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ORIGINAL ARTICLE

QTL mapping of carrot resistance to leaf blight with connected populations: stability across years and consequences for breeding

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Abstract

Key message **Combining biparental and multiparental connected population analyses was useful for the identification of 11 QTLs in two new genetic backgrounds of carrot resistance to *Alternaria dauci* and for breeding recommendations.**

Abstract Leaf blight due to the fungus *Alternaria dauci* is the major carrot foliar disease worldwide. Some resistance QTLs have been previously identified in one population, but the evaluation of additional genetic backgrounds with higher level of resistance would give opportunities for breeders to combine them by pyramiding. For this purpose, two segregating populations were evaluated twice across 4 years in the same environment (1) to compare the efficiency of the single vs. the connected populations approach for characterizing the new sources of carrot resistance to *Alternaria dauci*; (2) to evaluate the stability of QTLs over the years; and (3) to give recommendations to breeders for marker-assisted selection. Single and connected analyses were complementary; their combination allowed the detection of 11 QTLs. Connected analyses allowed the identification of common and specific QTLs among the two populations and the most favorable allele at each QTL. Important contrasts between allelic effects were observed with four and five most favorable alleles coming from the two resistant parental lines, whereas two other favorable alleles came from the susceptible parental line. While four QTLs were consistent across years, seven were detected within a single year. The heritabilities for both populations PC2 and PC3 were high (75 and 78 %, respectively), suggesting that the resistance of carrot to *A. dauci* was little affected by these environmental conditions, but the instability of QTL over years may be due to changing environmental conditions. The complementarity between these parental lines in terms of interesting allelic combinations is also discussed.

Introduction

Alternaria dauci is a fungus responsible for the major carrot foliar diseases worldwide, leading to serious damage in carrot production and the rejection of contaminated seed lots (Farrar et al. 2004). Previous studies reveal significant diversity in terms of aggressiveness and genetic variability among *A. dauci* isolates (Benichou et al. 2009; Rogers and Stevenson 2010; Boedo et al. 2012, to name only the most recent). Moreover, virulence studies suggest that host range is not restricted to cultivated and wild carrots and should be enlarged to other dicot species (Boedo et al. 2012). While Rogers and Stevenson (2010) report isolates by carrot variety interactions among American isolates and carrot varieties, no interaction was reported between cultivars and isolates exhibiting different geographical origins

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(Le Clerc et al. 2015). These findings should be carefully considered for resistance breeding programs and cultivar deployment strategies as they can impact the durability of the resistance. Partially resistant varieties of carrot have been available for many years, but selection for a higher level of resistance is still one of the first goals for carrot breeders. As no major gene for resistance has been discovered, one strategy to enhance resistance levels would be to accumulate several partial resistance mechanisms in one cultivar. This requires the precise identification of the QTLs responsible for different resistance factors likely to be efficient in different genetic backgrounds. The evaluation of genetic variation of carrot resistance to *Alternaria dauci* has been performed in different environments, mainly in field studies in Brazil (Vieira et al. 1991; Boiteux et al. 1993), the USA (Simon and Strandberg 1998), and France (Le Clerc et al. 2009, 2015) or in greenhouses and laboratories (Pawelec et al. 2006; Boedo et al. 2010). However, genetic loci associated with variation in resistance were only evaluated with a biparental population by Le Clerc et al. (2009). In that study, three QTLs involved in carrot resistance to *A. dauci* were identified confirming the polygenic character of this resistance as previously shown by Simon and Strandberg (1998). Comparing phenotyping results from controlled conditions in a tunnel with results from field evaluations, Le Clerc et al. (2009) also showed complementarity between environments in QTL detection with QTL specific to each environment and one common major QTL. However, these QTLs were only identified in one genetic background and the stability of the QTLs over environments has never been investigated.

Detecting new resistance factors will provide breeders many opportunities to combine them by pyramiding in one genotype. For this purpose, we analyzed new sources of carrot resistance to *Alternaria dauci* with a higher level of resistance than material previously analyzed in 2009 and originating from exotic material, i.e., potentially involving new resistance factors. To optimize QTL detection and characterization, we developed two connected populations. Multiparental connected populations are based on crossing designs composed of several biparental populations that are connected by common parents. Joint analyses of multiparental connected populations have proven their efficiency compared to single biparental population analyses for QTL detection (Blanc et al. 2006; Steinhoff et al. 2011). With a single population, only two alleles are considered and allelic effects are indeed specific to the population analyzed. QTL results are often not transferable to other materials, thus limiting their use for marker-assisted selection as mentioned by Würschum (2012). In contrast, in multiparental connected populations, it becomes possible to compare the allelic effects of different parental lines and to determine the most favorable allele for the trait under study. Multiparental connected population analyses also generally provide more precision than separate analysis for QTL location, leading to smaller support intervals as highlighted by Steinhoff et al. (2011) for the evaluation of grain yield and grain moisture in maize.

The objective of the present study was (1) to compare the efficiency of the single vs. the connected populations approach for characterizing the new sources of carrot resistance to *Alternaria dauci* in terms of number of QTLs, support intervals and allelic effects; (2) to evaluate the stability of QTLs over the years; and (3) to give recommendations to breeders.

Materials and methods

Plant material

Two connected populations, PC2 and PC3, were derived from crosses between I2 × H1 and K3 × H1, respectively, i.e., they were related by a common parental plant (H1). H1 is a single plant from a susceptible S3 line obtained from the HMClause breeding program and is of French origin. I2 and K3 are single plants from two partially resistant S1 Asiatic lines, both developed at Agrocampus Ouest (AO) as explained below. I2 and K3 are genetically different from the European lines previously used in Le Clerc et al. (2009). In comparison to commercial cultivars and previous breeding material, they present a higher level of quantitative resistance when evaluated in the field in France and in Brazil. Finally, they are genetically very different from each other, as revealed by a preliminary molecular study (Online Resource 1). I2 and K3 are issued from a breeding program begun in 1997 [accession numbers are 10002 (lot 1) and 10004 (lot 1), respectively, and are available for research under request].

From 1997 to 2000, more than 300 accessions from the Carrot gene bank of Angers, France, IPK-Gene Bank (Gatersleben, Germany), REGNGB (Nordic Gene Bank, Alnarp, Sweden), SASA (Scottish Agricultural Science Agency, Edinburgh, UK) and HRI GRU (Warwick, UK) gene banks were evaluated for their resistance to *Alternaria dauci*. From these several screenings, 22 accessions were preselected and introduced into a breeding program: crosses, self-pollinations or maternal lines were realized. After a few years of the breeding program, in 2004 and 2005, all the created material was evaluated for resistance to *Alternaria dauci* in tunnel in Agrocampus Ouest and 19 accessions were selected due to their high level of resistance. In 2006, these 19 accessions together with 3 additional accessions from HM Clause (namely, French-originated accessions 1, 4 and 5) were introduced into two location trials in Angers (artificial inoculation) and

Les Landes (natural infection). Five lines of these 22 were finally elected to become the funder lines of segregating populations necessary for the genetic study of resistance determinism. 194 F1 crosses between 61 parental plants of the five lines were realized. On the basis of the genetic distance between parents (Online Resource 1) and the quantity of harvested seeds, 35 F2 populations were evaluated in Brazil (high disease pressure), resulting in the final choice of (H1 × I2)-1 and (H1 × K3)-2. Around 180 individual plants belonging to each of these two F2 crosses were self-pollinated to derive the $F_{2:3}$ populations, with 181 and 142 self-pollinated individuals for populations PC2 and PC3, respectively.

Two commercial hybrids were included in each trial as reference varieties. Presto (Vilmorin) is a highly susceptible cultivar and Bolero (Vilmorin) offers a relatively high level of resistance to *A. dauci*. Each of the three parental plants, H1, I2 and K3, were self-pollinated to obtain the three parental lines as necessary controls for phenotyping trials.

Genotyping and genetic map

Genomic DNA was extracted from leaf tissue of each F_2 and parental plants using the extraction DNeasy 96 Plant Kit (Qiagen, Hilden, Germany). As public SSR were not available when we realized the genotyping (published by Cavagnaro et al. 2011), 100 SSR markers were kindly provided by Vilmorin Clause and Cie and tested for polymorphism on parental plants (Online Resource 1). PCR amplifications were done on a MyCycler (Bio-Rad, Hercules, California, USA). They were amplified in a 20 µl reaction mixture using 5 µl of 1/40 diluted DNA, 0.75× buffer, 2.5 mM MgCl2, 1 mM dNTP, 0.75 U Taq DNA polymerase (Interchim), 0.1 µM forward primer appended with the M13 tail 5′-CACGACGTTGTAAAACGAC3′, 0.4 µM reverse primer and 0.06 µM of the fluorescently labeled universal M13 primer. PCR cycling conditions were as follows: 94 °C (5 min), then 10 cycles at 94 °C (15 s)/55 °C (−0.5 °C/cycle) (15 s)/72 °C (30 s), followed by 30 cycles at 94 °C (15 s)/50 °C (15 s)/72 °C (30 s), and a final extension at 72 °C for 7 min. SSR were fractioned using the capillary electrophoresis system ABI 3730XL at the Gentyane platform (INRA Clermont-Ferrand). Genemapper® 3.7 (Applied Biosystems®) software was used to score the alleles.

Genetic maps were built using MAPMAKER/EXP 3.0 software (Lander et al. 1987) with the Kosambi mapping function to estimate the genetic distances (Kosambi 1944) and an LOD threshold of 6.0 for the establishment of the linkage groups (LG). A linkage map was built for each population with the 181 and 142 genotypes for PC2 and PC3, respectively. A consensus map was then generated with MAPMAKER software from the two connected populations. We used the same procedure as described in Schiex and Gaspin (1997): merging the two mapping data sets was done by building a new one that involved all the markers and individuals that appeared in the two original data sets. When allelic information for a given marker was not available in one of the original data sets, it was considered as missing in the new one. As shown by Schiex and Gaspin (1997), when a large amount of missing data exist in such joined data sets, software relying on multipoint maximum likelihood such as MAPMAKER is able to perform reliable parameter estimation. In the first consensus map obtained, when some markers were not more distant than 1 cM from each other, they were deleted to create a reliable framework map for QTL detection.

Experimental design and phenotypic evaluation

Field evaluation

In 2011, 145 and 141 $F_{2:3}$ progenies of PC2 and PC3, respectively, were evaluated in the field in Les Landes region (France), while 135 and 133 $F_{2:3}$ progenies were re-evaluated in 2014 in the same environment. For each population, replications of each $F_{2:3}$ progeny were distributed over two randomized blocks. Twenty replications of each resistant parental line (K3 and I2), 24 for H1, the susceptible parental line, and 20 replications for Presto and Bolero, the reference cultivars, were randomly introduced into the trial. Each plot corresponding to one parental line, one variety or one $F_{2:3}$ progeny included 160 plants. The susceptible cultivar Presto was sown all along the side of each block to check the uniformity of the infection. Plants were naturally infected without additional inoculation.

As previously done by Le Clerc et al. (2009), disease severity values (DSV) were visually scored on a 0–9 scale (Pawelec et al. 2006) for each replicate, with 0 corresponding to symptomless plants and 9 to plants that were totally blighted in the plot (Table 1), i.e., one single average score was given to the 160 plants of each replicate DSV scored in the beginning of October in 2011 and 2014 when the mean score of Presto reached approximately 7, leading to one unique assessment of the disease per population and per year.

Phenotypic data analyses

Scores were adjusted to take the environmental variation into account. For this purpose, analyses were done with R using the ASREML package (Butler et al. 2007). Several models were tested and the one with the lowest standard error of difference was retained. Multi-factor ANOVA model was written as

Table 1 Scale for the disease severity values used to evaluate Alternaria leaf blight

Scale	Symptoms
0	Symptomless plants
1	Very few spots
2	Very weak attack on the lower leaves
3	Low attack on lower leaves
4	Average attack on lower leaves and weak on the upper leaves
5	Strong attack on lower leaves and weak on the upper leaves
6	Strong attack on lower leaves and average on the upper leaves
7	Lower leaves completely blighted and strong attack on the upper leaves
8	Lower leaves completely blighted and very strong attack on the upper leaves
9	Plants totally blighted

y~mu + genotype + R+C + R*R + C*C + R*C where mu is the mean of the scores for one scoring method and R and C are row and column, respectively. Genotype effects of the model were estimated and scores were adjusted by the sum of mu and estimated genotype effects.

DSV of the progenies were analyzed using standard analysis of variance (ANOVA) performed with STATGRAPHICS Plus for Windows 3.1 after checking the postulates. The normality of the residues was evaluated with skewness and Kurtosis tests. The homogeneity of the variance of the residues was evaluated with Cochran's *C* test. For each population in 2011 and 2014, total variation was broken down into genotype, replicate and residual effects.

For both populations, the broad-sense heritability (h^2) was estimated as being the ratio of the genotypic variance (σ^2_G) to the phenotypic variance (σ^2_P), expressed in percentage. The phenotypic variance was calculated as follows: $\sigma^2_P = \sigma^2_G + \sigma^2_Y/n + \sigma^2_e/r$ where σ^2_Y is the variance of the year, σ^2_e is the residual variance, n is the number of year and r is the number of repetitions. The components of the phenotypic variance were calculated on the basis of the analysis of variance of the expected mean squares, as described by Nanson (1970).

QTL detection

QTL analysis was performed by regression interval mapping with MCQTL 4.0 software (Jourjon et al. 2005) on the single biparental populations and the multiparental connected population. The connected additive model and the iterative QTL mapping procedure (iQTLm) using genetic cofactors were used (Charcosset et al. 2001). Cofactor selection and testing of QTL effects were performed with the *F* test. For each trait, the cofactors were selected through a forward selection procedure after 1000 permutations. A QTL was inferred when the LOD exceeded the *F* threshold estimated for each trait after 1000 permutations. An additional detection of QTLs was done with manual choice of cofactors for some linkage groups suspected of harboring more than one QTL. A 1.5 LOD support interval (SI), the most appropriate for a 95 % support rate in an F2 population, was computed for each QTL (van Ooijen 1992). For one score, percentages of phenotypic variation explained by each QTL (R^2) and by all QTLs (global R^2) were calculated. Allelic effects at each QTL were also estimated. Based on the comparison between the size of LG on independent population maps and on a consensus map, the SI of each QTL was estimated on the consensus map. Two QTLs were considered to be the same when their SIs estimated on the consensus map were widely overlapping and when their maximal positions were not more distant than 15 cM. For easier reading, QTLs were coded by the number of the LG they belong to and if necessary a number was added when more than one QTL was detected on the LG.

Results

Genetic map

As explained before, some markers in the too highly covered regions were deleted to create a robust map for QTL detection. Indeed, little effect is observed with a marker density beyond 10 cM (Li et al. 2006). The resulting genetic framework maps of PC2 and PC3 contained 87 and 62 markers mapped on nine linkage groups per population as expected from the $2n = 18$ chromosomes for carrot. Out of these, 38 markers were common. The map lengths were estimated at 447.9 and 455.3 cM, with an average inter-marker distance of 5.1 and 7.3 cM, respectively, for PC2 and PC3. One linkage group (LG) for PC2 and two LGs for PC3 had low marker density coverage. The consensus map length was 614.8 cM, representing 96 SSR on nine linkage groups with one locus each of 6.4 cM on average.

Phenotypic evaluations

The residues were normally distributed for both populations, and all the DSV scores fitted a normal distribution (Fig. 1). The mean scores of the parental lines, I2 and K3, were quite similar to each other in 2011 (DSV ~ 4) and both were more resistant than our reference cultivar Bolero (mean DSV = 5). Standard error of means of the parental lines and reference cultivars was lower than 0.33. In 2014, the mean score of I2 was higher (5.4) than for K3 (4.2), and for both populations higher scores were observed in 2014 than in 2011. The mean DSV of Bolero (6.1) was also higher than in 2011 (mean = 5). According to ANOVA,

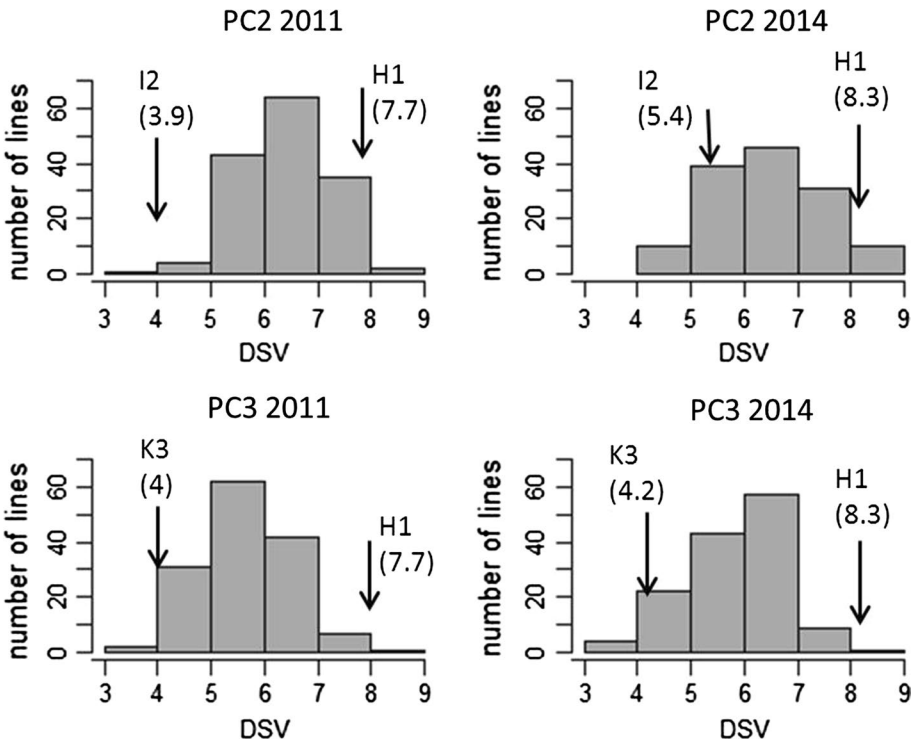

Fig. 1 Histograms of the mean disease severity scores (0–9 scale) in population PC2 and PC3 in 2011 and 2014. The *arrows* indicate the mean value (*in parenthesis*) of the parental lines. The standard error of the mean values of the parental lines were not higher than 0.3

the genotype had significant effects on the variance (*P* value <0.0001), and the year also had a significant effect for both populations. The heritabilities were high: 75 % for PC2 and 78 % for PC3 (Online resource 2). While relative humidity and mean temperatures were both favorable for disease development in 2011 and 2014, maximal temperatures were highest in 2014 between the 15th and 27th of July when the crop is highly susceptible to *Alternaria dauci* (~5–6 leaves). Moreover, wind speed was very low in 2014 (less than 2 m/s) during all the crop season, while it was in general higher than 4 m/s in 2011 and even more during the susceptible period described before (Online resource 3).

QTL analyses in single biparental populations

In 2011, three QTLs were detected on LG 1, 4 and 8 for population PC2, and three QTLs on LG 6, 8 and 9 for population PC3 (Table 2). The total percentage of phenotypic variation explained by all detected QTLs was higher for PC3 (52.8 %) than for PC2 (37.7 %). The QTL identified on LG 8 in PC3 was the one that explained the highest part of the phenotypic variation ($R^2 = 43.8$ %).

In 2014, the QTLs 1, 4-1 and 6-1 identified in PC2 and PC3, respectively, in 2011 were detected again. One additional QTL 9-2 was identified in both populations. The SI of this QTL overlapped with the one of the QTL 9-1 detected in 2011 in PC3, but its position was quite different.

Two additional QTLs 4-2 and 6-2 were detected in PC3. In PC2, the QTL on LG 8 had an SI (2.7–58.8 cM) similar to the one identified in 2011 (8–59.6 cM). However, the connected analysis suggested that it could also stand for a new QTL (8-2) with similar SI, but different position. In PC3, even with an overlapping SI and similar maximal positions with QTL 8-1 identified in connected analysis the same year, the QTL on LG 8 may stand for 8-1 but could also be a new one as suggested by the examination of the LOD values all along the linkage group (Online resource 4).

The contrasts between additive effects of alleles of the parental lines were important and varied from 0.40 to 0.90 for population PC2 and from 0.30 to 0.68 for population PC3. In each population, the most favorable alleles issued from the resistant parental line I2 or K3 conferred resistance except for the QTLs on LG 4 with the favorable allele coming from the parental line H1.

QTL analyses in multiparental connected populations

In 2011 compared to single biparental population analyses, two new QTLs were identified with the connected populations, one on LG 2 and one on LG 5 with low R^2, 6.6 and 4.8 %, respectively, and a very high SI for the one on LG 5 (1.6–114.8 cM). All the QTLs identified in 2011 in PC2 and PC3 were recovered in the connected analyses (Table 2).

In 2014, the QTLs 1, 4-1, 8-1 and 9-2 were identified as in single analyses. The QTLs 6-1 and 6-2 identified by the analysis of the single population PC3 were not found with

Table 2 QTLs detected in single or connected populations in Les Landes

Scoring year	Population	Code of the QTL[a]	1.5 LOD support interval (cM)[b]	Position (cM)	R^2 (%)	Global R^2 (%)	*F* test	Additive effect of allele		
								H1	K3	12
2011	PC2	1	0–22.4	11.5	16.8	37.7	6.2	0.23		0.23
		4-1	11.3–31.4	23.9	16.9		6.2	−0.23		−0.23
		8-1	8–59.6	27.3	15.9		5.8	0.20		0.20
	PC3	6-1	50.7–67.6	56.2	28.9	52.8	8.2	0.27	−0.27	
		8-1	35.5–39.1	37.7	43.8		13.3	0.34	−0.34	
		9-1	18.9–66.5	29.8	13.6		3.9	0.18	−0.18	
	PC2 and PC3 connected	1	0–20.5	10	19.2	52.5	10.4	0.29	0.07	−0.36
		2	34.1–49	49	6.6		3.3	0.11	0.06	−0.17
		4-1	10.5–27.4	14.8	12.6		6.6	−0.21	−0.00	0.21
		5	1.6–114.8	28.9	4.8		2.4	0.12	0.34	−0.46
		6-1	50.2–66.1	57	16.0		8.5	0.07	−0.38	0.31
		8-1	31.8–38.1	35.1	27		15.4	0.32	−0.30	−0.02
		9-1	29.4–51.7	40.2	8.7		4.5	0.19	−0.23	0.04
2014	PC2	1	10.5–24.1	17.2	34.8	52.7	12.4	0.45		−0.45
		4-1	10.5–39.6	18.3	18		16.1	−0.28		0.28
		8-2?	2.7–58.8	11	11		3.8	0.20		0.20
		9-2	36.5–68.4	52	12.1		4.2	0.21		0.21
	PC3	4-2	29.4–41.3	36.1	16.6	51.7	4.4	−0.20	0.20	
		6-1	44.2–67.6	60.1	14		3.7	0.18	−0.18	
		6-2	0–15.9	0.7	14		3.8	0.15	−0.15	
		8-1?	26.1–62.9	47	24		6.4	0.24	−0.24	
		9-2	49.7–65	59.4	18		4.8	0.20	−0.20	
	PC2 and PC3 connected	1	9.3–24.6	20	23.1	43	12.5	0.38	0.28	−0.66
		4-1	14.4–51	19.5	14		7.2	−0.30	−0.01	0.31
		4-2[c]	*15.2–39.6*	*34.5*	*10.6*		*5.5*	*−0.31*	*0.15*	*0.16*
		8-1	23.7–54.9	30	10.8		5.4	0.25	−0.20	−0.05
		8-2?	*2–52.5*	*5.7*	*10.2*		*5.2*	*0.28*	*0.01*	*−0.29*
		9-2	51.3–69.7	61.7	11.5		5.8	0.27	−0.17	−0.1

R^2 and global R^2 are the phenotypic part of the variation explained, respectively by one or all the QTLs detected at one scoring date

[a] Correspond to the number of the linkage group followed by a number when more than two QTLs were identified on the same linkage group

[?] Is added when affiliation is uncertain

[b] LOD confidence intervals and positions correspond to the ones on consensus map

[c] QTLs in italic were not taken into account in the calcul of global *R*2. QTLs identified with choice of manual cofactors

the connected analysis. Compared to 2011, two additional QTLs (4-2 and 8-2) were identified when the positions of QTLs 4-1 and 8-1 were used as cofactors for QTL analyses. The SI of QTL 4-2 was overlapping with the one of 4-1 identified in connected analysis, but their maximal positions were quite different (34.5 and 19.5 cM, respectively), while its position was very similar to the one of QTL 4-2 identified the same year in PC3 (36.1 cM) supporting the previous suggestion of two QTLs on LG 4. For the QTLs on LG 8, again, the SI were overlapping but their positions were also quite different (30 and 5.7 cM for 8-1 and 8-2, respectively) and the most favorable came one time from K3 (for 8-1) and the other time from I2 (for 8-2). This result also supported the previous suggestion that the QTL identified on LG 8 with connected analysis in 2014 probably stands for more than one QTL. Moreover, three QTLs could probably exist and explain the big support intervals of the QTLs identified on this linkage group. The two QTLs detected in 2011 on LG 2 and 5 were not recovered in 2014.

For 2011, the most favorable alleles for QTLs on LG 1, 2 and 5 were contributed by the resistant parental line I2, while the others for QTLs on LG 6, 8 and 9 were contributed by the resistant parental line K3 and the last for QTL on LG 4 by the susceptible parental line H1. For 2014, the most favorable alleles for the QTLs 1 and 8-2 came from

the resistant parental line I2. The most favorable alleles for QTLs on LG 4 came from H1. The two other most favorable alleles came from K3 (8-1 and 9-2). The additive effects were negative for alleles of I2 and K3 at the QTL on LG 8-1 whatever the year of analysis. They were also both negative for the QTL on LG 9-2 identified in 2014.

Discussion

Phenotypic evaluation between years

In the present study, the distance between field and laboratory led us to perform a unique score assessment when the susceptible reference cultivar reached the DSV = 7 (stage 7), in contrast to several papers we have already published on the concern of carrot resistance to *Alternaria dauci* (such as Pawelec et al. 2006; Le Clerc et al. 2009 or Lecomte et al. 2014). This unique scoring method has been proved in our past studies to be less costly and as efficient as AUDPC evaluation. This was due to the very high impact of this scoring data on AUDPC final value. Indeed when the susceptible reference cultivar reaches DSV = 7, it is the best time to discriminate the resistant and susceptible lines, i.e., where the difference between AUDPC curves are maximum after which the differences decrease.

The epidemic development between the years 2011 and 2014 was significantly different. The climatic data obtained from Meteo France explained this differential disease evolution. Indeed speed wind was very high in 2011 leading to reduce the free water on the leave requested by *A. dauci* spores for their germination and leaf penetration as reported by Strandberg in 1977. Free moisture was likely to be the limiting factor in disease incidence and severity. In 2014, low-speed wind combined with high temperatures during the most favorable development period for the fungus led to the highest disease incidence. As observed in Fig. 1, the DSV were globally higher for both populations in 2014 compared to 2011, i.e., a higher proportion of $F_{2:3}$ lines showed higher scores in 2014 than in 2011. This phenomenon concerned all genotypes, and no change in genotype range was observed.

From a lot of past evaluations, the resistance of the two I2 and K3 lines has been shown to mainly consist in a slower development of the disease (showed by visual assessments or qPCR) resulting in a lower score at stage 7. This was confirmed in the present study by a three-point difference of DSV between resistant parental lines compared to the susceptible Presto for both years. However until now, the mechanism underlying this lower progression has still not been elucidated. There is probably more than one mechanism involved, as suggested by previous results on these accessions or others (Le Clerc et al. 2009; Boedo et al. 2010; Lecomte et al. 2014) such as the limitation of fungal spore germination on different genotypes, the speed of colonization and the differential resistance to fungal toxins. More investigations are under current progress.

Single biparental vs. connected population analyses

With the single and the connected population analyses, an efficient QTL detection was obtained in the field for both years (Fig. 2). In 2011, two new QTLs among seven were detected with connected analyses, while five QTLs were identified with single population analyses. In 2014, six QTLs were detected in connected analyses versus eight with single analyses. These results are not very consistent with previous studies based on multipopulation connected analyses where a higher number of QTLs or new QTLs were always detected compared to single population analyses (Steinhoff et al. 2011; Espinoza et al. 2012; Liu et al. 2012; Pauly et al. 2012), leading several authors to suggest the use of connected analyses. In the present study, in 2014, QTLs 6-1 and 6-2 detected in single population analyses were not detected again with the connected analyses. This can be due to a dilution effect as already mentioned by Billotte et al. (2010) and Pauly et al. (2012). The QTL 6-2 was probably specific to the single cross PC3 and also specific to environmental conditions, as it was not identified in 2011 despite its high R^2 in 2014.

On the basis of previous studies, the joint analysis of connected populations generally leads to more precision concerning the LOD support interval due to a larger total population size. This was reported by Negeri et al. (2011) who studied the resistance of maize to *Cochliobolus heterostrophus* in three connected populations, and by Blanc et al. (2006) on maize. Our results were relatively consistent with these observations, especially when the SIs of the QTLs detected in the single population analyses were quite large, such as for the QTL 8-1 (SI = 51.6 cM) detected in 2011 in population PC2 compared to 6.3 cM, for the joint analysis.

For three linkage groups (LG 4, 8 and 9), the results of single biparental population analyses suggested for each of them the possibility of at least two QTLs instead of only one. The connected analyses reinforced or confirmed these suggestions. All these results highlighted the interest to combine individual and connected QTL analyses to identify specific or common QTLs.

As highlighted by Blanc et al. (2006), the different parental allele effects can be estimated simultaneously with a connected model, which makes it possible to identify the parental origin of favorable alleles at each QTL and an overall comparison of their effects in different genetic backgrounds. Through the connected populations analyses,

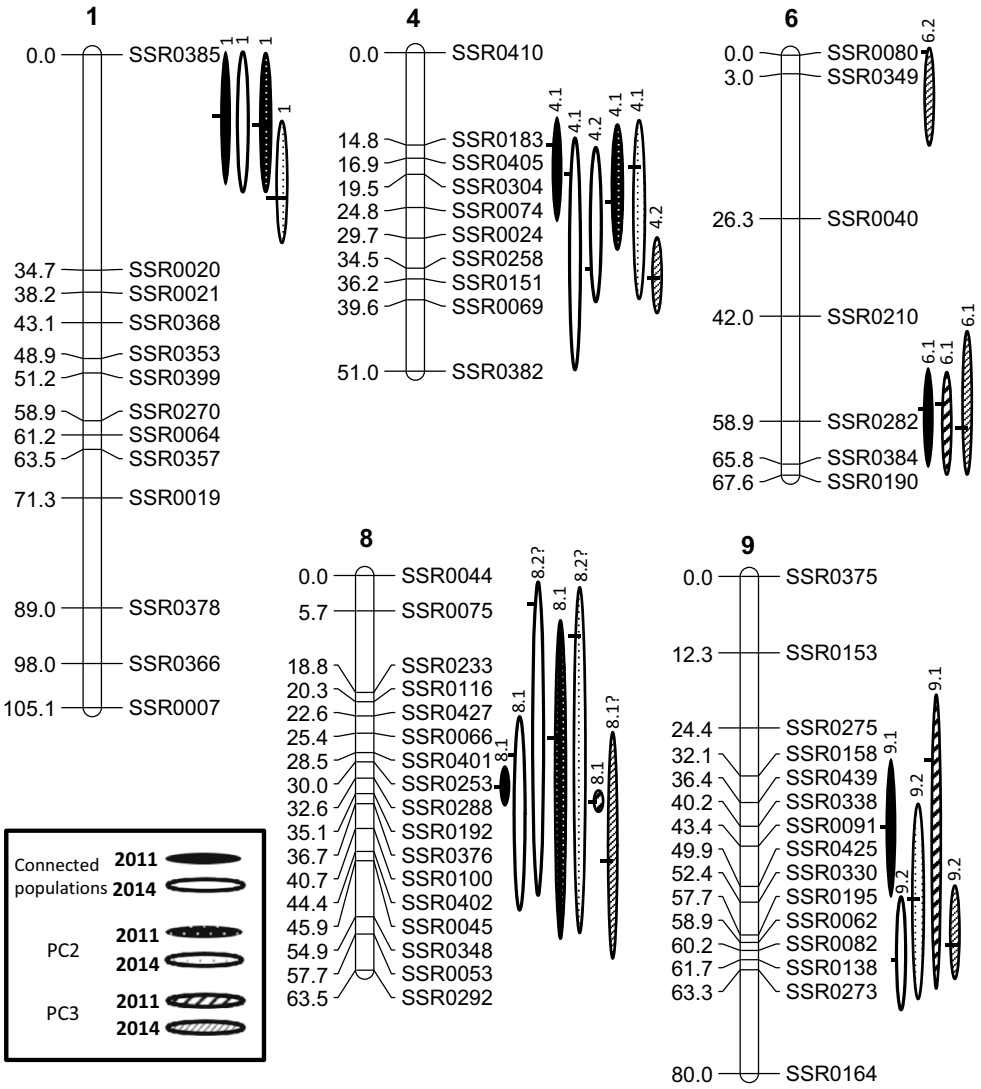

Fig. 2 Genomic localization of the main QTLs ($R^2 > 10$ %) of carrot resistance to *Alternaria dauci* identified in Les Landes in 2011 and 2014 with single or connected analyses of two segregating populations PC2 and PC3. The QTL length is the support interval in which LOD score is within 1.5 of its maximum. The code of the QTL as referenced in Table 2 is indicated above and the maximal position of the QTL is indicated by a *dash*

we observed important contrasts between allelic effects with three favorable alleles coming from I2 for QTLs 1, 2 and 5 in 2011, whereas three other most favorable alleles came from K3 for QTLs 6-1, 8-1 and 9-1. Those results suggested that most of the alleles underlying these QTLs were different even when QTls were common between both populations. In contrast, the alleles of the QTL 8-1 identified in 2011 and 2014 were probably the same in the two resistant lines, as they both conferred resistance. However, their effect in each genetic background I2 and K3 was quite different from the one from K3, being much more favorable. Similar alleles were also probably shared by I2 and K3 for QTL 9-2 in 2014 with the K3 allele being more favorable. Since the number of favorable alleles was balanced between the two partially resistant parents and one QTL at least could be specific to PC3 (on LG 6-2), this suggested a complementarity between the two resistant lines and the opportunity for breeders to make interesting allelic combinations. The favorable alleles for QTLs on LG 4 from the susceptible line H1 should also be considered in this strategy.

Stability of QTLs across years

Combining all single and connected analyses, 11 QTLs were detected. However while some QTLs were consistent across years, others were detected within a single year. Inconsistent detection of QTL across environments (years, locations, etc.) due to interaction between QTLs and environment has already been observed in many studies on different species as reported by Haggard et al. (2014). The differences observed in the present study may be due to changing environmental conditions between years. One hypothesis to explain QTL instability over years could be that these different QTLs among years could act on different components of quantitative resistance. As shown in

the wheat leaf rust pathosystem by Azzimonti et al. (2014), QTLs involved at the different stages of the epidemic were associated with different resistance components (infection efficiency, latent period, spore production per lesion, etc.). In 2014 a highest disease pressure could have led to reduce the latent period and favor secondary sporulations, eliciting QTLs able to reduce the efficiency of fungal penetration as found by Chung et al. (2010). On the contrary QTLs identified only in 2011 may delay the invasion and extension of the pathogen in the leaf.

Another hypothesis could be that the plants were facing different fungal strains in 2011 compared to 3 years later. Indeed, a very high diversity of *A. dauci* strains occurs in a carrot field (P. Simoneau, University of Angers, France, personal communication) and the different QTLs could harbor mechanisms with activities specifically dedicated against certain strains, but not others. In such case, one can imagine that all QTLs common to 2011 and 2014 were efficient against all strains, i.e., generalist QTLs, whereas the others would be more specialized against some strains, i.e., isolate-specific QTLs. Isolate-specific QTLs have already been identified in the resistance of pepper to *Phytophthora capsici* (Truong et al. 2012) and in barley, leading to partial resistance to *Puccinia hordei* (Marcel et al. 2007). However, we were not able to detect such QTLs in a tunnel experiment inoculated with the P2 *A. dauci* strain on the two present populations PC2 and PC3 (data not shown), as all the QTLs identified in this environment (one single strain) were also detected in the field (high diversity of strains). Our last study on the interaction between *A. dauci* isolate and carrot varieties suggested that carrot resistance to *A. dauci* was probably mainly explained by major QTLs that confer resistance to a large number of isolates and, potentially, some minor isolate-specific QTLs as well (Le Clerc et al. 2015). The analyses of other isolates than P2 in controlled conditions could confirm this hypothesis. A full characterization of *Alternaria dauci* natural populations in the field during the two experiments would have been informative: unfortunately, only few isolations were realized to check for the presence of *Alternaria dauci*. Anyway, the broad-sense heritabilities calculated here for both populations PC2 and PC3 were high, suggesting that the resistance of carrot to *A. dauci* was little affected by the environmental conditions. Vieira et al. in 1991 found lower or equal broad-sense heritability values, i.e., comprising between 45.6 and 81.9 % in four populations of carrots.

Consequences for breeding

Connected and single population analyses allowed identifying the most favorable alleles for the different QTLs. Some of them issued from I2 such as for QTLs 1, 2, 5 and 8-2, while some others issued from K3 such as for QTLs 6-1, 6-2, 8-1, 9-1 and 9-2. Finally, for the QTLs on LG 4, the favorable alleles came from H1, the susceptible parental line. As mentioned in the introductive paragraph, until yet no cultivar is completely resistant to leaf blight and even the most resistant accessions such as I2 or K3 are only partially resistant genotypes. Therefore to enhance the level of resistance of one genotype, the accumulation into this genotype of different alleles should be investigated. We could recommend breeders to focus first on conserved QTLs across years and genetic backgrounds such as QTLs 1, 4-1, 6-1, 8-1 with high R^2, as QTL stability across environments and genetic backgrounds are crucial for a successful MAS strategy. Special care has to be given to the most favorable alleles (e.g., K3 allele is more favorable than I2 allele for QTL 8-1). Secondly, specific QTLs could also be interesting to consider, as they could harbor a new mechanism of resistance; however, their efficiency when introgressed into new genetic backgrounds will have to be confirmed.

Conclusion

The present study underlines the complementarity between single population and multiparental population analyses, as some QTLs were only detected with one or the other method highlighting QTLs specific to one population or new QTLs. While we recommend detecting QTLs with both analyses, the complementarity between QTL detection across various years and environments as supported by previous results (Le Clerc et al. 2009) is also crucial. A higher number of QTLs were detected compared to the ones identified in 2009 in another population. A study to identify common or specific QTLs to all these different populations is under progress in our laboratory by genotyping the population of 2009 with SSR markers that were not available at that time. Higher genome coverage of the present maps is also considered, as it could be responsible for some inconsistencies observed among QTL detection. Indeed, it is likely that some QTLs exist, but were not detected due to poorly covered regions. Higher genome coverage will also allow reducing support intervals leading to the detection of more than one major QTL at a time. However, the integration of all these present results from different experimentations was efficient to better understand the genetic resistance of carrot to *Alternaria dauci* and to characterize two new complementary sources of resistance that should be considered to improve the current germplasm.

Author contribution statement VLC, MB, LB and RP designed the study. SM, AS, SH, LH and LV contributed to plant production. SM, AS, EA and VLC performed the experiments. VLC performed data analysis. VLC, MB, and MJ contributed to writing the paper.

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Conflict of interest The authors declare that they have no conflict of interest.

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