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# Resistant carrots to *Alternaria dauci* or tasty carrots: should we choose?

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## **Abstract**

**The carrot ideotype for carrot breeders could be a variety resistant to *Alternaria* leaf blight- the main foliar disease on carrot worldwide- with a low level of bitterness as consumers consider it as an undesirable taste of carrots. Our previous investigation on carrot resistance to *Alternaria dauci* allowed the identification of resistance QTLs (rQTLs). Based on these results, breeding for a higher level of resistance is possible but we could interrogate about the impact of selecting these genomic regions on carrot bitterness. To answer this question, we combined chemical and sensorial analyses of a set of resistant and susceptible carrot genotypes evaluated in different environments to identify the metabolites involved in bitterness. The genetic control and heritability**

**field experimentation and colocalizations between rQTLs and metabolite QTLs (mQTLs) were searched in order to evaluate the link between bitterness and resistance traits. Our results showed that selecting the genomic regions mainly involved in carrot resistance is possible without compromising carrot taste. However, the colocalization of rQTLs and mQTLs suggest that some genomic regions are involved in the expression of both traits and should be avoided or even counter selected.**

**Keywords:** root quality, bitterness, metabolite QTL, leaf blight, *Daucus carota*

## **INTRODUCTION**

While breeding for varieties highly resistant to *Alternaria dauci* is a top priority for carrot seed companies, consumers are more and more demanding on gustative quality, especially low bitter carrots. Among others, terpenes and polyacetylenes are considered key compounds for bitterness in carrots (Simon et al. 1980; Seljasen et al. 2001). Relying on sensory and chemical analyses, Kreuzmann et al. (2008) highlighted that a small number of metabolites, among them eight terpenes, could predict bitterness. However, while the genetic control of carrot resistance to *A. dauci* has already been investigated, leading to the identification of rQTLs (Quantitative Trait Loci involved in resistance) in different genetic backgrounds (Le Clerc et al., 2009; 2015), no information was available except very recently published by Keilwagen et al. (2017) on terpenes and polyacetylenes. Identifying the genomic regions involved in resistance and/or bitterness will be very helpful for breeders to develop resistant varieties while avoiding increasing bitterness. To answer this question, chemical and sensory analyses were performed on a set of five carrot genotypes during three years, in four environments to identify the terpenes involved in bitterness. These metabolites were characterized during two consecutive years in field trials in a population already segregating for resistance to *Alternaria dauci* and their genetic control was determined. The colocalization between previously identified rQTLs and mQTLs (QTLs involved in the accumulation of metabolites) were examined and recommendations were made for breeders.

## MATERIALS AND METHODS

### Plant material and experimental trials

During three consecutive years, two parental lines –H1 (susceptible to *Alternaria dauci*) and K3 (partially resistant) – of a segregating population named PC3; I2, another line partially resistant to *A. dauci* and two commercial hybrids (Boléro and Presto) were sown under a tunnel in Angers (Maine et Loire, France) and in the field in Lanxade (Dordogne, France) in 2014; in Field in Blagon (Gironde, France) and in Ychoux (Les Landes, France) in 2015 and 2016, respectively. For each trial, 180 seeds per genotype were sown on two meters in randomized blocks in duplicate as well as for the ~150 F<sub>2:3</sub> lines of the segregating population PC3 evaluated in 2015 and 2016 in the same fields. While in the tunnel plants were inoculated with an *A. dauci* strain (P2), the infection occurred naturally in the field.

### Chemical and sensory analyses

For chemical analyses, 15 to 20 plants per biological replicate and genotype were harvested at maturity and the central portions of the roots were shredded in a food processor, immediately frozen in liquid nitrogen and stored at -80 °C until analysis. The terpenes and polyacetylenes were separated and characterized on a Perkin Altus BEH C18 column (50 mm x 2.1 mm, i.d. 1.7 µm) using a Waters Acquity UPLC® system equipped with a photodiode array detector (Acquity PDA eλ).

For sensory analyses, 100 homogeneous and representative roots of each biological replication per genotype were pooled into one lot for the sensory panel in 2014 while in 2015 and 2016, the two biological replications were kept separated in 2015 and 2016. The panel was composed by more than 12 people specifically trained on carrot attributes including two attributes of basic taste (sweet, bitter) (Cottet and Navez, 2012). The sensory results of the five genotypes were then compared with the biochemical results to identify the metabolites likely to predict bitterness.

### Output data

Principal component analysis (PCA) was performed to describe the correlation between bitterness and volatile compounds in the set of five genotypes evaluated in four environments with R software version 3.3.2. Analyses were carried out with average biochemical values per year and scores of bitterness per year. The coefficients of variation were calculated from bitterness and disease means.

The accumulation value of each metabolite from each genotype replicate per year was autoscaled (i.e. centered-scaled, as described by van den Berg et al. 2006), according to the mean and standard deviation of the accumulation of the metabolite concerned per year. Thereafter, average data from autoscaled values were used to detect mQTLs in PC3 population, as previously described for rQTL analysis in Le Clerc et al. (2015). rQTLs had already been identified on a consensus map (Le Clerc et al., 2015). However as new SSRs were added, rQTL detection was repeated to check the positions of the rQTLs on a new map to compare mQTL and rQTL positions.

## RESULTS AND DISCUSSION

According to the sensory analysis of the five genotypes in the four environments, the range of bitterness values (min-max) was similar across environments and years and, genotype K3 was consistently the most bitter one while H1 and Presto the least bitter ones (Table 1). Seventeen terpenes and three polyacetylenes compounds were detected in common from the biochemical analysis over all three 2014-to-2016 years.

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Table 1. Disease score (*Alternaria dauci*), bitterness score and their coefficient of variation of five carrot genotypes evaluated in four environments.

Genotype	Varietal type	Disease score <sup>1</sup>		Bitterness				Adjusted mean <sup>3</sup>	CV <sup>2</sup>
		Mean	CV <sup>2</sup>	2014		2015	2016		
				Tunnel	Field	Field	Field		
K3	S2 line	2.2	0.52	6.3	7.6	3.0	5.2	5.0	0.36
I2	S2 line	4.1	0.04	5.5	6.0	1.3	3.5	3.5	0.57
H1	S3 line	7	0.22	0.8	1.6	0.3	0.9	0.8	0.58
Bolero	Hybrid	4	0.26	1.4	2.3	0.9	3.9	2.2	0.64
Presto	Hybrid	7.9	0.11	3.1	0.9	0.5	1.0	1.2	0.82
Mean bitterness score/environment				4.4	5.3	1.9	3.7	3.5	

<sup>1</sup> Mean disease score based on a 0-9 scale with 0 corresponding to symptomless plants and 9 to plants that were totally blighted

<sup>2</sup> CV: coefficient of variation

<sup>3</sup>: Mean were adjusted according to the number of biological repetitions per year

PCA components 1 and 2 explained 68% of the variance in the analysis of the five genotypes. Component 1 best described bitterness, with correlation values of 0.79 and the ten metabolites with correlation values greater than 0.6 (p.value <= 0.01) for component 1 were considered as good predictors of bitterness: limonene, sabinene,  $\alpha$ -terpinolene,  $\beta$ -pinene, faltarindiol,  $\beta$ -caryophyllene, myrcene, faltarindiol acetate, p-cymene-8-ol and bornyl acetate (Table 2). Our results confirmed that some terpenes and polyacetylenes are good predictors of bitterness as highlighted by Kreuzmann et al. (2008) who also identified faltarindiol, terpinolene, caryophyllene,  $\beta$ -pinene and sabinene among these predictors.

Table 2. Correlations between component one and bitterness (in bold) and correlations (>0.60) between component one and metabolite variables from PCA of the five genotypes in four environments.

Type of metabolite	Attribute and metabolites	Correlation with component 1	p.value
		<b>Bitterness</b>	<b>0.792</b>
Monoterpene	Limonene	0.952	0
Monoterpene	Sabinene	0.948	0
Monoterpene	$\alpha$ -terpinolene	0.912	0
Monoterpene	$\beta$ -pinene	0.907	0
Polyacetylene	Faltarindiol	0.866	0
Sesquiterpene	$\beta$ -caryophyllene	0.838	0
Monoterpene	Myrcene	0.804	0
Polyacetylene	Faltarindiol acetate	0.804	0
Monoterpene	p-cymen-8-ol	0.790	0
Monoterpene	Bornyl acetate	0.642	0.002

We identified 25 mQTLs, with one to three mQTLs per metabolite (Table 3). No mQTL was detected for p-cymen-8-ol. The part of the phenotypic variation explained by all the mQTLs identified for one metabolite was generally high, ranging from 12% for bornyl

acetate to 58% for falcarindiol and falcarindiol acetate, with a mean of 37 %. Among all the metabolites involved in bitterness, the two polyacetylenes presented the highest number of mQTLs with the highest total R<sup>2</sup> value for falcarindiol (~58%). The biggest mQTL for falcarindiol was identified on chromosome 9 (Figure 1). To date no rQTL has been identified on this chromosome, suggesting that counter-selecting this genomic region could reduce the perception of bitterness without compromising resistance.

While mQTLs were identified on eight chromosomes, a hotspot of mQTLs was identified on chromosome 4 representing 9 out of the 25 mQTLs.

Table 3. mQTLs detected for the metabolites predictors of bitterness, identified in a segregating carrot population evaluated in 2015 and 2016 in the field.

Metabolite	Chr <sup>1</sup>	R <sup>2</sup> (%)	Maximal position	CI <sup>2</sup>	Additive effect of allele		R <sup>2</sup> total
					H1	K3	
β-pinene	2	15.6	22.7	18.9-40	-0.23	0.23	49
	5	22.1	36.2	35.8-39.5	-0.36	0.36	
	4	22.6	50.8	39.5-51.5	-0.25	0.25	
limonene	8	19.6	3.1	0-12.4	-0.26	0.26	39
	4	33.5	20.4	15.8-20.9	-0.35	0.35	
sabinene	4	21.2	8.3	3-18.6	-0.29	0.29	47
sabinene	4	16.8	58.7	56.6-58.7	-0.23	0.23	
sabinene	9	18.4	26.8	4.4-29.8	0.27	-0.27	
α-terpinolene	8	21.4	3.1	0-12.9	-0.28	0.28	37
	4	29.2	20.4	15.2-21.2	-0.32	0.32	
myrcene	8	12.9	10.2	2.8-14.5	-0.22	0.22	27
	4	18.3	3.3	12-19.6	-0.27	0.27	
pcymen-8-ol	–						
bornyl acetate	6	12.3	34.3	24.4-40.2	-0.23	0.23	12.3
β-caryophyllene	4	18.4	16.4	7-48.7	-0.28	0.28	18.4
falcarindiol	2	8.7	33.6	16.1-39.2	-0.13	0.13	58
	4	23.6	57.9	50.7-58.7	-0.24	0.24	
	9	48.2	45.8	40-50.2	-0.45	0.45	
	6	15.3	24	15-25	-0.2	0.2	
falcarindiol acetate	3	24.5	68.9	58-69.7	-0.24	0.24	58
	8	27.2	4.1	3-7.7	0.29	-0.29	
	2	36.8	30.4	29.8-31	-0.33	0.33	
	4	15.7	0	0-6.1	0.18	-0.18	
	6	18	25.8	23-27.5	-0.22	0.22	

<sup>1</sup> Chr: chromosome

<sup>2</sup> CI: confidence interval

The upper and lower parts of this chromosome 4 presenting six and three mQTLs respectively colocalized with an rQTL whose favorable allele for resistance came from the K3 parental line (Le Clerc et al., 2015). Whatever the metabolite with an mQTL identified in this hotspot (except for the falcarindiol acetate), the alleles leading to higher contents were also supplied by the resistant K3 line. Therefore, when trying to increase resistance to *Alternaria dauci*, breeders should avoid this region because bitterness would also probably be increased. As explained by Carreno-Quintero et al. (2012)<sup>6</sup>, colocalization of QTLs related to different traits may indicate a common regulator that controls the variation of both traits, or the physical proximity of genes involved in different biological processes.

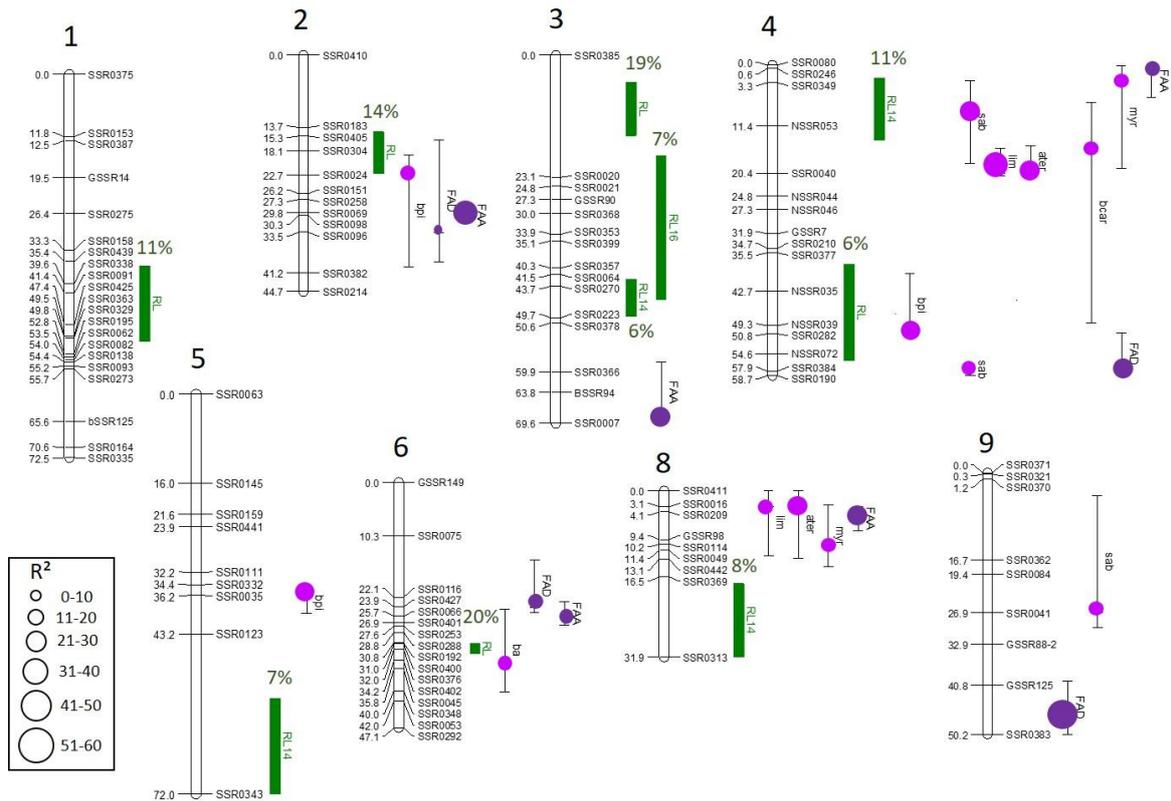


Figure 1. Resistance QTLs (RL) common to two or three years and RL11, RL14 and RL16 identified in 2011, 2014 and 2016 respectively identified only one year. RL are represented with 1-LOD support interval (solid bar) and R<sup>2</sup> in percentage and mQTLs of metabolites predictors of bitterness on the carrot chromosomes. For mQTL, maximal position is indicated by the circle and size of the circle is proportionnal to R<sup>2</sup>. Code of the metabolites: ater= α-terpinolene; ba= bornyl acetate; lim= limonene; sab= sabinene; FAA= faltarindiol acetate; bcar= β-caryophyllene; FAD= faltarindiol; bpi= β-pinene; myr=myrcene.

The three mQTLs on chromosome 2 are also partially colocalizing with an rQTL. However, these colocalizations result in favorable combinations of resistance and bitterness alleles in genotype H1 suggesting that breeder could increase resistance by selecting the rQTL of H1 on this chromosome while decreasing bitterness. Breeders could also select the biggest rQTL on chromosome 6 (R<sup>2</sup>~20%) as only one mQTL for bornyl acetate (R<sup>2</sup>~12%) is colocalizing and a low level of bitterness is expected in carrot as part of the typical carrot flavor (Simon, 1985). For the other rQTLs on chromosomes 1, 5 and 8, they can be used to increase resistance without problem as no mQTL involved in bitterness are colocalizing.

## CONCLUSION

In conclusion, breeding for resistance to *Alternaria dauci* without affecting root quality, i.e. without increasing bitterness is possible provided they select the favorable alleles for each trait.

-First study of the genetic determinism of carrot polyacetylenes has been done in the present study.

- Compared to Keilwagen et al. (2017), new mQTLs for terpenes have been identified and map on the carrot genome.
- Breeders can rely on rQTLs on chromosomes 1, 5, 8, 6 and 2 to increase resistance while decreasing bitterness under the conditions mentioned above.

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