

A grapevine (*Vitis vinifera* L.) deoxy-D-xylulose synthase gene colocalizes with a major quantitative trait loci for terpenol content

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Abstract Linalool, geraniol, nerol, citronellol and α -terpineol are isoprenoid molecules responsible for specific aromas found in grapes and wines. Total concentrations (free and bound forms) of these compounds were measured in the skins of mature berries during 2 successive years in two progenies obtained from Muscat Ottonel and Gewurztraminer selfings. Partial genetic maps based on microsatellite markers were constructed and several quantitative trait loci (QTLs) related to terpenol content were detected. A major QTL on linkage group (LG) 5 colocalized with a deoxy-D-xylulose synthase gene, coding for the first enzyme of the plastidial isoprenoid biosynthesis pathway. The number of favourable alleles at this locus determined the level of terpenol synthesis. A second QTL, on LG 10, was found to determine the balance linalool versus geraniol and nerol in the Muscat self-progeny plants.

Introduction

The complexity of wine flavours is partly due to molecules that are already present in grapes, at least as precursors, such as volatile phenols, sulphur compounds or terpenols. Linalool, geraniol, nerol, α -terpineol or citronellol are

monoterpenols (C₁₀) associated with floral flavours described as rose or lily of the valley (Ribéreau-Gayon et al. 1998). They are present in grapes and wines as free or glycosylated compounds (Günata et al. 1985a; Williams et al. 1982). Their concentrations vary according to year (Bureau et al. 2000; Reynolds et al. 1996), location (Dirninger et al. 1998; Reynolds et al. 1996), yield (Bureau et al. 2000) or canopy management (Belancic et al. 1997; Reynolds et al. 1996) but high concentrations are only found in the Gewurztraminer cultivar or in varieties of the “Muscat” group (Günata et al. 1985a; Ribéreau-Gayon et al. 1998; Wilson et al. 1986). Up to 4,400 names of varieties described to have a Muscat-like flavour have been gathered by Bronner (2003). This author identified many synonyms and proposed that these names corresponded actually to 1,042 varieties. Characterizing 18 varieties with 14 microsatellite markers, he also concluded that analysed Muscat varieties were relatives. For Crespan and Milani (2001), based on the genotype of 64 Muscat accessions at 25 microsatellite loci, Moscato bianco and Muscat of Alexandria could be the main progenitors of the Muscat family. Floral flavours can also appear in genotypes not related to the Muscat group. Indeed, heritable somatic mutations were shown to be responsible for the differences in terpenol content between clones of Savagnin (Savagnin rose, neutral and Gewurztraminer, aromatic) and clones of Chardonnay (clone 76, neutral and clone 809, aromatic) (Duchêne et al. 2008).

Terpenoids are essential for many aspects of plant biology and their biosynthesis pathway is well documented (Lange and Ghassemian 2003; Mahmoud and Croteau 2002). The first major steps of terpenoid biosynthesis lead to the synthesis of isopentenyl diphosphate (IPP). IPP is produced in cells via two pathways: the mevalonate pathway occurs in the cytosol while the methylerythritol pathway

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(MEP) takes place in plastids (Electronic Supplementary Material (ESM) S1). In the following step of the pathway geranyl diphosphate (GPP) is synthesized by condensation of IPP and its isomer dimethyl allyl diphosphate (DMAPP). GPP is the central substrate for all the monoterpene metabolism and over expression of deoxy-D-xylulose synthase (*DXS*) or deoxy-D-xylulose 5-phosphate reductoisomerase (*DXR*) genes, which code for the first enzymes of the pathway, have shown with transgenic plants that the MEP pathway is limiting for terpenoid synthesis (Carretero-Paulet et al. 2006; Enfissi et al. 2005; Estévez et al. 2001; Mahmoud and Croteau 2001; Munoz-Bertomeu et al. 2006). Two classes of *DXS* have been identified up to now in plants (Phillips et al. 2007; Walter et al. 2002). Class I *DXS* is involved in housekeeping functions while class II *DXS* transcripts synthesis is stimulated by fungi infection or elicitor treatment. Two class II *DXS* genes were shown to have distinct functions in Norway spruce. A third class of genes was found in *Arabidopsis thaliana* (Lange and Ghassemian 2003) or rice (Kim et al. 2005) by bioinformatic analysis, but not yet confirmed by functional analysis. In grapevine, the MEP pathway is also the dominant route for geraniol or linalool synthesis (Luan and Wüst 2002).

After GPP is synthesized, linalool synthase (*LIS*) (Cseke et al. 1998; Pichersky et al. 1995) or geraniol synthase (*GES*) (Iijama et al. 2004a; Yang et al. 2005) can utilize this substrate. In Sweet basil, the different linalool and geraniol concentrations in two cultivars were explained by the relative levels of transcripts coding for a linalool synthase and a geraniol synthase (Iijama et al. 2004a). In strawberry, linalool is synthesized by a linalool/nerolidol synthase coded by the *FaNES1* gene (Aharoni et al. 2004). The identification of an α -terpineol synthase (*ATS*) in the grapevine genome (Martin and Bohlmann 2004) also suggests an enzymatic control of α -terpineol synthesis.

If the general pathway of terpenoid biosynthesis is well described, little is known about the determinism of terpenol content in grapevine varieties. Terpenol content is a heritable trait and quantitative trait loci (QTL) detection has already been reported. In the progeny of a Moscato bianco \times *Vitis riparia* cross, Sevini et al. (2004) found QTLs for free and bound forms of linalool, nerol, citronellol and geraniol on three linkage groups (LGs) but only one of them (LG 2) can be precisely identified in the publication. Analysing only the concentrations of free terpenols in the offspring from a (Olivette \times Ribol) \times Muscat of Hamburg cross, Doligez et al. (2006b) located a major QTL on LG 5 and two QTLs with lower effects on LGs 2 and 13.

In the present study, our aims were to confirm QTLs for terpenol content in two different segregating populations, derived from Muscat Ottonel and Gewurztraminer, to collocate candidate genes with these QTLs and to assess allelic effects.

We present here QTLs for total terpenol content detected over 2 years in the greenhouse and in the vineyard for two segregating populations. We also show that a *DXS* gene collocate with a major QTL on LG 5.

Materials and methods

Populations and experimental conditions

Two populations were used in this study: progeny from a selfing of Muscat Ottonel grown in the greenhouse (S1 MO) and progeny from a selfing of Gewurztraminer grown in the vineyard (S1 GW).

For the first population, seeds were collected in 2001 from self-pollinated inflorescences of Muscat Ottonel clone 59 wrapped in paper bags. In total 8,552 seeds were collected. After germination of 5,000 seeds in 2002, 2,192 young plants were grown in 0.6 l pots. As they had approximately 7–8 leaves, 456 plants were transferred to 4 l pots. Twelve plants of the Muscat Ottonel parent and 12 plants of Chasselas blanc as a “non aromatic” control were added to the experiment at random positions to assess the environmental variance.

Before 2003 budbreak the plants were topped at the 30th node and a loop was made with the shoot. After budbreak, only the top two shoots were kept and stalked on nylon wires. After this experiment 212 living genotypes could be transferred in the vineyard.

For the Gewurztraminer self-progeny, seeds were collected in 1997 from self-pollinated inflorescences from clone 643 wrapped in paper bags. One thousand six hundred and fifty-eight seeds were sowed in 1998 and 245 genotypes were available in 1999. Grafts were prepared in 2000 with the Couderc 161–49 rootstock using a green grafting technique (Walter et al. 1990). One hundred and seventeen genotypes were planted in the INRA vineyard in Colmar in 2001. Each genotype was represented by three grafted plants per elementary plot. Plots were organized in a randomized design and ten plots with the Gewurztraminer parent were included in the experiment to assess the environmental variance.

Terpenol analysis

The objective of this study was to identify QTLs for berry terpenol content. Here, we quantified total concentrations in skins of linalool, α -terpineol, citronellol, nerol and geraniol, the main monoterpens found in grapes (Günata et al. 1985b; Razungles et al. 1993). We measured the total terpenol content in skins as concentrations per gram of fresh tissue, at least for Gewurztraminer and Muscat varieties, are higher than in mesocarps (Duchêne et al. 2008; Günata

et al. 1985b) and should then provide a more accurate evaluation of the trait. In our viticultural context, we are mainly interested in wine grapes where the free terpenol content has less significance than for table grapes. The study of the genetic determinism of the free to bound terpenol ratio was not part of our objectives and free and bound forms were consequently not analysed separately.

Sampling

In 2003 and 2004, for the S1 MO progeny in the greenhouse, véraison (onset of ripening) dates were recorded on all the genotypes. The low number of available berries for some genotypes of the progeny did not allow both a reliable evaluation of their sugar content and a reliable terpenol analysis. We then chose to collect berries for terpenol analysis 26 days after véraison for each genotype. The ripening stage is not a critical point to reveal the ability of cultivars to produce terpenols (Duchêne et al. 2008) and our objectives were more to discriminate genotypes than to evaluate their full aroma potential. For the S1 GW progeny in the vineyard, berries were picked as their sugar content reached 18°Brix. This value was chosen to allow a correct sampling even for late ripening genotypes in our conditions. However, when there were not enough berries for both sugar and terpenol analysis or when bunch rot would cause the loss of a genotype, berries were collected without preliminary evaluation of the sugar content. No statistical correlations were detected between the sugar content measured on the final sample and the concentrations of the five terpenols, either in 2004 ($R^2 = 0.004$, $n = 67$) or in 2005 ($R^2 = 0.003$, $n = 78$), suggesting that there was no bias in the sampling procedure. The mean sugar content of the analysed genotypes was 17.6 (± 2.0 standard deviation) °Brix in 2004, and 18.9 (± 1.6 standard deviation) °Brix in 2005.

For both experiments and years berries were immediately frozen after they were picked.

In the S1 MO progeny, 140 plants out of 456 had inflorescences in 2003 but berries could be collected only on 82 genotypes. Muscat Ottonel is a variety prone to poor fruit set, which means that in certain conditions, still unclear, no berries set after flowering. This unfavourable characteristic was found in the offspring of the S1 MO progeny where 44% of the inflorescences did not produce any berry. In 2004, berries were collected on 55 genotypes.

For the S1 GW population berries were collected on 81 genotypes in 2004 and 100 genotypes in 2005.

Isolation of free and bound monoterpenols by solid phase extraction (SPE)

Frozen berries were allowed to defrost, their mesocarp and their seeds were discarded. We obtained 3.5 g of skin per

genotype on average for the S1 MO population and 5 g for the S1 GW population. After weighing, skins were ground under liquid nitrogen and, after addition of 40 mg sodium sulphite, suspended in 40 ml of water. The solid phase extraction process was the same as described by Duchêne et al. (2008) with one supplementary step: 20 μ l of a 1 g/l 3-octanol solution in ethanol was added as external standard before centrifugation and filtration of the sample. Mean extraction yield of 3-octanol for ten Gewurztraminer samples in 2005, for example, was 63.8%, with a 8.1% standard deviation. Samples were stored at -20°C prior to gas chromatography analysis.

Terpenol identification

Terpenol identification was carried out by gas chromatography/mass spectrometry (GC/MS) as previously described (Duchêne et al. 2008).

Mass spectra of 17 samples, randomly chosen in the progenies, were compared and showed that the peaks corresponding to terpenols of interest were not ambiguous. We therefore assumed that retention times could be used for the identification, and then quantification, of these terpenols.

Terpenol quantification

Gas chromatography (GC) analyses were performed on a Hewlett-Packard 6890HP gas chromatograph in 2003 and on a ThermoQuest Trace GC in 2004 as reported by Duchêne et al. (2008). Signals were recorded and integrated by HP Chemstation in 2003 and by Chrom-Card for trace GC in 2004 and 2005.

Total amounts of 3-octanol, *m*-cresol, linalool, α -terpineol, citronellol, nerol and geraniol were determined using linear calibration curves built with standard molecules over a concentration range from 0 to 100 ng/ μ l. These calibration curves were calculated every time a new column was used in a chromatograph. Concentrations were set to 0 for statistical analysis when molecules were not detected.

Simple sequence repeat (SSR) analysis and gene mapping

Genomic DNA extraction and methods for SSR analysis were as in Merdinoglu et al. (2005). Sequences of the SSR primers used in this study can be found in NCBI (<http://www.ncbi.nlm.nih.gov>) databases (Nucleotide or UniSTS), excepted for VVI69 and VVI67 (Merdinoglu et al. 2005).

Genes participating in the terpenoid synthesis pathway were identified in the TGI public database (<http://compbio.dfci.harvard.edu/tgi>) or in bibliography (Martin and Bohlmann 2004; Iijama et al. 2004b) and primers were designed for 20 of them. Our objectives were to identify single nucleotide polymorphism (SNP) in the sequences of

Muscat Ottonel in order to map these genes. The process which was achieved for a *DXS* and an *ATS* gene is reported here. The availability of the grapevine genome sequence modified our approach for the other genes.

At least four paralogs of *DXS* gene can be identified in the current releases of the grapevine genome (Jaillon et al. 2007; Velasco et al. 2007) but ESTs can be found in databases for only three of them. A Clustalx analysis of the three corresponding proteins together with *DXS* proteins available in databases showed that grapevine identified *DXS* belong to class I, class II and to the putative class III (ESM S2).

Polymerase chain reaction primers were designed for the tentative mRNA consensus TC56417 (TGI project) corresponding to a class I *DXS* gene (*DXS1*) and for the Genbank accession numbers AY572986 and AY572987 (Martin and Bohlmann 2004) corresponding to an *ATS* gene.

Genomic DNA was amplified in a 20 μ l-vol reaction mixture containing 2.5 mM $MgCl_2$, 150 μ M dNTPs, 0.5 μ M of each primer, 0.025 U/ μ l of AmpliTaq Gold DNA polymerase, 0.5 ng/ μ l of grapevine DNA, 1 \times Gold Buffer. Amplifications were performed on a Perkin Elmer 9700 thermocycler programmed as follows for *DXS1*: 5 min at 94.5°C followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, 60 s at 72°C and a final step of 7 min at 72°C. Annealing temperature was set to 60°C for the *ATS* gene. Forward and reverse sequencing were performed on an ABI3100 Genetic analyser. Sequences were compared with accessions from public databases using Vector NTI 7 (InforMax Inc.).

A nucleotide polymorphism was identified in sequence chromatograms for *DXS1* in the fragments amplified by the primer pair (5'-caaacatggtggtgatggct-3', 5'-accaaacagctctgtactctgttc-3') for Muscat Ottonel (T/G) and the primer pair (5'-caaacatggtggtgatggct-3', 5'-gagagaatgaaagctgatgtatctgt-3') for Gewurztraminer (A/T) (see ESM S3 for their positions).

For *ATS*, SNPs were identified in the fragments amplified in Muscat Ottonel with the primers 5'-cagagactcgcacacagcat-3' and 5'-tgtaccataaacgtcgtaa-3'.

Single nucleotide polymorphisms were revealed in the populations using the SNaPshot[®] method (Applied Biosystems). Genomic PCR products were purified with 5 U of Shrimpalkaline phosphatase (SAP, USB Corporation, Cleveland, USA) and 2 U of Exonuclease I (EXO1, USB Corporation, Cleveland, USA) enzymes for 15 μ l of PCR product (1 h at 37°C, 15 min at 75°C). Primers 5'-aggtgaagctcactgaatctctacataaac-3' for Muscat Ottonel and 5'-aagtttgagttcctctttcataa-3' for Gewurztraminer were used to reveal the SNP in *DXS1*. The same methodology was used to map the *ATS* gene with the primer 5'-aatgtttttatgtctcct-3' for SNP identification.

The second PCR reaction was performed using 5 μ l of SNaPshot[®] Multiplex Ready Reaction mix, 3 μ l of 1/10 diluted PCR product, 1 μ l of primer and 1 μ l of water on a Perkin Elmer 9700 thermocycler programmed as follows: 5 min at 95°C, 25 cycles of 10 s at 96°C, 5 s at 50°C, 30 s at 60°C. The PCR product was purified with one unit of SAP per sample in the conditions described earlier.

Fragments were visualized with the same methodology as for SSR fragments with the use of a TAMRA-labelled 13–75 bp internal size standard.

Genetic analysis and construction of the genetic map

The broad sense heritability was calculated as the ratio of total genetic variation to the total phenotypic variation (Kearsey and Pooni 1996). The total phenotypic variance was calculated within the S1 MO and S1 GW populations. The environmental variance was evaluated with analysis of variance on the Muscat Ottonel and Chasselas blanc controls for the S1 MO progeny and with the variance of Gewurztraminer 643 for the S1 GW progeny. The genetic variance was obtained by the difference between the total phenotypic variance and the environmental variance.

A genetic map based on SSR markers and 121 genotypes bearing inflorescences was built for the S1 MO progeny. Primer pairs were chosen (1) to satisfy the condition of heterozygosity at the SSR locus in the parent (2) for their unambiguous electrophoretic profile (3) to cover the whole genome with a regular distribution. Map construction was performed with Carthagene (de Givry et al. 2005) with the parameters $LOD \geq 5$ and $r < 0.50$ for grouping followed by the “buildfw 2 2 1” command, i.e. with adding and removing thresholds set to 2. Markers order was ensured at $LOD 2$ using the “flips 5 2 0” command. Linkage groups were numbered according to the reference map of Riaz et al. (2004) with the modifications of Adam-Blondon et al. (2004). They will be mentioned in the text as MO05 for “Muscat Ottonel linkage group number 5” for example. JoinMap 3.0 (Van Ooijen and Voorrips 2001) was used to check for segregation distortion, to find genotyping errors and to draw the map.

For the S1 GW progeny, a genetic map for LG 5 was built with 115 genotypes as described earlier.

QTL detection

The QTL Cartographer package was used to identify QTLs (Basten et al. 2004). Simple interval mapping (SIM) and composite interval mapping (CIM) procedures were performed with Zmapqtl using a 2-cM step and a maximum of five background markers. The SRmaqtl program (forward-backward selection of markers, at $P = 0.05$ for introduction and elimination) was used to identify markers related to

quantitative traits. Permutation tests (1,000 permutations) were performed in order to determine the experimentwise significance levels and comparisonwise (locus by locus) probabilities. Maximum LOD values were used to estimate positions of the QTLs with a one-LOD support interval.

QTL Cartographer calculates an S statistic to test the normality of variables and residuals. The critical values for the rejection of the normality are 5.99 and 9.21 for tests at $P = 0.05$ and $P = 0.01$, respectively (Basten et al. 2004). In most of the cases, normality criteria were not satisfied with raw variables and they were therefore transformed with a neperian logarithm (Log) after addition a value of 1 to avoid null values.

Kruskal–Wallis non-parametric tests and analyses of variance were carried out using the *R* 2.6.2 package [Copyright (C) 2008 The R Foundation for Statistical Computing]. The model of analysis of variance used for analysing the effects at a locus was as follows: $y_j = \alpha_i + \varepsilon_j$ where y_j is the value of a given trait for the genotype j , α_i the effect of the marker α (depending on the genotype hh, hk or kk, of the individual j at this locus), ε_j the residual error. Interactions between markers were tested introducing a term for interactions in the model.

Results

Terpenol concentrations

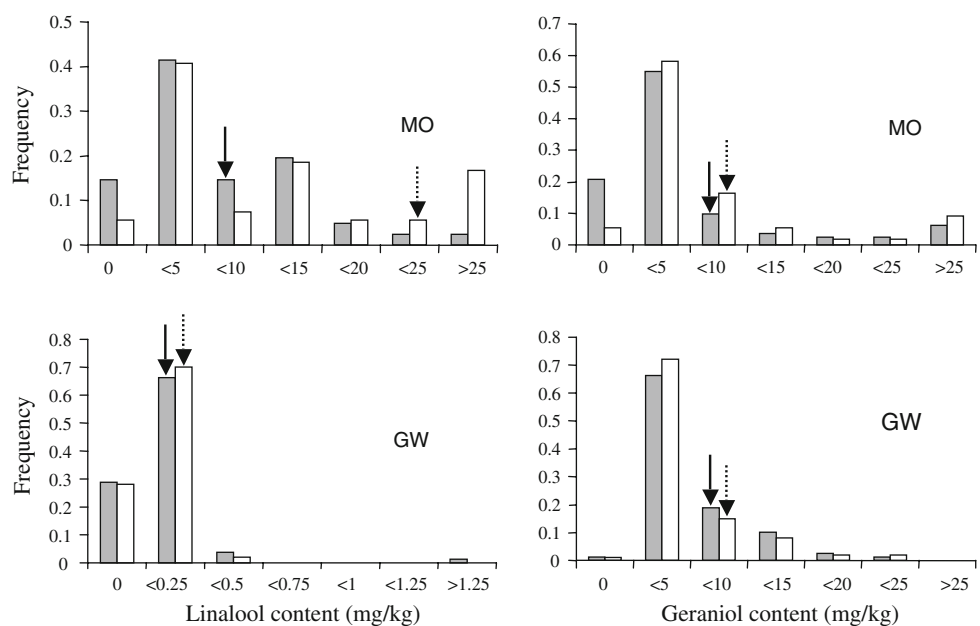
Linalool and geraniol content segregated (Fig. 1) and the distribution for geraniol was unimodal and continuous for both populations. Linalool concentrations were very low in GW as well as in all the S1 GW offspring. In the S1 MO

population, the distribution for linalool showed two modes. In our experimental conditions, heritabilities ranged from 67 to 99% for all the traits (Table 1), except for α -terpineol content in 2004 for the S1 GW progeny because of an unexpected high environmental variance. Even with very low concentrations, a heritability of 78% was calculated for linalool content in the S1 GW progeny.

Genetic map

Out of 252 SSR primer pairs tested on Muscat Ottonel, 216 gave a readable signal. Among them, 96 amplified a single fragment, i.e. Muscat Ottonel showed 56% of heterozygosity. One hundred and two markers were chosen to avoid expected redundancy and were encoded in the two possible phases before analysis with Carthagine. Eighty-four markers, including *DXS1*, were located on the Muscat Ottonel genetic map. Five of the 102 encoded SSR markers did not show any segregation in the progeny, while the others could not be linked to a group or were not retained after the procedure used under Carthagine to ensure markers order at LOD 2. Segregation distortion was significant at $P = 0.05$ for 41 markers, mainly located on LGs 1, 2, 4, 5, 8, 13, 15 and 18 (Fig. 2). No significant genotyping errors were identified with JoinMap. Nineteen linkage groups were identified, covering 568.7 cM Kosambi. There were on average 4.4 markers per linkage group and the mean distance between markers was 8.7 cM Kosambi. The position of the markers was in agreement with the reference map of Doligez et al. (2006a). For Gewurztraminer, a genetic map was built only for LG 5 (Fig. 3). Four markers were mapped, covering 27 cM Kosambi. SSR markers in the *DXS1* region, VVi52, VMC3b9, VVMD27 were homozygous for

Fig. 1 Distribution of linalool (left) and geraniol (right) concentrations in the Muscat Ottonel (top) and Gewurztraminer (bottom) self-progenies. Grey bars year 1. White bars year 2. Concentrations are expressed in milligram/kilogram of free and bound fractions in the skin. Arrows indicate the class of the parent (plain black year 1, dotted line year 2)



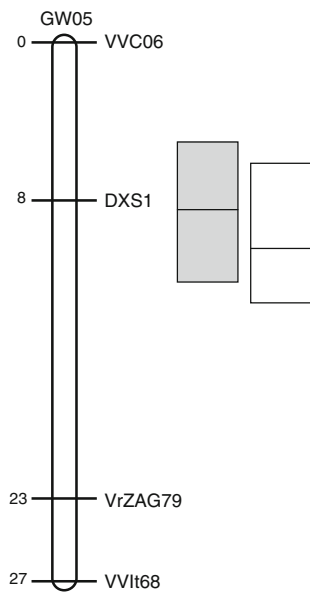


Fig. 3 Genetic map of linkage group 5 of Gewurztraminer and position of the QTL for the total concentration of five terpenols. Genetic distances are expressed in cM Kosambi. Confidence intervals are 1-LOD decrease (2004 in grey, 2005 in white)

from VVli52 and 1.8 Mb from VrZAG79 (ESM S3). The *ATS* gene sequence was found in the PN40024 genome but was not anchored to a chromosome.

No outcrossing event was observed as no unexpected allele appeared in either population.

QTL detection

In the S1 MO progeny, a QTL was detected on LG 5 for each of the five quantified molecules by the SIM procedure but the hypothesis of normality was not satisfied (data not shown) even after Log transformation. However, this condition could be met for the total concentration of the five terpenols and the same QTL detected (Table 2). This QTL had a maximum LOD at the *DXSI* position and explained 85 and 87% of the total variation in 2003 and

Table 3 Means of the terpenol concentrations according to the genotype at the *DXSI* locus in the S1 MO progeny and Kruskal–Wallis (KW) test

Terpenol	Year	<i>DXSI</i> genotype			KW test ^a
		TT	TG	GG	
Linalool	2003	0.92	11.35	12.13	<0.001
	2004	0.82	16.64	17.28	<0.001
α -Terpineol	2003	0.04	0.59	0.39	<0.001
	2004	0.14	0.44	0.37	<0.001
Citronellol	2003	0.00	0.29	0.18	<0.001
	2004	0.04	0.44	0.79	<0.001
Nerol	2003	0.06	5.99	6.74	<0.001
	2004	0.17	3.27	6.31	<0.001
Geraniol	2003	0.10	8.50	18.99	<0.001
	2004	0.27	8.31	28.21	<0.001
Five terpenols	2003	1.12	26.72	38.44	<0.001
	2004	1.43	29.10	52.97	<0.001

Concentrations are expressed in mg/kg

^a Significance level

2004, respectively. Non-parametric Kruskal–Wallis tests showed that the concentrations of all the analysed compounds were statistically linked to genetic variations at the *DXSI* locus for both years (Table 3).

For the S1 GW progeny, a QTL on LG 5, in the same region as for Muscat Ottonel (Fig. 3), explained 81 and 84% of the variation of the total concentration of the five terpenols in 2004 and 2005, respectively (Table 2). SIM was also appropriate for log-transformed geraniol and nerol concentrations in this population and QTLs for these traits were found at the same position as for the total concentration of the five terpenols (data not shown). These QTLs explained at least over the 2 years 67% of the variation of nerol concentrations and 80% of the variation of geraniol concentrations. Moreover, as for the S1 MO progeny, Kruskal–Wallis tests showed significant correlations between the genotype at the *DXSI* locus and the concentrations of

Table 2 Characteristics of the quantitative trait loci (QTL) for the concentration of the 5 terpenols detected on linkage group 5 of Muscat Ottonel and Gewurztraminer

Progeny	Year	LOD	Position (cM from top)	<i>P</i> -value ^a	Closest marker	CI ^b (cM)	<i>R</i> ²	<i>S</i> ^c
S1 MO	2003	34.7	0	<0.001	<i>DXSI</i>	0–2	0.87	3.96
	2004	22.5	0	<0.001	<i>DXSI</i>	0–1	0.85	2.90
S1 GW	2004	29.1	8.3	<0.001	<i>DXSI</i>	5–12	0.81	0.83
	2005	36.2	10.3	<0.001	<i>DXSI</i>	6–13	0.84	1.92

^a Experimentwise significance level

^b 1-LOD decrease confidence interval (CI)

^c *S* statistic for the normality of the residuals computed by QTLCartographer. Hypothesis of normality can be accepted at *P* = 0.05 for values under 5.99

Table 4 Means of the terpenol concentrations according to the genotype at the *DXS1* locus in the S1 GW progeny and Kruskal–Wallis (KW) test

Terpenol	Year	<i>DXS1</i> genotype			KW test ^a
		CC	AC	AA	
Linalool	2004	0.02	0.14	0.14	<0.001
	2005	0.00	0.05	0.11	<0.001
α -Terpineol	2004	0.11	0.33	0.29	<0.001
	2005	0.11	0.33	0.36	<0.001
Citronellol	2004	0.04	0.77	1.62	<0.001
	2005	0.03	0.58	1.24	<0.001
Nerol	2004	0.11	2.02	6.27	<0.001
	2005	0.06	1.77	6.05	<0.001
Geraniol	2004	0.32	6.01	10.30	<0.001
	2005	0.20	4.64	10.97	<0.001
Five terpenols	2004	0.62	9.27	18.62	<0.001
	2005	0.40	7.38	18.73	<0.001

Concentrations are expressed in mg/kg

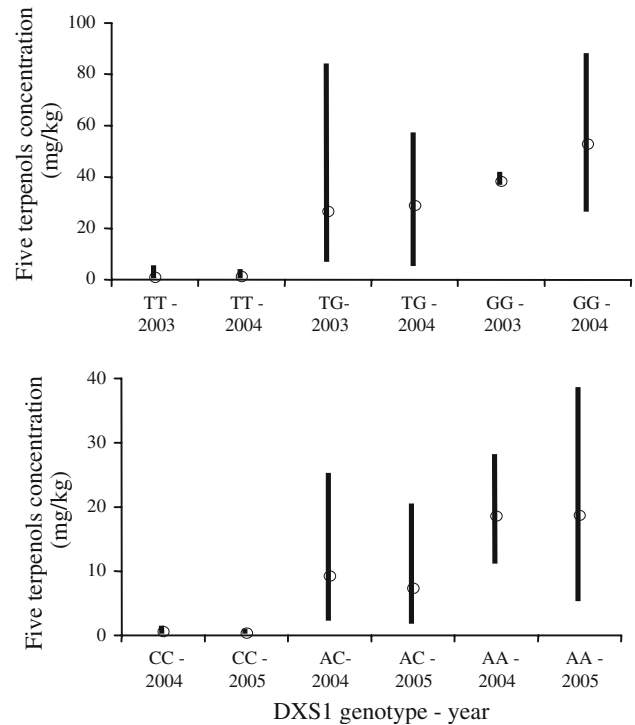
^a Significance level

each terpenol, even when present at very low levels as linalool or α -terpineol (Table 4).

Terpenol concentrations increased according to the number of alleles with a guanine (G) or a adenine (A) at the SNP position, for the S1MO and S1 GW progeny, respectively. There was a clear difference in terpenol content between genotypes with (*DXS1*+) or without (*DXS1*-) the favourable allele (Fig. 4). Whatever the progeny, terpenol synthesis level in *DXS1*- genotypes never reached the level obtained when at least one copy of the favourable allele was present.

Further analysis on the S1 MO progeny was performed with CIM after an automatic co-factor selection with the SRmapqtl program.

New QTLs, different from the major one on LG 5, were detected at $P = 0.05$ experimentwise for linalool content on MO10 for the 2 years and on MO15 in 2003, for geraniol content on MO01, for α -terpineol content on MO13 in 2003

**Fig. 4** Relationships between the genotype at the *DXS1* locus and the concentrations of the five analysed terpenols in the S1 MO progeny (top) and the S1 GW progeny (bottom). Bars indicate the range of observed values, circles indicate the mean value

(Table 5). The QTL for α -terpineol content colocalized with the *ATS* gene. These QTLs had low effects when compared to the QTL on LG 5. The QTL on MO10 was also detected for α -terpineol in 2003 and for geraniol in 2003 and 2004 with comparisonwise probabilities equal or lower than 0.005, but experimentwise significance levels failed to reach $P = 0.05$.

In order to enhance QTL detection and remove background noise of very low concentrations values, data analysis was restricted to *DXS1*+ genotypes. With 45 genotypes in 2003 and 35 in 2004 in the datasets, QTLs on MO02 for linalool, MO01 for nerol and geraniol, and MO10 for the

Table 5 Supplementary QTLs detected at $P = 0.05$ experimentwise with CIM in the S1 MO progeny (ln-transformed variables)

Terpenol	Year	Link. group	LOD	Position (cM from top)	P -value ^a	Closest marker	CI ^b (cM)	R^2	S^c
Linalool	2003	10	6.3	14.0	0.006	VrZAG64	10–16	0.12	0.22
		15	4.0	26.2	0.035	VVIp33	22–26	0.07	0.73
	2004	10	7.1	8.0	0.007	VMC3d7	0–14	0.32	0.02
α -Terpineol	2003	13	4.2	38.4	0.049	<i>ATS</i>	30–45	0.20	15.5**
Geraniol	2003	1	4.0	30.8	0.043	VVIIn61	28–33	0.06	12.1**

** Hypothesis of normality can be rejected at $P = 0.01$

^a Experimentwise significance level

^b 1-LOD decrease confidence interval (CI)

^c S statistic for the normality of the residuals computed by QTLCartographer

Table 6 Kruskal–Wallis (KW) tests and analysis of variance (Anova) for markers associated with QTLs detected for both years with the *DXS1+* genotypes of the S1 MO progeny

Terpenol	Year	Link. group	Marker	P-value	
				KW	Anova
Linalool	2003	2	VVIo55	0.009	0.005
		10	VrZAG64	<0.001	0.02
	2004	2	VVIo55	0.049	0.005
		10	VMC3d7	0.003	0.005
Nerol	2003	1	VVIn61	0.011	<0.001
		10	VrZAG64	0.002	<0.001
	2004	1	VVIm25	0.048	0.002
		10	VrZAG64	0.009	<0.001
Geraniol	2003	1	VVIn61	0.003	<0.001
		10	VrZAG64	0.011	0.002
	2004	1	VVIm25	0.043	0.009
		10	VrZAG64	0.008	0.009

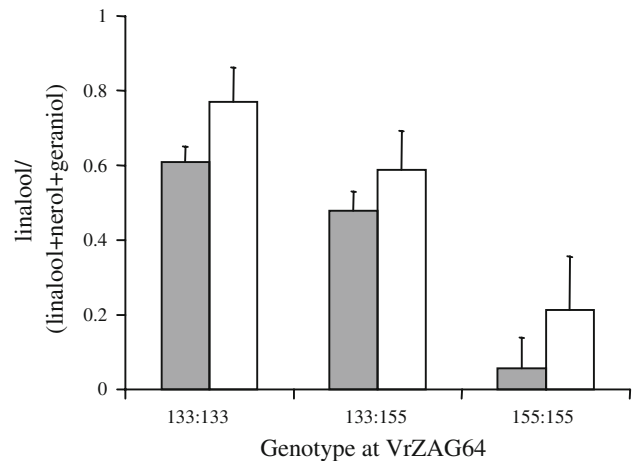
three molecules were detected with SIM for the 2 years, but often failed to reach a $P = 0.05$ significance level experimentwise or to satisfy the hypothesis of normality (data not shown). However, effects of the markers associated with these QTLs could be confirmed by Kruskal–Wallis tests and analyses of variance for the two seasons (Table 6). The QTL on MO10 had no significant effect on the total concentrations of linalool, nerol and geraniol ($P = 0.1$ in 2003, $P = 0.4$ in 2004), but had opposite effects on linalool on the one hand and geraniol and nerol on the other hand. When allele 133 was present at locus VrZAG64, linalool concentrations increased ($P < 0.001$ in 2003, $P = 0.04$ in 2004) whereas geraniol and nerol concentrations decreased ($P < 0.001$ in 2003, $P = 0.004$ in 2004), and consequently a higher relative content of linalool was observed (Fig. 5). Interestingly, the effect of this locus could also be detected in the same way in 2003 with *DXS1-* genotypes ($P = 0.002$).

Interactions between VVIn61 on MO01 and VrZAG64 on MO10 for geraniol and nerol concentrations were statistically significant in analyses of variance ($P = 0.03$ in 2003, $P = 0.02$ in 2004), but the number of genotypes in the different classes was not sufficient for a detailed analysis.

For linalool concentrations, analysis of variance allowed us to detect an effect of the alleles at the VVIo55 locus (MO02) at $P = 0.005$ in 2003 and 2004.

Discussion

Progeny from the selfing of Muscat Ottonel and Gewurztraminer was evaluated for terpenol content over two seasons and provided evidence that this trait segregates. Genetic maps allowed the detection of a major QTL, segregating

**Fig. 5** Relationships between the genotype at the VrZAG64 locus (MO10) and the ratio between the linalool concentration and the total concentration of linalool, nerol and geraniol in the *DXS1+* subset (grey bars 2003, white 2004). Vertical bars indicate standard errors

with a *DXS* class I gene on LG 5 in both populations. Supplementary QTLs on LGs 1, 2, 10, 13 and 15 were also identified in the Muscat Ottonel self-progeny.

The current Muscat Ottonel genetic map covers 568.7 cM Kosambi. Muscat Ottonel is apparently less heterozygous (56 %) than Riesling, Syrah or Grenache (69% according to Adam-Blondon et al. (2004)). It is consequently more difficult to find segregating loci and to expect a good coverage of the genome and some telomeric regions are not represented on our map. On the other hand, whenever comparisons are possible, distances between extreme markers are always shorter in our map than in the complete map built from five different populations (Doligez et al. 2006a): common markers cover 533 cM Kosambi in our case versus 872 cM Kosambi in the complete map. Heterogeneity between recombination rates could be observed among five populations (Doligez et al. 2006a) and differences with our mapping population are then not surprising.

Genotyping of the S1 MO population aimed to detect QTL for terpenol content of the berries and only fertile plants were chosen. A bias was induced by this choice as many loci of the MO map did not segregate with the expected ratio. Inbreeding depression seems clear in our case as many plants had a very low growth capacity and did not produce berries. It is noticeable that all the linkage groups where QTLs for terpenol content were detected, except LG 10, were affected by distortion of segregation. The known negative effects of terpenes on seed germination or seedling growth (Singh et al. 2002; Vaughn and Spencer 1993) may have amplified inbreeding depression. Markers with distorted segregation ratios were not observed for GW on LG 5.

Selfed populations have limitations but our results confirm in new genetic backgrounds previously detected QTLs

for terpenol content and moreover provide more quantitative information on allelic effects. Our data are consistent with results obtained by Doligez et al. (2006b). These authors found a major QTL for free geraniol, nerol and linalool on LG 5. This QTL explained a higher proportion of the phenotypic variance in our case (81–87 vs. 17–55%) presumably because of the type of progeny, a selfing in our case, and because we quantified both free and bound monoterpenols. The detection of a QTL for linalool content on LG 10 was previously mentioned (Battilana et al. 2005) but without sufficient data to compare with our results. The QTL for total linalool content we located on MO02 was in the same region as the QTL for free linalool content identified by Doligez et al. (2006b) and Sevini et al. (2004) detected a QTL for linalool content on LG 2 as well (LG 9 in their study). On MO13, we showed that an *ATS* gene colocalized with a QTL for α -terpineol content in the same region as a QTL for free geraniol and free nerol content previously detected (Doligez et al. 2006b).

In both studied genetic backgrounds, Muscat Ottonel and Gewurztraminer, the major detected QTL on LG 5 was linked to a *DXSI* gene. The effect of this QTL was found for all the terpenols analysed, which is consistent with the role of a *DXS* gene, expected to modify the availability of the main substrate for monoterpenol biosynthesis, GPP. For the 229 genotypes analysed in these two progenies, a favourable allele of this QTL was required to produce significant amounts of terpenols. Moreover, the type of genotypes obtained from the selfing process allowed us to show that this QTL had a clear additive effect (Fig. 4). *DXS* over expression experiments were shown to enhance the isoprenoid metabolism (Enfissi et al. 2005; Estévez et al. 2001; Munoz-Bertomeu et al. 2006). Thus, high terpenol concentrations in some grapevine genotypes could be consistent with a higher, still to be demonstrated, level of *DXSI* transcription. An alternative hypothesis would be a higher activity of the protein encoded by the favourable allele. In our study, the SNPs used to map *DXSI* are located in introns and their positions are not the same in Muscat Ottonel and Gewurztraminer, thus preventing us from providing a direct association between a molecular determinism in the *DXSI* gene and a high terpenol synthesis level. We confirm that this gene is a strong candidate, but further molecular analysis will be required to unravel its putative role.

A second QTL, on LG 10, had a clear effect on linalool content and when the allele favourable to linalool synthesis was present, geraniol and nerol were in lower concentrations. Final concentrations of each molecule are presumably under the control of enzymatic processes competing for GPP for the synthesis of linalool, geraniol and nerol. The presence of a linalool synthase in higher quantities or with a higher enzymatic activity could explain the increase in linalool concentrations. Interestingly, the effect of this

QTL was detected whatever the genotype at the *DXSI* locus (Table 5) and confirmed in *DXSI*– plants in 2003. This QTL was related to a higher level of linalool synthesis detectable even with a low GPP availability.

Other QTLs for terpenol content, with less characterized effects, were detected in the S1 MO progeny on LGs 1, 2, 13 and 15 and they are likely to interact with the QTLs on LGs 5 and 10. Observing segregating Muscat populations, Wagner (1967) proposed that up to six loci could be related to the determinism of terpenol content and this high number was confirmed by QTLs detection projects (Doligez et al. 2006b; Sevini et al. 2004).

On the basis of our present results, we can propose a two-step model. The first step would be a high production of substrate for terpenol synthesis, GPP, under the control of a QTL linked to a *DXSI* gene. No other favourable locus seems to be necessary for geraniol and nerol synthesis, which means that grapevine has the biochemical capacity of transforming GPP to geraniol and nerol. This model is consistent with our results with the S1 GW progeny where linalool was never detected in high concentrations. As the percentage of variations of the terpenol content explained by the *DXSI* QTL (Table 2) was similar to the heritability of the trait (Table 1), it also means that our genetic map of LG 5 allowed us to capture most of the genetic information in this progeny for this trait and that, if other QTLs exist, they have very low effects.

For Muscat Ottonel, the presence of a favourable allele at the *DXSI* QTL would increase GPP availability and, without the “linalool enhancer” allele on LG 10, geraniol and nerol synthesis. As for Gewurztraminer, the percentage of terpenol concentration variations explained by the *DXSI* QTL was of the same order as heritabilities. However, and this is the second step of the model, if an active “linalool enhancer” allele on LG 10 was present, part of the available GPP would be used for linalool synthesis and hence not for geraniol or nerol synthesis, leading to a terpenol profile where linalool is predominant. This model is consistent with the observed segregations, with unimodal distributions for geraniol content and two modes observed for linalool content in the S1 MO progeny (Fig. 1). The present results were obtained by determining the concentrations of free and bound terpenols in skins. There is no example in the literature of terpenols detected at high concentrations in skins and not in mesocarps. The QTL on LG 5, determining a general ability of terpenol synthesis should also be detected when analysing mesocarps. Although the proportion of linalool, nerol or geraniol might be different between skins and mesocarps (Günata et al. 1985b; Wilson et al. 1986), we are not aware of data where linalool could be detected in significant quantities in skins and not detected in mesocarps or vice versa. The effect of the QTL on LG 10 should also be detected for mesocarps.

To conclude, one favourable allele on LG 5 would be sufficient to obtain a Gewurztraminer-like terpenol profile whereas at least two favourable alleles, one on LG 5 and one on LG 10 would be necessary to obtain a Muscat-like terpenol profile. We have to date no evidence that the favourable allele of LG 5 is identical in Gewurztraminer and Muscat Ottonel.

Data for the S1 GW progeny were obtained in vineyard conditions whereas the S1 MO progeny was grown in the greenhouse. The QTL on LG 5 was detected for both experiments over 2 years and was identified as well in another genetic background in different growing conditions (Doligez et al. 2006b). We can consider that this QTL is stable and reliable enough to be used in current breeding process.

Conclusion

We have identified a major QTL for terpenol content on LG 5 closely related to a *DXSI* gene in two genetic backgrounds, Muscat Ottonel and Gewurztraminer, and two growing conditions. The synthesis of terpenols at a high level was possible only in plants bearing a favourable allele at this locus. Whereas no supplementary QTL was required for geraniol synthesis at a high level, a favourable allele at a QTL on LG 10 was necessary to observe high linalool concentrations in the Muscat self-progeny plants. Other QTLs with lower effects were detected as well as interactions between loci.

These results provide us with interesting molecular markers to be used in breeding process and also showed that relevant data can be obtained in the greenhouse with an efficient system to produce rapidly fruitful plants. They also open new perspectives for studying the molecular determinism of terpenol synthesis in grapevine.

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References

Adam-Blondon AF, Roux C, Claux D, Butterlin G, Merdinoglu D, This P (2004) Mapping 245 SSR markers on the *Vitis vinifera* genome: a tool for grape genetics. *Theor Appl Genet* 109:1017–1027

Aharoni A, Giri AP, Verstappen FWA, Berteaux CM, Sevenier R, Sun Z, Jongsma MA, Schwab W, Bouwmeester HJ (2004) Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. *Plant Cell* 16:3110–3131

Basten CJ, Weir BS, Zeng Z-B (2004) QTL Cartographer, version 1.17. Department of statistics. North Carolina State University, Raleigh

Battilana J, Madini A, Moreira FM, Costantini L, Velasco R, Grando MS (2005) QTL analysis of quality traits and disease resistance in

grape. In: Qiu WG, Kovacs L (eds) International grape genomics symposium, St Louis, Missouri, USA, pp 53–56

Belancic A, Agosin E, Ibacache A, Bordeu E, Baumes R, Razungles A, Bayonove C (1997) Influence of sun exposure on the aromatic composition of Chilean Muscat grape cultivars Moscatel de Alejandria and Moscatel rosada. *Am J Enol Vitic* 48:181–185

Bronner A (2003) Muscats et variétés muscatées. Inventaire et synonymie universels. des origines à nos jours, INRA Editions, Versailles

Bureau SM, Razungles AJ, Baumes RL (2000) The aroma of Muscat of Frontignan grapes: effect of the light environment of vine or bunch on volatiles and glycoconjugates. *J Sci Food Agric* 80:2012–2020

Carretero-Paulet L, Cairo A, Botella-Pavia P, Besumbes O, Campos N, Boronat A, Rodriguez-Concepcion M (2006) Enhanced flux through the methylerythritol 4-phosphate pathway in *Arabidopsis* plants overexpressing deoxyxylulose 5-phosphate reductoisomerase. *Plant Mol Biol* 62:683–695

Crespan M, Milani N (2001) The Muscats: a molecular analysis of synonyms, homonyms and genetic relationships within a large family of grapevine cultivars. *Vitis* 40:23–30

Cseke L, Dudareva N, Pichersky E (1998) Structure and evolution of linalool synthase. *Mol Biol Evol* 15:1491–1498

de Givry S, Bouchez M, Chabrier P, Milan D, Schiex T (2005) CARthagene: multipopulation integrated genetic and radiated hybrid mapping. *Bioinform* 21:1703–1704

Dirniger N, Duc D, Schneider C, Dumas V, Asselin C, Schaeffer A (1998) Qualité des vins et terroirs: incidence du milieu naturel sur l'expression aromatique du gewurztraminer. *Sci Aliments* 18:193–209

Doligez A, Adam-Blondon AF, Cipriani G, Di Gaspero G, Laucou V, Merdinoglu D, Meredith CP, Riaz S, Roux C, This P (2006a) An integrated SSR map of grapevine based on five mapping populations. *Theor Appl Genet* 113:369–382

Doligez A, Audiot E, Baumes R, This P (2006b) QTLs for muscat flavor and monoterpenic odorant content in grapevine (*Vitis vinifera* L.). *Mol Breed* 18:109–125

Duchêne E, Legras JL, Karst F, Merdinoglu D, Claudel P, Jaegli N, Pelsy F (2008) Variation of linalool and geraniol content within two pairs of aromatic and non aromatic grapevine clones. *Aust J Grape Wine Res* (in press)

Enfissi EMA, Fraser PD, Lois L-M, Boronat A, Schuch W, Bramley PM (2005) Metabolic engineering of the mevalonate and non-mevalonate isopentenyl diphosphate-forming pathways for the production of health-promoting isoprenoids in tomato. *Plant Biotech J* 3:17–27

Estévez JM, Cantero A, Reindl A, Reichler S, Leon P (2001) 1-Deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. *J Biol Chem* 276:22901–22909

Günata YZ, Bayonove CL, Baumes RL, Cordonnier RE (1985a) The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J Chromatogr* 331:83–90

Günata Z, Bayonove CL, Baumes RL, Cordonnier RE (1985b) The aroma of grapes. Localisation and evolution of free and bound fractions of some aroma components cv. Muscat during first development and maturation. *J Sci Food Agric* 36:857–862

Iijama Y, Davidovich-Rikanati R, Fridman E, Gang DR, Bar E, Lewinsohn E, Pichersky E (2004a) The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. *Plant Physiol* 136:3724–3736

Iijama Y, Gang DR, Fridman E, Lewinsohn E, Pichersky E (2004b) Characterization of geraniol synthase from the peltate glands of Sweet Basil. *Plant Physiol* 134:1–10

- Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C, Vezzi A, Legeai F, Huguency P, Dasilva C, Horner D, Mica E, Jublot D, Poulain J, Bruyere C, Billault A, Segurens B, Gouyvenoux M, Ugarte E, Cattonaro F, Anthouard V, Vico V, Del Fabbro C, Alaux M, Di Gaspero G, Dumas V, Felice N, Paillard S, Juman I, Moroldo M, Scalabrin S, Canaguier A, Le Clainche I, Malacrida G, Durand E, Pesole G, Laucou V, Chatelet P, Merdinoglu D, Delledonne M, Pezzotti M, Lechamy A, Scarpelli C, Artiguenave F, Pe ME, Valle G, Morgante M, Caboche M, Adam-Blondon AF, Weissenbach J, Quetier F, Wincker P (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449:U463–U465
- Kearsey MJ, Pooni HS (1996) The genetical analysis of quantitative traits, 1st edn. Chapman & Hall, London
- Kim BR, Kim SU, Chang YJ (2005) Differential expression of three 1-deoxy-D-xylulose-5-phosphate synthase genes in rice. *Biotechnol Lett* 27:997–1001
- Lange BM, Ghassemian M (2003) Genome organization in *Arabidopsis thaliana*: a survey for genes involved in isoprenoid and chlorophyll metabolism. *Plant Mol Biol* 51:925–948
- Luan F, Wüst M (2002) Differential incorporation of 1-deoxy-D-xylulose into (3S)-linalool and geraniol in grape berry exocarp and mesocarp. *Phytochemistry* 60:451–459
- Mahmoud SS, Croteau R (2001) Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. *Proc Natl Acad Sci USA* 98:8915–8920
- Mahmoud SS, Croteau RB (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends Plant Sci* 7:366–373
- Martin DM, Bohlmann J (2004) Identification of *Vitis vinifera* (α -terpineol synthase by in silico screening of full-length cDNA ESTs and functional characterization of recombinant terpene synthase. *Phytochemistry* 65:1223–1229
- Merdinoglu D, Butterlin G, Bevilacqua L, Chiquet V, Adam-Blondon AF, Decroocq S (2005) Development and characterization of a large set of microsatellite markers in grapevine (*Vitis vinifera* L.) suitable for multiplex PCR. *Mol Breed* 15:349–366
- Munoz-Bertomeu J, Arrillaga I, Ros R, Segura J (2006) Up-regulation of 1-deoxy-D-xylulose-5-phosphate synthase enhances production of essential oils in transgenic spike lavender. *Plant Physiol* 142:890–900
- Phillips MA, Walter MH, Ralph SG, Dabrowska P, Luck K, Uros EM, Boland W, Strack D, Rodriguez-Concepcion M, Bohlmann J, Gershenzon J (2007) Functional identification and differential expression of 1-deoxy-D-xylulose 5-phosphate synthase in induced terpenoid resin formation of Norway spruce (*Picea abies*). *Plant Mol Biol* 65:243–257
- Pichersky E, Lewinsohn E, Croteau R (1995) Purification and characterization of *S*-linalool synthase, an enzyme involved in the production of floral scent in *Clarkia breweri*. *Arch Biochem Biophys* 316:803–807
- Razungles A, Günata Z, Pinatel S, Baumes R, Bayonove C (1993) Etude quantitative de composés terpéniques, norisoprénoides et de leurs précurseurs dans diverses variétés de raisins. *Sci Aliments* 13:59–72
- Reynolds AG, Wardle DA, Dever M (1996) Vine performance, fruit composition, and wine sensory attributes of Gewürztraminer in response to vineyard location and canopy manipulation. *Am J Enol Vitic* 47:77–92
- Riaz S, Dangl GS, Edwards KJ, Meredith CP (2004) A microsatellite based framework linkage map of *Vitis vinifera* L. *Theor Appl Genet* 108:864–872
- Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D (1998) L'arôme des cépages. *Traité d'oenologie Tome 2 Chimie du vin, stabilisation et traitements*. Dunod, Paris, pp 239–245
- Sevini F, Marino R, Grando MS, Moser S, Versini G (2004) Mapping candidate genes and QTLs for aroma content in grape. *Acta Hort* 652:439–446
- Singh HP, Batish DR, Kaur S, Ramezani H, Kohli RK (2002) Comparative phytotoxicity of four monoterpenes against *Cassia occidentalis*. *Ann Appl Biol* 141:111–116
- Van Ooijen JW, Voorrips RE (2001) JoinMap 3.0. Software for the calculation of genetic linkage maps. Plant Research International, Wageningen
- Vaughn SF, Spencer GF (1993) Volatile monoterpenes as potential parent structures for new herbicides. *Weed Sci* 41:114–119
- Velasco R, Zharkikh A, Troglio M, Cartwright DA, Cestaro A, Pruss D, Pindo M, Fitzgerald LM, Vezzulli S, Reid J, Malacarne G, Iliev D, Coppola G, Wardell B, Micheletti D, Macalma T, Facci M, Mitchell JT, Perazzolli M, Eldredge G, Gatto P, Oyzerski R, Moretto M, Gutin N, Stefanini M, Chen Y, Segala C, Davenport C, Dematte L, Mraz A, Battilana J, Stormo K, Costa F, Tao Q, Siammour A, Harkins T, Lackey A, Perbost C, Taillon B, Stella A, Solovyev V, Fawcett JA, Sterck L, Vandepoele K, Grando SM, Toppo S, Moser C, Lanchbury J, Bogden R, Skolnick M, Sgaramella V, Bhatnagar SK, Fontana P, Gutin A, Van de Peer Y, Salamini F, Viola R (2007) A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS ONE* 2:e1326
- Wagner R (1967) Etude de quelques disjonctions dans des descendance de Chasselas, Muscat Ottonel et Muscat à petits grains. *Vitis* 6:353–363
- Walter B, Bass P, Legin R, Martin C, Vernoy R, Collas A, Vesselle G (1990) The use of a green-grafting technique for the detection of virus-like diseases of the grapevine. *J Phytopathol* 128
- Walter MH, Hans J, Strack D (2002) Two distantly related genes encoding 1-deoxy-D-xylulose-5-phosphate synthases: differential regulation in shoots and apocarotenoid-accumulating mycorrhizal roots. *Plant J* 31:243–254
- Williams PJ, Strauss CR, Wilson B, Massy-Westropp RA (1982) Use of C18 reverse-phase liquid chromatography for the isolation of monoterpene glycosides and nor-isoprenoid precursors from grape juice and wines. *J Chromatogr* 235:471–480
- Wilson B, Strauss CR, Williams PJ (1986) The distribution of free and glycosidally-bound monoterpenes among skin, juice, and pulp fractions of some white grape varieties. *Am J Enol Vitic* 37:107–111
- Yang T, Li J, Wang HX, Zeng Y (2005) A geraniol-synthase gene from *Cinnamomum tenuipilum*. *Phytochemistry* 66:285–293