linked the two maps.

QTL detection

The QTL detection was carried out with the QTL cartographer software package (Basten et al. 2000). With an F_2 population and dominant markers, two genotypic classes are expected and additive and dominance effects are merged. QTL cartographer was thus not able to reliably estimate these two parameters and hence to detect QTLs. A back-cross model was used to overcome this problem. QTL detection was performed separately for each parental map. With the parental S269 map (respectively, R268 map), we thus tested differences between RS + SS and RR genotypes (respectively, RS + RR and SS genotypes), where R and S were alleles from R268 and S269, respectively.

QTLs were mapped with the simple interval mapping (SIM) method (Lander and Botstein, 1989) and the composite interval mapping (CIM) method (Zeng 1993; 1994). A walking speed of 2 cM was chosen and the percentage of total phenotypic variation explained (R^2) was estimated for each QTL.

CIM is a multiple regression procedure that corrects for background effects of markers (cofactors) other than those in the interval being tested. To choose cofactors, we used the "SRmapqtl" function with forward-backward elimination (FB). For each score, markers with the highest *F* values were used for the CIM analysis. There were 2–4 cofactors according to the genetic map and trait. The window size around the test interval, i.e., the region not considered as a background cofactor, was set at 10 cM.

The LOD thresholds were determined for each parental map with the 1000 permutations test. The confidence interval of each QTL was determined by the LOD drop-off one method.

Results

In the parent experiment, the parental DSV were significantly different from each other for each score (Fig. 1). DSV for R268 and S269 were, respectively, two and five for score one; 3.5 and eight for score two; 5.5 and nine for score three. The greatest difference between parent DSV was obtained for score two, with a differential value of 4.5. S269 (susceptible parent) had a higher DSV than cv Presto, whereas R268 (resistant parent) and cv Bolero had similar disease severity levels.

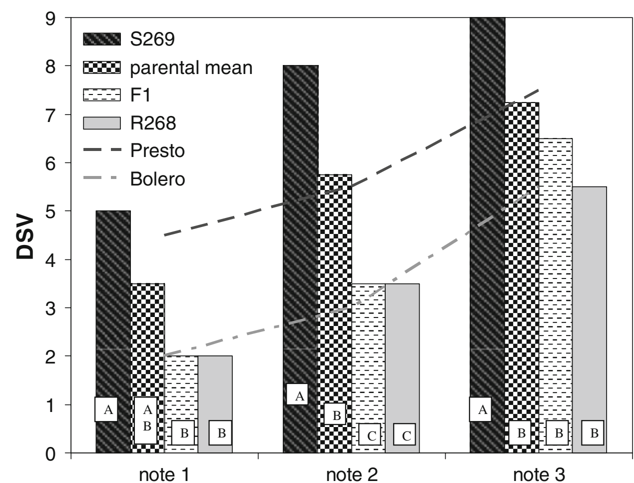

Fig. 1 Comparison of the disease severity values (DSV) calculated for the R268 and S269 parents, the F_1 hybrid, the parental mean and the reference cultivars for three screening dates. The parental mean is the arithmetical mean between the two parent DSVs. **a–c** are the Student-Newman–Keuls groups calculated for each score

Irrespective of the score, the F_1 hybrid and R268 DSV were not significantly different, and the DSV for the F1 hybrid was always lower than the DSV for the parental mean (average between R268 and S269 DSV), but significantly different only for score two.

In the $F_{2:3}$ experiments, the $F_{2:3}$ DSV distributions were continuous for both field and tunnel experiments (Fig. 2). They were significantly normally distributed ($P \leq 0.05$) except for score2T which showed a skewed distribution toward resistance. For each environment, field or tunnel, the DSV distribution showed that the symptom severity increased between the two screening dates. In tunnel, DSV ranged from four to seven, with a mean of 5.3 for score1T, and from five to 8.5, with a mean of 6.3 for score2T. The phenotypic variance was 0.4 for both screening dates. In the field, DSVs were similar to the levels obtained in tunnel and ranged from 4.1 to 7.1, with a mean of 5.3 for score1F, and from 5.7 to nine, with a mean of 7.3 for score2F. Phenotypic variance was 0.3 for both screening dates.

Within each environment, the two scores were significantly correlated ($P \leq 0.01$), with values of 0.58 in the field and 0.52 in tunnel. Between environments, correlations were lower and ranged from 0.30 to 0.47.

The heritability values were 0.46 and 0.47 for score1T and score2T, and 0.32 and 0.29 for score1F and score2F.

Linkage analysis

A total of 203 markers was obtained, i.e., 154 AFLP, 28 ISSR, and 21 APF-RGA markers, with a mean of 12.8

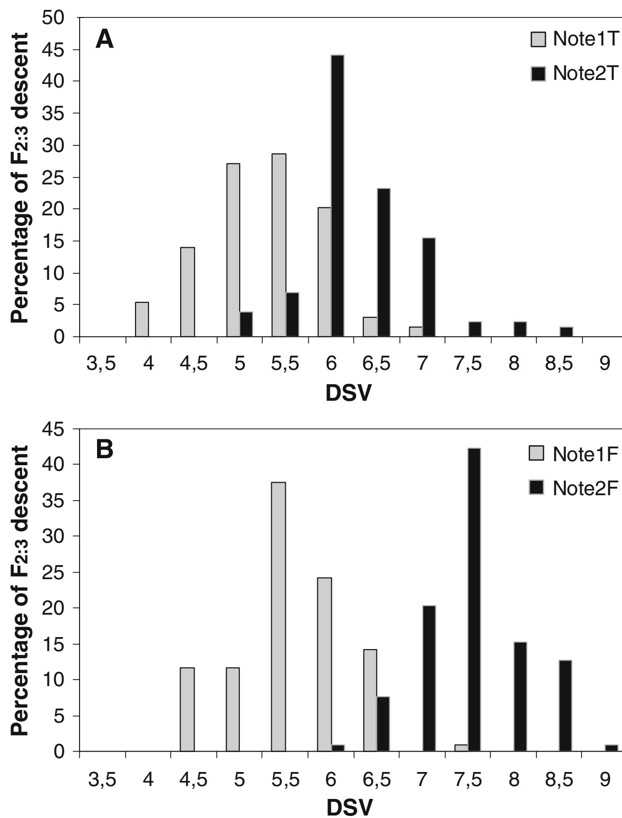

Fig. 2 The disease severity value (DSV) frequency distributions for the $F_{2:3}$ progenies **a** in the tunnel experiment, and **b** in the field experiment

AFLP, 9.3 ISSR, and seven APF-RGA markers per primer pair. Twenty-seven markers (13.3% of the markers) exhibited significant distortion ($P < 0.01$). Of the 203 markers, 139 were mapped (Fig. 3), i.e., 68.5%. Among these 139 markers, four markers showed distorted segregation ($P < 0.01$).

Seventeen linkage groups were obtained (Fig. 3). The S269 map had 9 linkage groups, with a total size of 652.3 cM, 69 markers mapped and a mean marker interval of 9.5 cM. The R268 map had eight linkage groups, with a total size of 636.2 cM, 70 markers and a mean marker interval of 9.1 cM. A chi-square test showed no difference at 5% in marker composition between maps. Except for the 1S group from S269 map, not associated with a putative 1R group from the R268 map, all the linkage group may be joined by pairs: one group from S269 and the corresponding one from R268 map.

QTL detection

Four QTLs were found with score2T, three with score2F, and one with score1T. No QTLs were found for score1F and all QTLs were detected on three linkage groups of the R268 map.

In the 2R group, the QTL of score1T, two of score2T, and one of score2F colocalised. Indeed, their confidence intervals overlapped. The groups 5R and 8R had only one score2F QTL and one score2T QTL, respectively (Fig. 3). QTLs found by SIM were also found by CIM (Table 2). The R^2 were similar for the results obtained by the SIM and CIM methods for score2F QTLs in group 5R, with values of 10.6 and 10%, and for score2T QTLs in group 8R, with values of 17.4 and 16.6%. In group 2R, when CIM was used, the score2T R^2 increased from 16.9 to 23.4%, and two other QTLs were found for score1T and score2F, with R^2 of 8.6 and 10.5%.

Discussion

Several years are needed to obtain a mapping population. In carrot, an F_2 population provides a fast and easy way to get a mapping population, as compared to a RIL or a back-cross population. The F_2 population requires only one cross and one self-pollination, whereas one cross between the parents and seven self-pollinations are needed to obtain a RIL population with 1.6% of residual heterozygoty. This procedure would take 16 years since carrot is a biennial species. Moreover, this procedure would be hazardous because of a high inbreeding depression due to successive self-pollinations. A back-cross population requires only two crosses, but self-pollination must be avoided, which is difficult in carrot. These are the reasons why F_2 populations have been used in all carrot mapping studies to date (Vivek and Simon 1999; Santos and Simon 2002; Just et al. 2007).

Phenotypic evaluations

DSV differences between parents (R268 and S269) were greater than between the two reference hybrids (Presto and Bolero). Although it could seem rather low (three for score one; 4.5 for score two, and 3.5 for score three), it was enough to obtain a clear differentiation between progenies. In some cases, studies on other pathosystems with partial resistance were based on similar or even lower differences. For example, in the wheat/*Stagonospora nodorum* pathosystem, which was also evaluated on a 0–9 scale, the difference between parents in a doubled-haploid population was 2.8 (Arseniuk et al. 2004). The parents of our population, and therefore the offspring, appeared suitable to study the genetic factors underlying *A. dauci* resistance.

Complete dominance for *A. dauci* resistance was identified for score two since the DSV of the resistant parent and F_1 hybrid were identical and since the mean parent DSV and F_1 DSV were significantly different.

In $F_{2:3}$ progenies, the continuous DSV distributions for all dates were consistent with the behavior of the partially

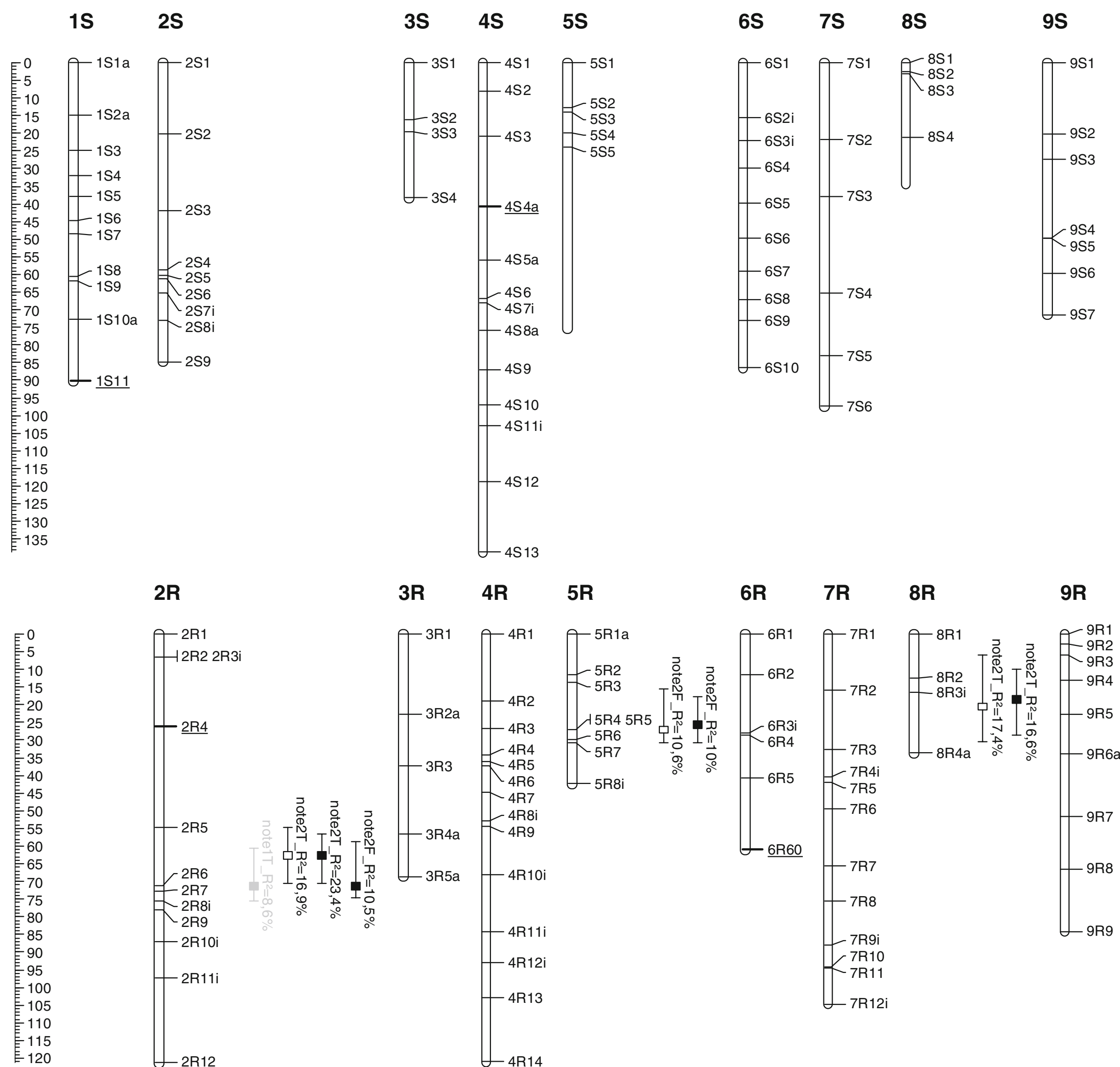

Fig. 3 Genetic map and QTL detection. *S* and *R* represents the S269 and R268 maps, respectively. Linked groups have the same group number. APF-RGA markers end with an *a*, ISSR markers with an *i*, and no extension correspond to AFLP markers. Distorted markers ($P \geq 1\%$) are *underlined*. QTLs are shown on the right side of the linkage groups. The *box* is the QTL position and the lines give the confidence interval. When the QTLs were detected by simple interval mapping, the *box* is empty (*open square*) whereas when the QTLs were detected by composite interval mapping, the *box* is full (*filled square*). R^2 is the phenotypic part of the variation explained by the QTL

resistant carrot cultivars obtained by breeders. It also indicated that this trait is quantitative in Nantes type carrots. Therefore, even if the present Nantes resistance source clearly differs from the Brasilia source used by Boiteux et al. (1993), both are probably based on polygenic systems.

The broad sense heritabilities were moderate as compared to those found in other partial resistance studies. Moreover, DSV correlations between environments were significant but moderate (under 0.5). The environment thus plays a role in resistance to *A. dauci*, as shown with respect to other pathogens. For example, for *Setosphaeria turcia* (a fungus) resistance in maize, four out of nine QTLs found had a significant genotype × environment interaction (Welz et al. 1999). As expected, the heritabilities were higher in tunnel than in the field, the environment effect being best controlled in tunnel. It was thus important to study the plants in tunnel so as to optimize the QTL detection.

Linkage analysis

In the present study, most of the markers used to build the genetic map are dominant markers. They have the advantage of quickly providing information about many loci simultaneously and do not need sequencing, contrary to

Table 2 QTL properties

Linkage group	QTL detection method	Trait	LOD	QTL position (cM)	Confidence interval (cM)	R^2(%)
2R	CIM	Score1T	2.9	71.3	60.7–75.4	8.6
	SIM	Score2T	2.6	62.7	54.7–70.7	16.9
	CIM	Score2T	4.6	62.7	56.7–70.7	23.4
	CIM	Score2F	3.4	71.3	58.7–74.6	10.5
5R	SIM	Score2F	2.9	27.1	15.6–30.6	10.6
	CIM	Score2F	3.2	25.6	17.6–30.6	10.0
8R	SIM	Score2T	4.3	20.5	6.0–30.5	17.4
	CIM	Score2T	5.2	18.5	10.0–28.5	16.6

The phenotypic effect represents the difference between the mean DSV of SS genotypes and the mean DSV of RS + RR genotypes. R^2 is the phenotypic part of the variation explained by the QTLs

codominant markers such as SSR. Here 203 markers were obtained from 18 primer pairs. To obtain the same number of SSR markers, around 200 primers pairs would have been required, thus seriously increasing the number of PCRs and migrations. Moreover, only a few SSR sequence were available in database at the beginning of our study.

Recombination fraction estimates and locus order are reliable only when dominant markers are in the coupling phase (Mather 1936; Mester et al. 2003). In contrast, the estimation of locus groups is seldom problematic with mixed-phase markers (Knapp et al. 1995). Therefore, in the present study, two coupling phase maps were built to map dominant markers in our F_2 population. For the S269 map, nine linkage groups were obtained, probably representing the nine carrot chromosomes. Only eight groups were obtained for R268. The absence of a 1R group is probably due to the unsaturated map. About 31.5% of the markers were not mapped, which was high compared to other studies, e.g., 19.5% for the chickpea map (Flandez-Galvez et al. 2003), and 18% for ryegrass (Bert et al. 1999). The Mendelian markers could be unlinked because they were located in an uncovered map region.

The two maps had similar properties: map size, number of markers, and marker composition. The marker densities were under 10 cM and, according to Asins (2002), having more markers beyond a density of one every 15 cM does not substantially enhance QTL detection. These results suggested that our two parental coupling maps could be used to detect QTLs. However, each map had a size of around 650 cM, covering around 72% of the genome, whereas it was estimated to be 900 cM in length (Vivek and Simon 1999). Two parental coupling maps were already obtained by Just et al. (2007) in an F_2 population with dominant markers. Each of their parental maps had nine linkage groups and their sizes were around 1100 and 1200 cM. Consequently, R268 and S269 maps covered between 53 and 60% of the maps of Just et al. (2007), thus confirming that the R268 and S269 maps appeared to be unsaturated but nevertheless useful for a first QTL detection.

QTL detection

Instead of using the F_2 model that is well suited for codominant markers, the BC model was chosen to analyze F_2 population data with dominant markers. Two genotypic classes were expected regardless of the population pattern. Additive and dominance effects were thus combined for both of these populations. However, a BC model takes two genotypic classes at an equal ratio into account (e.g., RS and SS), whereas an F_2 population studied with dominant markers has two genotypic classes with a 3:1 ratio (e.g., 3/4 of RS + RR and 1/4 SS). RS + RR in F_2 are thus taken into account like RS with the BC model, which leads to over-estimation of the additive effect. With this model, all QTLs detected by SIM were also found by CIM. However, three QTLs were found with the SIM whereas two more QTLs were therefore detected with the CIM method. Similar results were obtained in other QTL detection studies, e.g., on grain yield and grain-related traits in maize (Marsan et al. 2001) and on broomrape resistance in faba bean (Roman et al. 2002).

According to Young (1996), between three and five QTLs are commonly found in resistance studies whereas finding 10 or more QTLs is much more uncommon. In another review, Kover and Caicedo (2001) reported that one to seven resistance QTLs were found in different studies. Hence, the three QTLs found in this study was a small number, but consistent. This small QTL number could be due to moderate heritabilities but also to the unsaturated map. Indeed, regions playing a role in ALB resistance could be located in the uncovered genomic regions. The population size should also be considered. To detect QTLs with the same detection power, the population

size needs to be higher with dominant markers than with codominant markers. An optimal F_2 population size should range from 700 to 6,300 according to the dominance (Soller et al. 1976). The carrot population could not reach this optimal size because of the experimental constraints. Indeed, $F_{2:3}$ progenies are required for phenotypic evaluations but their production is time consuming and very expensive. Moreover, field or tunnel evaluations of these progenies is also time consuming and requires considerable space. It is very hard to assess more than 200 progenies.

Among the three QTL regions, one was field specific and one was tunnel specific suggesting that QTL detection is probably affected by the fungal strain, soil and/or climatic conditions which are supposed to be different between the two locations. In the present study, the strain and other environmental effects could not be separated. The strain effect was shown in other studies through the detection of strain-specific and broad-spectrum QTLs. This was, for example, the case for *Venturia inaequalis* in apple (Calenge et al. 2004) and *Pyricularia grisea* in rice and barley (Chen et al. 2003). In the near future, it would thus be interesting to compare the resistance of our progenies to different *A. dauci* strains under similar soil and climatic conditions.

As similar heritabilities were obtained for both scores in field and tunnel, respectively, similar QTL R^2 sums were also expected but this was not the case (i.e., 8.6% for score1T against 40% for score2T in tunnel with CIM). This suggested that a QTL of score one with a greater effect was not detected because it was in an uncovered genomic region or that there were many loci with small effects involved in score one with effects that were too low to be individually detected.

The QTL regions found in the 5R and 8R linkage groups were specific to the second screening date, contrary to the 2R group which had score one and score two QTLs. The presence of score two specific QTLs suggests that genes involved in plant resistance may change during plant development or may depend on the delay after infection. A plant stage dependent response was reported in several studies. In barley leaf rust resistance, distinct QTLs were identified for the seedling stage and the adult plant stage (Qi et al. 1998). Three QTLs involved in resistance of rapeseed to *Sclerotinia sclerotiorum* were detected at the seedling stage and three other QTLs were detected in the mature plant stage (Zhao and Meng 2003). In the present carrot study, scores one and two were both at the vegetative stage. However, score one could have been, for example, to genes involved in the plant ability to fight against the spread of the pathogen into the tissues, whereas score two could represent the ability to make new leaves.

In this study, we found that resistant alleles came only from R268. Similar results were obtained by Foolad et al. (2002) for early blight (*Alternaria solani*) resistance in tomato. However, resistant alleles from susceptible parent are quite commonly found in other pathosystems. These alleles may lead to transgressive genotypes (de Vicente and Tanksley 1993). In 2003, 67 other $F_{2:3}$ progenies of the same R268 × S269 cross were tested and some of them were transgressive genotypes: eight were more resistant than the resistant parent and one was more susceptible than the susceptible parent (data not shown). This suggests that S269 could have resistant alleles that we did not detect. Pilet et al. (1998) reported that the susceptible parent effect was often weak and small QTLs may be undetected.

In conclusion, this is the first analysis of the genetic architecture of carrot factors involved in partial resistance to ALB. It confirmed that this trait is polygenic and that the environment could play a role. The findings also revealed that the genes involved may depend on the delay after infection and that no major QTLs were involved. Moreover, we describe a method to detect QTLs in an F_2 population with dominant markers. Although this method is not perfect, it could be useful as a first step when investigating a species such as carrot that has not been the focus of many studies.

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