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Genetic Determinants of Grape Volatile Compounds and Their Influence on Wine Aroma

By

JERRY LIN
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

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Abstract

From its center of domestication in the forests of the Caucasus to vineyards on every inhabited continent, the grapevine, *Vitis vinifera* subsp. *vinifera*, has been valued for the organoleptic properties of its fruit and wine. Among these properties, aroma has been especially important, inspiring extensive cultural, agricultural, and scientific interest. Wine aroma arises from a complex mixture of volatile compounds that may originate in the grape itself, be released from non-volatile precursors during fermentation and aging, or be introduced through winemaking practices and environmental sources. Considerable research has examined the chemical composition of grapes and wine, the volatile products of yeast and bacterial fermentation, the contribution of oak-derived compounds, and the transformations that occur during wine aging. Only recently, however, have researchers begun to investigate the genetic basis of aroma compound biosynthesis and metabolism.

This dissertation examines the genetic basis of grape aroma variation and its translation into wine aroma with an emphasis on monoterpenes. Chapter 1 reviews the current understanding of genes and pathways involved in grape and wine aroma, focusing on major volatile compound classes including monoterpenes, sesquiterpenes, norisoprenoids, methoxypyrazines, furan derivatives, volatile sulfur compounds, lipoxygenase pathway products, and phenylpropanoid-derived compounds. By compiling functionally characterized aroma-related genes and placing them in the context of grapevine genome resources, we identify both the progress made in aroma genetics and the remaining gaps in linking genetic variation to aroma biosynthesis and metabolism.

For the second chapter, we investigated the genetic architecture of berry aroma traits in a Riesling x Cabernet Sauvignon F1 mapping population. A chromosome-scale diploid Riesling genome assembly was used to anchor genotyping-by-sequencing markers and construct a genetic map for quantitative trait locus (QTL) analysis. Free volatile compounds and monoterpene glycosides were profiled using mass spectrometry-based metabolomics, and QTL mapping was used to identify genomic regions associated with aroma-related metabolites. From the QTLs identified, we selected a major (3*S*)-linalool-associated locus for further study. To resolve candidate genes within this locus, we integrated metabolite variation with haplotype-resolved gene expression data. These analyses connected differences in genetic regulation at a major linalool-associated locus with berry monoterpene accumulation and established the basis for examining its consequences in wine.

Chapter 3 examines whether the effects of genetic variation associated with berry monoterpene accumulation were retained through winemaking and expressed as differences in wine aroma. Wines were produced from selected Riesling × Cabernet Sauvignon progeny carrying contrasting alleles at the chromosome 10 (3*S*)-linalool-associated locus. Subsequently, wine volatile composition was analyzed using HS-SPME-GC-MS, and sensory impacts were assessed by descriptive analysis. Multivariate and correlation-based approaches were then used to relate genotype, berry metabolites, wine volatile composition, and sensory attributes. Collectively, these chapters connect prior knowledge of grape aroma genetics with experimental analyses of berry metabolites, candidate gene regulation, wine chemistry, and sensory expression.

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When I embarked on this journey over nine years ago, I had little idea where it would lead me, what I would learn, and how many wonderful connections I would build along the way. I recall starting my masters degree in Dario's lab with a firm conviction that I would never do a PhD. Now, as I close this chapter of my life nearly a decade later, I want to thank all the people that have helped me get here.

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REVIEW ARTICLE

Open Access

The genetic basis of grape and wine aroma

Jerry Lin¹, Mélanie Massonnet¹ and Dario Cantu¹ 

Abstract

The grape is one of the oldest and most important horticultural crops. Grape and wine aroma has long been of cultural and scientific interest. The diverse compound classes comprising aroma result from multiple biosynthetic pathways. Only fairly recently have researchers begun to elucidate the genetic mechanisms behind the biosynthesis and metabolism of grape volatile compounds. This review summarizes current findings regarding the genetic bases of grape and wine aroma with an aim towards highlighting areas in need of further study. From the literature, we compiled a list of functionally characterized genes involved in berry aroma biosynthesis and present them with their corresponding annotation in the grape reference genome.

Introduction

With nearly 8 million hectares of vineyards worldwide and a global annual production of around 90 million tons, the grapevine is one of the most important horticultural crops (<http://faostat.fao.org>). The majority of cultivated grapes belong to *Vitis vinifera* subsp. *vinifera* (hereafter *V. vinifera*); however, cultivars and hybrids of other *Vitis* species, as well as the related genus *Muscadinia* are also grown, particularly in regions where climate and/or disease pressure preclude the cultivation of *V. vinifera*^{1–3}. Over its long history of domestication and usage for wine and consumption as fresh and dried fruit, *V. vinifera* has undergone selection for desirable traits such as hermaphroditism, sugar content, berry color, and berry size^{4–6}. Attempts to utilize wild non-*vinifera* species, whether as interspecific crosses with *V. vinifera* or as selected cultivars, to overcome environmental and biotic limitations on grape cultivation have been complicated by the presence of negatively perceived flavors, especially in wine production^{2,3,7}. On the other hand, there is enormous genetic diversity in cultivated and wild species of grape and with it the potential to generate novel flavor combinations by utilizing that diversity^{8–10}.

The aroma, i.e., the olfactory component of flavor, of grapes and wine has been an area of intense interest for many decades. Much has been written regarding topics

such as the volatile profiles of different cultivars, the sensory significance of individual compounds, environmental effects on the accumulation of aroma compounds, and the impacts of viticultural and enological treatments on fruit and wine aroma. Advances in analytical techniques together with sensory methodologies have dramatically increased our understanding of the composition and interactions within the complexity that is grape and wine aroma¹¹. While variations in aromatic potential between cultivars have long been documented, for many reasons the study of genetic explanations for these differences has lagged behind our knowledge of grape volatile constituents.

Only fairly recently have we been able to unravel the role of specific genes in the origins of grape and wine aroma. Molecular markers have been invaluable in helping researchers understand the inheritance of a number of viticulturally and enologically significant traits including those related to flavor and aroma¹⁰. Expressed sequence tags (ESTs) have also been instrumental in the identification of genes involved in secondary metabolism^{12,13}. More recently, the publication of the Pinot noir and PN40024 genomes has greatly facilitated the identification and characterization of genes involved in many metabolic pathways^{14–18}. Ongoing technological advances in sequencing, omics, biotechnology, and phenotyping will further enable researchers to understand the genetic mechanisms behind grape and wine aromas.

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The purpose of this review is to summarize the state of our knowledge regarding grapevine genetics in relation to the biosynthesis and modification of volatile compounds. The current review is not intended to be a complete listing of the volatile compounds found in wine but to inform the reader on what has so far been elucidated regarding their genetic basis and to highlight areas that need further study. Although the impact of environmental conditions on the biosynthesis and metabolism of volatile compounds can be considerable, genotype by environment interactions have not yet been thoroughly explored in grape. A number of excellent reviews have been written regarding the diversity of wine and grape aroma compounds, environmental impacts on their biosynthesis, and their chemistry, to which we refer the reader for more information in those areas^{11,19–25}. An emphasis has been made here on genes that have been functionally characterized in grapevine. Where little study has been conducted on grapevine, comparable systems in other plants have been provided.

Grape aroma compounds

The predominate compounds contributing to the aroma profile of grape berries fall into the following categories: mono- and sesquiterpenes, methoxypyrazines, furan derivatives, lipoxygenase pathway products, and phenylpropanoid pathway products. Two classes of compounds, norisoprenoids and volatile sulfur compounds, are important in wine but are generally found as nonvolatile precursors in berries and require further modifications to be perceived^{2,3,11,21,26}. During winemaking and subsequent aging processes, volatile compounds and their precursors can undergo enzyme-catalyzed modifications and spontaneous chemical transformations. Further chemical diversity is introduced by wine microorganisms and aging vessels²¹.

This complexity, along with extremely low concentrations of some compounds, significant environmental and temporal effects on volatile concentration and composition, and conjugation of volatile compounds into non-volatile precursors has made research into the aroma components of grape berries challenging^{11,21,27}. The difficulty is compounded in the characterization of genes encoding enzymes that produce aroma compounds or their precursors. In the subsequent sections of this review, we summarize the current knowledge regarding the major groups of grape aroma compounds and genes involved in their biosynthesis. The genes which have been functionally characterized and shown to produce aroma compounds or their precursors are listed in Table 1.

Terpenoids

Terpenoids represent an extremely diverse class of natural compounds with over 40,000 identified from

organisms in every kingdom of life. In plants, multiple processes such as photosynthesis, membrane construction, and growth regulation utilize terpenoids^{21,28,29}. The volatile terpenoids are important constituents of plant aromas and play major roles in plant-animal interactions, general defense, and responses to abiotic stresses. In addition, they contribute key components to the fragrance of many flowers, fruit, and herbs^{28,30}. Terpenoids consist of multiple C₅ isoprene units and, despite their diversity, share isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) as precursors²⁹. Biosynthesis of terpenoids occurs both in the cytoplasm via the mevalonic acid (MVA) pathway and in the plastid through the methyl-erythritol-phosphate (MEP) pathway^{21,28}. Several excellent reviews of both pathways and their elucidation have been written^{31–33}. Many of the genes involved in these pathways remain to be identified and functionally characterized in grape²¹. The terpenoids of major importance in grape and wine aroma are the monoterpenes, sesquiterpenes, and indirectly, carotenoids^{11,21,22}.

Monoterpenes

Twenty two different monoterpenes have been identified in grapes and wine; half are linalool derivatives²². Monoterpenes are characteristic components of berries during early development but in most cultivars, levels decline below the detection threshold after véraison and are not major contributors to the aroma of ripe berries^{11,34,35}. Muscats represent a major exception as their characteristic floral aroma is due to high concentrations of monoterpenes with linalool, geraniol, nerol, α -terpineol, and hotrienol being the most important. Small amounts of other terpenes contribute to varietal differences. Interactions between combinations of monoterpenes and their metabolites result in a spectrum of flavor intensities among Muscat varieties^{23,36}.

Grape cultivars can be divided into three general groupings based on total free monoterpene concentration, as well as by monoterpene profile: neutral varieties with very low concentrations such as *V. vinifera* cv. Chardonnay, “aromatic” cultivars with 1–4 mg/L of monoterpenes e.g., *V. vinifera* cv. Riesling, and Muscat types like *V. vinifera* cv. Muscat blanc and cv. Muscat of Alexandria with as much as 6 mg/L of free monoterpenes^{37,38}. Profiling 15 monoterpenes is sufficient for discriminating between Riesling and five of its offspring³⁸. Overall, monoterpenes are mainly concentrated in the skin with differential distribution depending on the compound. Geraniol and nerol are much more abundant in the skin than in the flesh, while linalool is more generally distributed throughout the fruit³⁹. The genetic factors responsible for increasing the accumulation of monoterpenes have long been of interest to grape breeders.

Table 1 Genes involved in the biosynthesis and metabolism of grape berry and wine aroma compounds and precursors

Compound class	Gene name ^a	Function	GenBank accession number	Gene ID ^b	References
Monoterpenes	WiDXS	1-Deoxy-D-xylulose 5-phosphate synthase	MA161553	VT_05s0020g02130	Battilana et al. ⁴² , Duchêne et al. ⁵³
	WiTPS52 <i>VviGer</i>	Geraniol synthase	HM807399	VT_12s0134g00140	Martin et al. ¹⁴ , Matarese et al. ⁵⁴
	WiTPS54 <i>VviLinNer1</i>	(3S)-Linalool/(E)-Nerolidol synthase	HM807391	VT_00s0385g00020	Martin et al. ¹⁴ , Matarese et al. ⁵⁴
	WiTPS56 <i>VviLinNer2</i>	(3S)-Linalool/(E)-Nerolidol synthase	HM807392	VT_00s0271g00060	Martin et al. ¹⁴ , Zhu et al. ⁶³
	WiTPS57 <i>VviLNG1</i>	Linalool/(E)-Nerolidol/(EE)-Geranyl linalool synthase	HM807394	VT_00s0372g00040	Martin et al. ¹⁴
	WiTPS58 <i>VviLNG3</i>	Linalool/(E)-Nerolidol/(EE)-Geranyl linalool synthase	HM807396	VT_00s0372g00070	Martin et al. ¹⁴
	WiTPS61 <i>VviLNG4</i>	Linalool/(E)-Nerolidol/(EE)-Geranyl linalool synthase	HM807397	VT_00s0372g00070	Martin et al. ¹⁴
	WiTPS63 <i>VviLNG2</i>	Linalool/(E)-Nerolidol/(EE)-Geranyl linalool synthase	HM807395	VT_00s0847g00020	Martin et al. ¹⁴
	WiTer	(-)-α-Terpineol synthase	AA579351	VT_13s0067g00370	Martin et al. ¹²
	CYP76F14	(E)-8-Carboxylinalool synthase	CAB85635	VT_02s0025g04880	Ilc et al. ⁶⁹
Sesquiterpenes	WiGT7	Glucosyltransferase	XM_002276510.2	VT_16s0050g01580	Li et al. ⁵⁸
	WiGT14	Glucosyltransferase	XM_002285734.2	VT_18s0001g06060	Bönisch et al. ⁷² , Li et al. ⁵⁸
	WiGT15a	Glucosyltransferase	XM_002281477.2	VT_06s0004g05780	Bönisch et al. ⁷² , Li et al. ⁵⁸
	WiGT15b	Glucosyltransferase	XM_002281477.2	VT_06s0004g05780	Bönisch et al. ⁷² , Li et al. ⁵⁸
	WiGT15c	Glucosyltransferase	XM_002281477.2	VT_06s0004g05780	Bönisch et al. ⁷² , Li et al. ⁵⁸
	WiTPS24 <i>VviGuaS</i>	Selinene synthase polymorphic variant	XM_002282452	VT_19s0014g02590	Drew et al. ⁸⁴
	WiSTO2	Sesquiterpene oxidase α-guaiene 2-oxidase	XM_010646246	VT_19s0015g00110	Takase et al. ⁸¹
	WiCCD1.1	Carotene cleavage dioxygenase	KF008001	VT_13s0064g00810	Lashbrooke et al. ¹⁶ , Mathieu et al. ¹⁰⁰
	WiCCD1.2	Carotene cleavage dioxygenase	AY856353	VT_13s0064g00840	Mathieu et al. ¹⁰⁰
	WiCCD4a	Carotene cleavage dioxygenase	KF008002	VT_02s0087g00910	Lashbrooke et al. ¹⁶
Methoxypyrazines	WiCCD4b	Carotene cleavage dioxygenase	KF008003	VT_02s0087g00930	Lashbrooke et al. ¹⁶
	WiOMT1	S-Adenosyl-L-Met (SAM)-dependent O-methyltransferase	GQ357167	VT_12s0059g01790	Dunlevy et al. ¹³⁵ , Hashizume et al. ¹³⁴
	WiOMT2	S-Adenosyl-L-Met SAM)-dependent O-methyltransferase	GQ357168	VT_12s0059g01750	Dunlevy et al. ¹³⁵
	WiOMT3	S-Adenosyl-L-Met SAM)-dependent O-methyltransferase	KC243500	VT_03s0038g03090	Dunlevy et al. ¹³³ , Guillaumie et al. ¹²⁹
	WiOMT4	S-Adenosyl-L-Met SAM)-dependent O-methyltransferase	KC243503	VT_03s0038g03080	Dunlevy et al. ¹³³ , Guillaumie et al. ¹²⁹
Furans	WiQR ^c	Enone reductase	Not available	VT_01s0127g00740	Sasaki et al. ¹³⁸
	WiGlyT_07 <i>UGT85K14</i>	Furaneol glucosyltransferase	XM_002268601.2	VT_00s0324g00050	Sasaki et al. ¹³⁸
	WiGST3	Glutathione synthase	EF469244	VT_12s0028g00920	Kobayashi et al. ¹⁵⁷
	WiGST4	Glutathione synthase	AY971515	VT_04s0079g00690	Kobayashi et al. ¹⁵⁷
	WiGST ^c	γ-Glutamyltransferase	Not available	VT_11s0016g02830	Kobayashi et al. ¹⁵⁷
	WiLOXA	Type II lipoygenase	XM_002285538 NM_001281094.1	VT_06s0004g01510	Podolyan et al. ¹⁵²
	WiLOXO	Type II lipoygenase	XM_002273222.2	VT_09s0002g01080	Podolyan et al. ¹⁵²
	WiHPL1	13-hydroperoxidase	HM627632	VT_12s0059g01060	Zhu et al. ¹⁵⁵
	WiHPL2	9-hydroperoxidase	HM627633	VT_03s0063g01830	Zhu et al. ¹⁵⁵
	WiADH1	Alcohol dehydrogenase	AF194173	VT_18s0001g15410	Qian et al. ¹⁵⁶ , Tesnière et al. ¹⁶³
Lipoygenase	WiADH2	Alcohol dehydrogenase	AF194174	VT_04s0044g01110	Qian et al. ¹⁵⁶ , Tesnière et al. ¹⁶³
	WiADH3	Alcohol dehydrogenase	AF194175	VT_18s0001g15450	Tesnière et al. ¹⁶³
	WiAAT	Alcohol acyltransferase	AAW22989	VT_09s0018g01490	Qian et al. ¹⁵⁶
	AMAT	Anthraniloyl-coenzyme A (CoA): methanol acyltransferase	AY705388	VT_09s0018g01490	Wang and Luca ⁸

Note: All genes have been functionally characterized and shown to be expressed in berries during development unless noted

^aPN40024 alleles are listed for genes for which multiple alleles have been characterized

^bBest hit in PN40024 V1 annotation

^cIdentified but not functionally characterized

Using simple sequence repeat (SSR) markers, Doligez et al. identified a major quantitative trait locus (QTL) on linkage group (LG) 5 associated with linalool, nerol, and geraniol content within an 8.3 cM interval between markers VRZAG79 and VVC6⁴⁰. Battilana et al. and Duchêne et al. confirmed the location of a QTL on LG 5 associated with total monoterpene levels in two Muscat × neutral mapping populations and two populations of selfed aromatic varieties (*V. vinifera* cv. Muscat Ottonel and cv. Gewürztraminer), respectively⁴¹. A *V. vinifera* 1-deoxy-D-xylulose 5-phosphate synthase (*VviDXS*) gene was identified as the gene responsible for Muscat character due to its co-localization with the LG 5 QTL associated with monoterpene content⁴². Overall, the *VviDXS*-associated QTL was found to explain 17–93% of the variation in linalool, nerol, and geraniol concentrations^{40–42}. This is consistent with the role of *VviDXS*, which as the first enzyme of the MEP pathway catalyzes the formation of the substrates used for monoterpene production.

The *VviDXS* sequence contains a total of 4790 bp in 10 exons and 9 introns and encodes a protein with 716 amino acids⁴³. *DXS* is highly conserved in plants and bacteria due to its importance in the MEP pathway^{44,45}. *VviDXS* expression in Muscat blanc increases at véraison and peaks at 12 weeks post flowering. Expression of the gene is significantly correlated with the accumulation of free and bound monoterpenes, and both *VviDXS* transcript levels and free monoterpene concentrations decrease late in fruit ripening while bound monoterpenes continue to increase⁴⁶.

Analysis of the *VviDXS* sequence among 148 Muscat, Muscat-like aromatic, and neutral cultivars identified 95 Single Nucleotide Polymorphisms (SNPs), four of which were predicted to affect protein function⁴³. A G/T SNP results in replacement of the lysine (K) at position 284 with asparagine (N). Ninety five percent of the Muscat cultivars examined by Emanuelli et al. possessed the K284N mutation with 68 of the 69 Muscat cultivars having the allele in a heterozygous state. The highly aromatic Gewürztraminer and its offspring *V. vinifera* cv. Siegerrebe share a C/T SNP resulting in replacement of arginine with cysteine in one allele of *VviDXS* (R306C). Two aromatic “musqué” clones of otherwise neutral varieties were also found to have non-neutral mutations within *VviDXS*: *V. vinifera* cv. Chasselas musqué has an A/G SNP causing a deletion of amino acid residues 285–289 while a musqué clone of Chardonnay has a T/C SNP replacing the serine at position 272 with proline. The four mutations are located in a region corresponding to the active site of *DXS* in *Deinococcus radiodurans*⁴³. Of the identified mutant *VviDXS* alleles, the Muscat N284 allele has received the most attention from researchers.

To determine the effect of the N284 mutation on *VviDXS* function, Battilana et al. expressed both the N284

and K284 *VviDXS* variants in *E. coli*⁴⁶. Recombinant *VviDXS* N284 exhibited twice the catalytic efficiency compared with *VviDXS* K284 with minimal changes in substrate affinity. Transformation of the model *V. vinifera* cv. Microvine 04C023V0006⁴⁷ with the Muscat *VviDXS* N284 allele resulted in increase of monoterpene production in berries despite lower expression of the Muscat *VviDXS* N284 allele^{46,48}. These findings suggesting that greater monoterpene production is mostly due to the higher catalytic efficiency of the N284 *VviDXS* variant.

Comparison of *VviDXS* haplotypes between Muscat and neutral varieties showed a reduced sequence diversity within the haplotypes carrying the N284 mutation, implying that the Muscat mutation arose once and has spread through selective pressure and breeding practices⁴³. This is in agreement with studies showing that the Muscats are a distinct group descended from two progenitors: Muscat blanc and Muscat of Alexandria, itself the result of a cross between Muscat blanc and *V. vinifera* cv. Axina de tres bias^{49–52}. Emanuelli et al. suggest a history of selection for Muscat flavor followed by crosses between Muscat and neutral cultivars to develop cultivars with other desired phenotypic characters⁴³. The large number of direct descendants of Muscat blanc and Muscat of Alexandria supports this; the two cultivars are the direct parents of 58 and 255 cultivars, respectively, according to the *Vitis* International Variety Catalog (VIVC, www.vivc.de).

A number of cultivars unrelated to Muscats have been characterized for their Muscat flavor, e.g., Malvasias⁴⁹, suggesting that other mutations within the MEP pathway may confer increased monoterpene biosynthesis. In addition to the major QTL containing *VviDXS*, numerous QTLs with smaller effects have been identified in marker-trait analyses of different mapping populations^{40–42,53}. They may co-locate with downstream genes involved in regulation of monoterpene synthesis and metabolism.

Monoterpene synthesis and modifications

With 69 putative genes identified, the terpene synthase (*TPS*) gene family is greatly expanded in *Vitis vinifera* and represents all of the angiosperm terpene synthase subfamilies except for *TPS-f*¹⁴. Seventeen *VviTPS* belonging to the *TPS-b* and *TPS-g* subfamilies have been functionally characterized as monoterpene synthases. The majority are multi-product enzymes¹⁴. In this section, we describe the genes functionally characterized as involved in the synthesis, i.e., *VviTPS*, and/or modification of the major monoterpenes geraniol, α -terpineol, and linalool.

In aromatic cultivars, geraniol concentrations peak early in berry development followed by a decline until véraison, at which point the concentration increases dramatically⁵⁴. *VviTPS52*, a geraniol synthase characterized by Martin et al.¹⁴, was shown to correlate with geraniol

accumulation in ripening *V. vinifera* cv. Aleatico berries and to a smaller extent in Muscat blanc, indicating its importance in the biosynthesis of geraniol in ripe fruit⁵⁴. Experiments with deuterium-labeled geraniol in *V. vinifera* cv. Scheurebe berries revealed that geraniol can be

converted to nerol by an unknown isomerase. In addition, geraniol can be enzymatically reduced to (*S*)-citronellol by an unidentified reductase (Fig. 1). (*S*)-citronellol then undergoes hydroxylation and cyclization to form *cis* and *trans*-rose oxide⁵⁵. *Cis*-rose oxide predominates in

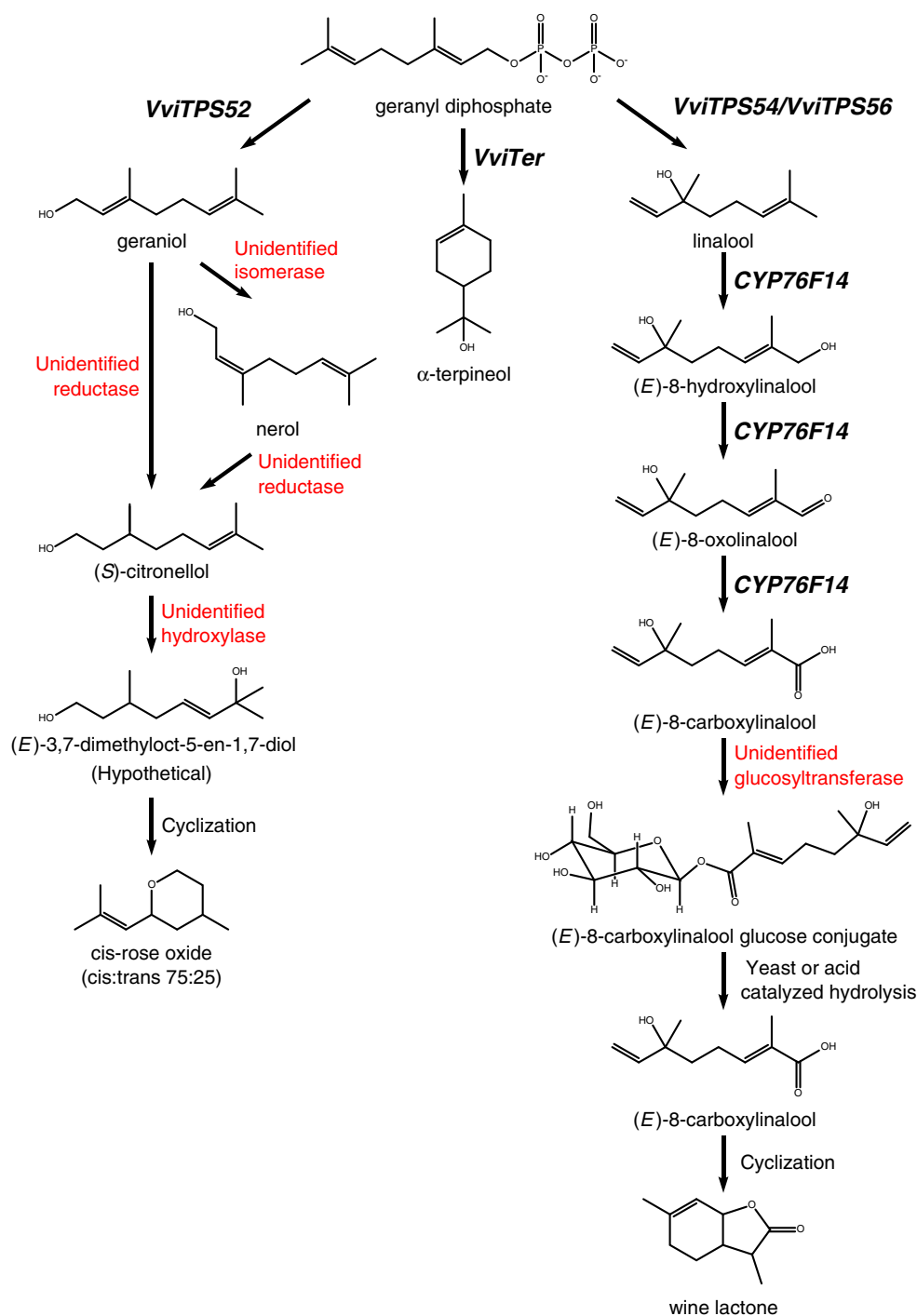


Fig. 1 Proposed biosynthetic pathways of a selection of monoterpenes and monoterpene-derived metabolites in grapevine. Gene names are included where functionally characterized. (Adapted from refs. ^{55,59,69})

Gewürztraminer and Scheurebe wines and is a key component of the varietal character of Gewürztraminer^{56–58}.

Although no enzymes responsible for the formation of rose oxides from (*S*)-citronellol have been identified, the process may occur in a similar manner as in rose geranium (*Pelargonium*) and roses (*Rosa damascena*)⁵⁵. Wüst et al. proposed a mechanism of diol formation via a cytochrome oxidase with low specificity followed by acid-catalyzed cyclization of two of the four resulting diols as shown in Fig. 1^{59,60}. Enantiomeric ratios of *cis*-rose oxides in grape, rose geranium, and rose all indicate the action of as yet unidentified enzymes. It is interesting to note that during fermentation, yeast can also produce both (–) and (+) enantiomers of *cis*-rose oxide from citronellol in sufficient quantities to impact wine aroma⁶¹. Therefore, the final impact of *cis*-rose oxide in wine depends not only on the amount synthesized by the grape but also on the level of citronellol present at harvest. The conversion rate of geraniol to citronellol increases in late berry development, indicating that the final stages of ripening are important in developing the characteristic aroma of Gewürztraminer and Scheurebe⁵⁵.

As one of the more abundant monoterpenes in grape, α -terpineol is an important component of the character of aromatic varieties²³. In addition to contributions from the rearrangement of other monoterpenes, α -terpineol is synthesized in the grape berry: a *TPS-b* subfamily α -terpineol synthase (*VviTer*) was one of the first monoterpene synthases identified and functionally characterized in grape¹². A candidate gene association study of 61 grape cultivars found two SNPs in coding regions of the *VviTer* gene correlated with higher α -terpineol concentrations. These mutations result in non-synonymous amino acid substitutions (I38S and T519I); however, additional studies are needed to establish the sensory impact of these point mutations⁶².

Linalool is the predominant monoterpene in Muscats and plays a major role in their varietal aroma⁵⁸. Despite having similar gain-of-function mutations in the active site of *VviDXS*, Muscat berries have a higher linalool content compared with Gewürztraminer⁵⁸. While linalool is present in the berries of Gewürztraminer during the early developmental stages, concentrations decline rapidly as berries mature. In contrast, linalool concentrations in Riesling and Muscat blanc decrease initially followed by a very large increase after véraison^{54,63}. QTLs associated with linalool content have been identified in the same region of LG 10 between SSR markers VrZAG 64 and VVIH01^{42,53}. Duchêne et al. proposed a two-step model for Muscat aroma: one mutation in *VviDSX* conferring higher geranyl diphosphate (GPP) biosynthesis and another favorable allele near VrZAG64 shifting the monoterpene profile towards linalool⁴¹. No genes have been shown to co-locate with the QTL, but a number of possible candidates are located on chromosome 10.

One of the linalool synthases annotated by Martin et al.¹⁴ falls on chromosome 10, but most were located on unmapped scaffolds. The VCost.v3 annotation of the 12x.v2 genome assembly places many of the previously characterized linalool synthases on chromosome 10⁶⁴. A (3*S*)-linalool synthase located on the chromosome, *VviTPS56*, has been shown to convert GPP to (3*S*)-linalool as a major product^{14,63}. However, gene expression of *VviTPS56* in aromatic cultivars does not correlate with linalool accumulation during berry ripening^{54,63}. On the other hand, *VviTPS54*, another linalool synthase, is expressed much more highly during the later stages of ripening (76–116 days after flowering) in Muscat blanc and Aleatico⁵⁴. *VviTPS54* is still unmapped but has also been shown to produce (3*S*)-linalool from GPP in vitro and may be responsible for linalool synthesis in ripening berries^{14,54}. Additional linalool synthases identified and characterized by Martin et al.¹⁴ as linalool/(*E*)-nerolidol/(*E,E*)-geranyl linalool synthases (*VviTPS57*, *58*, *61*, and *63*) have been proposed to be involved solely in nerolidol synthesis as they lack the plastid transit signal peptide characteristic of other monoterpene synthases⁶⁴. Curiously, despite the abundance of annotated linalool synthases on chromosome 10, a genome-wide association study of 96 accessions composed of *V. vinifera*, *V. amurensis*, and *V. labrusca* cultivars and hybrids found QTLs associated with linalool content only on linkage groups 1, 5, 7, and 19⁶⁵.

The oxidation products of linalool comprise a large proportion of the monoterpenoid diversity in wines²². Linalool is subject to a number of enzymatic reactions during fruit ripening that can generate monoterpenoids of higher oxidation states such as (*E*)-8-hydroxylinalool⁶⁶. (*E*)-8-hydroxylinalool can then be further oxidized to (*E*)-8-carboxylinalool (Fig. 1). Under wine aging conditions (*E*)-8-carboxylinalool can undergo a slow acid-catalyzed cyclisation resulting in the production and accumulation of wine lactone, a bicyclic monoterpene lactone, over time⁶⁷. With its extremely low odor threshold (10 ng/L), wine lactone is an important contributor to the aroma of white wines, particularly those made from Gewürztraminer, despite its low abundance (100 μ g/L)⁵⁷. Wine lactone is also found in some red wines⁶⁸. Ilc et al. identified CYP76F14, a CYP76 enzyme highly expressed in ripe berries, that is capable of oxidizing linalool to (*E*)-8-carboxylinalool when expressed in *Saccharomyces cerevisiae* and *Nicotiana benthamiana*. While two other CYP76s expressed in ripening berries can also metabolize linalool, only CYP76F14 catalyzes the entire oxidative cascade from linalool to (*E*)-8-carboxylinalool⁶⁹.

Marker-trait analysis of a Riesling x Gewürztraminer population showed that (*E*)-8-carboxylinalool concentration is associated with three major QTLs: one containing *VviDXS*, a QTL colocalizing with several terpene

synthases that have since been assigned to chromosome 10, and finally one on chromosome 2 containing *CYP76F14*. These data provide support for the importance of *CYP76F14* in (*E*)-8-carboxylinalool synthesis⁶⁹. Ilc et al. also found no difference in the product profiles of *CYP76F14* cloned from Muscat Ottonel and the non-aromatic *V. vinifera* cv. PN40024⁶⁹. This, along with the slow conversion rate of (*E*)-8-carboxylinalool to wine lactone suggests that increased monoterpene production due to a *VviDXS* mutation is a prerequisite for wine lactone concentrations exceeding the sensory threshold. Presumably, some amount of linalool synthesis is required as well, although it is interesting to note that wine lactone occurs at suprathreshold levels in red wine cultivars that accumulate only modest amounts of linalool⁶⁸. The (*E*)-8-carboxylinalool precursor has been detected in berries only as a glucose conjugate but no grape UDP glucosyl-transferases have been identified that catalyze production of the glucoside^{69,70}.

Glycosides represent the majority of the monoterpene pool in grapes. They can undergo hydrolysis during winemaking and aging and contribute to the aroma over time^{11,27}. In grape, glycosides of aroma compounds occur as monosaccharides bound to a β -d-glucose moiety (glucosides) or as disaccharides consisting of glucose and rhamnose, apiose, or arabinose²⁷. In *V. vinifera*, anthocyanins occur only as monosaccharides, suggesting that the glycosylation of aroma compounds in that species occurs through a different pathway¹¹. Glycosyl-transferases (GTs) are ubiquitous enzymes that play important roles in many metabolic processes. Although a large number of GTs have been identified in plants, only a small fraction has been functionally characterized. Grape GTs were among the first monoterpene-specific GTs to be characterized⁷¹.

Three GTs, *VviGT7*, *VviGT14*, and *VviGT15* have been functionally characterized in *V. vinifera* and are expressed in conjunction with the accumulation of monoterpene glycosides in ripening berries^{58,72,73}. All three enzymes share geraniol, nerol, and citronellol as major substrates and exhibit activity towards other aroma compounds known to occur as glycosides in berries such as lipoxigenase and phenylpropanoid pathway products. In addition, *VviGT14* is able to glucosylate linalool^{72,73}. Li et al. found *VviGT14* to be particularly highly expressed in Muscat blanc, a cultivar with high linalool concentrations⁵⁸. However, Bönisch et al. found that *VviGT15* was more highly expressed in Muscat blanc instead⁷². Expression of the three GTs has been shown to vary significantly between cultivars along with concentrations of free and bound monoterpenes, perhaps contributing to the differences in aromatic profile between cultivars, vintages, and locations⁵⁸. Four alleles of *VviGT7*, two functional and two inactive, are present in Muscat

blanc⁷³. In contrast, *VviGT14* exists as a single functional allele in Muscat blanc and Gewürztraminer⁵⁸. While these GTs are capable of glucosylating a wide variety of substrates, over 200 aroma compound glycosides have been isolated from grape and much remains to be learned about the genes involved in their formation^{58,71–73}.

Sesquiterpenes

Compared with monoterpenes, sesquiterpenes in grapes and wine have received less attention due to their lower volatility and higher detection thresholds^{11,74}. In their review on the extraction and identification of grape-derived sesquiterpenes, Petronilho et al. recorded a total of 91 sesquiterpenes isolated from must, wines, distillates, and other grape products. 57 of these were identified from berries. Grape sesquiterpenes comprise predominately of hydrocarbons with some ketones, oxides, and alcohols⁷⁵. Like monoterpenes, sesquiterpenes are synthesized from DMAPP and IPP, which in turn result from two parallel pathways, the cytosolic MVA and the plastidial MEP pathways. Although monoterpenes are largely synthesized from the MEP pathway, and sesquiterpene biosynthesis is thought to occur via the cytosolic MVA pathway, there is evidence of movement of intermediates from the plastid to the cytosol in a number of plants^{25,28,76}.

The immediate precursor molecule for sesquiterpenes is farnesyl diphosphate, which is synthesized from one DMAPP molecule and two IPPs. In grape, both MVA-derived and MEP-derived DMAPP and IPP are incorporated into sesquiterpenes⁷⁶. In Microvines transformed with the Muscat *VviDXS* N284 allele, sesquiterpene biosynthesis is increased in flowers compared with the wild type, albeit to a smaller extent than monoterpenes⁴⁸. Since *VviDXS* is part of the MEP pathway and not the MVA pathway, this supports the possibility of some level of exchange between the two pathways. As of yet no genes encoding transporters involved in exchanging terpenoid intermediates have been identified.

Thirty putative sesquiterpene synthases have been identified from the PN40024 genome sequence¹⁴. The *VviTPS-a* subfamily represents a highly expanded group of predominantly sesquiterpene synthases on chromosomes 18 and 19. Thirteen members of the subfamily have been functionally characterized by Martin et al., and the majority of these synthesize multiple products from the FPP substrate¹⁴. However, the characterized sesquiterpene synthases have not been shown to produce the entire range of sesquiterpenes found in grape berries²⁵. Biosynthesis of sesquiterpenes in berries appears to be restricted to the skin, which is consistent with their absence in the mesocarp and high concentration in the epicuticular wax of the exocarp⁷⁶. Sesquiterpenes are also produced by grape anthers during bloom as pollinator attractants or herbivore deterrents. Martin et al.

functionally characterized a (+)-valencene synthase (*VviVal*) expressed in grapevine flowers that produces (+)-valencene and (–)-7-epi- α -selinene as major products. Like many other TPS, *VviVal* expression decreases after bloom but increases again during late berry development in Gewürztraminer berries¹². However, a survey of the sesquiterpene profiles of several varieties did not detect either product of *VviVal* in Gewürztraminer berries⁷⁷.

The most prominent sesquiterpene identified from wine so far is rotundone. An important component of the aroma of *Piper nigrum*, rotundone is extraordinarily potent with detection thresholds of 16 ng/L in red wine and 8 ng/L in water⁷⁸. Although notable as an impact compound in *V. vinifera* cv. Syrah wines where it can reach concentrations of 220 ng/L, rotundone has also been detected in wines made from a number of other varieties. Some other varieties contain relatively high levels of rotundone as well, such as *V. vinifera* cv. Durif, cv. Graciano, cv. Vespolina, and cv. Grüner Veltliner^{74,79,80}. Rotundone accumulation begins at véraison and continues through the ripening period⁸⁰.

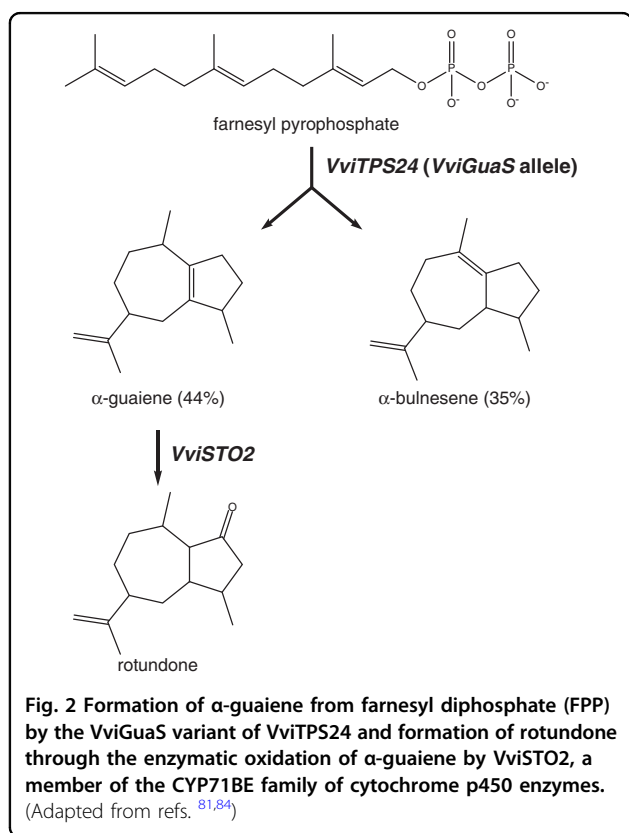
The precursor of rotundone is α -guaiene, a 5,7 bicyclic sesquiterpene found in high concentrations in Syrah berry skins, as well as in other plants that produce rotundone (Fig. 2)^{81,82}. Concentrations of α -guaiene peak at 12 weeks

post-flowering, while rotundone levels reach their maximum 14 weeks after anthesis⁸¹. Of the 13 sesquiterpene synthases functionally characterized by Martin et al., just one, *VviTPS24*, encodes an enzyme (*VviPNSeInt*) that produces α -guaiene and only as a minor product (3.5% of the total)¹⁴. *VviTPS24* is significantly upregulated during véraison in Syrah berries⁸³. Drew et al. transformed *N. benthamiana* with *VviTPS24* cDNA isolated from Syrah berries and were able to detect α -guaiene and δ -guaiene as major products (44% and 35%, respectively)⁸⁴. Due to the divergent range of products, the enzyme encoded by the Syrah cDNA was named *VviGuaS*. The transcript sequence of the *VviGuaS* variant of *VviTPS24* is 99.5% identical to that of the previously characterized *VviPNSeInt* variant. The amino acid sequences of the two gene products differ at six positions, two of which, corresponding to T414S and V530M substitutions in *VviPNSeInt*, are located in the active site.

Site-directed mutagenesis of *VviGuaS* cDNA altered the product profile towards that reported for *VviPNSeInt*, with the *VviGuaS*-T414S/V530M double mutant having the most similar profile to *VviPNSeInt* and very little α -guaiene production⁸⁴. The *VviGuaS* variant of *VviTPS24* is absent from the PN40024 genome; however, the *V. vinifera* cv. Pinot Noir genome contains ambiguous base calls at four of the six positions that are polymorphic between the two variants of *VviTPS24*. This suggests that Pinot Noir is heterozygous for both *VviGuaS* and *VviPNSeInt* and that the *VviGuaS* allele was lost during breeding for homozygosity to produce PN40024^{17,84}.

The existence of an allele with a major effect on the volatile profile of a cultivar and its absence in the reference genome illustrates a limitation of the PN40024 genome particularly as it derives from a genotype for which there is limited phenotypic data¹⁸. Recently, Smit et al. conducted a functional characterization of *VviTPS-a* genes expressed in the inflorescences of several *V. vinifera* cultivars and found cultivar-specific structural variations with major impacts on gene function and floral sesquiterpene profile⁸⁵. It is likely that structural variations are also important contributors to differences in berry and wine aroma profiles. More research is needed to confirm if Pinot Noir is indeed heterozygous for the *VviGuaS* allele and to determine if Syrah is homozygous for the same allele. Heterozygosity of the *VviTPS24* gene in Pinot Noir, a variety with low levels of rotundone, implies the existence of other factors impacting the conversion of α -guaiene to rotundone.

Huang et al. demonstrated that α -guaiene undergoes aerial oxidation to rotundone⁸². However, *VviSTO2*, a cytochrome p450 enzyme located on chromosome 19 and belonging to the CYP71BE family, has also been shown to catalyze a one-step oxidation of α -guaiene to rotundone. *VviSTO2* is more highly expressed in berry skins than in



the pulp and at much greater levels in Syrah than in *V. vinifera* cv. Merlot, a cultivar not known for accumulating rotundone. Expression is also concurrent with the accumulation of rotundone and peaks at 14 weeks after flowering. In vitro assays with VviSTO2 protein exhibited enzyme activity towards α -guaiane and (+)-valencene only. Since neither (+)-valencene nor its oxidation product β -nootkatol have yet been isolated from ripe Syrah berries and no other sesquiterpenes were utilized as substrates, it is likely that the primary role of VviSTO2 is in rotundone production⁸¹. Low VviSTO2 expression may account for the lack of rotundone in varieties with the VviGuaS variant of VviTPS24.

Norisoprenoids

Norisoprenoids are a diverse group of widespread compounds derived from the oxidative breakdown of carotenoids^{28,86}. They are important components of the aroma of many plants and plant products including roses, tomato (*Solanum lycopersicum*), tea (*Camellia sinensis*), saffron (*Crocus sativus*), watermelon (*Citrullus lanatus*), and osmanthus (*Osmanthus fragrans*)^{87–89}. Although C₁₃ norisoprenoids are most abundant in plants, compounds with 9 to 11 carbons occur as well⁸⁹. A large number of norisoprenoids have been identified in grape berries and wine; however, only a few have been established to have sensory significance. As with monoterpenes, the majority are found in the berry as non-volatile glycosides^{19,21,90}. Multiple precursors, each with various glycoside conjugates, are possible for each norisoprenoid⁹¹.

The most important norisoprenoids for wine aroma are β -ionone, β -damascenone, vitispirane, actinidol, 4-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB), 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), and 2,2,6-trimethylcyclohexanone (TCH)¹⁹. In wine, TCH has been isolated only from Port and is the only C₉ with significant sensory importance identified so far⁹². Since little is known about its biosynthesis, it will not be discussed further. β -ionone, a compound with a “woody” or “violet” aroma is widespread in plants and occurs around or above its detection threshold of 90 ng/L in the wines of many grape cultivars^{93,94}. β -damascenone is ubiquitous in wines and many fruit products, and, despite only rarely exceeding detection thresholds, may have important indirect effects on wine aroma^{95,96}. β -damascenone is also found in cultivars of *V. rotundifolia* and is particularly abundant (5 μ g/kg) in *V. labrusca* cv. Concord⁹⁷. TDN is characteristic of Riesling wines, while TPB was identified more recently. High levels of both TDN and TPB can contribute negative effects to wine aroma. The sensory contributions of vitispirane and actinidol are less clear¹⁹.

Norisoprenoid formation from carotenoids can be non-enzymatic or catalyzed by a number of oxidative enzymes⁸⁶. However, the abundance of C₁₃

norisoprenoids in grapes, as well as the preservation of carotenoid asymmetric centers in norisoprenoid products suggests an enzymatic pathway^{21,98}. The general mechanism of formation begins with cleavage of the carotenoid by a dioxygenase followed by enzymatic transformation of cleavage products to non-volatile precursors and ultimately conversion of the precursors to volatile norisoprenoids through acid catalyzed reactions (Fig. 3). Only β -ionone does not require further modification to be volatile¹⁹. Norisoprenoids and their precursors are subject to a large number of possible reactions that adds enormous complexity to the study of their biosynthesis²¹. The carotenoid precursors of norisoprenoids are extremely important pigments with essential roles in many plant functions such as photosynthesis, flower and fruit color, and phytohormone biosynthesis²⁸. While carotenoids play a major role in the skin color of many ripe fruits, the carotenoid profile in grape berries is more similar to that of leaves than other fruit⁹⁹. During berry ripening, there is a simultaneous increase in norisoprenoid content and decrease in carotenoid content⁹⁸.

Like monoterpenes, carotenoids are synthesized in the plastids via the MEP pathway²¹ and share DXS as a rate-limiting enzyme in their biosynthesis. Nonetheless, there have been limited studies comparing the carotenoid or norisoprenoid levels between grape cultivars with N284 and K284 VviDXS^{48,100}. Although, overexpression of K284 VviDXS, as well as transformation with the Muscat N284 VviDXS, causes upregulation of some genes involved in carotenoid production during anthesis and véraison, the effect on carotenoid content is unclear. Conversely, the overexpression of *E. coli* DXS in tomato results in greater accumulation of carotenoids and chlorophylls¹⁰¹.

The initial cleavage of carotenoid molecules to yield C₁₃ norisoprenoid skeletons is carried out by 9,10,(9',10')-carotenoid cleavage dioxygenases (CCDs, Fig. 3). Although, nine CCD members have been identified in *Arabidopsis*, only CCD1 and CCD4 have been shown to cleave carotenoids at the 9,10 and 9',10' double bonds to yield two C₁₃ ketone end groups and one C₁₄ aldehyde. CCD4s have been functionally characterized in *Crocus sativus*, *Malus x domestica*, and *Chrysanthemum x morifolium* and cleave β -carotene to give β -ionone^{88,102,103}. Mathieu et al. identified VviCCD1 from a Syrah EST database. When expressed in *E. coli*, the recombinant protein catalyzed the cleavage of zeaxanthin and lutein to form 3-hydroxy- β -ionone but did not generate any cleavage products with β -carotene^{100,104}. In addition, unlike plastid-localized CCD4s, CCD1s are found in the cytosol where their carotenoid substrates are much less abundant¹⁰⁵. Nevertheless, the strong correlation between CCD1 expression and volatile C₁₃ norisoprenoid production in rose and petunia (*Petunia x atkinsiana*)

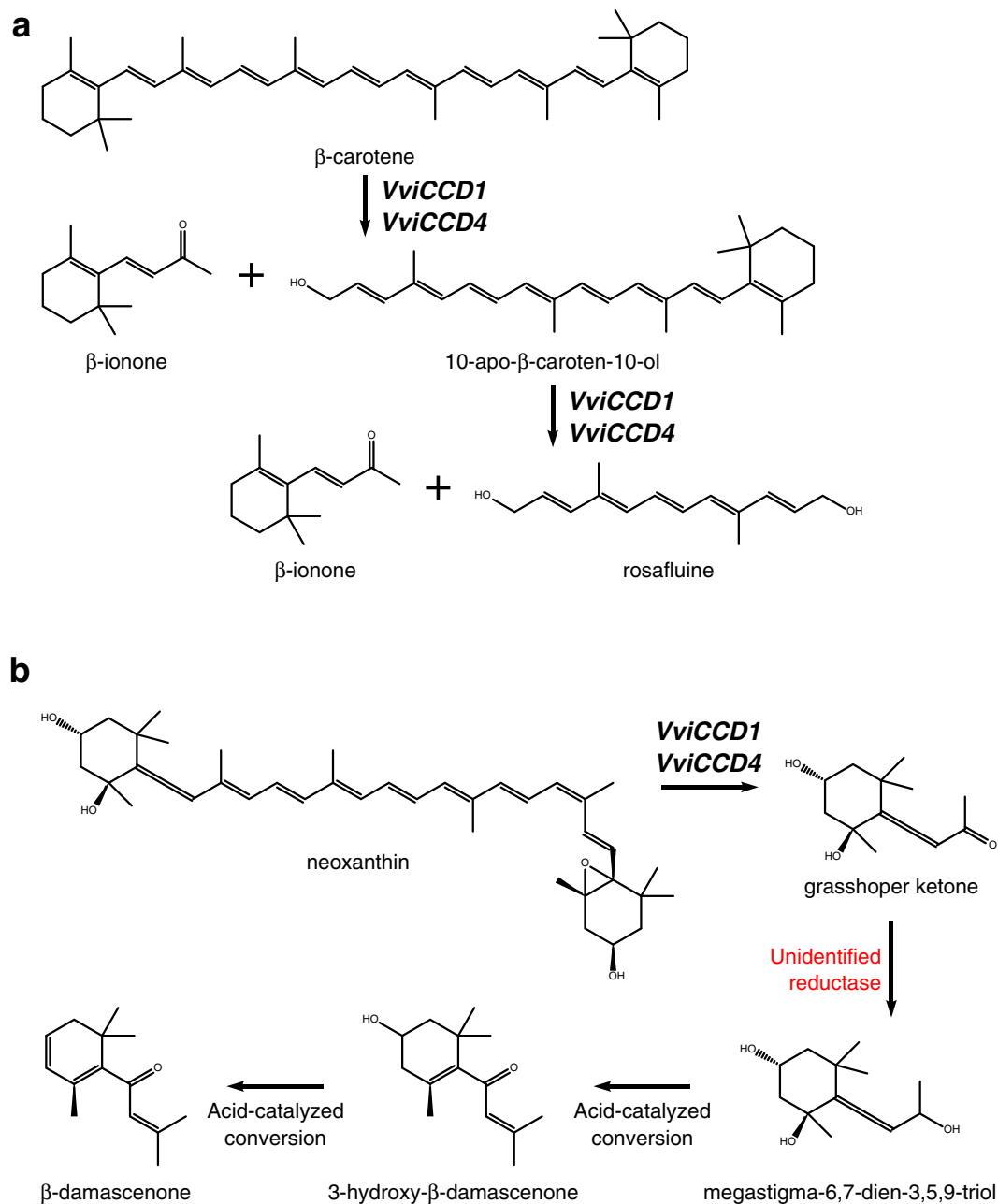


Fig. 3 Formation of β -ionone and β -damascenone in grape. Cleavage of (a) β -carotene and (b) neoxanthin by 9,10(9',10')-carotenoid cleavage dioxygenases (CCDs) and proposed subsequent formation of β -damascenone from the neoxanthin cleavage product grasshopper ketone. (Adapted from ref. ¹⁹)

suggests that the gene plays a role in the production of norisoprenoids in those flowers^{106,107}.

Overall, 72 non-redundant genes putatively involved in carotenoid metabolism in *V. vinifera* were identified by Young et al. and Leng et al.^{15,108}. Of these, seven were annotated as CCDs: *VviCCD1.1*, *VviCCD1.2*, *VviCCD4a*, *VviCCD4b*, *VviCCD4c*, *VviCCD7*, and *VviCCD8*. In Pinot noir, *VviCCD1* exists as a tandem duplication on

chromosome 13, while there are three paralogues of *VviCCD4* located on chromosomes 2 and 16 with highly conserved amino acid sequences^{15,16}. Functional characterization of *VviCCD1*, *VviCCD4a*, and *VviCCD4b* isolated from *V. vinifera* cv. Pinotage determined that they were all able to catalyze 9,10(9',10') cleavage from a variety of carotenoid substrates, with *VviCCD1* being the sole CCD to produce β -ionone from β -carotene¹⁶. The

expression patterns of *VviCCD1*, *VviCCD4a*, and *VviCCD4b* supports the possibility of their role in norisoprenoid biosynthesis.

VviCCD1 expression has been found to increase several fold at véraison in a number of grape cultivars, while *VviCCD4a* and *VviCCD4b* expression peaks during ripening^{15,16,104,108}. *VviCCD1* and *VviCCD4* transcript levels are negatively correlated with carotenoid content in Fujiminori, a tetraploid table grape with *V. vinifera* and *V. labrusca* parentage, and Sauvignon blanc^{15,108}. Similarly, norisoprenoid content peaked 1 week after induction of *VviCCD1* expression in Muscat of Alexandria and Syrah¹⁰⁰. Norisoprenoid profiles of the two cultivars were dominated by modified (via an oxidase or reductase) products of carotenoid cleavage. A grape cell suspension culture was also shown to metabolize β -ionone into oxygenated, reduced, and glycosylated derivatives, but the enzymes responsible have not yet been identified¹⁰⁹. On the other hand, the direct cleavage product of zeaxanthin and lutein by *VviCCD1*, 3-hydroxy- β -ionone, is a major component of the free norisoprenoids in Muscat of Alexandria¹⁰⁰.

Although the preceding in vitro experiments and expression patterns suggest a role for *VviCCD1* in the cleavage of carotenoids, transformation experiments give a more complicated picture. Overexpression of *VviCCD1* in *V. vinifera* cv. Sultana did not affect leaf carotenoid nor norisoprenoid concentrations¹⁶, while in tomato (*Solanum lycopersicum*), silencing of *LeCCD1* resulted in a 50% decrease fruit β -ionone concentrations without reducing carotenoid levels¹¹⁰. When incubated in vitro with various carotenoids and apocarotenoids, *OsCCD1*-overexpressing rice (*Oryza sativa*) endosperms metabolized apocarotenoids more effectively than carotenoids¹¹¹. Ilg et al. proposed that the apocarotenoid cleavage activity observed with *OsCCD1* is due to a function of CCD1s as scavengers of non-enzymatic cleavage products of carotenoids resulting from high light stress¹¹¹. Similarly, the role of *VviCCD1* may be to catalyze the cleavage of apocarotenoids that have been exported into the cytoplasm after partial metabolism by other CCDs such as CCD4s, whose expression is upregulated during berry development¹⁶.

Unlike β -ionone, β -damascenone is not the direct product of dioxygenase cleavage of a carotenoid. Concentrations in berries are generally much lower than in wine, indicating that further chemical transformations are necessary to generate free β -damascenone⁹⁶. The formation of β -damascenone is complex and has been reviewed in detail by Sefton et al.⁹⁶. A major proposed precursor of β -damascenone is grasshopper ketone, a ketodiol resulting from dioxygenase cleavage of neoxanthin^{19,112}. The ketodiol is then enzymatically reduced to megastigma-6,7-dien-3,5,9-triol which may then undergo acid-catalyzed

conversion to β -damascenone (Fig. 3b)^{89,96,113}. The two compounds have been isolated from grape juice as glycoside conjugates, supporting their role as the precursors for β -damascenone formation^{114,115}. While no candidate enzymes have been proposed for the reduction of grasshopper ketone to form the triol, a rose CCD1 (*RdCCD1*) has been shown to produce grasshopper ketone from neoxanthin¹⁰⁷. It remains to be determined if any *VviCCDs* have the same activity.

In contrast to the fairly ubiquitous β -ionone and β -damascenone, only a few genotypes produce wines with aromatically significant quantities of TDN. TDN is a characteristic component of Riesling wines and reaches concentrations as high as 200 $\mu\text{g/L}$ ^{116,117}. Very little research on TDN content in cultivars besides Riesling has been carried out, but small amounts of TDN have been identified from juices and wines of other cultivars^{117,118}. As TDN is produced from the slow hydrolysis of glycosylated precursors during wine fermentation and aging^{116,119}, this suggests that Riesling has higher concentrations of TDN precursors than other cultivars; however, the precursors are not completely absent in other grapes. Although a number of possible precursors have been isolated, neither the enzymes involved, nor the starting carotenoids have been identified.

Compared with the other major norisoprenoids, the formation of vitispiranes, actinidols, TPB, and their precursors is still poorly understood. TDN concentrations have been found to correlate positively with vitispirane, although it is possible that TDN can be reduced to the latter compound by yeast during fermentation¹²⁰. As with TDN, a number of norisoprenoid precursors are possible for TPB. Three, including the actinidols, occur as glycoconjugates and are hydrolyzed to form TPB during wine aging¹²¹. It is interesting that while Riesling, a cultivar with moderate levels of monoterpenes, has an abundance of TDN and vitispirane, Muscat cultivars rich in monoterpenes do not. Riesling is also not significantly higher in β -damascenone or β -ionone⁹⁶. This suggests the existence of genetic mechanisms not found in other cultivars that increase the formation of TDN and vitispirane precursors.

Methoxypyrazines

3-alkyl-2-methoxypyrazines are a widespread class of potent odorants found in many kingdoms^{122–124}. They are often characterized by extremely low detection thresholds (2–16 ng/L in wine), as well as by aromas described as herbaceous, green, vegetal, and earthy^{123,125}. Methoxypyrazines have been detected in a number of *Vitis* species such as *V. amurensis*, *V. cinerea*, *V. riparia*, *V. rupestris*, and *V. vinifera*^{2,3}. Their high concentration in unripe fruit, toxic plants, and aposematic insects suggests a protective role for developing seeds¹²⁶. These compounds constitute an integral element of the aromas of some

French *V. vinifera* cultivars such as Cabernet Sauvignon and Carménère^{11,123}. Smaller quantities of methoxypyrazines have also been found in other cultivars: Grenache, Pinot noir, Riesling, and Syrah^{24,127}. Although they can be perceived negatively when present in excess in wine, methoxypyrazines are desirable in small quantities, particularly for white wines¹¹. The most abundant methoxypyrazine in grapes and wine is 3-isobutyl-2-methoxypyrazine (IBMP), a compound also responsible for the characteristic aroma of green bell pepper. Unlike IBMP, the next two most abundant methoxypyrazines, 3-isopropyl-2-methoxypyrazine (IPMP) and 3-sec-butyl-2-methoxypyrazine (SBMP), rarely occur above detection threshold in grape berry¹²³.

Methoxypyrazines reach their highest concentrations in the vegetative organs of the grapevine. In contrast to its low abundance in berries, IPMP reaches concentrations of 8000 ng/L in roots. On the other hand, IBMP is most abundant in mature basal leaves¹²³. In berries, methoxypyrazines show a distinct pattern of accumulation in early development until the onset of véraison followed by rapid decrease until harvest regardless of final concentration^{124,127,128}. For varieties with low methoxypyrazine levels in mature fruit, prévéraison concentrations are a fraction of those in high-methoxypyrazine varieties and decline to undetectable levels after véraison^{127,129}. The mechanisms for this decline have not been resolved and may be due to a dilution effect from berry expansion or demethylation to a nonvolatile hydroxypyrazine^{124,127}.

A number of biosynthetic pathways have been postulated for the production of methoxypyrazines. A mechanism for bacterial synthesis starting with amidation of a branched chain amino acid followed by condensation with glyoxal was first proposed in 1970. However, the reaction is chemically unfavorable and glyoxal has not been isolated in plants^{130,131}. A second pathway involving the condensation of two amino acids to form the cyclic backbone has been proposed as well, but neither have been demonstrated in plants^{123,132}. Only the final O-methylation of the nonvolatile 3-alkyl-2-hydroxypyrazine precursors has been elucidated in grapevine¹²³. 3-isobutyl-2-hydroxypyrazine (IBHP) occurs in comparable concentrations in pre-véraison berries of low-methoxypyrazine and high-methoxypyrazine varieties, indicating that differences in accumulation are not due to a lack of precursor molecules^{127,133}.

Methylation of the precursors is catalyzed by O-methyltransferases (OMTs), which use S-adenosyl-L-methionine as the methyl donor. Four OMTs have been identified in grapevine (*VviOMT1*, *VviOMT2*, *VviOMT3*, and *VviOMT4*)¹²⁹. The first grape OMT with activity towards hydroxypyrazines was isolated in 2001 from Cabernet Sauvignon berries¹³⁴. The gene encoding this OMT was subsequently identified by Dunlevy et al. as

VviOMT1 along with another OMT with a similar gene sequence (*VviOMT2*). Recombinant *VviOMT1* and *VviOMT2* products exhibited activity towards hydroxypyrazine substrates with *VviOMT1* having greater catalytic efficiency. However, hydroxypyrazines may not be their main substrate, as both enzymes had 100-fold greater activity towards caffeic acid and quercetin^{134,135}. To identify additional loci affecting methoxypyrazine accumulation, QTL analysis was performed on several mapping populations consisting of progeny between Cabernet Sauvignon, a high-IBMP cultivar, and a low-IBMP producing parent^{129,133}.

Analyzing IBMP production in an F₁ population derived from Cabernet Sauvignon and a dwarf vine derived from *V. vinifera* cv. Pinot Meunier showed that all progeny produced IBMP at varying concentrations. This led Dunlevy et al. to hypothesize that production of IBMP is a dominant trait for which Cabernet Sauvignon is homozygous. A 3:1 segregation ratio of IBMP production in a derived F₂ population confirmed their hypothesis. In addition, a methoxypyrazine-associated QTL was located on chromosome 3 in the F₂ population. This locus contained 261 putative genes from which *VviOMT3* and *VviOMT4* were identified¹³³. Guillaumie et al. conducted a separate marker-trait association on a population of Cabernet Sauvignon x *V. riparia* cv. Gloire de Montpellier. They identified five QTLs accounting for 41% of the variance in IBMP content, including the QTL encompassing *VviOMT3* and *VviOMT4* along with another QTL colocalizing with *VviOMT1* and *VviOMT2*¹²⁹.

Functional characterization of *VviOMT3* and *VviOMT4* by Dunlevy et al. found that Cabernet Sauvignon *VviOMT3* has a catalytic efficiency 150-fold to 7000-fold greater than *VviOMT1*, 2, and 4. Interestingly, *VviOMT3* encoded by an allele from the low-methoxypyrazine cultivar Pinot noir was found to be only slightly less efficient than Cabernet Sauvignon, which is in contrast to Guillaumie et al.'s findings^{129,133}. *VviOMT3* is relatively specific for hydroxypyrazines, and substrate specificity between alleles from different cultivars is not significantly different^{129,133}. Together with the similar catalytic efficiency of *VviOMT3* alleles from low-methoxypyrazine varieties, this suggests that varietal differences in methoxypyrazine levels is not due to altered enzyme function but instead due to variations in gene expression.

Several studies have shown that the expression of *VviOMTs* peaks early in berry development^{124,129,133,136}. A lack of differences in *VviOMT1* and *VviOMT2* transcript levels between high-methoxypyrazine and low-methoxypyrazine cultivars suggests that these enzymes are not responsible for differences in IBMP concentration, but may produce the small amount measured in low-methoxypyrazine cultivars. In contrast, *VviOMT3* is many

orders of magnitude more highly expressed in Carménère and Cabernet Sauvignon than in Pinot noir or Petit Verdot^{129,133}. The upregulation of *VviOMT3* in high-methoxypyrazine genotypes and its lack of expression in other genotypes suggests that differences in transcription are responsible for variations in methoxypyrazine content.

To determine the mechanisms associated with *VviOMT3* expression variation, Dunlevy et al. examined the non-coding regions of the gene in Cabernet Sauvignon and Pinot noir¹³³. Three alleles were identified: one for which Cabernet Sauvignon is homozygous, and two in Pinot noir. Although the exact mechanism suppressing *VviOMT3* expression in Pinot noir is not yet known, the authors identified polymorphisms among the three alleles including a transposon in one Pinot noir allele. All Cabernet Sauvignon × Pinot Meunier F₂ progeny that contain at least one Cabernet Sauvignon *VviOMT3* allele accumulate methoxypyrazines. This indicates that a single Pinot allele is insufficient for repressing the Cabernet Sauvignon allele. Association mapping of 91 *V. vinifera* cultivars found that cultivars with the highest methoxypyrazine concentrations possess at least one copy of the Cabernet Sauvignon *VviOMT3* allele¹³³. Interestingly, Cabernet Sauvignon, Carménère, and Merlot are all high-methoxypyrazine cultivars, suggesting a positive selection for this distinctive aroma trait likely inherited from their common parent Cabernet Franc¹³⁷.

Furan derivatives

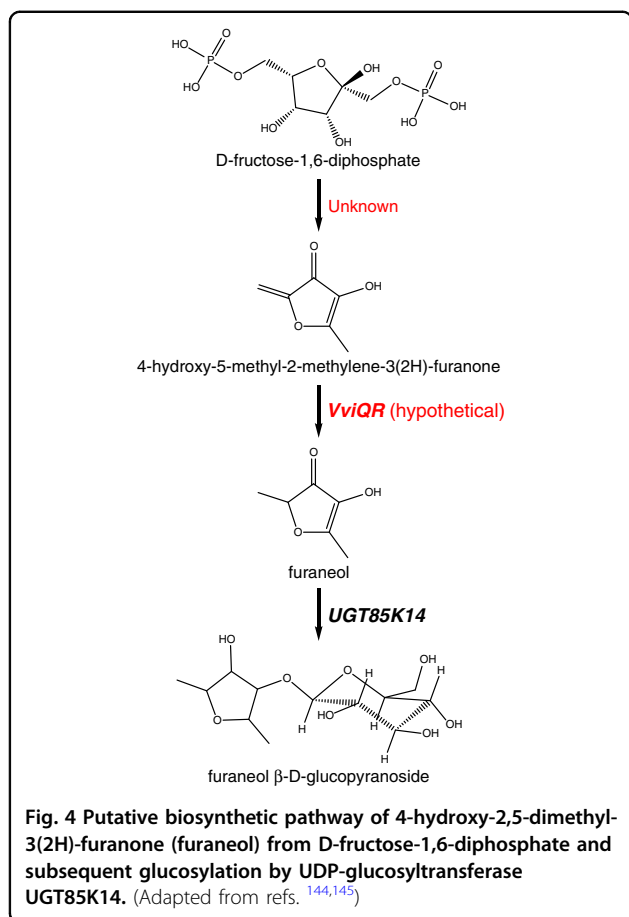
Furanones are pentose-derived and hexose-derived odorants, often with extremely low odor detection thresholds, that constitute important components of the aromas of some fruits. The most prominent of these is 4-hydroxy-2,5-dimethyl-3(2H)-furanone or furaneol, a compound described as having a strawberry or caramel aroma at low and high concentrations, respectively²⁸. Furaneol is the most abundant compound in the free and bound volatile profiles of *Muscadinia rotundifolia* and a major component of the characteristic aroma of *V. labrusca*. The majority of furaneol in *M. rotundifolia* berries exists as non-volatile furaneol glucopyroside²⁶. Ninety five percent of the furaneol content and 72% of furaneol glucopyranoside in berries of the interspecific hybrid cultivar Muscat Bailey A are contained in the flesh¹³⁸. The most common furan derivatives in *V. vinifera* wine, e.g., furfural and sotolon, are introduced during fermentation and aging¹¹. However, furaneol has also been identified from the wines of a number of *V. vinifera* cultivars. Concentrations are much lower than in *M. rotundifolia* and *V. labrusca* hybrids, but can still significantly exceed the detection threshold of 5–37 µg/L^{56,139,140}. Low concentrations of furaneol may contribute to the fruitiness of *V. vinifera* wines^{140,141}.

The biosynthetic pathway for furaneol has not been elucidated in grape, and only a few key enzymes have been functionally characterized in strawberry (*Fragaria x ananassa*) and tomato¹⁴². In strawberry, D-fructose-1,6-diphosphate is first converted into 4-hydroxy-5-methyl-2-methylene-3(2H)-furanone (HMMF) by a yet unknown pathway¹⁴³. HMMF is then converted into furaneol by an enone oxidoreductase (*FaEO*). An enone oxidoreductase bearing 71% amino acid identity with *FaEO* was identified in tomato and shown to have similar activity towards HMMF when expressed in *E. coli*. This, along with an enone oxidoreductase identified in furaneol-rich pineapple (*Ananas comosus*), suggests that the furaneol biosynthetic pathway may occur in *Vitis* as well^{142,144}.

An enone reductase sharing 73% identity with *FaEO* was identified among the PN40024 predicted proteins but has not yet been shown to catalyze furaneol formation from HMMF¹⁴⁵. Like strawberries, *V. labrusca* hybrids accumulate large amounts of furaneol glucoside. Small amounts of the glucoside are found in *V. vinifera* berries as well and can act as precursors for furaneol during wine fermentation and aging¹³⁸. A UDP-glucose:furaneol glucosyltransferase (*UGT85K14*) with 98% sequence identity between *V. labrusca* and *V. vinifera* was identified from Muscat Bailey A by Sasaki et al.¹⁴⁵. Heterologous expression of *UGT85K14* produced a protein that catalyzed the production of furaneol glucoside from furaneol and UDP-glucose (Fig. 4). Despite the major differences in furaneol and furaneol glucoside concentrations between *V. labrusca* and *V. vinifera* cultivars, *UGT85K14* is expressed in berries from both species and expression is not significantly different between *V. labrusca* cv. Concord and Cabernet Sauvignon¹⁴⁵.

Lipoxygenase pathway products

The products of the lipoxygenase pathway, primarily C₆ and C₉ aldehydes and alcohols, are involved in plant wound responses and defense strategies against pests and diseases²⁸. They comprise a major percentage of total grape and wine volatiles, ranging from 40 to 97% in *V. vinifera* and *V. amurensis* cultivars and between 25 and 57% in *V. labrusca* cultivars and hybrids^{2,146–148}. Lipoxygenase products are often described as having an herbaceous, leafy “green” aroma and can be a negative contributor to wine aroma^{149,150}. However, they contribute a non-negligible supply of substrates necessary for the formation of desirable esters during fermentation¹⁵⁰. C₆ aldehydes may also be involved in the production of volatile thiols, as described in the following section. Like many volatile compounds found in grapes, a large proportion of C₆ compounds are glycosylated. Functionally characterized monoterpenol glucosyltransferases (GTs) were shown to exhibit in vitro catalytic activity towards C₆ alcohols, but it is unknown if they have this role in vivo⁷².



In many plants, the lipoxygenase pathway is activated upon tissue damage or pathogen infection; however, the presence of C_6 compounds in undamaged ripe berries suggests that the pathway is active to some extent³⁴.

The production of C_6 alcohols and aldehydes from free fatty acids is initiated by lipoxygenases (LOXs, Fig. 5a). LOXs are a ubiquitous family of fatty acid dioxygenases that target polyunsaturated fatty acids such as linoleic and α -linolenic acids. Plant LOXs can be classified into two groups by their target oxygenation site at either the 9th (9-LOXs) or 13th (13-LOXs) carbon of the fatty acid. 13-LOXs can be further classified by the presence (Type II) or absence (Type I) of a plastidic transit peptide^{28,151}. Podolyan et al. identified 12 potential genes from the PN40024 reference genome based on sequence similarity with known plant LOXs¹⁵². Four of the identified LOXs, two Type II 13-LOXs (*VviLOXA* and *VviLOXO*) and two 9-LOXs (*VviLOXC* and *VviLOXD*), are expressed in Sauvignon blanc berries. Expression patterns of the four LOX genes vary across berry tissue type and development and are affected by wounding and botrytis infection¹⁵². Recombinant *VviLOXA* and *VviLOXO* proteins were shown to oxygenate unsaturated fatty acids in an

in vitro assay, yielding two hydroperoxides: 13(*S*)-hydroperoxy-(9*Z*,11*E*,15*Z*)-octadecatrienoic acid (13(*S*)-HPOT) from α -linolenic acid and 13(*S*)-hydroperoxy-(9*Z*,11*E*)-octadecadienoic acid (13(*S*)-HPOD) from linoleic acid (Fig. 5a)¹⁵².

Hydroperoxides then undergo cleavage by hydroperoxide lyases (HPLs) to form C_6 and C_9 aldehydes and oxoacids²⁸. HPLs are CYP74B/C cytochrome P450 enzymes that act on the hydroperoxy functional group and can be divided into three classes based on their substrate specificity. 9-HPLs and 13-HPLs are specific to 9(*S*)-hydroperoxides and 13(*S*)-hydroperoxides, respectively, while 9/13-HPLs are capable of catalyzing cleavage of both hydroperoxides^{21,28}. *VviHPL1* and *VviHPL2*, located on chromosomes 12 and 3, respectively, catalyze the cleavage of (13(*S*)-HPOD) into hexanal and 13(*S*)-HPOT to form (*Z*)-3-hexenal and (*E*)-2-hexenal in *V. vinifera*. The latter product is likely the result of isomerization of (*Z*)-3-hexenal either via a 3*Z*,2*E*-enal isomerase or spontaneous rearrangement²⁸. Although as of yet uncharacterized in grape, 3*Z*,2*E*-enal isomerases responsible for converting (*Z*)-3-hexenal to (*E*)-2-hexenal have recently been characterized in cucumber (*Cucumis sativus*) and pepper (*Capsicum annuum*)^{153,154}.

While *VviHPL1* is restricted to 13(*S*)-hydroperoxides, *VviHPL2* is also capable of utilizing 9(*S*)-hydroperoxides as a substrate, producing C_9 aldehydes. *VviHPL1* has a much higher catalytic activity towards 13(*S*)-HPOT compared with *VviHPL2*¹⁵⁵. In several cultivars, both genes are expressed at a low level until véraison when they increase rapidly in expression followed by a peak near maturity. Expression of both *VviHPL* genes correlates with the accumulation of hexanal and the two hexenals until the 10th week post-flowering, after which their concentrations decrease while HPL expression continues to increase^{1,34,155,156}. The predominance of (*E*)-2-hexenal over hexanal immediately after véraison in Cabernet Sauvignon led Kalua and Boss to suggest that the α -linolenic acid to 13(*S*)-HPOT to (*E*)-2-hexenal pathway is favored³⁴. Decreases in C_6 aldehydes during fruit ripening may be due to conjugation with glutathione or reduction to alcohols by alcohol dehydrogenases (ADHs)^{28,157}.

Like in many other angiosperms, grape ADHs are encoded by a small multi-gene family^{158–160}. Six ADH isoforms, *VviADH1-6*, have been identified on chromosomes 4 and 18. Only *VviADH1-3* have so far been shown to be expressed in berries^{161,162}. *VviADH1* and *VviADH3* increase in expression from fruit set until véraison upon which expression levels decline¹⁶³. This is consistent with the accumulation of aliphatic alcohols in berries of the interspecific hybrid Kyoho¹. In contrast, concentrations of hexanol in Cabernet Sauvignon berries increase dramatically at véraison and continue increasing through ripening³⁴. This difference in alcohol accumulation may be due

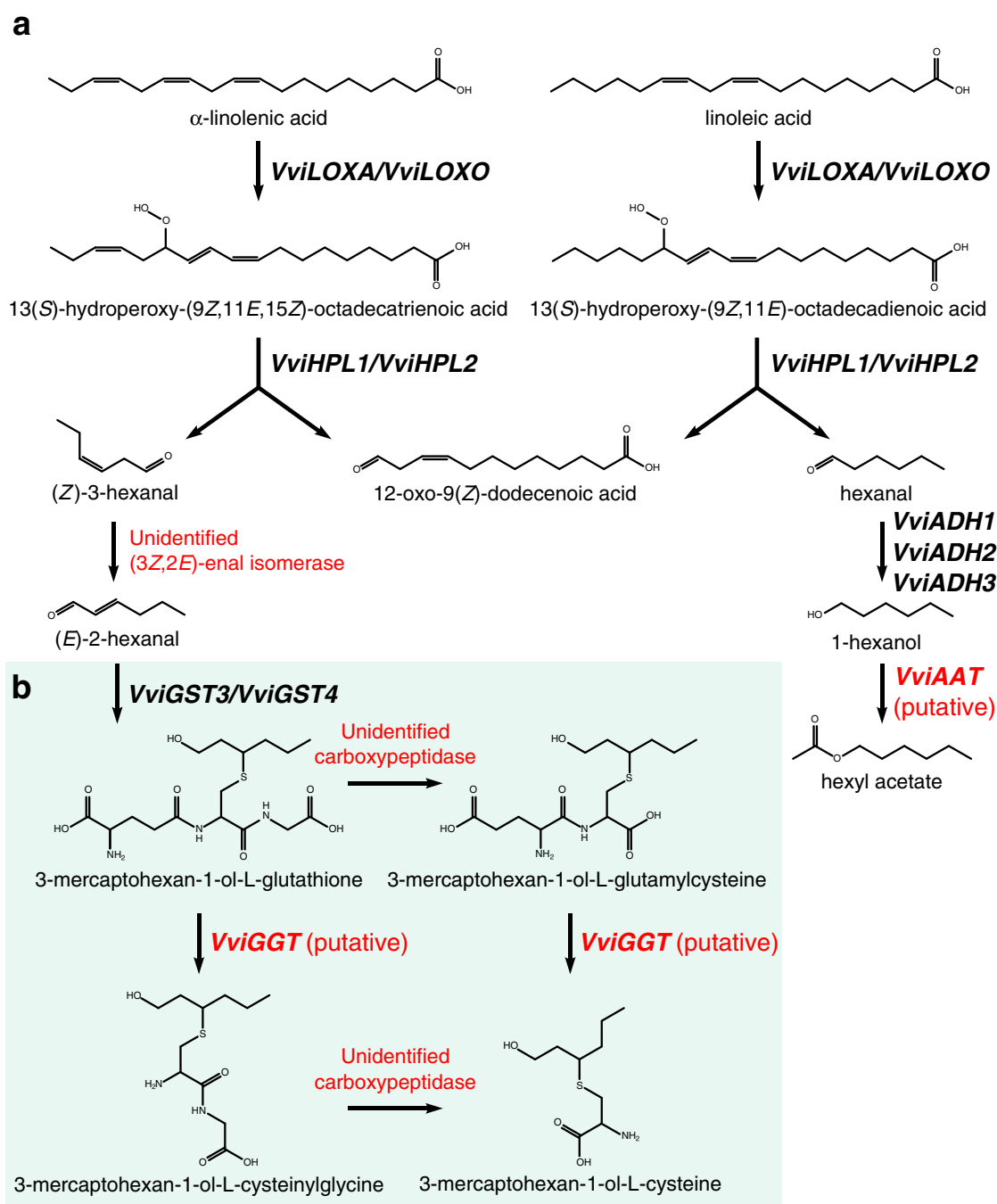


Fig. 5 Proposed biosynthesis of volatile lipoxigenase pathway products and polyfunctional thiol precursors. a Schematic representation of lipoxigenase pathway metabolism of free fatty acids in grape berries. Not all products are shown. Conversion of 1-hexanol to hexyl acetate by VviAAT is putative and has not been shown experimentally. **b** Hypothetical 3-mercaptohexan-1-ol precursor formation from E-2-hexenal. Only VviGST3 and VviGST4 have been functionally characterized (Adapted from^{21,152,155,180})

to their utilization as substrates for ester production in Kyoho.

Unlike *VviADH1* and *VviADH3*, *VviADH2* shows very low expression prior to véraison followed by a rapid increase in expression and enzyme activity. At its peak,

VviADH2 expression is considerably higher than the maxima of the other two ADH isoforms^{1,156,163,164}. In a number of varieties, *VviADH2* is downregulated in late ripening; however, enzyme activity continues to increase, indicating that there is little turnover in the enzyme¹⁶³.

Although grapes are a non-climacteric fruit, the promoter of *VviADH2* contains ethylene response elements, and ADH activity is increased in *V. vinifera* cells grown in suspension in the presence of ethylene-generating compounds¹⁶¹. Despite significantly altered ADH activity, under-expression and overexpression of *VviADH2* in *V. vinifera* cv. Portan caused minimal changes to the concentrations of volatile compounds in mature berries¹⁶⁴. This is in contrast to similar experiments in tomato where increased ADH activity resulted in higher concentrations of hexanol and (Z)-3-hexenol¹⁶⁵.

As the most highly expressed *VviADH* in ripe berries, *VviADH2* may also be important for the production of wines through carbonic maceration¹⁶⁶. ADH is necessary for the regeneration of NAD⁺ during alcoholic fermentation, and the upregulation of *ADH* is a common response to anaerobic conditions in plants^{167,168}. Although research on gene expression during anaerobic metabolism of grapes is limited, anaerobic conditions were shown to induce the production of ADH protein in cultured cells but not whole berries¹⁶⁹. Carbonic maceration has been shown to increase the concentration of higher alcohols in grape berries^{170,171}. This may be due to the action of *VviADH2* after the onset of anaerobic metabolism. More research is necessary to elucidate the role of *VviADH2* in the development of volatile compounds associated with carbonic maceration.

Alcohols produced by ADH can contribute to grape and wine flavor on their own or as substrates for ester production by endogenous and yeast alcohol acyltransferases (AAT)¹⁵⁰. Despite their predominance in wine, most wine esters are the product of yeast metabolism¹⁷². Endogenously produced esters are not an important component of ripe *V. vinifera* berries. In contrast, at 33% to 73% of total volatile compounds, esters constitute a major proportion of the volatile profile of *V. labrusca*, *V. riparia* and *M. rotundifolia* berries^{26,148,173}. The predominant esters in *V. labrusca* and *M. rotundifolia* cultivars and hybrids are ethyl acetate, ethyl butanoate, ethyl 2-butenate, ethyl hexanoate, and ethyl 2-hexenoate^{1,26,148}.

Like many other aroma compounds, esters accumulate in berries beginning at véraison and rapidly increase in concentration until ripening. Ji et al. found that the rapid post-véraison increase in ester concentration coincided with dramatic upregulation of *VviAAT* expression in Kyoho¹. Although ester concentrations in *V. vinifera* berries are typically very low, *VviAAT* expression and ester accumulation during berry development parallels that of Kyoho in some cultivars, albeit at lower amounts and with a predominance of C₆-acetates¹⁵⁶. Concentrations of (Z)-3-hexenyl acetate and (Z)-3-hexenyl butanoate are comparable with those of monoterpenes during the early development of Cabernet Sauvignon berries.

However, ester concentrations decline to low levels after véraison. This suggests that *VviAAT* is present only during early development³⁴.

VviAAT has not yet been experimentally shown to have enzymatic activity⁸. AATs belong to the BAHD superfamily of acyltransferases. They share the ability to act on multiple alcohols and acyl-coAs, producing a wide range of products^{28,174}. AATs in kiwi (*Actinidia chinensis*) and apple can utilize a range of substrates deriving from the lipoxygenase pathway and amino acid metabolism with the products determined by enzyme specificity and relative substrate concentrations^{175,176}. The only studied grape AAT encodes an anthraniloyl-coenzyme A:methanol acyltransferase (AMAT) and was isolated from *V. labrusca* cv. Concord. This AMAT bears 81% sequence similarity to *VviAAT*⁸. AMAT catalyzes the production of methyl anthranilate, a compound responsible for “labrusca character,” from anthraniloyl-coA and methanol. The enzyme has been shown to utilize multiple different alcohols as a substrate and may also play a role in the biosynthesis of butanoate and hexanoate esters in *V. labrusca* berries⁸. However, a study on the inheritance of “labrusca character” found no correlation between methyl anthranilate and total ester concentrations¹⁷⁷. Methyl anthranilate is an important component of the varietal character of *V. labrusca* cultivars; however, it is derived from the phenylpropanoid pathway and will be further discussed in the corresponding section.

Volatile sulfur compounds

Volatile sulfur compounds range from intensely malodorous to pleasantly fruity and are integral to the aromas of many plants such as passionfruit, black currant, garlic, and asparagus. They are also important volatile components of meat, mushrooms, and wine^{11,21,178}. Until the discovery of thiols that contribute positive aromas to wines, sulfur compounds were generally associated with wine defects¹⁷⁸. The most common volatile sulfur compounds in wine are thiols, hydrogen sulfide, dimethylmercaptans, and methylthioesters. Many of these are the products of microbial metabolism during fermentation; however, polyfunctional thiols and methylthioesters are crucial components of “varietal character” in some varieties and originate from precursors found in the berry. Sauvignon blanc is most well-known for aroma characters derived from thiols, but sulfur compounds are also important contributors to *V. vinifera* cultivars such as Muscat blanc, Gewürztraminer, Riesling, Chenin blanc and Semillon^{11,179}. Polyfunctional thiols and their precursors have been previously reviewed in excellent detail^{20,178,180}. Aside from a brief overview on the most important thiols and their precursors, this section will focus on their biosynthesis and what little is known about the genes involved.

4-mercapto-4-methylpentan-2-one (4MMP), a compound with the aroma of box tree (*Buxus*), was the first sulfur compound identified as an important contributor to the aroma of Sauvignon blanc¹⁸¹. Subsequently, Tominaga et al. identified 3-mercaptohexan-1-ol (3MH) and its acetate ester (3MHA), often described as “grape-fruit” and “passionfruit”, respectively, from Sauvignon blanc wine^{182,183}. Although generally present at lower concentrations, 3MH and 3MHA were found to also contribute to perceived fruitiness in wines made from Cabernet Sauvignon and Merlot^{184,185}. 4-mercapto-4-methylpentan-2-ol (4MMPOH) contributes to the citrus elements of the aromas of Muscat blanc and Sauvignon blanc^{179,183}. These four compounds represent the major contributors to Sauvignon blanc character and have perception thresholds of 0.8–60 ng/L²⁰. Compounds such as 2-furanmethanethiol, 2-methyl-3-furanthiol, and 2-sulfanylethyl acetate that contribute smoky, roasted meat, or coffee aromas to wines have also been isolated, but the sources of these compounds remain unclear^{178,186,187}.

Unlike the majority of volatile compounds important for grape aroma, 3MH, 3MHA, 4MMP, and 4MMPOH do not exist in volatile form nor as glycosylated conjugates in the berry. Rather, the metabolic action of yeast is necessary to convert the precursors to the volatile compounds^{20,188}. 3MHA is formed during fermentation through the esterification of 3MH by yeast enzymes and will not be discussed further¹⁸⁹. The exact source of thiol precursors in wine remains disputed with several hypotheses involving cysteine and/or glutathione conjugates of grape origin. Alternatively or simultaneously, thiols may be generated through the addition of a sulfhydryl functional group from hydrogen sulfide to an aliphatic compound like (*E*)-2-hexenal¹⁹⁰. For the latter hypothesis, the (*E*)-2-hexenal starting compound originates from the lipoxygenase pathway and its biosynthesis has been discussed in that section of this review.

Glutathione and cysteine conjugates of 3MH and 4MMP have been observed from grape juice. Both glutathione-conjugated and cysteine-conjugated precursors can undergo β -lyase-catalyzed cleavage by yeast to release the free polyfunctional thiol^{157,180,191,192}. However, several studies have found limited correlation between juice concentrations of precursors and thiol levels in the resulting wine^{193,194}. The addition of precursors does have a significant impact on final thiol concentrations but only a small amount of precursor is actually converted to a volatile form^{188,192}. In addition, no correlation between the biosynthesis of 3MH and 4MMP has been found¹⁸⁸. It is possible that there are several sources of volatile thiols in Sauvignon blanc wines. While the exact pathway responsible for the formation of volatile thiols remains unresolved, what limited genetic evidence exists supports

some contribution from glutathione and cysteine precursors. Certainly, the occurrence of volatile thiols including 3MH in ripe guava (*Psidium guajava*) and yellow passionfruit (*Passiflora edulis* f. *flavicarpa*) implies the existence of a biosynthetic pathway for these molecules in plants^{195,196}.

The proposed glutathione precursor of 3MH (3MH-glut) appears to result from conjugation of glutathione with (*E*)-2-hexenal through the action of a glutathione *S*-transferase (GST, Fig. 5b). GST expression can be induced by the presence of (*E*)-2-hexenal and other aldehydes. This links production of thiols to the lipoxygenase pathway^{157,197,198}. Increases in glutathione and cysteine precursors have been found to coincide with greater accumulation of glutathione and (*E*)-2-hexenal immediately before harvest^{35,199,200}. In plants, GSTs are a highly expanded gene family with diverse functions often related to detoxification and abiotic and biotic stress responses^{201,202}. GSH conjugation of C_6 aldehydes may be a mechanism to minimize cellular damage caused by the electrophilic nature of the compounds²⁰³.

Kobayashi et al. found that following exposure to UV-C radiation or downy mildew (*Plasmopara viticola*) infection, 3MH-glut accumulated in grape leaves and berries in correlation with the upregulation of *VviGST1*, *VviGST3*, and *VviGST4*¹⁵⁷. Recombinant *VviGST3* and *VviGST4* catalyzed the formation of 3MH-glut from glutathione and (*E*)-2-hexenal, indicating that the two genes may be responsible for thiol precursor production in grapes¹⁵⁷. However, more studies are necessary to determine if cultivar variations in *VviGST* sequences or expression are responsible for differences in thiol concentration. Interestingly, infection with *Botrytis cinerea* dramatically increases 4MMP and 3MH levels in Semillon^{179,198}. *Botrytis* infection has been shown to cause upregulation of the lipoxygenase (LOX) pathway genes *VviLOXC* and *VviLOXO*, as well as 50 GST genes, including *VviGST3* and *VviGST4* in Semillon berries^{152,204}. This supports the hypothesis that lipoxygenase pathway products are the precursors for glutathione conjugates of polyfunctional thiols.

The combined actions of two enzymes, a γ -glutamyl-transpeptidase (GGT) and a carboxypeptidase, may convert glutathione conjugated thiols to their cysteine conjugates. Dubourdieu and Tominaga proposed that GGT catalyzes the removal of glutamate from 3MH-glut, resulting in the production of *S*-3-mercaptohexanol-L-cysteinylglycine (3MH-cysgly). This is followed by carboxypeptidase-catalyzed removal of the glycine to yield cysteine-conjugated 3MH (3MH-cys)¹⁷⁸. Alternatively, a carboxypeptidase can react with 3MH-glut first, yielding a glutamylcysteinylated conjugate (3MH-glucys). This can then react with GGT and produce 3MH-cys (Fig. 5b). 3MH-cysgly has been shown to occur in grape juice, and

the existence of 3MH-glucys was inferred by the production of 3MH-cys following the passage of Sauvignon blanc juice through a column containing immobilized GGT^{191,205}.

3MH-glucys and 3MH-cysgly have also been found in the juice of yellow passionfruit, a fruit with high concentrations of 3MH¹⁹⁶. Although no genes encoding a GGT or carboxypeptidase have been functionally characterized in grape, similar pathways in other organisms and the existence of the appropriate intermediates in grape suggest that they may be involved in the production of thiol precursors^{157,178,180,206}. GGT-mediated catabolism of glutathione conjugates is well-documented in mammals and has also been shown to occur in plants; however, glutathione catabolism in plants is complicated and incompletely elucidated. GGTs are encoded by multiple genes in *Arabidopsis*, barley (*Hordeum vulgare*), and maize (*Zea mays*)²⁰⁶. In *Arabidopsis*, knockout mutants of *GGT3* (At4g29210) are unable to metabolize GSH conjugates in the roots²⁰⁷. Kobayashi et al. measured an increase in *VviGGT* expression in grape leaves following UV-C exposure but were unable to express a recombinant protein and test its catalytic activity¹⁵⁷. Blanco-Ulate et al. found that two putative carboxypeptidases and a GGT are upregulated during *Botrytis* infection²⁰⁴. More research is necessary to determine which genes are responsible for thiol precursor biosynthesis and how their activity and expression differs between cultivars.

Even though very little is known about the genes involved in production of thiol precursors, many of the cultivars known for thiol content share parent-offspring or sibling relationships^{5,56,178,208}. The shared ancestry between many thiol-rich cultivars suggests that there may be common alleles responsible for increased production of thiol precursors. Alternatively, the potential for thiol formation may be present in a number of unrelated varieties and has gone unnoticed due to their low concentration.

Phenylpropanoids

The phenylpropanoid pathway is well known in grapes as the starting point for the biosynthesis of flavonoids, anthocyanins, stilbenes, hydroxycinnamates, and other important compounds^{209,210}. Less well studied is the biosynthesis of volatile phenylpropanoids despite their abundance in the glycosidically bound fraction of volatile compounds in grape juice¹¹. As with lipoxygenase pathway compounds, grape monoterpene glycosyltransferases have been shown to generate glucosides with phenylpropanoid compounds as substrates; however, it is not known how much they contribute to the pool of bound phenylpropanoids in the berry⁷². As a group, phenylpropanoid-derived volatile compounds comprise up to 20% of volatile aglycones in enzymatically treated Chardonnay juice,

albeit individual compounds generally do not exceed detection threshold. They may still contribute to the overall sensory properties of a wine since some, such as phenylethanol and eugenol, are also produced by microbes during fermentation and aging^{11,211}. Although the mechanisms for their formation in grapevine have not been well studied, the phenylpropanoid volatiles found in grape are not unique to the genus, and it is likely that the biosynthetic pathways are similar²¹.

Eugenol is a characteristic volatile component of cloves and is also widespread in many plant species including grape^{11,212}. In basil (*Ocimum basilicum*), eugenol is synthesized from coniferyl acetate and NADPH²⁸. Although present in *V. vinifera* berries, eugenol is more abundant in non-*vinifera* species³. While a eugenol synthase gene has not been isolated and characterized in grape, transcript levels of an enzyme identified from a *M. rotundifolia* proteome as eugenol synthase were found to increase during ripening²¹³. However, this was not accompanied by data regarding eugenol concentrations.

Of the phenylpropanoid-derived aroma compound in grapes, the biosynthesis of methyl anthranilate is best studied. Methyl anthranilate is an important component of the odor of *V. labrusca* cultivars and contributes to the “foxy” character of *V. labrusca* cultivars and hybrids despite its relatively low concentration compared with other esters^{3,8}. Although primarily associated with American *Vitis* species and their hybrids, methyl anthranilate has also been detected from Pinot noir wine along with ethyl esters of anthranilic and cinnamic acids²¹⁴. In *V. labrusca*, methyl anthranilate is synthesized from anthraniloyl-coA and methanol by an anthraniloyl-coenzyme A: methanol acyltransferase (*AMAT*). *AMAT* expression begins at véraison and peaks 16 weeks after flowering in the berry mesocarp, paralleling the enzyme activity, as well as the accumulation of methyl anthranilate and its precursor anthranilic acid⁸.

Anthranilic acid is produced by anthranilate synthase (AS) in the first step of tryptophan biosynthesis from chorismate²¹⁵. Tryptophan concentration increases during ripening along with the concentration of anthranilic acid; however, it is unknown whether the increase in anthranilic acid is due to higher AS activity or tryptophan metabolism^{8,216}. To produce methyl anthranilate, anthranilic acid is esterified with methanol supplied by methylesterase-mediated hydrolysis of pectins. Although other *V. labrusca* cultivars have similar *AMAT* expression and enzymatic activity to Concord, methyl anthranilate concentration appears to be limited by the rate of formation of the anthraniloyl-coA and methanol precursors⁸. Concord had the highest methanol content and pectin-methylesterase activity among five *V. labrusca* cultivars and hybrids studied²¹⁷. The segregation ratio of methyl anthranilate concentrations in two families of crosses

between cultivars with high and low methyl anthranilate content suggests that three dominant complementary genes are responsible¹⁷⁷. Tryptophan metabolism is also important in the production of 2'-aminoacetophenone (2-AAP), another compound responsible for the “foxy” aroma of *V. labrusca* and *M. rotundifolia*.

The presence of methyl anthranilate, 2-AAP, and high total ester content in *V. labrusca* and *M. rotundifolia* and their relative absence in other grape species may be an adaptation for mammalian seed dispersal. Methyl anthranilate and 2-AAP act as bird repellents and olfactory attractants to mammals^{218,219}. 2-AAP has also been implicated as the cause of “untypical aging off-flavor” in wines made from *V. vinifera*. Described as having an odor of “wet wool”, “furniture polish”, or “mothballs”, 2-AAP in wine is thought to be the result of oxidative degradation of indole-3-acetic acid (IAA), an important tryptophan-derived auxin^{216,220}. Despite its presence in wine, 2-AAP has not been reported from *V. vinifera* berries and the biosynthetic pathway in *V. labrusca* and *M. rotundifolia* has not been explored to date.

Conclusions

Although there are still many unanswered questions regarding the biosynthesis of aroma compounds, our understanding of the genes involved is steadily improving. A number of genes related to secondary metabolism have been functionally characterized in the years since Dunlevy et al.'s excellent 2009 review on aroma biosynthesis in grapes²¹. Knowledge of the genes involved in the biosynthesis of a particular aroma compound can help breeders select for desirable genotypes and raises the possibility of modulating the expression of these genes to attain a desired volatile profile in the resulting wine.

While the publication of the PN40024 reference genome has been invaluable in the identification of grapevine genes, it is not without its limitations. Reliance on a single genome sequence of a genotype for which we have no volatile compound data can be misleading due to structural variants that result in altered gene expression and function in other genotypes^{85,221}. Drastic changes in aroma profile due to small mutations in key genes such as *VviDXS* or the *VviGuaS* variant of *VviTPS24* that are absent in PN40024 exemplify the limitations of a single reference genome^{43,84,222}. Moreover, we cannot assume that a *V. vinifera* genome sequence is adequate for non-*vinifera* cultivars and hybrids. The recent publication of additional high-quality *V. vinifera* genomes will undoubtedly facilitate the discovery and characterization of genes and alleles responsible for varietal characteristics^{221,223–225}.

Unlike many domesticated annual crops, *V. vinifera* cultivars have retained much of the diversity of wild grapevines, and the majority of cultivars are likely only a

few generations removed from the wild progenitor^{5,226,227}. Clonal propagation of selected grape cultivars has allowed for the spread of desirable genotypes; however this has also limited the distribution of potentially valuable rare mutations such as the REN1 powdery mildew (*Erysiphe necator*) resistance allele found in a few *V. vinifera* cultivars^{4,228}. Over half of the accessions in the USDA germplasm collection are connected by first-degree relationships⁵. Worldwide, viticulture is characterized by the increased plantings of a small number of elite cultivars, resulting in limited exploitation of the genetic diversity available⁹. Not only does this underutilization of genetic resources result in vineyards susceptible to attack by insects and disease, genes for novel flavor compounds could exist in rare cultivars and wild species⁵. An understanding of the genetic bases for aroma can facilitate the application of *Vitis* genetic diversity towards developing a sustainable and dynamic grape and wine industry.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter 2: Haplotype-specific expression of a terpene synthase underlies linalool variation in the grapevine cultivar Riesling

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Abstract

Grapevine cultivars vary widely in monoterpenoid content, yet the genetic and regulatory mechanisms underlying this variation remain poorly characterized beyond highly aromatic Muscat types. We profiled free volatiles and monoterpenoid glycosides in a Riesling × Cabernet Sauvignon F1 mapping population, revealing extensive variation and transgressive segregation consistent with multigenic control. QTL mapping identified 59 significant loci associated with 47 volatile compounds and monoterpene glycosides, including two major QTLs explaining 33.8% and 32.4% of phenotypic variance in (3*S*)-linalool accumulation. Integration of haplotype-resolved transcriptomics with metabolite data, enabled by a chromosome-scale diploid Riesling genome assembly, resolved a (3*S*)-linalool/nerolidol synthase cluster on chromosome 10 and identified *VviTPS54* as the strongest candidate underlying linalool variation. *VviTPS54* exhibited haplotype-specific expression strongly correlated with (3*S*)-linalool accumulation across genotypes, while no QTL was detected at the *VviDXS1* locus and *VviDXS1* expression was not correlated with terpene levels, indicating that regulatory variation within terpene synthase clusters, rather than methylerythritol phosphate (MEP) pathway flux, drives monoterpenoid composition in this population. These results establish regulatory variation of terpene synthases as a key mechanism underlying monoterpenoid diversity in grapevine and demonstrate that resolving such variation

requires haplotype-phased genome assemblies coupled with haplotype-resolved transcriptomics to detect allele-specific expression differences at complex, heterozygous loci.

Keywords

Grapevine, berry aroma, monoterpenoids, linalool, haplotype-resolved genome, volatile metabolomics, terpene synthases

Introduction

Disease pressure has driven the introgression of resistance traits from wild grape species into domesticated grape (*Vitis vinifera* ssp. *vinifera*; hereafter *V. v. vinifera*) backgrounds, while ongoing and projected climatic challenges have renewed interest in traits such as drought tolerance, heat tolerance, and altered phenology.¹ However, wild germplasm often introduces undesirable aroma and flavor attributes, and its effects on the volatile profile and resulting organoleptic properties of fruit and wine remain difficult to predict.² For example, crosses of *V. v. vinifera* with *V. x labruscana* yield progeny with elevated ester levels absent in *V. v. vinifera*³, while hybrids with *V. amurensis* and *V. thunbergii* show reduced volatile diversity.^{4,5} Difference accessions of *V. cinerea* and *V. riparia* exhibit wide variation in methoxypyrazine content, further affecting their utility in breeding programs.⁶ Compounding this challenge, grapevine genomes are highly heterozygous and harbor recessive deleterious alleles, which complicate the recovery of desirable traits through backcrossing.⁷ Identifying quantitative trait loci (QTLs) and tightly linked genetic markers for key organoleptic traits would enable early selection of seedlings, accelerate breeding cycles, and improve parental selection strategies.

Volatile aroma compounds are key determinants of wine sensory properties that arise from grapes, microbial metabolism during fermentation, and aging and storage conditions.⁸ Grape-derived aroma compounds can largely be grouped into eight major classes based on biosynthetic

origin: lipoxygenase pathway products (e.g., C6 volatiles such as green leaf aldehydes), monoterpenoids, sesquiterpenoids, norisoprenoids, furan derivatives (e.g., furaneol), methoxypyrazines, volatile sulfur compounds, and phenylpropanoid-derived compounds.^{8,9}

Compared to fruits such as apples, strawberries, and peaches, most grape species have relatively low levels of esters and terpenoids. Notable exceptions include *V. x labruscana*, whose aroma is characterized by esters such as methyl anthranilate, and “aromatic” *V. v. vinifera* cultivars that accumulate higher concentrations of monoterpenoids.⁶ Within *V. v. vinifera*, cultivars can be broadly classified into three groups based on monoterpenoid content: “neutral” or “non-aromatic” cultivars (e.g., Chardonnay, Cabernet Sauvignon) with very low levels; intermediate “aromatic” cultivars such as Riesling (1-4 mg/L); and highly aromatic Muscat cultivars, which can exceed 6 mg/L of total monoterpenoid content.^{10,11}

Monoterpenoids are among the most important contributors to grape and wine aroma. Even in cultivars not typically classified as aromatic, monoterpenoids, together with related terpenoids such as sesquiterpenoids and norisoprenoids, contribute to overall bouquet and to fruit, floral, and herbal sensory descriptors. In grape, monoterpenes are synthesized via the methylerythritol phosphate (MEP) pathway, with 1-deoxy-D-xylulose-5-phosphate synthase (DXS) catalyzing the first committed step.¹² Several terpenoid-associated QTLs have been identified in *V. v. vinifera*, including a locus on chromosome 5 containing *VviDXS* associated with increased monoterpenoid accumulation in Muscat cultivars and aromatic mutants of otherwise neutral backgrounds.^{13,14} Structural diversity among monoterpenes is generated by terpene synthases (TPSs), which form a large, often tandemly duplicated gene family in grapevine and show substantial cultivar-specific variation.^{15–18} However, it remains unclear whether variation in monoterpenoid accumulation in

moderately aromatic cultivars is primarily driven by structural variation at terpene biosynthetic loci, differences in gene dosage, or regulatory variation affecting gene expression.

Previous studies of monoterpenoid production in *V. v. vinifera* have focused largely on highly aromatic cultivars such as Muscat blanc and its derivatives, whereas the genetic basis of moderate monoterpenoid accumulation in cultivars such as Riesling and Viognier remains poorly characterized.⁹ Most analyses of grape volatile composition have relied on interspecific hybrids or table grape crosses, often with limited genetic resolution and without genome references, constraining the identification of causal genes and regulatory mechanisms. In contrast, relatively few studies have examined aroma variation in intraspecific wine grape populations. This gap is notable given the genetic divergence between wine and table grapes, the greater contribution of *V. v. sylvestris* ancestry in wine grapes, and the structural complexity of grapevine genomes.^{7,19–21} Together, these limitations have hindered a mechanistic understanding of the genetic architecture of aroma traits in wine grapes.

Riesling and Cabernet Sauvignon represent contrasting aroma profiles within *V. v. vinifera*. Riesling is defined by moderate monoterpenoid levels and a strong contribution of norisoprenoids such as 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) to its varietal profile,^{22–24} whereas Cabernet Sauvignon accumulates low levels of monoterpenes and is characterized by methoxypyrazines.^{23,25} Both cultivars are widely planted, genetically representative of Western European germplasm, and extensively used in breeding, making them well-suited for dissecting the genetic basis of aroma variation.²⁶

To dissect the genetic basis of aroma variation in wine grape, we present a chromosome-scale diploid genome assembly of Riesling and use it to anchor genotyping-by-sequencing and high-density linkage mapping in an F1 Riesling × Cabernet Sauvignon population. Coupling this

framework with semi-targeted metabolomics of free and glycosidically bound volatiles, we map QTLs associated with a broad spectrum of berry aroma compounds. Integration with haplotype-resolved expression data identified two major loci controlling (3*S*)-linalool accumulation, with haplotype-specific expression of *VviTPS54* emerging as a key determinant of monoterpenoid variation between the parental cultivars and their progeny.

Materials and Methods

RxCS Mapping Population

The population used in this study consisted of 138 full-sibling progeny resulting from a cross between *V. v. vinifera* cv. Riesling clone FPS 24/GM 110 and *V. v. vinifera* cv. Cabernet Sauvignon clone FPS 08 planted at the Viticulture and Enology Department Experimental Station (Oakville, Napa, CA) in 2018. This planting was previously used for identifying QTLs related to microbiome recruitment, cluster architecture, and yield components.^{27,28} The population was planted as a randomized complete block vineyard consisting of three biological replicates of each genotype in three adjacent blocks for a total of 9 vines per genotype, including both parent varieties. The experimental vines were completely surrounded by a buffer of Cabernet Sauvignon vines to minimize edge effects. Rows were oriented northwest-southeast with 2.4 meters between rows and 1.8 meters between vines. Training consisted of bilateral cordons in a modified vertical shoot positioning system pruned to two-bud spurs. Water was supplied through drip irrigation. Pesticide applications and irrigation were scheduled at the discretion of the vineyard manager.

Genome Assembly and Annotation

The genome assembly of Riesling was scaffolded at chromosome level using contigs generated previously.²⁹ Using a high-density consensus map and HaploSync, the contigs were phased and anchored into 19 sets of diploid chromosomes after 7 runs of HaploSplit and 4 runs of

HaploFill. Gene models were functionally annotated concatenating the results from blastp v.2.7.1+³⁰ and interproscan v.5.27-66.0³¹ using blast2go³² as described previously.³³ The parentally phased Cabernet Sauvignon genome assembly and annotation were obtained from.³⁴

Genotyping, Map Construction, and QTL Analysis

A consensus linkage map was generated from the Riesling × Cabernet Sauvignon (RxCS) genotyping-by-sequencing (GBS) data previously obtained by Flörl et al.²⁷ GBS data were aligned to the Riesling FPS24 Hap1 genome sequence using BWA-MEM (ver. 0.7.17-r1188) with default parameters.³⁵ SNPs were filtered with Tassel 5 using the following criteria: a coverage depth of >6, minor allele frequency of >0.1, and missing data <10% .³⁶ SNPs within 64 base pairs were merged. Map construction followed the method published in Flörl et al.²⁷ and Lopez-Moreno et al.³⁷ using the Kosambi mapping function in the ASMap package.³⁸ Collinearity between the genetic map and physical map was assessed by calculating the Spearman correlation coefficient. Mapchart v.2.2 was used to draw linkage groups and QTL intervals.³⁹ QTL analysis was performed using MapQTL v.6⁴⁰ on genotype means. The maximum likelihood mixture model was used to locate QTLs and permutation tests of 1000 permutations were used to identify genome-wide significance thresholds at 0.05 significance level. The physical boundaries of significant QTLs were located on the haplotype 1 of the Riesling genome using the coordinates of two flanking markers roughly 1-2 LOD from the peak.⁴¹

Sample Collection and Processing

Berries from the RxCS population were collected for volatile and glycoside analysis from 128 genotypes in 2020 and from a subset of 8 genotypes in 2021. Berries were sampled from the two parent cultivars in both years. For the 2020 data set, berries were sampled once or twice a week starting 11 weeks post-anthesis to track sugar accumulation. Collection for chemical analysis

occurred when the average Brix reached 21 Brix or when sugar levels plateaued for three consecutive samplings. Approximately 150g of berries were collected by snipping off individual berries from multiple clusters. Each block replicate consisted of pooled berries from the three replicate vines of each genotype. Berries were frozen in liquid nitrogen and stored at -80° until further processing. The 2021 samples were collected in the same manner at two time points: 14 weeks post anthesis (Preharvest) and at full ripeness as determined with the same methodology as in 2020 (Harvest).

Homogenized frozen tissue for HS-SPME-GC-MS and UHPLC-QTOF/MS analysis was prepared according to the protocol described by.⁴² Approximately 60g of whole frozen berries with pedicels removed were weighed and cooled in liquid nitrogen to facilitate grinding. An A11 Basic Grinder (IKA, Staufen, Germany) was used to homogenize the berries to a fine powder. Subsequently, the powder was transferred into 50mL Falcon tubes and refrozen in liquid nitrogen. Homogenized samples were then stored at -80°C until extraction. Samples were collected for RNA extraction in 2021 from the eight progeny genotypes and both parent cultivars concurrently with collection for metabolite analysis. Three replicates of each genotype were collected, one per block. Each sample consisted of a pool of 30 berries collected from three vines. Berries were kept cool during transport. Pedicels and seeds were removed and samples frozen in liquid nitrogen before storage at -80°C until extraction.

Headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) analysis

Volatile compounds were analyzed semi-quantitatively using the HS-SPME-GC-MS protocol used by.⁴³ Analysis of all samples used an Agilent 6890N gas chromatograph coupled to a 5975 mass selective detector (Agilent, Santa Clara, CA, USA) with an MPS2 autosampler

(Gerstel, Mülheim an der Ruhr, Germany). Berry samples were analyzed in duplicate for a total of 6 samples per genotype (2 technical replicates of the 3 block replicates). For each sample, 20 ($\pm$ 0.20) g of frozen homogenized powder were measured into 50mL polypropylene centrifuge tubes with 5 mL of 1.0 M sodium citrate adjusted with HCl to pH 6. The mixture was homogenized and allowed to thaw completely before centrifuging for 15 min at 4°C and 5,000 rpm. Two 10mL aliquots of supernatant were pipetted into 20 mL amber headspace vials with 5 mg of NaCl and spiked with 50 μ L each of 10 mg/L 2-octanol and 2-undecanone dissolved in 100% ethanol as internal standards. All samples were immediately capped with 20 mm crimp caps with PTFE/silicone septa (Supelco, Bellefonte, PA, USA) and analyzed within 24 hours of extraction. Sample order was randomized for each run.

The MPS2 sampling sequence was programmed so that berry samples were equilibrated at 30°C for 5 min with agitation in the MPS2 incubator prior to extraction using a 23-gauge syringe, 1 cm polydimethylsiloxane (PDMS) SPME fiber for 45 minutes at 30°C and 250 rpm. The fiber was thermally desorbed in the GC inlet at 260°C operating in splitless mode. Chromatographic separation utilized a DB-Wax ETR capillary column (30 m, 0.25 mm i.d., 0.25 μ m film thickness) with helium as the carrier gas at a constant flow of 0.90 mL.min⁻¹. The oven temperature program began at 40°C for 5 min, ramped at 3°C.min⁻¹ to 180°C, then 30°C.min⁻¹ to 260°C with a final hold of 10 min. The MS transfer line, ion source, and quadrupole were maintained at 260°C, 230°C, and 150°C, respectively. Mass spectra were acquired in scan mode over an *m/z* range of 30–300 with a 2 min solvent delay. Compounds were identified using NIST mass spectrum matches and Kovats retention indices (RI). Analysis of GCMS data was performed on Agilent MassHunter Qualitative and Quantitative packages (versions 10.0). Relative abundances were

calculated by normalizing compound peak areas to the peak area of the octan-2-ol internal standard.

Solid-Phase-Extraction (SPE) of Monoterpene Glycosides

Samples for glycoside analysis were extracted according to the solid-phase extraction (SPE) method used by Hjelmeland et al.⁴² Five g of previously prepared homogenized berry powder were added to 5 mL of 50 mM sodium citrate (pH 5) and vortexed. After thawing, samples were centrifuged at 4°C and 4100 x g for 15 minutes. 5 mL of supernatant were transferred to clean Falcon tubes with 50 µL of 10,000 mg/L decyl-β-D-glucopyranoside internal standard. Solid phase extractions were performed in 96-well C18 phase Agilent plates with 100 mg bed mass per plate using a 96-well acrylic vacuum manifold. The plates were conditioned with methanol and 18 MΩ water, and the samples were washed and eluted as outlined with 1 mL of methanol. Samples were then stored at -80°C until analysis. Analysis of glycoside samples occurred no more than two weeks after extraction.

UHPLC-QTOF/MS Analysis

UHPLC-QTOF/MS analysis was conducted on an Agilent 1290 UHPLC coupled to an Agilent 6545 QTOF mass spectrometer with an Agilent Dual ESI Jet Stream source in negative ion mode. Separation was achieved using an Agilent Poroshell 120 phenyl-hexyl column (2.1x 150 mm, 2.7 µm) with an Agilent Poroshell 120 phenyl-hexyl (2.1 x 5 mm, 2.7 µm) guard column at 40°C. Mobile phases consisted of water with 0.1% acetic acid (A) and acetonitrile with 0.1% acetic acid (B) delivered at 0.42 mL.min⁻¹. The gradient program increased from 5% B to 20% over 5 minutes, held at 20% B for 13 minutes, then ramped up to 90% B over 4 minutes before returning to initial conditions for equilibration of the column. Samples were injected at a volume of 9 µL. Mass spectra were acquired in negative ion mode over an *m/z* range of 100–1000 using the Agilent

“All ions” workflow and collision energies of 15 and 30 V. Continuous reference mass correction using proton abstracted purine ($m/z = 119.0362$) and hexakis acetate adduct ($m/z = 980.016375$) was applied to maintain mass accuracy. Target compounds were extracted using a ± 20 ppm mass window and retention time tolerance of ± 0.5 min. Monitoring of instrument performance was done regularly using a control sample (E&J Gallo Brandy (Modesto, CA, USA)). Glycoside data were normalized to decyl- β -D-glucopyranoside equivalents prior to analysis and were therefore semiquantitative.

RNA extraction, sequencing, and analysis

Samples for RNA extraction were processed on the collection day as described in Blanco-Ulate et al.^{44,45} Briefly, deseeded berries were frozen using liquid nitrogen and ground with a mechanical mill. Two g of tissue were used for RNA extraction. Total RNA extraction was performed on 60 samples (10 genotypes at two timepoints and three biological replicates). Extraction and library preparation, were performed as in Vondras et al.⁴⁶ RNA-seq libraries were sequenced in 75 single-end mode, using an Illumina NextSeq sequencer (DNA Technology Core Facility, University of California, Davis).

Transcript abundance quantification

The pipeline nf-core/rnaseq v.3.13.2 was used to perform gene expression analysis using default parameters.^{47,48} Briefly, raw RNA-Seq reads were quality-filtered using TrimGalore (<https://github.com/FelixKrueger/TrimGalore>). Filtered reads were then pseudo-aligned using Salmon⁴⁹. The output salmon.merged.gene_tpm.tsv was used for the subsequent analyses. To aid in the identification of genes related to aroma compound metabolism, differential expression analysis was carried out on samples from the parent cultivars at both sampling timepoints using the R package DESeq2 v.1.42.1.⁵⁰ Protein sequence motifs and domains were identified using

TargetP 2.0 (<https://services.healthtech.dtu.dk/services/TargetP-2.0/>) and InterPro 108.0 (<https://www.ebi.ac.uk/interpro/>).^{51–53}

Statistical Analysis

Multivariate analysis of variance (MANOVA) and univariate analysis of variance (ANOVA) were used to test for statistical significance for all HS-SPME-GC-MS and UHPLC-QTOF/MS data with a significance threshold of 0.05. Correlations between metabolite and RNA-Seq data were assessed through Spearman's rank-order correlation using *cor.test* in R Statistical Software (v4.1.2, R Core Team 2021). Significance of correlation was determined by Benjamini-Hochberg adjusted *P*-value calculated using the *corTest* function from the R package psych v 2.5.6.⁵⁴ Multivariate ordination analyses were carried out using the FactoMineR v. 2.13 and factoextra v. 1.0.7 packages.^{55,56} Partial least squares discriminant analysis (PLS-DA) of parental metabolites was conducted on Metaboanalyst 6.0 (<https://www.metaboanalyst.ca/home.xhtml>).⁵⁷

Results

Segregation of volatile compounds within the Riesling × Cabernet Sauvignon population

Ripe berries from Riesling, Cabernet Sauvignon, and their F1 progeny were analyzed by HS-SPME-GC-MS to characterize volatile composition. Two genotypes did not produce fruit, and an additional 10 genotypes yielded insufficient material for analysis. In total, 186 volatile compounds were detected, of which 110 differed significantly among genotypes. Aldehydes and alcohols represented the largest fraction of detected compounds (**Supplementary Tables S1 and S2; Supplementary Fig. 1**). Additional classes included acids, alkanes, aromatic compounds, esters, furans, ketones, mono- and sesquiterpenes, norisoprenoids, and a small group of miscellaneous compounds. Overall, alcohols and aldehydes dominated the volatile profile.

Riesling and Cabernet Sauvignon showed clear differences in volatile composition. Partial least squares discriminant analysis (PLS-DA) identified major discriminating compounds, including C6 and C7 alcohols and related metabolites, mono- and sesquiterpenes, one norisoprenoid, two acetate esters, and two phenylpropanoid-derived compounds (**Fig. 1A-B**). These largely overlap with the compounds previously reported to distinguish the two cultivars.²³ Although (3*S*)-linalool was not detected in that study, Riesling was distinguished by higher monoterpene content, a pattern confirmed here. In the present dataset, total monoterpenes were substantially higher in Riesling (**Fig. 1C**), and composition also differed between cultivars, with (3*S*)-linalool predominating in Riesling and geraniol in Cabernet Sauvignon (**Supplementary Table S1**).

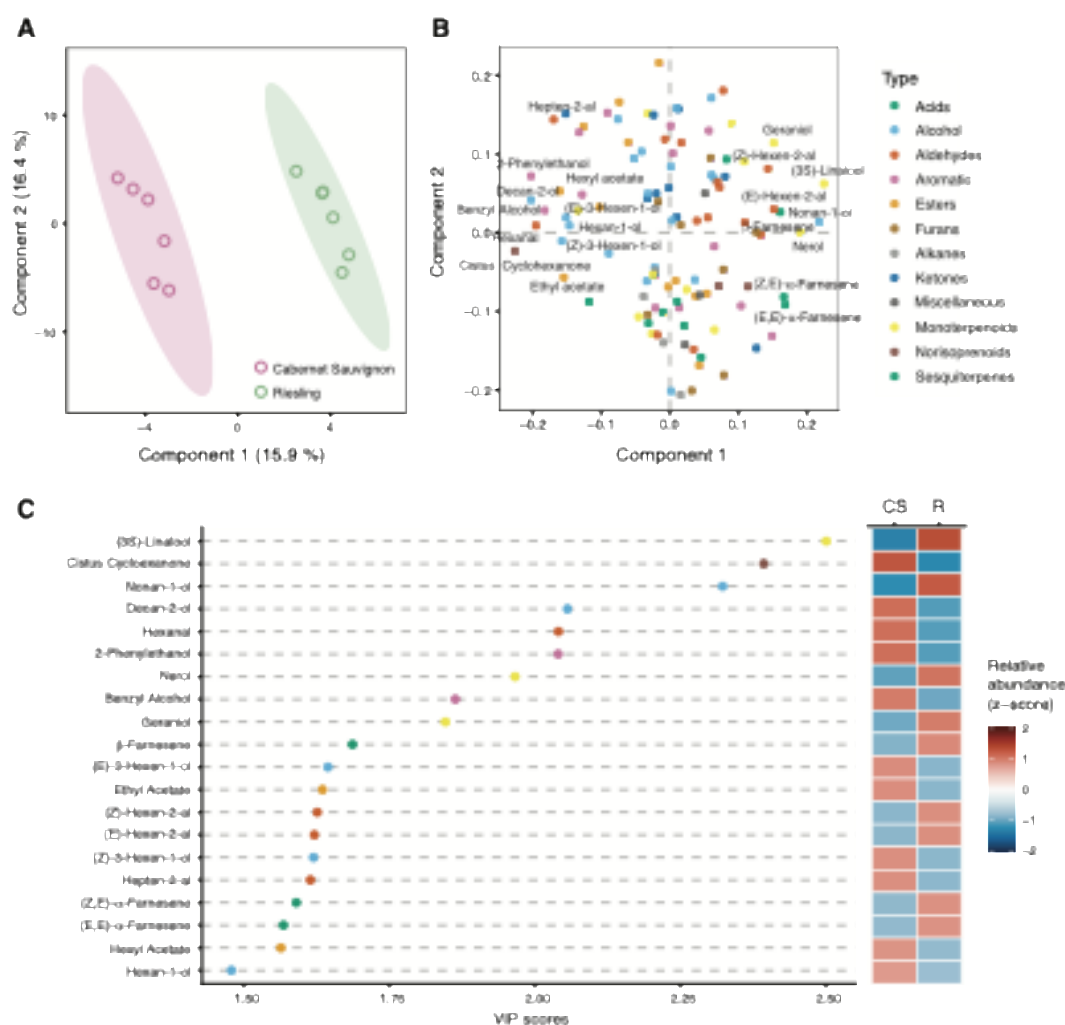


Figure 1. Partial least squares discriminant analysis (PLS-DA) of volatile compounds in Riesling and Cabernet Sauvignon berries. (A) PLS-DA score plot showing separation between cultivars. Points represent individual samples; ellipses indicate 95% confidence intervals for each group. (B) Loading plot showing the contribution of individual volatile compounds to sample separation. Points are colored by compound class. (C) Variable importance in projection (VIP) scores for the top 20 compounds contributing to cultivar discrimination. Points are colored by compound class. The accompanying heatmap shows scaled relative abundance (z-score) of each compound in Cabernet Sauvignon (CS) and Riesling (R).

Hierarchical clustering by Euclidean distance and complete linkage of metabolite relative abundance across genotypes yielded nine clusters (**Supplementary Table S1**). Clusters broadly reflected biosynthetic relationships, with lipoxygenase-derived compounds grouping together (clusters A, B, and E) and terpenoids forming distinct clusters, including a monoterpene-rich cluster (cluster I) containing (3S)-linalool, geraniol, and nerol. Sesquiterpenes and norisoprenoids co-clustered (clusters D and F), whereas remaining clusters were more heterogeneous but still

showed partial organization by chemical structure. Overall, the population exhibited broad variation in metabolite abundance, with many genotypes showing strongly positive or negative Z-scores across clusters. These patterns indicate segregation of volatile traits within the population and suggest that subsets of metabolites are subject to shared genetic control.

The abundance of representative compounds from each cluster across parents and progeny is shown in **Fig. 2**. Several compounds, including linalool, exhibited transgressive segregation within the mapping population. Notably, (3*S*)-linalool (cluster I) showed clear differentiation between parental genotypes and extensive segregation in the progeny, with some individuals exceeding Riesling levels. Broad-sense heritability (H^2) was estimated for each significant compound (**Supplementary Table S2**), ranging from 0.15 for β -myrcene to 0.96 for (*Z*)-theaspirane. Monoterpenes showed consistently high heritability, with H^2 values of 0.90 for (3*S*)-linalool and geraniol, and 0.73 for nerol, indicating strong genetic control of monoterpenoid biosynthesis.

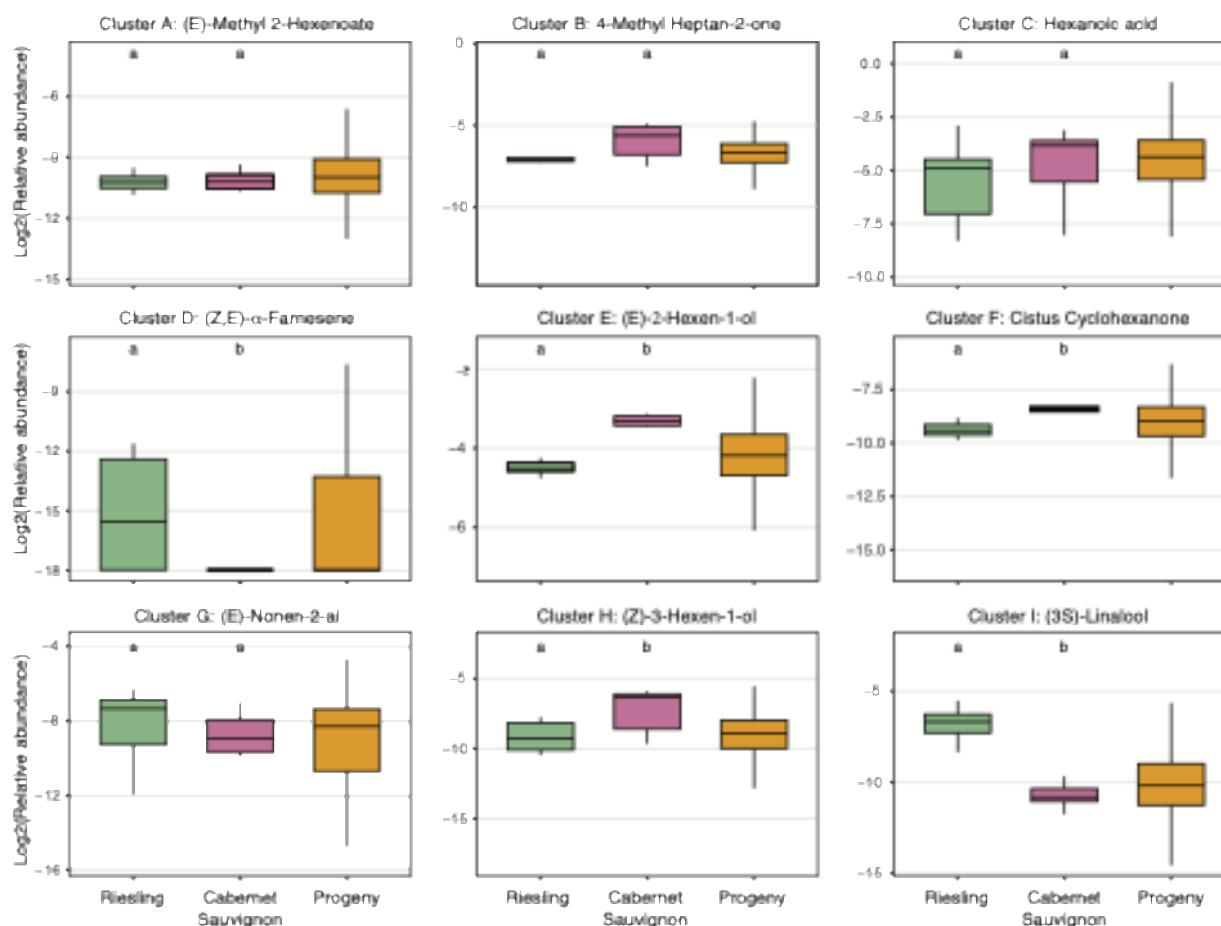


Figure 2. Distribution of representative volatile compounds from hierarchical clusters in Riesling, Cabernet Sauvignon, and their F1 progeny. Boxplots show log₂-transformed relative abundance of representative compounds from clusters A-I identified by hierarchical clustering based on compound abundance across genotypes. Colors indicate genotype groups (Riesling, Cabernet Sauvignon, and progeny). Titles denote compound identity and cluster assignment; compound classes are indicated by panel titles. Letters above boxplots represent Tukey's HSD groupings ($P < 0.05$).

Segregation of monoterpene glycosides within the RxCS population

Because most monoterpenes in grape occur as nonvolatile glycosides,⁵⁸ we quantified the relative abundance of 14 monoterpenoid glycosides in the RxCS population (**Table 1**). Monoterpene glycosides were classified as pentose-hexose conjugates (PH; PentHex-MT-ol) and deoxyhexose-containing conjugates (DH; DeoxyHex-MT-ol).

Principal component analysis (PCA) revealed substantial variation in glycoside accumulation, with the first two components explaining 66.3% of total variance (**Fig. 3**). Riesling and Cabernet Sauvignon were clearly separated along PC1, while progeny were distributed

between parental extremes. PC1 primarily reflected overall monoterpene glycoside abundance, driven largely by pentose-hexose conjugates PH5-PH9. In contrast, separation along PC2 was associated with glycoside composition, with PH1-PH4 showing strong positive correlations and contributing most to this axis. The correlations between monoterpene glycosides and free monoterpenoids are shown in **Fig. 4**. Most glycosides were significantly correlated with at least one aglycone (adjusted $P < 0.05$), although correlations were generally modest ($r < 0.7$). Geraniol showed weak associations, limited to PH10 and PH12. In contrast, (3*S*)-linalool exhibited strong correlations ($r > 0.8$) with PH5, PH7, and DH1, indicating tight coupling between glycoside pools and free linalool levels. Notably, PH1 and PH2 showed little to no correlation with any detected monoterpenoids despite their relatively high abundance in some genotypes.

Table 1. Relative abundance of monoterpene glycosides. Mean relative abundances in decyl- β -D-glucopyranoside equivalents are shown for 12 pentose-hexose monoterpenol glycosides (PH) and two deoxyhexose monoterpenol glycosides (DH). RT, retention time (min); R and CS, mean relative abundance in Riesling and Cabernet Sauvignon parents, respectively; Progeny, mean relative abundance across the RxCS population; Min and Max, minimum and maximum relative abundance observed among the progeny.

Monoterpenol Glycoside	RT	R Mean	CS Mean	Progeny Mean	Min	Max
PentHex-MT-ol 1 (PH1)	8.97	2.02	0.186	0.925	0.0133	7.868
PentHex-MT-ol 2 (PH2)	9.71	0.84	0.136	0.853	0.0059	8.936
PentHex-MT-ol 3 (PH3)	10.38	0.16	0.020	0.077	0.0010	0.584
PentHex-MT-ol 4 (PH4)	10.48	0.32	0.020	0.086	0.0016	1.471
PentHex-MT-ol 5 (PH5)	10.90	4.14	0.001	0.685	0.0010	13.530
PentHex-MT-ol 6 (PH6)	11.59	0.43	0.002	0.060	0.0003	1.050
PentHex-MT-ol 7 (PH7)	11.90	0.82	0.000	0.087	0.0002	2.504
PentHex-MT-ol 8 (PH8)	12.18	1.14	0.066	0.277	0.0628	1.594
DeoxyHex-MT-ol 1 (DH1)	12.23	2.24	0.000	0.296	0.0001	7.157
PentHex-MT-ol 9 (PH9)	12.72	0.68	0.003	0.069	0.0034	1.312
PentHex-MT-ol 10 (PH10)	13.39	2.60	0.275	1.075	0.1443	5.860
PentHex-MT-ol 11 (PH11)	14.06	0.24	0.035	0.072	0.0021	0.297
PentHex-MT-ol 12 (PH12)	14.36	0.31	0.030	0.105	0.0007	0.739
DeoxyHex-MT-ol 2 (DH2)	14.46	0.15	0.002	0.031	0.0004	0.307

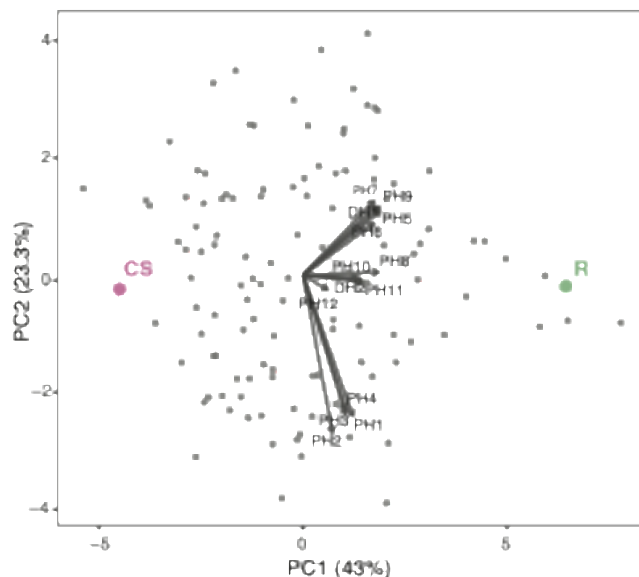


Figure 3. Principal component analysis of monoterpene glycosides in the RxCS population. Genotype means of individual progeny are shown as gray points, with parental genotypes highlighted (Riesling, R; Cabernet Sauvignon, CS). The first two principal components explain 66.3% of total variance (PC1: 43.0%; PC2: 23.3%). Arrows indicate loadings of individual glycosides (PH and DH), with direction and length reflecting their contribution to each principal component. PC1 primarily captures variation in total glycoside abundance, whereas PC2 reflects differences in glycoside composition.

Assembly and annotation of the Riesling genome and linkage map construction

The genome of *Vitis vinifera* cv. Riesling clone FPS24 was assembled to chromosome scale using HaploSync⁵⁹ in combination with a high-density consensus map.⁶⁰ The two phased haplotypes (Haplotype 1, RHap1; Haplotype 2, RHap2) were comparable in size (**Table 2**), each consisting of ~48.4% repetitive sequence and over 31,000 annotated protein-coding gene loci. Unplaced sequences were more repeat-rich (57.4%) and accounted for fewer than 10% of the annotated genes.

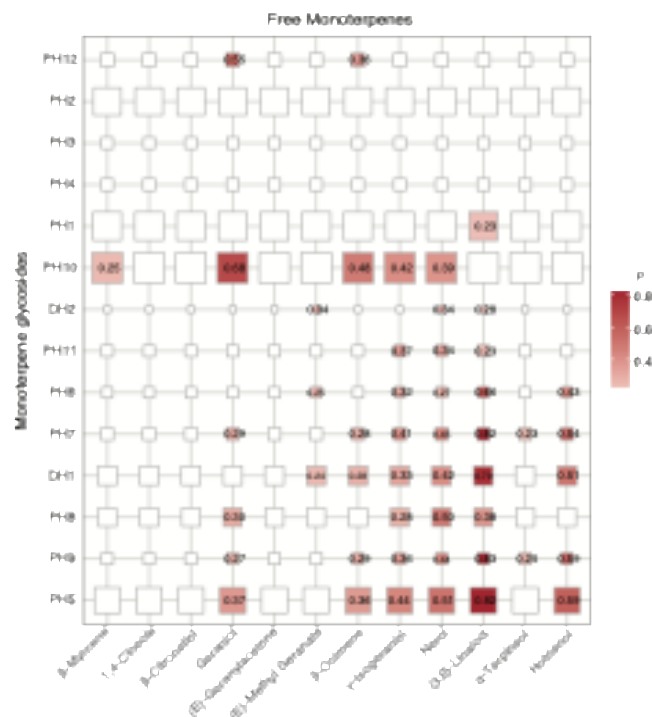


Figure 4. Correlation between monoterpene glycosides and volatile monoterpenes in the RxCS population. Heatmap showing pairwise Spearman's rank correlation coefficients (ρ) between monoterpene glycosides (rows) and free monoterpenes (columns). Color intensity reflects the strength of positive correlations, and values are shown within each cell. Only significant correlations (adjusted $P < 0.05$) are displayed. Rows are ordered based on PCA structure (PC1 and PC2) shown in Fig. 3. Tile size is proportional to the mean relative abundance of each glycoside in the population.

Table 2. Summary statistics of the diploid genome assembly of Riesling

	Haplotype 1 (RHap1)	Haplotype 2 (RHap2)	Unplaced
Sequence length (Mb)	489.62	494.15	108.17
Number of sequences	19	19	1,842
N50 (bp)	27,222,099	27,741,044	66,238
Repeats (%)	48.48	48.45	57.37
Genes	31,292	31,521	6,049

To enable QTL mapping of aroma traits in the segregating population, a genetic linkage map was constructed using GBS data. A total of 134,411 100-bp reads were obtained, with a mean read depth of 15.1x (median 2.9x; SD 90.2). After filtering for read depth, missing data, and minor allele frequency, 7,680 high-quality SNPs were retained for map construction. A consensus



Figure 5. Linkage map and QTLs associated with volatile aroma compounds.

Genetic linkage map of the Riesling × Cabernet Sauvignon F1 population showing 19 linkage groups corresponding to the 19 grapevine chromosomes. Marker density is represented along each linkage group, with positions in centimorgans (cM). Horizontal bars indicate the positions of QTLs associated with individual volatile compounds, with labels denoting compound identity. QTL intervals correspond to confidence intervals defined from the mapping analysis. The scale bar (left) indicates genetic distance (cM).

parental map was generated for QTL analysis of aroma compounds (**Fig. 5**). The final linkage map comprised 5,186 markers across 19 linkage groups, spanning 1,346.36 cM with an average inter-marker distance of 0.26 cM. The largest gap between markers was 13.56 cM (**Supplementary Table S3**). The genetic map showed high collinearity with the physical map (**Supplementary Fig. S2**), with an average Spearman correlation coefficient of 0.98.

Detection of QTLs associated with volatile and bound aroma compounds

QTL analysis identified genomic regions associated with variation in aroma compound abundance (**Fig. 5**). A total of 40 volatile compounds were associated with significant QTLs, with individual loci explaining 16.4% (2-phenylethanol) to 65.5% (δ -cadinene) of phenotypic variance, both on chromosome (Chr) 2. Most compounds (33) were associated with a single QTL, while seven showed multiple QTLs, for a total of 49 loci distributed across the genome. No significant QTLs were detected on Chr 3, 4, 5, 8, or 13, whereas Chr 2 harbored the highest number (nine loci). Monoterpenes accounted for the largest number of QTLs (13 loci). No significant QTLs were detected for furans or miscellaneous compounds. Compounds with significant QTLs are listed in **Table 3**, together with their physical positions in the RHap1 assembly. QTLs frequently colocalized for compounds sharing structural or biosynthetic relationships. For example, the aliphatic alcohols octan-1-ol, nonan-1-ol, and (*Z*)-3-nonen-1-ol mapped to the same region on Chr 15, while phenylalanine-derived volatiles (benzyl alcohol and 2-phenylethanol) colocalized on Chr 2.

Most sesquiterpenes and all three norisoprenoids were associated with significant QTLs. (*Z*)-theaspirane and dihydroactinidiolide mapped to single loci on Chr 1 and 17, respectively, whereas cistus cyclohexanone was associated with three QTLs on Chr 11, 12, and 18. Among sesquiterpenes, significant QTLs were detected for (*E*)- β -farnesene on Chr 1, 9, and 14, while

cyclic sesquiterpenes mapped to Chr 2 and 19. Overlapping QTLs on Chr 18 were identified for germacrene D and dihydroedulan, and δ -cadinene was associated with a single major QTL on Chr 2.

Of the 12 monoterpenes detected, eight were associated with significant QTLs on Chr 2, 6, 10, 12, and 15. Geraniol and its derivatives (*E*-methyl geranate and γ -isogeraniol) colocalized on Chr 15, with an additional shared QTL on Chr 12. Geraniol, (*E*)- β -ocimene, and β -citronellol mapped to the same region on Chr 6. QTLs associated with the abundance of (3*S*)-linalool, hotrienol, and nerol colocalized on Chr 10, and (3*S*)-linalool and hotrienol also shared a QTL on Chr 2. Both (3*S*)-linalool QTLs were major, explaining 33.8% and 32.4% of the variation.

Seven of the 14 monoterpene glycosides were associated with significant QTLs. PH1-PH4 colocalized on Chr 13, despite the absence of QTLs for free monoterpenes in this region. In contrast, PH5 mapped to two major QTLs overlapping those identified for (3*S*)-linalool, jointly explaining 81% of phenotypic variation, with the Chr 10 locus alone accounting for 53.6%. Given the major QTL effects and its central role in Riesling aroma, subsequent analyses focused on the genetic basis of (3*S*)-linalool production.

Table 3. Significant quantitative trait loci (QTLs) associated with volatile compounds and monoterpene glycosides in the RxCS population. QTL intervals were defined by the positions of flanking markers corresponding to a 0.5-LOD drop on either side of the peak. Only QTLs exceeding the 95th percentile of the genome-wide LOD threshold are shown.

Class	QTL Number	Compound	Chr	Start (bp)	End (bp)	Start (cM)	End (cM)	Peak LOD	Peak Marker (RHapI)	Percent Variance	Genes in QTL
Acid	1	n-Decanoic acid	11	3,048,855	5,902,730	5.04	28.91	4.99	CHR11_3519187	17	290
	2	(E)-2-hexen-1-ol	1	694,440	5,665,110	4.29	22.89	5.63	CHR01_2204154	19	496
	3	(Z)-3-hexen-1-ol	15	13,488,830	17,681,341	35.31	56.13	8.15	CHR15_16690679	26.3	404
	4	Nonan-1-ol	15	15,004,444	16,946,524	43.22	51.13	9.7	CHR15_15875293	30.5	201
	5	Octan-1-ol	7	26,315,802	27,899,218	61.56	66.56	5.56	CHR07_29254069	18.8	174
Alcohol	6	(Z)-3-nonen-1-ol	12	19,238,044	21,186,879	48.35	51.2	8.35	CHR12_20514952	26.9	133
	7	(Z)-3-nonen-1-ol	1	14,963,421	18,073,634	41.85	43.99	5.89	CHR01_14905497	19.8	92
	8	Decan-1-ol	15	15,570,482	17,009,996	43.95	52.55	7.4	CHR15_16157018	24.2	152
	9	(E,E)-2,4-heptadienal	19	19,881,449	21,925,778	48.87	52.2	7.93	CHR19_20030878	25.7	134
	10	(E)-hexen-2-al	19	15,804,112	21,733,333	44.69	52.2	6.88	CHR19_20748095	22.7	286
Aldehyde	11	(Z)-hexen-2-al	19	17,399,164	21,925,778	45.05	52.2	5.14	CHR19_20030878	17.5	242
	12	(E)-nonen-2-al	1	2,676,209	3,653,671	9.65	15.75	8.68	CHR01_2720122	27.8	100
	13	Ethylbenzene	2	11,073,778	19,214,008	47.83	60.84	5.53	CHR02_15537002	18.7	284
	14	Toluene	2	19,214,008	21,275,965	60.84	66.36	5.8	CHR02_20358829	19.5	110
	15	Benzyl alcohol	2	11,566,036	19,138,182	47.83	57.52	4.8	CHR02_15851855	16.4	265
Aromatic	16	2-Phenylethanol	2	14,128,650	21,083,470	55.1	64.07	6.52	CHR02_20358829	21.7	287
	17	Methyl hex-2-enoate	16	4,593,278	5,959,435	6.09	6.65	5.13	CHR16_5620349	17.5	47
	18	(E)-methyl hex-2-enoate	12	9,262,853	11,969,757	29.29	35.49	8.02	CHR12_10293679	25.9	259
	19	Ethyl acetate	12	8,693,726	10,063,473	27.14	29.29	5.56	CHR12_9262853	18.8	160

	20	(E)-ethyl but-2-enoate	10	8,393,861	10,470,145	15.36	21.1	4.98	CHR10_9422814	17	194
	21	Pentyl acetate	18	2,190,801	3,906,155	4.91	7.22	5.07	CHR18_2243935	17.3	153
Hydrocarbon	22	Tetradecane	2	11,363,813	14,717,925	47.83	54.29	5.14	CHR02_13179708	17.5	100
	23	4,6-dimethyl-heptan-2-one	14	5,819,037	10,973,137	11.07	19.48	5.69	CHR14_6754203	19.2	235
Ketone	24	(E)-methyl hex-2-enoate	2	20,300,502	21,206,376	64.07	65.22	6.19	CHR02_20852996	20.7	48
	25	Octan-3-one	14	18,823,692	23,119,473	29.94	38.05	5.73	CHR14_23482094	19.3	203
	26	Geraniol	6	4,724,259	6,544,650	19.05	27.79	11.65	CHR06_4994369	35.3	188
	27	Geraniol	12	8,138,436	11,657,909	27.14	35.49	5.74	CHR12_8540953	19.3	354
	28	Geraniol	15	15,798,851	17,017,873	44.67	51.84	6.6	CHR15_16157018	21.9	136
	29	(E)- β -ocimene	6	5,042,115	7,687,998	20.61	29.24	5.06	CHR06_6544650	17.2	287
	30	(E)-methyl geranate	12	10,063,405	15,681,332	29.29	41.56	6.49	CHR12_11337672	21.6	336
	31	(E)-methyl geranate	15	12,931,290	15,408,224	28.46	42.51	4.94	CHR15_13509146	16.9	219
Monoterpene	32	Hotrienol	2	5,077,251	6,341,438	27.28	33.3	5.58	CHR02_5291224	18.9	126
	33	Hotrienol	10	4,774,999	7,018,428	12.87	12.87	6.38	CHR10_6908938	21.3	219
	34	Nerol	10	3,870,491	5,568,232	12.85	12.87	6.76	CHR10_3995724	22.4	206
	35	γ -Isogeraniol	15	9,619,429	13,331,325	16.86	33.87	5.39	CHR15_11680187	18.3	258
	36	β -Citronellol	6	3,258,658	6,277,243	13.19	29.24	7	CHR06_4994369	23	297
	37	(3S)-Linalool	2	5,026,521	5,375,133	27.28	27.64	11	CHR02_5198993	33.8	41
	38	(3S)-Linalool	10	3,972,907	7,475,551	12.85	13.98	10.46	CHR10_5431663	32.4	368
Norisoprenoid	39	(Z)-Theaspirane	1	3,653,671	4,103,100	15.75	17.18	25.07	CHR01_4060900	60.9	36
	40	Dihydroactinidiolide	17	13,721,527	24,127,437	48.78	56.64	11.11	CHR17_24082626	34	327

	41	Cistus cyclohexanone	11	316,912	783,312	1.47	2.88	5.46	CHR11_338773	18.5	55
	42	Cistus cyclohexanone	12	1,668,330	6,362,655	8.64	20.09	5.8	CHR12_1668330	19.5	255
	43	Cistus cyclohexanone	18	6,636,103	7,977,021	20.09	25.92	7.43	CHR18_6525848	24.3	153
	44	(Z,E)- α -Farnesene	9	7,083,270	7,644,375	35.29	35.29	7.25	CHR09_7215558	23.8	30
	45	(Z,E)- α -Farnesene	14	28,849,280	31,295,985	58.79	63.8	5.12	CHR14_30214461	17.4	295
	46	(Z,E)- α -Farnesene	1	5,204,981	6,064,966	21.82	22.89	14.59	CHR01_5394295	42.1	83
	47	Dihydroedulan	18	2,147,296	4,008,254	4.91	6.85	11.01	CHR18_2688697	33.8	173
	48	Germacrene D	18	1,441,513	3,020,876	3.68	4.91	8.94	CHR18_2688697	28.4	125
	49	δ -Cadinene	2	6,997,965	9,134,829	37.34	41.37	5.55	CHR02_7523961	65.5	125
	50	PH1	13	20,312,618	22,962,230	47.37	51.7	23.93	CHR13_22872934	61.6	119
	51	PH2	13	20,313,457	22,962,230	47.37	51.7	29.08	CHR13_21136279	68.8	137
	52	PH3	13	19,225,568	23,236,187	44.51	52.77	17.54	CHR13_19871765	50.5	172
	53	PH4	13	16,798,759	22,962,230	43.8	51.7	14.68	CHR13_19603418	44.4	254
	54	PH5	2	5,077,251	5,566,371	27.28	27.64	8.01	CHR02_5198993	27.4	46
	55	PH5	10	3,995,724	5,748,943	12.86	12.87	19.15	CHR10_5486459	53.6	162
	56	PH6	2	1,379,492	6,341,438	8.16	33.3	6.87	CHR02_2008551	24.1	544
	57	PH6	10	3,903,774	8,393,861	12.85	15.36	5.75	CHR10_5422073	20.6	467
	58	PH6	15	14,702,159	16,690,679	42.51	48.99	7.01	CHR15_16157018	24.5	205
	59	PH8	5	22,463,101	24,772,382	53.93	57.42	5.54	CHR05_24327860	19.9	124

Sesquiterpene

**Monoterpene
Glycoside**

Confirmation of QTL effects on linalool and linalool glycoside abundance

Two major QTLs were identified for the accumulation of free (3*S*)-linalool and the highly correlated glycoside PH5 on Chr 2 and 10 (**Fig. 6A**). The Chr 2 QTL spanned ~0.34 Mb for both traits (0.35 cM), whereas the Chr 10 QTLs were broader, spanning 3.5 Mb for (3*S*)-linalool and 1.8 Mb for PH5 (1.13 cM). To assess allelic effects, we examined the relationship between compound abundance and genotype at peak markers within each QTL. On Chr 2, both traits shared the same peak marker (CHR02_5198993). On Chr 10, peak markers differed slightly (CHR10_5431663 for (3*S*)-linalool and CHR10_5486459 for PH5) within a broad interval encompassing >200 markers above the genome-wide significance threshold.

Linear models were used to estimate the proportion of variance explained by the markers. Modeling revealed strong additive effects and modest but significant interactions between markers. For (3*S*)-linalool, the additive model explained 38.4% of phenotypic variance, increasing to 44.4% when interactions were included. For PH5, marker effects were stronger, with the additive model explaining 61.8% of the variance. In both cases, the Chr 10 locus contributed more to phenotypic variation than the Chr 2 locus. For (3*S*)-linalool, CHR10_5431663 and CHR02_5198993 showed strong effects ($F = 87.1$ and 69.9 , respectively; $P < 10^{-15}$). For PH5, CHR10_5431663 was the primary predictor ($F = 322.0$; $P = 8.76 \times 10^{-50}$), followed by CHR02_5198993 ($F = 87.1$; $P = 2.08 \times 10^{-18}$). Given that the aglycone of PH5 was not experimentally confirmed, subsequent analyses focused on loci associated with free (3*S*)-linalool.

Genotype effects were consistent with additive contributions from both loci. Individuals heterozygous at both loci (AC/CT at CHR02_5198993 and CHR10_5431663) exhibited the highest (3*S*)-linalool levels, significantly exceeding all other genotype combinations. Heterozygosity at a single locus (AC/TT or AA/CT) resulted in intermediate levels (**Fig. 6B**).

Riesling is homozygous (AA) at CHR02_5198993, whereas Cabernet Sauvignon is homozygous (TT) at CHR10_5431663, suggesting that the C allele at these loci is derived from RHap2 (**Fig. 6C**). A subset of homozygous and heterozygous genotypes was selected for transcriptome and metabolite profiling in 2021.

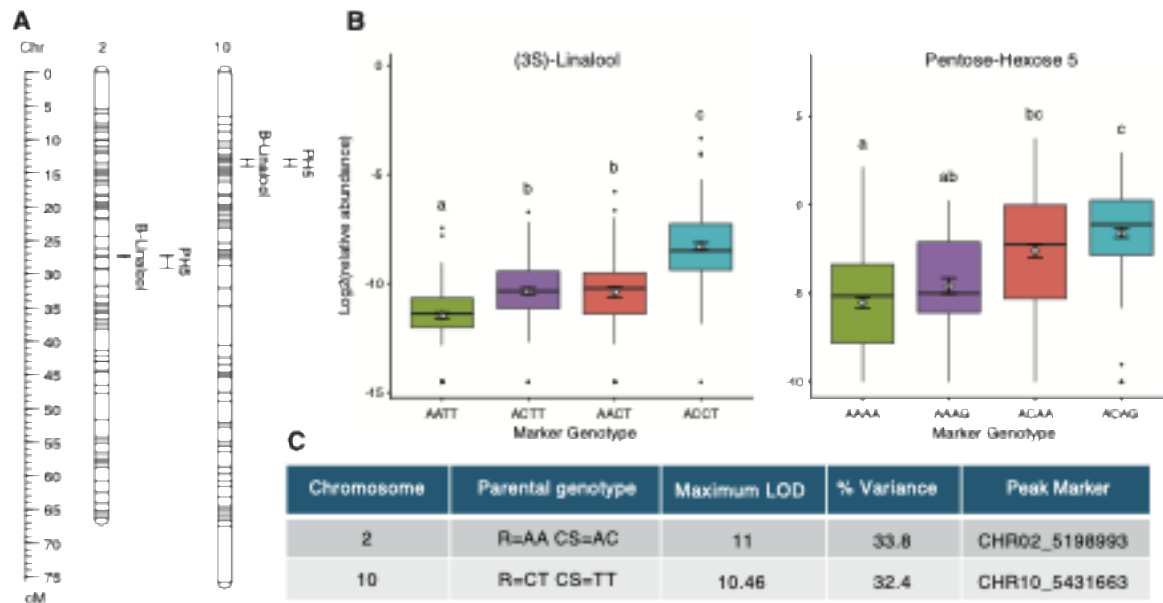


Figure 6. Marker effects of (3S)-linalool QTLs in the RxCS population. (A) Genetic maps of chromosomes 2 and 10 showing the positions of QTLs for (3S)-linalool and pentose-hexose glycoside 5 (PH5), with 95% LOD confidence intervals. (B) Distribution of (3S)-linalool (left) and PH5 (right) relative abundance across the RxCS population, grouped by genotype at peak markers within each QTL interval. Relative abundances for (3S)-linalool and PH5 are in octan-2-ol and decyl- β -D-glucopyranoside equivalents, respectively. Boxplots show median and interquartile range; points represent individual genotypes. Different letters indicate significant differences among genotype classes (Tukey's HSD, $P < 0.05$). (C) Summary of QTL statistics for (3S)-linalool, including chromosome location, parental genotype configuration, maximum LOD score, percentage of explained variance, and peak marker.

Integration of transcriptome and metabolite data to identify candidate genes for linalool accumulation

To aid in the identification of candidate genes associated with linalool accumulation, we sequenced berry transcriptomes of both parents and eight progeny in fall 2021 at two time points: 14 weeks post-anthesis (preharvest) and at harvest (15-17 weeks post-anthesis). Progeny were selected based on marker genotype and fruit pigmentation. At preharvest stage, 18,789 genes were

differentially expressed between Riesling and Cabernet Sauvignon ($|\log_2FC| > 1$, adjusted $P < 0.01$), increasing to 20,490 at harvest (**Supplementary Table S4**).

Berry samples collected at the same time points were analyzed for volatile and glycosylated compounds using HS-SPME-GC-MS and UHPLC-QTOF/MS. A total of 77 volatile compounds were detected, of which 65 differed significantly among genotypes (**Supplementary Table S5A, Figure 7**). 13 monoterpene glycosides were detected (**Supplementary Table S5B, Figure 7**). All compounds were detected at both developmental stages. Hierarchical clustering based on monoterpenoid and monoterpene glycoside abundance separated genotypes into three groups. Genotypes heterozygous (CT) at the CHR10_5431663 marker formed distinct clusters from homozygous individuals, although the individuals 95 and 106 grouped closer to TT genotypes. Nearly all monoterpenoid glycoside abundances were strongly correlated between years ($r^2 = 0.83-0.97$, adjusted $P < 0.003$, **Supplementary Table S6A**). Fifty-five volatile compounds were identified in both 2020 and 2021. With an r^2 value of 0.92 and adjusted P value of 0.0002, (3*S*)-linalool was also highly correlated between seasons, indicating consistent genotype-dependent abundance across years (**Supplementary Table S6B**). Over half of the other volatile compounds were moderately to strongly correlated between years ($r^2 = 0.42-0.76$) but many correlations did not remain significant after correcting for multiple testing (**Supplementary Table S6B**). A total of 31 compounds differed significantly between preharvest and harvest stages, with most increasing in abundance at harvest (**Supplementary Fig. S3, Supplementary Table S7**). Only five compounds were more abundant at preharvest, including the norisoprenoid (*Z*)-theaspirane and four lipoxygenase-derived compounds (e.g., (*Z*)-3-hexen-1-ol).

To integrate metabolite and transcriptome data, volatile and glycosylated monoterpenoid abundances were combined with gene expression data within the Chr 10 (3*S*)-linalool QTL interval

across all four haplotypes using multiple factor analysis (MFA). The first two dimensions explained 32.9% and 17.4% of the total variance, respectively (**Fig. 8A**). Individuals formed three clusters corresponding to marker genotypes at the Chr 2 and Chr 10 QTLs, with genotypes homozygous at CHR10_5431663 grouping together. The variable projection (**Fig. 8B**) showed strong alignment of monoterpenoids and monoterpene glycosides with multiple genes from RHap2, indicating coordinated variation between gene expression and metabolite accumulation. Among these, *VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680*, annotated as a (3S)-linalool/-nerolidol synthase, showed the strongest association with monoterpenoid abundance. In contrast, most genes from Cabernet Sauvignon haplotypes and RHap1 were projected in the opposite direction, suggesting haplotype-specific regulation.

We further assessed gene-metabolite relationships using Spearman correlation with (3S)-linalool content. Given previous reports linking MEP pathway genes to linalool accumulation in Muscat cultivars,⁶¹ we initially examined this pathway. However, *VviDXS1* expression was not significantly correlated with linalool levels. Only two genes showed weak positive correlations: CDP-ME kinase (*CMK*; *VITVvi_vRiesFPS24_v1.1.hap2.chr06.ver1.0.g409230*) and geranyl-diphosphate synthase (*GPPS*; *VITVvi_vRiesFPS24_v1.1.hap1.chr15.ver1.0.g235820*), at harvest and preharvest, respectively (**Supplementary Table S8**).

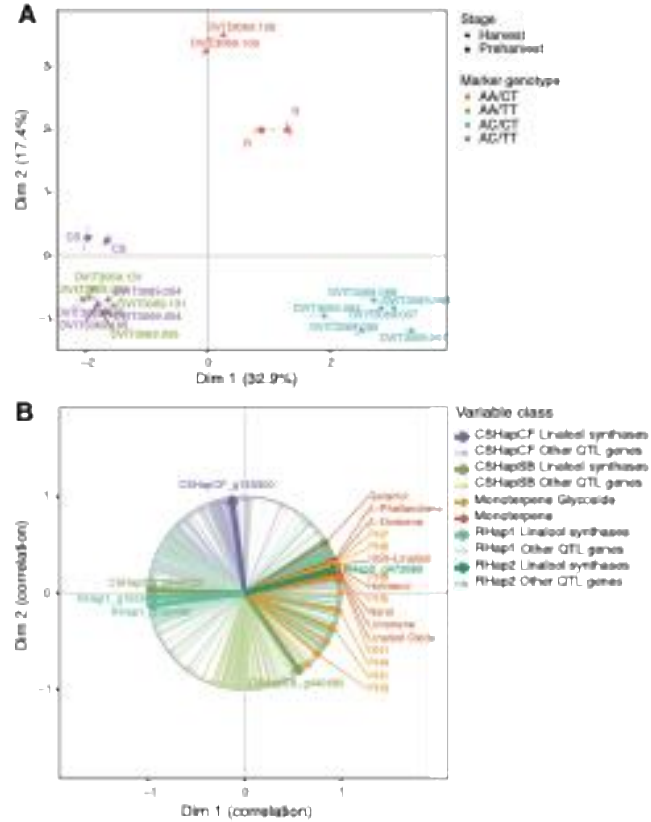


Figure 8. Multiple factor analysis (MFA) integrating monoterpenoids, monoterpene glycosides, and haplotype-specific gene expression within the linalool QTL on chromosome 10. (A) MFA individuals plot showing the distribution of RxCS progeny. Points represent individual samples, colored by marker genotype at Chr 2 and Chr 10 QTLs and shaped by developmental stage (preharvest or harvest). The first two dimensions explain 32.9% and 17.4% of total variance, respectively. (B) MFA variables plot showing correlations between metabolites and gene expression variables within the Chr 10 QTL interval. Only the top contributing monoterpenoids and monoterpene glycosides are labeled for clarity. Genes encoding (3S)-linalool/(E)-nerolidol synthases are highlighted in bold. Variable classes are color-coded by haplotype and functional category.

Identification of a candidate TPS gene within the Chr 10 (3S)-linalool QTL

The number of annotated genes within the Chr 10 QTL interval ranged from 299 to 397 across haplotypes (**Fig. 9A**). Each haplotype contained a cluster of 10-12 terpene synthase genes, annotated as (3S)-linalool/nerolidol synthases. After filtering out lowly expressed genes (TPM < 2 in at least 12 samples), 109-124 genes remained expressed within the interval across haplotypes. Spearman correlation analysis revealed marked differences among haplotypes in the number of genes associated with (3S)-linalool content (**Fig. 9B**). Ten or fewer genes from RHap1 and both Cabernet Sauvignon haplotypes³⁴ showed significant correlations, whereas 66 genes from RHap2

were significantly correlated, including 36 with strong associations ($\rho > 0.7$; adjusted $P < 4.2 \times 10^{-9}$; **Fig. 9C**; **Supplementary Table S9**). Genes with higher expression variance across samples tended to show stronger correlations, suggesting that transcriptional variability contributes to metabolite variation within the population.

The gene with the highest correlation across time points was *VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680* ($\rho = 0.86$; adjusted $P = 2.3 \times 10^{-16}$), annotated as a (3*S*)-linalool/nerolidol synthase. Both Spearman correlation and MFA independently identified this gene as the strongest candidate within the Chr 10 QTL. Although several neighboring genes showed similarly high correlation coefficients, they lack functional annotation related to terpene biosynthesis and are likely co-inherited within the same haplotype block. In contrast, none of the 20-25 genes in the Chr 2 QTL of each haplotype was significantly associated with (3*S*)-linalool (**Supplementary Table S10**).

Alignment of the *VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680* protein sequence against the monoterpene synthases annotated in *V. v. vinifera* PN40024 line showed 98.2% sequence identity with VIT_00s0385g00020 (VviTPS54/VviPNLinNer1), a functionally characterized (3*S*)-linalool/(*E*)-nerolidol synthase belonging to the TPS-g subfamily (VviTPS54 in Martin et al,¹⁵ (**Supplementary Table S11**). Multiple sequence alignment with Cabernet Sauvignon homologs and VIT_00s0385g00020 revealed high conservation at the amino acid level, with no major structural differences. However, the RHap1 homolog (*VITVvi_vRiesFPS24_v1.1.hap1.chr10.ver1.1.g155480*) appeared truncated at the N-terminus.

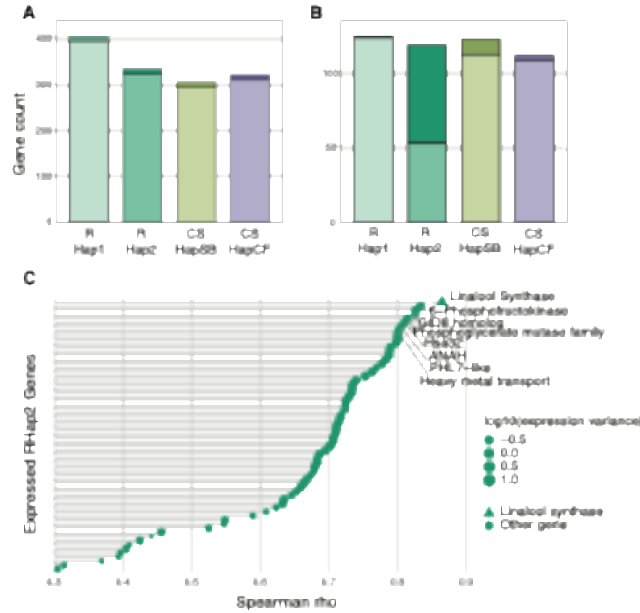


Figure 9. Identification of candidate genes associated with linalool accumulation within the chromosome 10 QTL interval. (A) Number of genes located within the Chr 10 QTL interval for each parental haplotype (RHap1, RHap2, CSHapSB, CSHapCF). (B) Number of genes expressed in berry tissue (TPM ≥ 2 in at least 12 samples) within each haplotype-specific interval. Darker segments indicate genes significantly correlated with linalool abundance (Spearman; adjusted $P < 0.05$). (C) Spearman correlation between gene expression and (3S)-linalool abundance for expressed RHap2 genes (TPM ≥ 2 in at least 12 samples). Each point represents a gene ranked by correlation coefficient (ρ). Point size reflects $\log_{10}$ -transformed expression variance across samples. Genes with $\rho > 0.3$ and adjusted $P < 0.05$ are shown; the top eight genes are labeled with functional annotations.

Analysis of N-terminal sequences across all four homologs did not identify a plastid-targeting peptide (**Supplementary Table S12**), consistent with previous observations that targeting signals are poorly conserved in grape TPS-g proteins despite their predicted plastid localization.¹⁵ All sequences retained the conserved DDxxD and NSE/DTE motifs characteristic of terpene synthases (**Supplementary Figure S4**).⁶²

Together, these results indicate that functional differences in (3S)-linalool accumulation are unlikely to be driven by major changes in protein sequence. Instead, the strong association between (3S)-linalool levels and expression of *VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680* suggests that transcriptional regulation is a key driver of the phenotype. To explore potential regulatory mechanisms, we analyzed transcription factor-binding sites within 3 kb up- and downstream of the gene across haplotypes

(Supplementary Table S13). Although differences in predicted binding site composition were observed for the RHap2 allele, the distribution of binding sites for known regulators of grape monoterpenoid biosynthesis such as bHLH and MYB family members was similar for the majority of haplotypes.^{63,64} However, correlation analysis identified several transcription factor genes differentially expressed between Riesling and Cabernet Sauvignon that were strongly associated with both (3S)-linalool content and expression of *VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680* (Table 4).

Table 4: Top 10 transcription factor genes exhibiting significant Spearman correlation (ρ) with (3S)-linalool content. All genes are significantly differentially expressed between Riesling and Cabernet Sauvignon.

Gene ID	Function	ρ	Adjusted <i>P</i>
VITVvi_vRiesFPS24_v1.1.hap1.chr10.ver1.0.g159690	trihelix transcription factor ASIL2-like	0.77	2.69 x10 ⁻³
VITVvi_vRiesFPS24_v1.1.hap1.chr10.ver1.0.g162350	S-adenosyl-L-methionine-dependent methyltransferases superfamily	0.79	1.20 x10 ⁻⁴
VITVvi_vRiesFPS24_v1.1.hap1.chr19.ver1.0.g303420	Probable WRKY transcription factor 31	0.79	4.30 x10 ⁻⁴
VITVvi_vRiesFPS24_v1.1.hap2.chr08.ver1.0.g434550	Transducin WD40 repeat-like superfamily	0.85	3.50 x10 ⁻⁴
VITVvi_vRiesFPS24_v1.1.hap2.chr08.ver1.0.g434460	MYB SANT-like DNA-binding domain	0.84	1.00 x10 ⁻⁴
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g471280	Heavy metal transport detoxification superfamily	0.82	3.90 x10 ⁻⁴
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g472350	transcription factor ILR3-like	0.8	1.10 x10 ⁻³
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g471950	MYB family transcription factor PHL7-like	0.79	1.76 x10 ⁻³
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g474280	Transcription factor	0.76	3.30 x10 ⁻³
VITVvi_vRiesFPS24_v1.1.hap2.chr19.ver1.0.g623080	probable RNA polymerase II transcription factor B subunit 1-1	0.77	4.00 x10 ⁻⁴

Discussion

The RxCS population segregated for a wide range of volatile compounds, including key grape monoterpenoids, sesquiterpenes, norisoprenoids, and phenylpropanoid derivatives. QTL analysis identified 49 significant loci associated with 40 volatile compounds, with an additional 10 loci linked to seven monoterpene glycosides. As expected, Riesling accumulated substantially higher monoterpenoid levels than Cabernet Sauvignon,²³ and several compounds exhibited transgressive segregation, consistent with multigenic control of volatile biosynthesis in grape.^{18,65}

QTLs for structurally or biosynthetically related compounds frequently colocalized, suggesting shared genetic control at multiple steps within biosynthetic pathways.

Much of the genetic variation in monoterpene accumulation in grape has been attributed to a gain-of-function mutation in *VviDXS1* on Chr 5, which increases precursor supply through the MEP pathway.⁶⁶ However, monoterpene biosynthesis is clearly multigenic, with multiple QTLs identified beyond this locus across different populations.^{18,65,67–69} Notably, 75% of aromatic non-Muscat cultivars lack functional *VviDXS1* mutations, indicating that other loci make substantial contributions to monoterpenoid accumulation.⁶⁹ In the RxCS population, no QTL on Chr 5 was associated with monoterpenoid content, and *VviDXS1* expression was not significantly correlated with terpene levels. This is consistent with prior work showing that introduction of the Muscat *VviDXS1* allele increases some monoterpenes without inducing linalool synthase expression or linalool accumulation⁶⁸ and with genetic evidence that linalool content can be inherited independently of total monoterpene content.¹³ Together, these findings support a two-step model in which precursor supply and monoterpene composition are controlled by distinct loci and confirm that Riesling's intermediate monoterpenoid accumulation is not explained by a *VviDXS1* gain-of-function allele.

The two major (3*S*)-linalool QTLs on Chr 2 and Chr 10 overlap loci consistently reported across diverse grapevine populations,^{3,13,65,67} suggesting they represent conserved genetic determinants of linalool accumulation. Each QTL explained more than 30% of phenotypic variation. Linear modeling of corresponding marker genotypes further showed that the markers accounted for 38.4% of variance in an additive model, increasing to 44.4% when marker interactions were included. Despite their repeated identification, candidate genes within these intervals had not previously been proposed, likely due to the highly duplicated and structurally

variable nature of TPS genes and the limitations of haploid reference genomes in resolving heterozygous loci.

Terpene synthases comprise a large and structurally complex gene family in grape, with 80 to over 200 members per cultivar.^{15,18,70} High heterozygosity and tandem duplication have historically hindered placement of individual TPS genes, leaving key linalool synthases *VviTPS54* and *VviTPS56* unplaced in earlier reference genomes.^{15,71} Using phased diploid assemblies, a *TPS-g* gene cluster including *VviTPS54* and *VviTPS56* was resolved on Chr 10,⁷¹ corresponding precisely to the Chr 10 QTL identified here. Functional assays have shown that *VviTPS56* alone is sufficient to produce (3*S*)-linalool, with *VviDXS1* coexpression increasing yield but not being required,⁶¹ supporting a model in which downstream terpene synthase activity, rather than *VviDXS1*-driven precursor supply, is the primary determinant of linalool accumulation in non-Muscat aromatic cultivars.

To identify candidate genes within the Chr 10 interval, we examined correlations between linalool and haplotype-resolved gene expression across the QTL region. The number of genes significantly correlated with (3*S*)-linalool content differed dramatically between haplotypes: 66 RHap2 genes showed significant correlation compared to ten or fewer in RHap1 and both Cabernet Sauvignon haplotypes. The gene with the highest correlation coefficient across both timepoints was the RHap2 homolog of *VviTPS54/VviPNLinNer1*, a functionally characterized (3*S*)-linalool/(*E*)-nerolidol synthase,¹⁵ a result corroborated by MFA. In Riesling, *VviTPS54* expression correlates with linalool content while *VviTPS56* declines toward the end of ripening,⁷² and both genes are expressed during later stages of berry development in parallel with linalool accumulation in aromatic cultivars,^{73,74} further supporting *VviTPS54* as the primary driver of linalool accumulation at harvest.

Protein sequence alignment across all four haplotypes revealed high conservation at key functional sites of *VviTPS54* (**Supplementary Figure 5**), including the DDxxD and NSE/DTE metal-binding motifs, with no major substitutions predicting altered enzymatic activity.^{16,75} The RHap1 homolog appeared truncated at the N-terminal domain, likely rendering it non-functional. Together these observations suggest that sequence divergence does not explain linalool accumulation differences between Riesling and Cabernet Sauvignon. Instead, the strong haplotype-specific correlation between *VviTPS54* transcript abundance and linalool content points to regulatory variation as the primary mechanism. Consistent with this, exogenous salicylic acid increased *VviTPS54* expression and (3S)-linalool content in Muscat Hamburg,⁷⁶ overexpression of *VvbZIP61* in *V. amurensis* calli increased monoterpenoid production,⁵ and *VvbZIP61* was differentially expressed in aromatic versus non-aromatic cultivars,⁵ suggesting that trans-acting regulators of TPS expression vary between cultivars.

The molecular basis for elevated *VviTPS54* expression in RHap2 remains unresolved. Transcription factor-binding site analysis in the 3 kb flanking regions of all four homologs identified differences proximate to RHap2, but none with established roles in monoterpenoid regulation.^{63,64} Several transcription factor genes differentially expressed between Riesling and Cabernet Sauvignon correlated with both (3S)-linalool content and *VviTPS54* expression, suggesting possible trans-regulatory control. Transcription factors located on Chr 2 belonging to the bHLH family have been shown to correlate with (3S)-linalool accumulation; however, these lie outside the QTL interval.⁶³ Epigenetic mechanisms such as differential DNA methylation could also contribute to haplotype-specific expression and cultivar- or clone-specific variation in monoterpenoid accumulation,⁷⁷ and copy number variation within the Chr 10 TPS cluster may further contribute to differences in total linalool synthase activity.¹⁸

Although no candidate gene was identified within the Chr 2 interval, this locus has been independently detected across multiple populations^{3,13,65,67,78} and made a significant additive contribution alongside Chr 10. One possibility is that it acts through a regulatory mechanism operating at an earlier developmental stage than those sampled here; additional time points spanning early berry development could help clarify its role. We observed a slight bias toward higher (3*S*)-linalool in unpigmented genotypes, suggesting a possible link to anthocyanin biosynthesis. However, *VviMYBA1* and *VviMYBA2* also map outside the Chr 2 QTL interval, and the same locus was identified in selfed progeny of Muscat Ottonel,¹³ which are all unpigmented, ruling out anthocyanin status as the source of variation at this locus. The bias toward unpigmented genotypes may instead reflect independent upregulation of linalool synthases: in Tempranillo blanco, *VviTPS54*, *55*, *58*, *60*, and *64* are upregulated relative to the pigmented Tempranillo tinto, with *VviTPS55* and *VviTPS60* also responding to UV-B during ripening.^{79,80} Whether anthocyanin status directly influences *VviTPS54* expression in the RxCS population could not be resolved with our RNA-Seq sample size and warrants further investigation.

The distribution of monoterpenoid content beyond (3*S*)-linalool reflects a more complex genetic architecture involving multiple loci and biosynthetic steps. Hotrienol and nerol oxide, which arise through enzyme-mediated oxidation and rearrangement of linalool,^{81,82} co-mapped with the Chr 2 and Chr 10 linalool QTLs, consistent with their metabolic relationship. Geraniol and its derivatives mapped to distinct loci on Chr 6, 12, and 15, indicating separate genetic control of different monoterpene end products. The strongly correlated glycoside PH5 shared both linalool QTLs, suggesting coordinated regulation of biosynthesis and glycosylation, while the Chr 13 glycoside QTL shared by PH1-4, lacking a corresponding free monoterpenoid QTL, may reflect variation in glycosyltransferase activity and should be addressed in future work.

Volatile QTL mapping in the full population was conducted using a single season of phenotypic data. Because volatile abundance in grape berries can be strongly influenced by environmental and vintage effects,^{83,84} multi-year phenotyping would help evaluate QTL stability.⁸⁵ However, linalool abundance measured in a second year in the transcriptome subset was strongly correlated with 2020 data, the detected QTLs overlap loci reported in other populations and environments, and the convergence of metabolite, transcriptomic, and comparative QTL evidence supports the Chr 10 locus as a recurrent genetic determinant of linalool variation rather than a season-specific artifact.

This study links (3*S*)-linalool accumulation in an intraspecific wine grape population to a terpene synthase cluster on Chr 10 and identifies *VviTPS54* as a strong candidate gene driven by regulatory rather than coding sequence variation. The phased Riesling genome assembly was critical for resolving this highly duplicated and heterozygous locus and demonstrates the value of haplotype-resolved references for dissecting complex quantitative traits in grape. Together, the integration of untargeted volatile metabolomics, high-density QTL mapping, and haplotype-resolved transcriptomics provides a framework for marker-assisted selection of aroma traits and for understanding monoterpenoid diversity across wine grape cultivars.

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Author contribution

DC and JL: conceptualization; DC and SE: supervision; JL and DC: writing; DC: funding acquisition; JL: experimentation; JL: data analysis and visualization; JL, MD-L: sampling; JL, MM, NC, LD-G: bioinformatic analysis; LL, JL: chemical analysis; RF-B: library preparation.

Conflict of interest

The authors declare no conflict of interest.

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Data Availability

All raw RNA-seq data are available on NCBI SRA under BioProject PRJNA1452220. The phased diploid genome assembly of Riesling and its structural annotation are deposited on NCBI under BioProject numbers PRJNA1453061 (Haplotype 1 and unplaced sequences) and PRJNA1453060 (Haplotype 2). Mass spectrometry data are available on Zenodo (DOI: 10.5281/zenodo.19747775).

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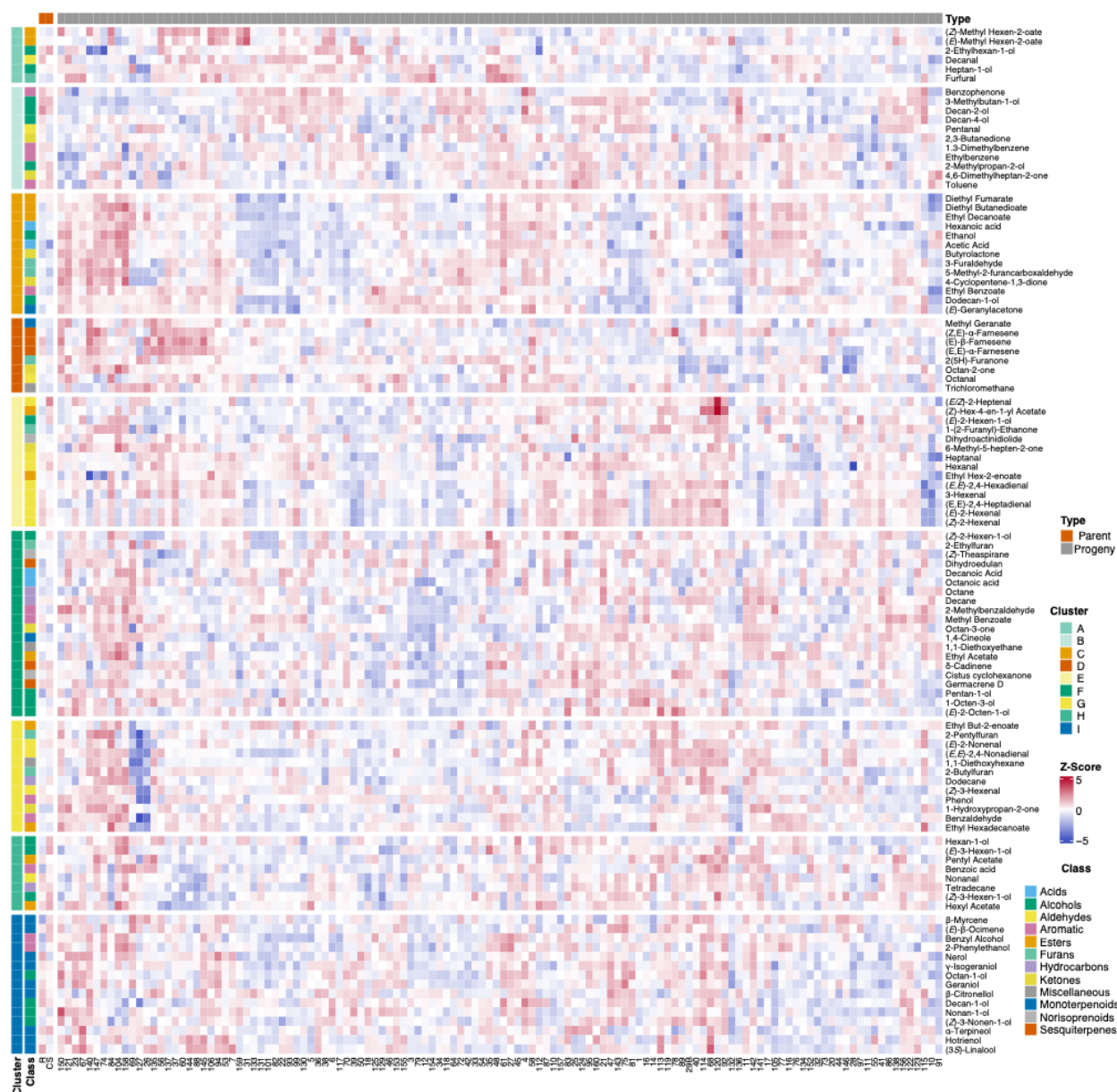
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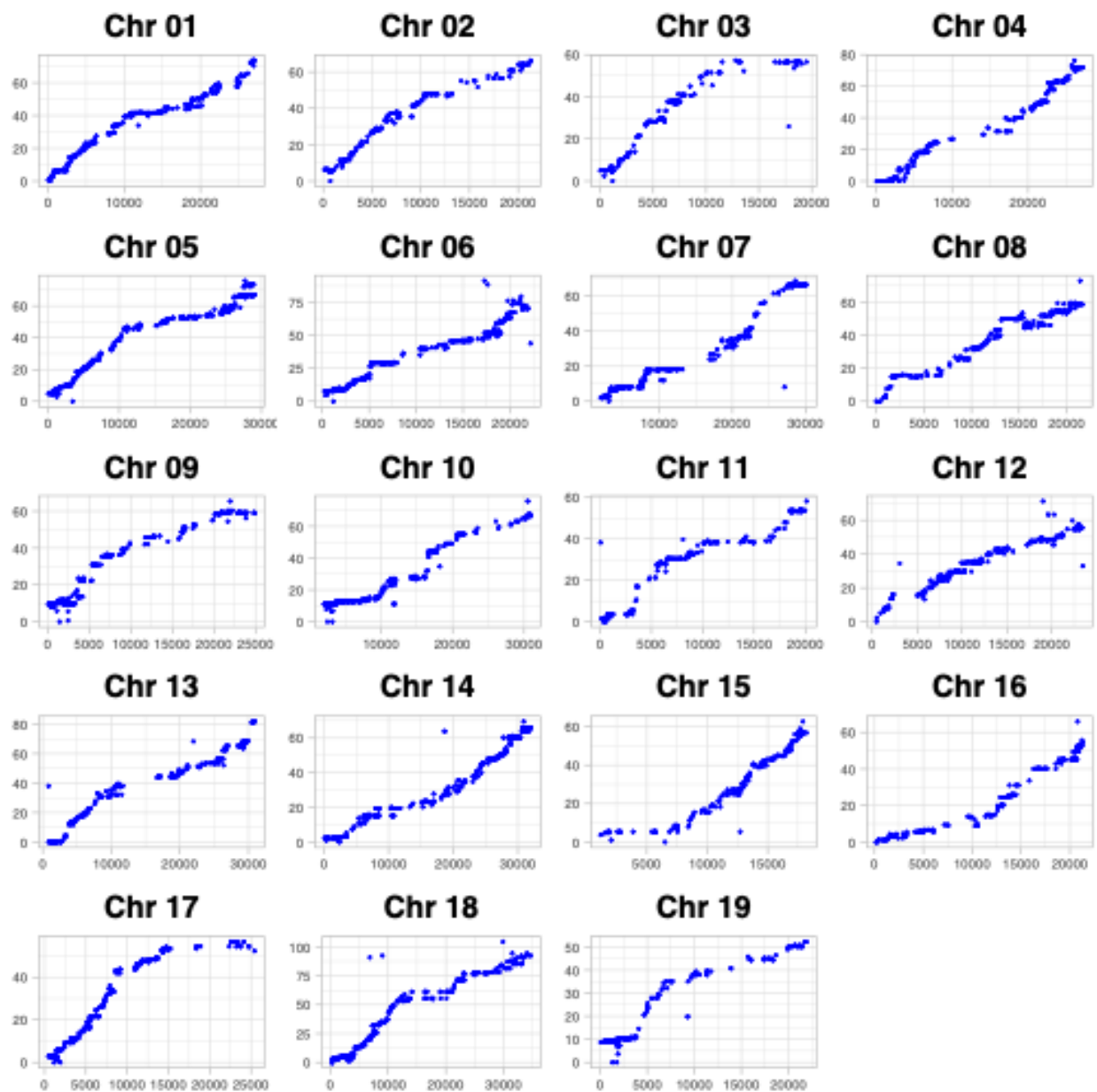
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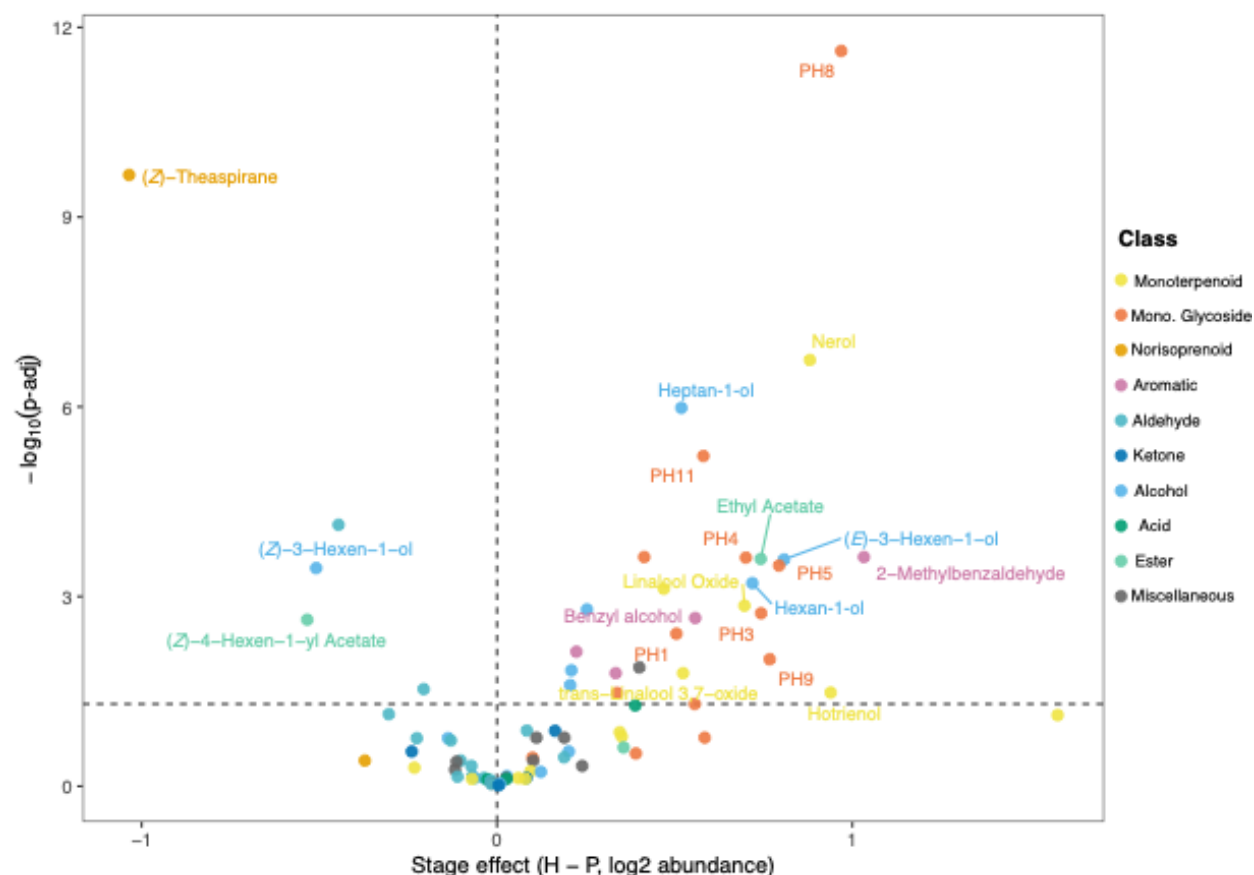
Supplementary Figures



Supplementary Figure S1: Z-score heatmap of 2020 berry volatile metabolites in the Riesling x Cabernet Sauvignon mapping population. Parental and progeny genotypes and volatile compounds are depicted in columns (n = 130) and rows (n = 110), respectively. Metabolites were grouped (Clusters A-I) by hierarchical clustering based on relative abundance across genotypes. Hierarchical clustering was performed using Euclidean distance and complete linkage. Metabolites are colored based on compound class.



Supplementary Figure S2: Relationship between physical and genetic positions of single-nucleotide polymorphism (SNP) markers across all 19 chromosomes. Genetic position (cM) is plotted against physical position (kb) for each chromosome. Physical coordinates are based on the *Vitis vinifera* cv. Riesling clone FPS24 Hap1 reference genome.



Supplementary Figure S3: Volcano plot showing the effect of ripening stage on volatile compound and monoterpene glycoside relative abundance across the Riesling $\times$ Cabernet Sauvignon population. The x-axis shows the $\log_2$ fold change in abundance between harvest and pre-harvest stages (H – P), while the y-axis shows $-\log_{10}$ adjusted p-values from statistical tests evaluating stage effects. Points represent individual volatile compounds, with labels shown for selected metabolites with strongest stage effects. Compounds are colored by chemical class. Positive values indicate compounds that increase in abundance at harvest, whereas negative values indicate compounds more abundant at pre-harvest.

VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	1	MACSCGFYVWSIPAVGSKNFREIGKTNFT	RIVPMNTHKWSIAHDHTLVS	50
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	1	MACSCGFYAWSIPAVGPNFREFIGKTNFT	RIVPMNTHKWSIAHDHTLVS	50
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	1	MPSCGLYAWSIPAVGPNKREFIGKTNFT	RIVPMNTHKWSIAHDHTLVS	50
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	1	MACSCGFYAWSIPAVGPNKREFIGKTNFT	RIVPMNTHKWSIAHDHTLVS	50
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	51	IPLKKDNHSPELGSGTDEFYNEHAQKLKEVKHVF	SKLGEDAMESMMMIDA	100
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801				
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	51	IPLKKDNHSPELGSGTDEFYNEHAQKLKEVKHVF	SKLGEDAMESMMMIDA	100
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	51	IPLKKDNHSPELGSGTDEFYNEHAQKLKEVKHVF	SKLGEDAMESMMMIDA	100
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	101	IQRLGIDHHFEEEEIEAVLQKQYMKSSIHGDCDEDLY	EVALRFRLLRQEGY	150
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	1	-----MKY	SIHGDCDEDLYEVALRFRLLRQEGY	28
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	101	IQRLGIDHHFEEEEIEAVLQKQYMKSSIHGDCDEDLY	EVALRFRLLRQEGY	150
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	101	IQRLGIDHHFEEEEIEAVLQKQYMKSSIHGDCDEDLY	EVALRFRLLRQEGY	150
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	151	TPADVLLNNFKNKEGKFKQNLREDIRGLMGLYEA	SQLSIGEDILEEAGNF	200
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	29	TPADVLLNNFKNKEGKFKQNLREDIRGLMGLYEA	SQLSIGEDILEEAGNF	78
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	151	TPADVLLNNFKNKEGKFKQNLREDIRGLMGLYEA	SQLSIGEDILEEAGNF	200
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	151	TPADVLLNNFKNKEGKFKQNLREDIRGLMGLYEA	SQLSIGEDILEEAGNF	200
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	201	SSLLLNACQLHLDHRHQAAYVKNTLEPHPHKSLAR	FMTKNFLTDFQGTNGW	250
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	79	SSLLLNACQLHLDHRHQAAYVKNTLEPHPHKSLAR	FMTKNFLTDFQGTNGW	128
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	201	SSLLLNACQLHLDHRHQAAYVKNTLEPHPHKSLAR	FMTKNFLTDFQGTNGW	250
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	201	SSLLLNACQLHLDHRHQAAYVKNTLEPHPHKSLAR	FMTKNFLTDFQGTNGW	250
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	251	INALQELAKIDFNVMVKSVHQKEMLQISKWVKDLGLT	EELKFARDQPLKWF	300
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	129	INALQELAKIDFNVMVKSVHQKEMLQISKWVKDLGLT	EELKFARDQPLKWF	178
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	251	INALQELAKIDFNVMVKSVHQKEMLQISKWVKDLGLT	EELKFARDQPLKWF	300
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	251	INALQELAKIDFNVMVKSVHQKEMLQISKWVKDLGLT	EELKFARDQPLKWF	300
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	301	MWPMAIIPNPRLSQRILELTKPISLIYIIDDFD	VGGTLDLFT EAVN	350
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	179	MWPMAIIPDPRLSQRILELTKPISLIYIIDDFD	VGGTLDLFT EAVN	228
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	301	MWPMAIIPDPRLSQRILELTKPISLIYIIDDFD	VGGTLDLFT EAVN	350
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	301	MWPMAIIPDPRLSQRILELTKPISLIYIIDDFD	VGGTLDLFT EAVN	350
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	351	RWDLSAFKELPEYMKMCFKALDDITNEISTKVHK	EHKWNPVGLSRKAWAS	400
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	229	RWDLSAFKELPEYMKMCFKALDDITNEISTKVHK	VHK-NPVGLSRKAWAS	277
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	351	RWDLSAFKELPEYMKMCFKALDDITNEISTKVHK	EHKWNPVGLSRKAWAS	400
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	351	RWDLSAFKELPEYMKMCFKALDDITNEISTKVHK	EHKWNPVGLSRKAWAS	400
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	401	LCNAFLVEAKWFA SGHVPKAE EYLKNGAVSSGVHVV	LVHLFLLGHGITK	450
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	278	LCNAFLVEAKWFA SGHVPKAE EYLKNGAVSSGVHVV	LVHLFLLGHGITK	327
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	401	LCNAFLVEAKWFA SGHVPKAE EYLKNGAVSSGVHVV	LVHLFLLGHGITK	450
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	401	LCNAFLVEAKWFA SGHVPKAE EYLKNGAVSSGVHVV	LVHLFLLGHGITK	450
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	451	DNVDLVDDFPFGIISSTATILRLWDDLGS	AKDEVDQDHDGSGYVECYLKEHR	500
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	328	DNVDLVDDFPFGIISSTATILRLWDDLGS	AKDEVDQDHDGSGYVECYLKEHR	377
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	451	DNVDLVDDFPFGIISSTATILRLWDDLGS	AKDEVDQDHDGSGYVECYLKEHR	500
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	451	DNVDLVDDFPFGIISSTATILRLWDDLGS	AKDEVDQDHDGSGYVECYLKEHR	500
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	501	GSSVENARQIVAHMISDMWKR LNKECLSPNPFST	SFTKGS LN IARMVPLM	550
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	378	GSSVENARQIVAHMISDMWKR LNKECLSPNPFST	SFTKGS LN IARMVPLM	427
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	501	GSSVENARQIVAHMISDMWKR LNKECLSPNPFST	SFTKGS LN IARMVPLM	550
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	501	GSSVENARQIVAHMISDMWKR LNKECLSPNPFST	SFTKGS LN IARMVPLM	550
VviTPS54/VviPNLinNer1				
VITVvi_vRiesFPS24_v1.1.hap2.chr10.g4726801	551	YSYDDQQLPLGLEEHMKSLLLENLPYRNAQKIN	-	583
VITVvi_vRiesFPS24_v1.1.hap1.chr10.g1554801	428	YSYDDQQLPLGLEEHMKSLLLENLPYRNAQKIN	E	461
VITVvi_vCabSauv08_v2.0.hapCF.chr10.g145220	551	YSYDDQQLPLGLEEHMKSLLLENLPYRNAQKIN	E	583
VITVvi_vCabSauv08_v2.0.hapSB.chr10.g440600	551	YSYDDQQLPLGLEEHMKSLLLENLPYRNAQKIN	E	584
VviTPS54/VviPNLinNer1				

Domains	Conserved Motifs
 PF01397: Terpene Synthase, N-Terminal Domain	RIVPMNTHKWS RR(X ₈)W motif
 PF03936: Terpene Synthase, metal-binding Domain	DDIFD DDxxD motif
	LWDDLGS AKDE NSE/DTE motif

Supplementary Figure S4: Multiple sequence alignment of TPS54 protein homologs across Riesling and Cabernet Sauvignon haplotypes. The functionally characterized (3S)-linalool synthase VvTPS54/VviPNLinNer is included as a reference. TPS54 homologs were identified using GMAP and phylogenetic placement of BLASTP results. Conserved terpene synthase domains are highlighted, including the N-terminal domain (yellow) and the C-terminal metal-binding domain (orange). Additional conserved motifs are indicated by boxed regions.

Supplementary Table S1: Volatile compounds detected in 2020 RxCS berry samples by headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS). Only compounds that differed significantly between genotypes in ANOVA (p-adj <0.05) are shown. Compounds are grouped by clusters formed during hierarchical clustering (Supplementary Figure S2). Kovats RI and retention time are averaged across all samples. Parental means and progeny min/max values are given in octan-2-ol equivalents and are therefore semiquantitative only.

Cluster	Compound Name	CAS #	Formula	MW	Class	Kovats RI	Retention Time	Quantifier	Qualifiers	Retking Mean	CS Mean	Progeny Min	Progeny Max	Broad-Sense Heritability
A	(E)-Methyl 2-Hexenoate	13894-63-8	C7H12O2	128.084	Ester	1294	17.72	55.1	41.1, 55.1, 97.1	8.66E-04	8.79E-04	2.39E-04	1.74E-02	0.72
	(Z)-Methyl 2-Hexenoate	2396-77-2	C7H12O2	128.084	Ester	1293	17.78	55	41, 55, 87	1.11E-03	8.32E-04	2.96E-04	2.67E-02	0.61
	1-Heptanol	111-70-6	C7H16O	116.12	Alcohol	1464	25.54	70.1	41.1, 56.1, 70.1	1.44E-03	1.79E-03	1.16E-05	3.81E-03	0.49
	Furfural	98-01-1	C5H4O2	96.021	Furan	1472	25.91	96.1	39.1, 95.1, 96.1	1.88E-04	1.75E-04	8.00E-06	2.12E-01	0.58
	2-Ethylhexan-1-ol	104-76-7	C8H18O	130.136	Alcohol	1497	27.11	57.1	41.1, 43.1, 57.1	1.52E-02	2.06E-02	5.94E-03	2.05E-02	0.7
	Decanal	112-31-2	C10H20O	156.151	Aldehyde	1502	27.36	57.1	41.1, 43.1, 57.1	5.24E-03	2.20E-03	7.59E-04	7.93E-03	0.43
	2-Methylpropan-2-ol	75-65-0	C4H10O	74.073	Alcohol	910	3.29	59.1	31.1, 43.1, 59.1	4.71E-03	9.52E-03	1.43E-04	1.63E-02	0.73
	Pentanal	110-62-3	C5H10O	86.073	Aldehyde	985	4.86	44	44, 57, 58	3.92E-03	3.50E-03	1.44E-03	1.24E-02	0.2
	2,3-Butanedione	431-03-8	C4H6O2	86.037	Ketone	994	4.99	43	42, 43, 86	3.32E-02	2.27E-02	2.30E-04	3.62E-01	0.23
	Toluene	108-88-3	C7H8	92.063	Aromatic	1042	6.66	91	65, 91, 92	2.76E-03	3.18E-03	7.51E-04	4.93E-03	0.61
B	1,3-Dimethylbenzene, Ethylbenzene	108-38-3	C8H10	106.078	Aromatic	1165	10.2	91.1	91.1, 105, 106.1	9.35E-04	1.00E-03	2.46E-05	2.71E-03	0.61
	4-Methylheptan-2-one	100-41-4	C8H16O	106.078	Aromatic	1138	10.5	91	50, 91, 106	9.26E-04	7.34E-04	7.91E-05	2.59E-03	0.61
	3-Methylbutan-1-ol	123-51-3	C5H12O	88.089	Alcohol	1214	13.75	43	43, 58, 59	1.14E-02	1.99E-02	2.68E-03	8.78E-02	0.52
	Decan-4-ol	2051-31-2	C10H22O	158.167	Alcohol	1584	31.01	55.1	55.1, 73.1, 97.1	6.32E-01	3.75E-01	7.72E-05	5.53E-01	0.61
	Decan-2-ol	1120-06-5	C10H22O	158.167	Alcohol	1526	32.2	45.1	45.1, 55.1, 69.1	9.89E-03	4.72E-03	1.74E-04	1.05E-02	0.53
	Benzophenone	119-61-9	C13H10O	182.073	Aromatic	2284	55.84	105	77.1, 105, 182.1	8.82E-03	4.42E-03	3.87E-05	8.97E-03	0.44
	Ethanol	64-17-5	C2H6O	46.042	Alcohol	936	3.85	31.1	31.1, 45.1, 46.1	1.86E-01	1.92E-01	1.09E-01	5.49E-01	0.71
	3-Furaldehyde	498-60-2	C5H4O2	96.021	Furan	1472	25.45	95.1	39.1, 95.1, 96.1	9.97E-04	7.48E-04	1.71E-04	3.53E-01	0.42
	Acetic acid	64-19-7	C2H4O2	60.021	Acid	1461	25.52	43	43, 45, 61	5.60E-01	2.72E-01	3.13E-01	1.32E-01	0.57
	5-Methyl-2-Furancarboxaldehyde	620-02-0	C6H8O2	110.037	Furan	1578	30.53	110.1	53.1, 109.1, 110.1	1.93E-04	8.06E-06	8.06E-06	1.06E-01	0.37
C	2-Cyclopentene-1,4-dione	930-60-9	C5H4O2	96.021	Ketone	1590	31.03	96.1	42.1, 68.1, 96.1	2.98E-04	1.32E-04	7.74E-06	2.01E-01	0.26
	Butyrolactone	96-48-0	C4H6O2	86.037	Ketone	1626	32.57	42	41, 42, 56	1.41E-03	8.20E-03	7.05E-04	1.76E-01	0.54
	Ethyl Decanoate	110-38-3	C12H24O2	200.178	Ester	1639	33.34	88.1	41.1, 88.1, 101.1	1.43E-03	4.08E-03	5.21E-04	2.15E-02	0.48
	Diethyl Fumarate	623-91-6	C8H12O4	172.074	Ester	127	33.72	127	99, 126, 127	1.73E-03	1.72E-03	5.53E-04	2.55E-03	0.52
	Ethyl Benzoate	93-89-0	C9H10O2	150.068	Aromatic	1668	34.32	105	77, 105, 122	2.16E-03	2.05E-03	1.61E-03	3.65E-03	0.71
	Diethyl Butanedioate	123-25-1	C8H14O4	174.089	Ester	1684	34.54	101	57, 101, 129	1.25E-03	1.65E-03	1.11E-03	2.62E-03	0.51
	Hexanoic acid	142-62-1	C6H12O2	116.084	Acid	1843	40.99	60	41, 55, 60	4.42E-02	6.01E-02	1.23E-02	1.94E-01	0.3
	(E)-Geranylacetone	3796-70-1	C13H22O	194.167	Monoterpene	1849	41.66	43.1	41.1, 43.1, 69.1	2.65E-03	8.67E-03	1.10E-03	1.16E-01	0.46
	Dodecan-1-ol	112-53-8	C12H26O	186.198	Alcohol	1975	45.75	55	43, 55, 69	2.02E-03	1.78E-03	9.17E-04	5.26E-03	0.39
	Trichloromethane	67-66-3	CHCl3	117.914	Miscellaneous	1031	6.2	83.1	47, 83.1, 85.1	7.46E-03	8.22E-03	5.68E-03	1.76E-02	0.48
D	Octan-2-one	111-13-7	C8H16O	128.12	Ketone	1287	17.48	43	43, 58, 71	2.29E-02	2.10E-02	1.30E-02	9.40E-02	0.5
	Octanal	124-13-0	C8H16O	128.12	Aldehyde	1290	17.7	43	41, 43, 44	1.80E-03	2.10E-03	9.64E-04	1.79E-00	0.27
	(E)- β -Farnesene	18794-84-8	C15H24	204.188	Sesquiterpene	1672	34.48	69	41, 69, 93	9.74E-04	1.24E-04	1.58E-05	2.41E-02	0.57
	(E)-Methyl Geranate	1189-09-9	C11H18O2	182.131	Monoterpene	1699	35.2	69.1	41.1, 69.1, 83.1	3.79E-04	3.20E-04	9.84E-06	4.54E-03	0.45
	(Z,E)- α -Farnesene	26560-14-5	C15H24	204.188	Sesquiterpene	1731	36.88	93	41, 69, 93	1.11E-04	3.90E-06	3.90E-06	2.31E-03	0.67
	2-(5H)-Furanone	497-23-4	C4H4O2	84.021	Furan	1748	37.66	55.1	39.1, 54.1, 55.1	7.44E-03	7.15E-03	1.64E-04	2.20E-01	0.34
	(E,E)- α -Farnesene	502-61-4	C15H24	204.188	Sesquiterpene	1749	37.73	93.1	55.1, 69.1, 93.1	2.19E-03	1.38E-04	7.09E-06	1.84E-02	0.85
	Hexanal	66-25-1	C6H12O	100.089	Aldehyde	1088	8.26	44	41, 44, 56	1.78E-00	2.49E-00	7.01E-01	3.22E+00	0.7
	Hexen-3-ol	4440-65-7	C6H12O	98.073	Aldehyde	1145	10.76	41	39, 41, 69	2.29E-02	1.53E-02	3.63E-03	4.68E-02	0.76
	Heptanal	111-71-7	C7H14O	114.104	Aldehyde	1188	12.75	70.1	41.1, 43.1, 70.1	2.72E-03	2.92E-03	1.03E-03	4.26E-03	0.69
E	(Z)-Hexen-2-ol	505-57-7	C6H12O	98.073	Aldehyde	1204	13.57	41	39, 41, 55	1.82E-02	1.30E-02	3.98E-03	2.83E-02	0.92
	(E)-Hexen-2-ol	6728-26-3	C6H12O	98.073	Aldehyde	1219	14.24	41	39, 41, 42	1.59E-00	1.18E-00	3.22E-01	2.19E+00	0.91
	(4Z)-4-Hexenyl Acetate	42125-17-7	C8H14O2	142.099	Ester	1321	19.37	43	43, 67, 82	9.84E-04	1.88E-02	2.96E-04	2.89E-01	0.73
	(E,Z)-Hepten-2-ol	18829-55-5	C7H12O	112.089	Aldehyde	1325	19.44	41	39, 41, 55	9.75E-04	3.35E-04	3.16E-02	3.16E-02	0.44
	6-Methyl-5-Hepten-2-one	110-93-0	C8H14O	126.104	Ketone	1341	20.04	43	41, 43, 69	2.11E-03	2.32E-03	1.12E-03	8.93E-03	0.51
	Ethyl 2-Hexenoate	1552-67-6	C8H14O2	142.099	Ester	1347	20.4	55.1	55.1, 73.1, 97.1	8.08E-03	1.05E-02	2.32E-03	3.62E-02	0.73
	(E,E)-2,4-Hexadienal	142-83-6	C6H8O	96.058	Aldehyde	1404	22.96	81.1	39.1, 81.1, 96.1	2.75E-02	2.24E-02	4.26E-03	7.40E-02	0.75
	(E)-2-Hexen-1-ol	928-95-0	C6H12O	100.089	Alcohol	1412	23.31	57	41, 57, 82	4.45E-02	9.81E-02	1.67E-02	1.74E-01	0.92
	(E,E)-2,4-Heptadienal	3/513	C7H10O	110.073	Aldehyde	1461	25.84	81	39, 53, 81	2.48E-03	2.03E-03	5.10E-04	4.68E-03	0.79
	1-(2-Furanyl)-Ethanol	1192-62-7	C6H6O2	110.037	Furan	1510	27.61	95.1	39.1, 95.1, 96.1	6.88E-04	3.26E-04	1.15E-05	3.63E-02	0.49
	Dihydroxyindriolide	17092-92-1	C11H16O2	180.115	Norsoprenoid	2247	54.31	111.1	109.1, 111.1, 137.1	1.87E-03	1.46E-03	6.25E-04	6.10E-03	0.81

Supplementary Table S1 (cont.)

Cluster	Compound Name	CAS #	Formula	MW	Class	Kovats RI	Retention Time	Quantifier	Qualifiers	Rising Mean	CS Mean	Progeny Min	Progeny Max	Broad-Sense Heritability
F	Octane	111-65-9	C8H18	114.141	Hydrocarbon	859	2.28	43.1	41.1, 43.1, 85.1	5.27E-03	9.95E-03	3.50E-03	7.63E-02	0.38
	1,1-Diethoxyethane	108-57-7	C6H14O2	118.099	Miscellaneous	902	3.14	45	43, 45, 103	4.93E-03	5.61E-03	5.30E-05	8.10E-02	0.87
	Ethyl Acetate	141-78-6	C4H8O2	88.052	Ester	901	3.17	43	43, 45, 61	6.61E-03	1.46E-02	2.04E-03	1.72E-01	0.78
	2-Ethylfuran	3208-16-0	C6H8O	96.058	Furan	957	4.1	81	53, 81, 96	9.19E-04	2.74E-03	6.49E-06	1.22E-02	0.61
	Decane	124-18-5	C10H22	142.172	Hydrocarbon	1006	5.33	57	43, 57, 71	2.69E-02	4.46E-02	2.20E-03	9.37E-02	0.89
	1,4-Cineole	470-67-7	C10H18O	154.136	Monoterpene	1172	12.17	43.1	43.1, 69.1, 71.1	6.42E-03	4.84E-03	5.32E-04	2.67E-02	0.67
	Pentane-1-ol	71-41-0	C5H12O	88.089	Alcohol	1254	15.99	42.1	41.1, 42.1, 57.1	7.49E-04	1.71E-03	7.09E-04	2.95E-03	0.39
	Octan-3-one	106-68-3	C8H16O	128.12	Ketone	1255	16.01	57.1	43.1, 57.1, 72.1	2.73E-03	1.80E-03	5.20E-04	4.20E-03	0.38
	Cistus Cyclohexanone	2408-37-9	C9H16O	140.12	Norisoprenoid	1310	18.63	82	56, 69, 82	1.56E-03	2.85E-03	2.90E-04	1.06E-02	0.92
	(1)-2-Hexen-1-ol	928-94-9	C6H12O	100.089	Alcohol	1415	23.67	57	41, 57, 82	1.15E-02	3.40E-02	2.01E-05	6.09E-02	0.61
	1-Octen-3-ol	3391-86-4	C8H16O	128.12	Alcohol	1458	25.38	57	43, 57, 72	9.53E-03	7.80E-03	4.15E-03	2.58E-02	0.88
	Thienatriene	36431-72-8	C13H22O	194.167	Norisoprenoid	1516	27.1	138	41, 82, 138	9.36E-03	8.34E-03	1.80E-04	7.85E-02	0.96
	Dihydrooctalin II	41678-32-4	C13H22O	194.167	Sesquiterpene	1514	27.52	179	41, 43, 179	4.68E-04	4.16E-03	1.33E-04	2.99E-02	0.77
	(E)-2-Octen-1-ol	18409-17-1	C8H16O	128.12	Alcohol	1623	32.4	57	41, 54, 57	2.60E-03	3.03E-03	1.02E-03	1.43E-02	0.8
	Methyl Benzoate	93-58-3	C8H8O2	136.052	Aromatic	1624	32.47	105	77, 105, 136	3.66E-03	2.86E-03	7.38E-04	7.16E-03	0.74
	2-Methylbenzaldehyde	529-20-4	C8H8O	120.058	Aromatic	1646	32.95	91	91, 119, 120	7.19E-03	6.04E-03	2.83E-03	4.55E-02	0.78
	Germaene D	23986-74-5	C15H24	204.188	Sesquiterpene	1702	35.77	161	79, 105, 161	2.37E-03	3.90E-03	1.31E-05	1.38E-02	0.91
	δ -Cadinene	483-76-1	C15H24	204.188	Sesquiterpene	1748	37.8	161.1	83.1, 119.1, 161.1	1.13E-03	1.30E-03	7.06E-05	4.68E-03	0.66
	Octanoic Acid	124-07-2	C8H16O2	144.115	Acid	2066	48.5	60	43, 60, 73	1.03E-02	8.80E-03	5.36E-04	1.30E-01	0.67
	n-Decanoic Acid	334-48-5	C10H20O2	172.146	Acid	2229	53.86	60	41, 60, 129	8.12E-03	9.43E-03	1.30E-03	1.59E-01	0.27
G	2-Butylfuran	4466-24-4	C8H12O	124.089	Furan	1136	10.31	81	81, 82, 124	3.88E-04	3.12E-04	1.26E-05	1.97E-03	0.46
	(Z)-Hexen-3-ol	6789-80-6	C6H10O	98.073	Aldehyde	1148	10.95	41	41, 55, 69	9.39E-03	6.14E-03	2.61E-05	2.87E-02	0.46
	Ethyl-(E)-2-Butenoate	623-70-1	C6H10O2	114.068	Ester	1162	11.48	69	39, 41, 69	5.25E-04	3.76E-04	2.11E-05	4.83E-03	0.45
	Dodecane	112-40-3	C12H26	170.203	Hydrocarbon	1199	13.22	57	43, 57, 71	2.27E-03	2.19E-02	9.69E-06	2.87E-02	0.25
	2-Pentylfuran	3777-69-3	C9H14O	138.104	Furan	1235	14.98	81	53, 81, 82	4.35E-03	4.42E-03	6.56E-06	1.31E-02	0.4
	1,1-Diethoxyhexane	3658-93-3	C10H22O2	174.162	Miscellaneous	1238	15.13	103.1	47, 75, 103.1	2.64E-02	2.98E-02	1.58E-05	9.04E-02	0.47
	1-Hydroxypropan-2-one	116-09-6	C3H6O2	74.037	Ketone	1305	18.29	43	31, 42, 43	2.14E-03	1.63E-03	5.47E-04	4.04E-00	0.42
	Benzaldehyde	100-52-7	C7H6O	106.042	Aromatic	1522	28.25	77	77, 105, 106	2.10E-03	2.49E-03	3.71E-04	5.42E-03	0.77
	(E)-Nonen-2-ol	18829-56-6	C9H18O	140.12	Aldehyde	1537	28.83	41	41, 43, 55	5.09E-03	3.14E-03	1.39E-04	1.27E-02	0.46
	(E,E)-2,4-Nonadienal	5910-87-2	C9H14O	138.104	Aldehyde	1703	35.69	81	39, 41, 81	2.76E-03	1.87E-03	4.53E-06	7.59E-03	0.53
H	Phenol	108-95-2	C6H6O	94.042	Aromatic	2014	47.1	94	65, 66, 94	4.07E-03	4.10E-03	1.57E-05	2.53E-02	0.47
	Ethyl Hexadecanoate	628-97-7	C18H36O2	284.272	Ester	2225	53.66	88.1	55.1, 73.1, 88.1	7.11E-04	4.14E-04	1.49E-05	1.28E-01	0.46
	Pentyl Acetate	628-63-7	C7H14O2	130.099	Ester	1176	12.4	43.1	43.1, 61, 70.1	1.37E-03	8.05E-04	2.60E-05	7.34E-03	0.41
	Hexyl Acetate	142-92-7	C8H16O2	144.115	Ester	1276	17.02	43	43, 55, 56	1.22E-02	1.60E-02	5.83E-04	5.72E-02	0.47
	Hexan-1-ol	111-27-3	C6H14O	102.104	Alcohol	1359	20.85	56	43, 55, 56	1.17E-01	2.44E-01	5.62E-02	3.52E-01	0.9
	(E)-3-Hexen-1-ol	928-97-2	C6H12O	100.089	Alcohol	1378	21.37	41	41, 67, 69	1.26E-03	5.33E-03	5.75E-04	2.03E-02	0.52
	(Z)-3-Hexen-1-ol	928-96-1	C6H12O	100.089	Alcohol	1455	22.25	67	41, 55, 67	2.28E-03	9.94E-03	3.05E-04	5.02E-02	0.5
	Nonanal	124-19-6	C9H18O	142.136	Aldehyde	1397	22.61	57	41, 56, 57	6.25E-02	3.25E-02	4.60E-03	1.14E-01	0.38
	Tetradecane	629-59-4	C14H30	198.235	Hydrocarbon	1403	22.88	57.1	41.1, 43.1, 57.1	5.94E-02	3.14E-02	2.18E-03	1.26E-01	0.44
	Benzoic acid	65-85-0	C7H6O2	122.037	Aromatic	2267	55.59	105	51.1, 105, 122	2.40E-04	1.42E-03	3.64E-05	5.50E-02	0.18
I	β -Myrcene	123-35-3	C10H16	136.125	Monoterpene	1166	11.68	41	41, 69, 93	3.85E-04	1.87E-03	4.01E-04	6.33E-03	0.15
	β -Ocimene	3779-61-1	C10H16	136.125	Monoterpene	1255	15.91	93	79, 91, 93	5.98E-04	8.52E-04	1.19E-05	2.65E-03	0.49
	(3S)-Linalool	78-70-6	C10H18O	154.136	Monoterpene	1556	29.64	71	55, 71, 93	1.04E-02	6.53E-04	1.33E-04	7.23E-02	0.9
	Octan-1-ol	111-87-5	C8H18O	130.136	Alcohol	1563	29.97	56.1	41.1, 55.1, 56.1	9.07E-03	8.11E-03	3.08E-03	2.16E-02	0.85
	Horienol	29957-43-5	C10H16O	152.12	Monoterpene	1616	32.23	71	43, 71, 82	1.16E-03	1.68E-04	1.74E-05	8.81E-03	0.71
	Nonan-1-ol	143-08-8	C9H20O	144.151	Alcohol	1666	34.2	56	41, 55, 56	7.46E-03	4.32E-03	2.97E-03	2.85E-02	0.92
	(Z)-3-Nonen-1-ol	10340-23-5	C9H18O	142.136	Alcohol	1644	35.14	55	41, 55, 68	5.38E-04	7.68E-04	2.06E-04	2.41E-03	0.71
	α -Terpinol	98-55-5	C10H18O	154.136	Monoterpene	1699	35.5	59.1	59.1, 93.1, 121.1	2.13E-03	2.42E-03	6.33E-04	5.45E-03	0.32
	Decan-1-ol	112-30-1	C10H22O	158.167	Alcohol	1759	38.2	55.1	41.1, 55.1, 70.1	4.73E-03	3.36E-03	5.55E-04	5.75E-02	0.5
	β -Citronellol	106-22-9	C10H20O	156.151	Monoterpene	1762	38.41	69	41, 67, 69	4.05E-03	2.08E-03	2.97E-04	3.92E-02	0.73
	γ -Isogeraniol	13066-51-8	C10H18O	154.136	Monoterpene	1779	39.17	69.1	41.1, 69.1, 111.1	2.04E-03	1.26E-03	1.09E-04	3.40E-03	0.74
	Nerol	106-25-2	C10H18O	154.136	Monoterpene	1789	39.64	69	41, 68, 69	5.24E-03	1.01E-03	4.13E-04	8.81E-03	0.73
	Geraniol	106-24-1	C10H18O	154.136	Monoterpene	1843	41.43	69	41, 68, 69	3.96E-02	2.58E-02	2.48E-03	1.75E-01	0.9
	Benzyl alcohol	100-51-6	C7H8O	108.058	Aromatic	1873	42.37	79	77, 79, 108	7.98E-04	2.55E-03	6.41E-04	4.49E-03	0.65
	2-Phenylethanol	60-12-8	C8H10O	122.073	Aromatic	1913	43.62	91	91, 92, 122	3.11E-03	6.01E-03	1.94E-03	1.68E-02	0.68

Supplementary Table S2: Volatile compounds detected by HS-SPME-GC-MS in 2020 RxCS berry samples that did not differ significantly between genotypes. Chemical identifiers (CAS number, formula, molecular weight), retention indices (Kovats RI), retention times, and quantifier and qualifier ions are shown for each compound.

Compound	Cas #	MW	Retention Time	Kovats RI	Quantifier	Qualifiers
Acetone	67-64-1	C3H6O	2.4	821.3	43.1	58.1,42.1,39.1
2,2,5,5-Tetramethyltetrahydrofuran	C8H16O	C8H16O	2.8	NA	113	44.1,70.1,41.1
2,4-Dimethyl-1-heptene	19549-87-2	C9H18	3	882.5	43.1	44.1,70.1,41.1
Nonane	111-84-2	C9H20	3.2	906.5	43.1	41.1,57.1,85.1
3-Methylbutanal	590-86-3	C5H10O	3.6	981.6	44.1	32,43.1,41.1
α -Thujene	52/67	C10H16	5.9	1022	93	91,92,77
Ethyl 2-Methylbutanoate	7452-79-1	C7H14O2	7.1	1050	57	29,85,102
α -Fenchene	471-84-1	C10H16	7.4	1056.3	93	91,92,77
Ethyl 3-Methylbutanoate	108-64-5	C7H14O2	7.7	1069	88	57,29,85
3-Methylbutyl acetate	123-92-2	C7H14O2	10	1132	43	70,55,73
2-Methylbutyl acetate	624-41-9	C7H14O2	10	1123	43	70,73,74
o-Xylene	95-47-6	C8H10	12.5	1180.4	91	106,105,79
α -Terpinene	99-86-5	C10H16	12.2	1178	121	93,136,91
p-Xylene	106-42-3	C8H10	12.6	1180.5	91	106,32,105
Limonene	138-86-3	C10H16	13	1178	68	93,67,94
Eucalyptol	470-82-6	C10H18O	13.4	1195	43.1	81.1,71.1,108.1
Ethyl Hexanoate	123-66-0	C8H16O2	15.1	1220	88	43,29,27
4,6-dimethylheptan-2-one	19549-80-5	C9H18O	15.5	1243.4	43	58,69,84
δ -Octalactone	698-76-0	C8H14O2	20.2	NA	42	57,41,55
Pent-1-ene	109-67-1	C5H10	16.1	1254.5	42	55,41,70
p-Cymene	99-87-6	C10H14	16.6	1261	119	134,91,120
(E)-2-(1-pentenyl)furan	20992-69-2	C9H12O	18.4	1304.9	79	68,136,107
1-Octen-3-one	4312-99-6	C8H14O	18.3	1301.7	55.1	70.1,97.1,48
4-Methylhexan-2-ol	2313-61-3	C7H16O	19.4	1324.6	45.1	59.1,43.1,55.1
Cyclamal	103-95-7	C13H18O	19.8	1332.3	119	133.1,190.1,105
3-Methylheptan-3-ol	5582-82-1	C8H18O	20.4	1346.9	73	45,55,101
cis-Rose oxide	16409-43-1	C10H18O	20.5	1338	139	55,41,69
2-Ethylhexyl Acetate	103-09-3	C10H20O2	22.3	1385.9	43.1	70.1,57.1,55.1
Octan-3-ol	589-98-0	C8H18O	22.8	1402.2	59.1	55.1,83.1,101.1
1,3-bis(1,1-Dimethylethyl)benzene	1014-60-4	C14H22	24.2	1428	175.1	55.1,57.1,190.1
Ethyl Octanoate	106-32-1	C10H20O2	24.6	1436	88	57,101,127
Linalool Oxide	5989-33-3	C10H18O2	25	1443	59	43,68,94
(Z)-3-Hexenyl Butanoate	16491-36-4	C10H18O2	25.7	1463.3	67.1	43.1,71.1,82.1
(E,E)-2,4-Heptadienal	3/5/13	C7H10O	25.8	1466.8	81	53,39,110
Nerol Oxide	1786-08-9	C10H16O	26	1479	68	67,83,85
Bicyclosesquiphellandrene	157477-72-0	C15H24	26.4	1488.3	119	105,161,93
Nonan-2-ol	628-99-9	C9H20O	28.4	1524.3	45	43,69,41
Propanoic acid	79-09-4	C3H6O2	29.3	1549.5	74	30,57,45
(E,Z)-2,6-Nonadienal	557-48-2	C9H14O	31	1586.2	41	39,69,70
Caryophyllene	87-44-5	C15H24	31.2	1594	93	133,91,41
2-Naphthyl methyl ketone	93-08-3	C12H10O	31.6	NA	170.2	155.1,127.1,141.1
Benzonitrile	100-47-0	C7H5N	31.8	1608.8	103	76,50,104
β -Cyclocitral	432-25-7	C10H16O	32.4	1598	41	39,137,152
2,5-Dihydro-3,5-dimethyl-2-furanone	1000196-88-1	C6H8O2	33.5	1650.5	41	39,112,69
Acetophenone	98-86-2	C8H8O	33.6	1650.5	105	120,77,51
Levomenthol	2216-51-5	C10H20O	33.3	1644.5	41	71,81,95
Dodecanal	112-54-9	C12H24O	36.2	1711.6	57.1	82.1,41.1,55.1
Citral	5392-40-5	C10H16O	36.9	1715	69	41,39,84
3-Hydroxymethylene-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	15051-75-9	C11H16O2	37.1	1736.4	138.1	83.1,95.1,41.1
2,5-Dimethyl-2,5-hexanediol	110-03-2	C8H18O2	38.5	1777.6	43	59,70,113
β -Phellandren-8-ol	65293-09-6	C10H16O	38.4	1768.6	59	94,79,0
Methoxyphenyloxime	67160-14-9	C8H9NO2	38.6	NA	133	151,135,134
p-Mentha-1,5-dien-7-ol	19876-45-0	C10H16O	39.4	1794.3	79	91,108,81
Tridecan-2-one	593-08-8	C13H26O	39.9	1798.9	58	43,59,71
3,5-Dimethylcyclohexanol	5441-52-1	C8H16O	40.3	1809	57	91,108,81
β -Damascenone	23726-93-4	C13H18O	40.3	1813	69	121,41,105
3-hydroxy-2,2,4-trimethylpentyl isobutyrate	77-68-9	C12H24O3	42.2	1871.7	71	56,89,43
(E)- β -Ionone	79-77-6	C13H20O	44.5	1912	177	43,91,135

Supplementary Table S2 (cont.)

Compound	Cas #	MW	Retention Time	Kovats RI	Quantifier	Qualifiers
Tetradecanal	124-25-4	C14H28O	44.1	1923.9	57	43,41,82
4-Morpholineethanamine	3/1/38	C6H14N2O	45.1	NA	100	101,56,43
Benzothiazole	95-16-9	C7H5NS	45.2	1951	135	108,69,82
(<i>E</i>)-2-Hexenoic acid	13419-69-7	C6H10O2	45.8	1980.7	73.1	41.1,55.1,42.1
γ -Amylbutyrolactone	104-61-0	C9H16O2	47.7	2042	85	41,43,29
Pentadecanal	316249	C15H30O	47.8	2029.9	57.1	82.1,43.1,41.1
(<i>E</i>)-Methyl cinnamate	1754-62-7	C10H10O2	49.3	2081.5	131.1	103,176,77
Ethyl Cinnamate	103-36-6	C11H12O2	51.1	2139	131	103,176,77
Hexadecanal	629-80-1	C16H32O	51.3	2134.6	57	82,43,41
Nonanoic acid	112-05-0	C9H18O2	52.3	2167.9	60	57,41,55
Methyl Anthranilate	134-20-3	C8H9NO2	53.5	2194	119	92,151,120
Ethyl Anthranilate	87-25-2	C9H11NO2	53.9	2232	119	57,206,192
5-Hydroxy-, 2,4-di-t-butylphenyl Pentanoate	166273-38-7	C19H30O3	54.1	2238.2	191	57,206,192
Geranic acid	459-80-3	C10H16O2	54.6	2230.8	69	41,100,39
(<i>E</i>)-Farnesol	106-28-5	C15H26O	54.6	2350	41	69,81,67
Methyl Vanillate	3943-74-6	C9H10O4	57	2607	151	182,123,152
Apocynin	498-02-2	C9H10O3	57.3	2640	151	166,123,43
1-Hexadecanol	36653-82-4	C16H34O	59.4	2419.3	43	83,69,97
Butyl Hexadecanoate	111-06-8	C20H40O2	59.7	2428.9	43	73,57,55

Supplementary Table S3: Summary statistics of the RxCS composite linkage map: The number of markers, total length in centimorgans (cM), mean inter-marker interval, and maximum gap between adjacent markers are shown for each chromosome.

Chromosome	Markers	Length (cM)	Mean interval	Max gap
1	324	73.65	0.23	4.7
2	227	66.36	0.29	5.49
3	168	57.27	0.34	4.34
4	306	76.31	0.25	5.02
5	378	75.8	0.20	5.05
6	335	91.34	0.27	9.03
7	309	68.8	0.22	6.46
8	275	72.94	0.27	13.56
9	235	65.42	0.28	7.28
10	289	75.77	0.26	8.21
11	205	58.23	0.29	6.2
12	281	71.28	0.25	7.92
13	243	81.75	0.34	12.48
14	402	69.55	0.17	3.61
15	201	62.69	0.31	3.59
16	205	65.93	0.32	10.27
17	284	56.64	0.20	5.78
18	333	104.43	0.31	9.75
19	186	52.2	0.28	5.29
Total	5186	1346.36	0.26	13.56

Supplementary Table S4. Differentially expressed genes in Riesling vs. Cabernet Sauvignon at pre-harvest (P) and harvest (H). Columns show functional description, baseMean, log₂ fold change, standard error, test statistic, P-values, and adjusted P-values for each timepoint. For space reasons, the table will be omitted here and available online

Supplementary Table S5A: Volatile compounds detected in 2021 berry samples at preharvest (14 weeks post anthesis, P) and harvest (15-17 weeks, H) timepoints. Compound class, chemical identifiers (CAS number, formula), retention time (min) and Kovats retention index (RI), quantifier and qualifier ions, and mean concentrations in parental cultivars (Riesling and Cabernet Sauvignon) and progeny (8 genotypes) are shown for each timepoint. Significance of genotype effects was determined by one-way ANOVA with P-adj <0.05. Abundances are shown in octan-2-ol equivalents.

Compound Class	Compound	CAS	Formula	Retention Time	RI	m/z	Qualifiers	R Mean (P)	R Mean (H)	CS Mean (P)	CS Mean (H)	Progeny Mean (P)	Progeny Mean (H)	Progeny Min (P)	Progeny Min (H)	Progeny Max (P)	Progeny Max (H)	Significant
Esther	Ethyl Acetate	141-78-6	C4H8O2	3.17	907	43	61,45,29	1.14E-02	2.56E-02	5.48E-02	2.00E-01	3.58E-02	4.12E-02	9.42E-03	1.98E-01	1.60E-02	9.81E-02	Yes
Aldehyde	Pentanal	110-62-3	C5H10O	4.74	988	44	41,58,57	4.60E-02	3.33E-02	1.63E-01	5.87E-02	6.02E-02	7.00E-02	2.79E-02	1.83E-01	2.53E-02	3.68E-01	Yes
Miscellaneous	Decane	124-18-5	C10H22	5.17	1006	57	43,71,41	1.64E-02	1.98E-02	1.66E-02	3.57E-02	1.70E-02	2.61E-02	2.99E-02	7.37E-02	7.85E-03	9.37E-03	Yes
Miscellaneous	Trichloromethane	67-66-3	CHCl3	6.08	1027	83,1	85,1,48,47	1.30E-02	1.81E-02	1.81E-02	2.28E-02	1.80E-02	1.95E-02	1.12E-02	1.39E-02	1.39E-02	3.15E-03	Yes
Aromatic	Toluene	108-88-3	C7H8	6.54	1039	91	92,65,63	2.39E-02	2.33E-02	4.20E-02	3.74E-02	3.14E-02	3.74E-02	1.57E-02	6.36E-02	2.14E-02	6.05E-02	Yes
Aldehyde	Hexanal	66-25-1	C6H12O	8.19	1084	44	56,41,43	1.83E-01	1.86E-01	2.18E-01	2.63E-01	2.07E-01	2.19E-01	1.50E-01	2.76E-01	1.48E-01	3.28E-01	Yes
Aromatic	1,3-Dimethyl-benzene	108-38-3	C8H10	10.20	1130	91,1	106,1,105,65,1	4.86E-03	4.98E-03	9.51E-03	5.40E-03	4.15E-03	4.70E-03	4.82E-04	1.56E-02	4.82E-04	8.29E-03	Yes
Furan	2-Ethylfuran	4466-24-4	C8H12O	10.31	1134	81	82,12,4,53	1.35E-02	8.73E-03	1.64E-02	1.97E-02	1.43E-02	1.78E-02	4.59E-03	2.38E-02	6.39E-03	3.22E-03	Yes
Aromatic	Ethylbenzene	100-41-4	C8H10	10.50	1130	91	106,105,50	3.02E-02	2.76E-03	1.27E-02	7.39E-03	5.03E-03	4.61E-03	1.81E-03	1.47E-02	2.42E-03	1.08E-02	Yes
Aldehyde	(E)-3-Hexenal	100-41-4	C6H10O	10.58	1141	41	69,39,55	2.47E-01	1.83E-01	1.83E-01	1.20E-01	3.48E-01	2.34E-01	1.65E-01	9.72E-01	1.04E-01	3.58E-01	Yes
Aldehyde	(Z)-3-Hexenal	6789-80-6	C6H10O	11.00	1150	41	39,69,55	2.60E-02	3.43E-02	1.77E-02	2.54E-02	3.38E-02	2.70E-02	1.47E-02	6.40E-02	2.52E-04	4.90E-02	Yes
Monoterpene	B-Myrcene	123-35-3	C10H16	11.68	1145	41	93,69,39	1.25E-02	2.71E-02	5.40E-03	6.34E-03	4.75E-03	8.31E-03	1.53E-03	2.04E-02	1.53E-03	4.71E-02	Yes
Monoterpene	1,4-Cineole	479-67-7	C10H18O	12.17	1179	43,1	69,1,111,1,71,1	3.26E-02	4.55E-02	1.55E-01	4.84E-02	4.96E-02	3.39E-02	4.28E-04	4.15E-01	4.28E-04	1.13E-01	Yes
Aldehyde	Heptanal	111-71-7	C7H14O	12.53	1185	70,1	43,1,44,1,41,1	4.71E-02	4.67E-02	5.51E-02	4.89E-02	5.23E-02	5.95E-02	3.25E-02	8.86E-02	3.55E-02	8.35E-02	Yes
Miscellaneous	p-Xylene	106-42-3	C8H10	12.62	1181	91	106,32,105	1.20E-02	1.25E-02	2.10E-02	1.92E-02	1.46E-02	1.49E-02	1.05E-02	2.61E-02	1.10E-02	2.20E-02	Yes
Monoterpene	Limonene	138-86-3	C10H16	12.98	1178	68	93,53,79	3.97E-03	1.11E-02	9.69E-03	2.10E-04	1.63E-03	2.75E-03	9.69E-05	1.32E-02	9.69E-05	2.08E-02	Yes
Aldehyde	(Z)-2-Hexenal	506-57-7	C6H10O	13.25	1202	41	83,69,55	3.10E-01	3.25E-01	3.05E-01	1.49E-01	3.00E-01	2.44E-01	1.20E-01	4.89E-01	1.28E-01	3.99E-01	Yes
Ketone	4-Methylheptan-2-one	6137-06-0	C8H16O	13.75	1206	43	58,85,79	2.62E-02	4.69E-02	4.69E-02	2.35E-02	2.53E-02	2.33E-02	6.19E-03	9.52E-02	8.82E-03	4.25E-02	Yes
Aldehyde	(Z)-Hexen-2-ol	6728-26-3	C6H12O	14.00	1201	41	55,69,83	2.27E-01	2.34E-01	2.27E-01	1.20E-01	2.11E-01	1.95E-01	3.41E-01	1.02E-01	1.02E-01	2.87E-01	Yes
Alcohol	3-Methylbutan-1-ol	123-51-3	C5H12O	14.04	1205	55	70,42,41	2.09E-01	2.23E-01	2.18E-01	1.12E-01	2.02E-01	1.86E-01	7.10E-02	9.30E-02	3.24E-01	9.30E-02	Yes
Furan	2-Pentylfuran	3777-69-3	C9H14O	14.72	1233	81	82,138,53	4.65E-02	3.12E-02	6.73E-02	4.62E-02	5.09E-02	5.18E-02	9.09E-03	1.00E-01	1.00E-01	1.04E-01	Yes
Miscellaneous	1,1-Dithoxyhexane	3658-93-3	C10H22O2	14.83	1235	103,1	129,1,47,75	5.99E-02	6.11E-02	6.30E-02	7.01E-02	6.86E-02	6.40E-02	3.70E-02	1.58E-01	4.19E-02	9.30E-03	Yes
Monoterpene	(E)-p-Citronene	3779-61-1	C10H16	15.91	1253	93	91,79,80	5.25E-03	1.58E-02	4.41E-04	2.83E-04	3.12E-03	4.67E-03	1.77E-04	1.57E-02	1.77E-04	2.97E-02	Yes
Alcohol	Pentan-1-ol	71-41-0	C5H12O	15.99	1258	42,1	55,1,57,1,41,1	1.00E-02	8.12E-03	1.13E-02	1.97E-02	1.16E-02	1.30E-02	5.40E-03	2.35E-02	5.91E-03	3.08E-02	Yes
Ketone	Octan-3-one	106-68-3	C8H16O	16.01	1251	57,1	43,1,72,1,42,1	8.49E-03	7.42E-03	1.13E-02	1.05E-02	1.06E-02	1.08E-02	3.34E-03	1.98E-02	4.11E-03	1.64E-02	Yes
Esther	Heptyl Acetate	142-92-7	C8H16O2	17.02	1270	43	56,69,61	3.17E-03	3.43E-03	6.07E-03	1.49E-02	9.20E-03	6.66E-03	6.54E-04	5.35E-02	6.54E-04	1.69E-02	Yes
Aldehyde	Octanal	124-13-0	C8H16O	17.70	1280	43	44,41,56	2.01E-02	1.56E-02	1.99E-02	1.64E-02	1.83E-02	1.86E-02	8.90E-03	3.33E-02	6.02E-03	3.29E-02	Yes
Ketone	1-Octen-3-one	4312-99-6	C8H14O	18.30	1302	55,1	70,1,97,1,48	2.22E-02	1.61E-02	2.36E-02	1.14E-02	1.82E-02	1.67E-02	3.74E-03	5.80E-02	4.26E-03	4.87E-02	Yes
Nonisoprenoid	Citrus Cyclohexanone	2408-37-9	C9H16O	18.63	1309	82	60,140,41	9.88E-04	1.75E-03	1.08E-03	1.06E-03	1.41E-03	1.15E-03	1.70E-04	4.38E-03	1.70E-04	5.11E-03	Yes
Esther	(Z)-4-Hexen-1-yl Acetate	42125-17-7	C8H14O2	19.37	1320	43	67,82,41	3.09E-02	1.79E-02	9.22E-02	8.23E-02	9.42E-02	5.21E-02	2.04E-02	3.42E-01	1.06E-02	1.25E-01	Yes
Aldehyde	(E)-Hepten-2-ol	18829-55-5	C7H12O	19.44	1322	41	27,39,55	4.66E-02	4.14E-02	4.40E-02	2.52E-02	4.47E-02	3.88E-02	1.14E-02	8.22E-02	6.80E-03	8.86E-02	Yes
Ketone	6-Methyl-5-Hepten-2-one	110-93-0	C8H14O	20.04	1339	43	41,108,69	1.05E-02	1.30E-02	1.03E-02	1.56E-02	1.35E-02	1.41E-02	6.45E-03	2.76E-02	5.49E-03	2.42E-02	Yes
Alcohol	Hexan-1-ol	111-27-3	C6H14O	20.85	1360	56	43,41,55	4.63E-01	4.32E-01	3.44E-01	1.44E-00	6.15E-01	9.11E-01	7.55E-02	2.51E-00	1.21E-01	2.36E-00	Yes
Alcohol	(E)-3-Hexen-1-ol	928-97-2	C6H12O	21.37	1368	41	67,69,82	3.25E-03	5.35E-03	4.01E-03	2.25E-02	6.03E-03	7.88E-03	1.03E-03	2.55E-02	1.94E-03	1.64E-02	Yes
Alcohol	(Z)-3-Hexen-1-ol	928-96-1	C6H12O	22.25	1395	67	41,39,55	5.66E-02	2.17E-02	1.80E-01	1.56E-01	1.67E-01	1.24E-01	1.50E-02	7.41E-01	2.08E-02	5.34E-01	Yes
Aldehyde	Nonanal	124-19-6	C9H18O	22.61	1394	57	41,43,56	4.51E-02	4.82E-02	3.74E-02	3.09E-02	4.16E-02	3.95E-02	1.90E-02	7.12E-02	2.04E-02	6.14E-02	Yes
Alcohol	Octan-3-ol	389-98-0	C8H18O	22.79	1402	59,1	83,1,55,1,101,1	5.45E-03	6.65E-03	8.92E-03	5.54E-03	6.98E-03	6.71E-03	2.77E-03	1.20E-02	2.46E-03	1.22E-02	Yes
Aldehyde	(E)-2,4-Hexadial	142-83-6	C6H8O	22.96	1403	81,1	39,1,96,1,67,1	3.24E-01	3.49E-01	3.63E-01	2.09E-01	3.33E-01	3.21E-01	1.10E-01	6.32E-01	1.34E-01	5.47E-01	Yes
Alcohol	(E)-2-Hexen-1-ol	928-95-0	C6H12O	23.31	1400	57	41,82,67	2.32E-01	2.37E-01	3.90E-01	6.45E-01	4.15E-01	3.64E-01	5.60E-02	1.83E-01	1.10E-01	8.38E-01	Yes
Monoterpene	(Z)-Limonol Oxide	5989-33-3	C10H18O2	25.31	1474	59	43,94,68	8.53E-03	8.53E-03	2.99E-04	2.99E-04	6.09E-03	9.84E-03	2.99E-04	4.48E-02	2.99E-04	7.44E-02	Yes
Alcohol	1-Octen-3-ol	3391-96-4	C8H16O	25.38	1449	57	43,72,85	6.75E-02	6.17E-02	5.91E-02	7.75E-02	5.34E-02	6.44E-02	3.25E-02	9.90E-02	3.94E-02	8.78E-02	Yes
Acid	Acetic acid	64-19-7	C2H4O2	25.52	1450	43	45,60,29	6.56E-02	9.38E-02	8.05E-01	3.15E-01	9.34E-02	1.31E-01	1.40E-02	2.20E-01	3.50E-02	6.94E-01	Yes
Aldehyde	Heptan-1-ol	111-70-3	C7H16O	25.84	1460	70,1	56,1,55,1,41,1	1.58E-02	2.42E-02	1.32E-02	2.10E-02	2.42E-02	3.31E-02	7.70E-03	5.86E-02	9.17E-03	7.64E-02	Yes
Aldehyde	(E)-2,4-Heptadial	4313-03-5	C7H10O	25.84	1467	81	110,39,53	4.39E-02	6.85E-02	4.56E-02	1.66E-02	4.57E-02	4.56E-02	1.69E-02	1.30E-01	7.77E-03	1.42E-01	Yes
Monoterpene	(E)-Limonol 3'-oxide	39028-58-5	C10H18O2	26.03	1731	68	59,94,93	2.39E-02	2.56E-02	1.09E-04	4.04E-04	3.71E-03	4.32E-03	1.09E-04	2.56E-02	1.09E-04	2.80E-02	Yes
Alcohol	2-Ethylhexan-1-ol	104-76-7	C8H18O	27.11	1495	57,1	41,43,1,55,1	3.24E-02	3.48E-02	3.69E-02	3.75E-02	3.78E-02	3.84E-02	2.90E-02	6.10E-02	2.94E-02	5.77E-02	Yes
Nonisoprenoid	(Z)-Theophrine	36431-72-8	C13H22O	27.72	1530	138	41,82,96	3.15E-02	1.43E-02	9.40E-03	2.85E-02	2.85E-02	1.72E-02	3.25E-02	8.24E-02	5.98E-04	6.03E-02	Yes
Aromatic	Benzaldehyde	100-52-7	C7H6O	28.25	1495	77	106,105,51	2.46E-02	2.55E-02	2.97E-02	3.05E-02	2.72E-02	3.21E-02	1.46E-02	5.55E-02	1.90E-02	5.13E-02	Yes

Supplementary Table S5A (cont.)

Compound Class	Compound	CAS	Formula	Retention Time RI	m/z	Qualifiers	R Mean (P)	R Mean (H)	CS Mean (P)	CS Mean (H)	Progeny Mean (P)	Progeny Mean (H)	Progeny Min (P)	Progeny Max (P)	Progeny Min (H)	Progeny Max (H)	Significant
Monoterpene	(3S)-Linalool	78-70-6	C10H18O	28.56	1537	71 93,80,43	5.82E-01	2.10E+00	1.42E-03	1.49E-03	2.76E-01	5.47E-01	2.98E-04	2.08E+00	2.98E-04	4.40E+00	Yes
Aldehyde	(E)-Nonen-2-al	18829-56-6	C9H16O	28.83	1534	41 70,55,43	9.02E-03	1.12E-02	6.05E-03	4.73E-03	7.51E-03	6.85E-03	1.66E-03	2.51E-02	1.62E-03	1.58E-02	Yes
Alcohol	Octan-1-ol	111-87-5	C8H18O	29.97	1562	56,1 84,1,41,1,69,1	4.79E-02	7.22E-02	3.58E-02	4.26E-02	7.53E-02	8.13E-02	2.94E-02	1.69E-01	2.93E-02	1.40E-01	Yes
Monoterpene	Horitolol	29957-43-5	C10H16O	32.23	1611	71 43,67,82	7.20E-03	2.38E-02	4.73E-05	4.73E-04	3.05E-03	8.54E-03	4.73E-05	2.29E-02	4.73E-05	7.45E-02	Yes
Aromatic	2-Methylbenzaldhyde	529-20-4	C8H8O	33.40	1646	91 119,120,65	5.65E-02	7.71E-02	4.34E-02	7.41E-02	4.06E-02	7.63E-02	6.16E-03	1.13E-01	1.59E-02	2.00E-01	Yes
Alcohol	Nonan-1-ol	143-06-8	C9H20O	34.20	1665	56 55,41,69	2.61E-02	3.00E-02	1.52E-02	1.45E-02	3.68E-02	3.72E-02	1.47E-02	7.90E-02	1.29E-02	7.71E-02	Yes
Alcohol	(Z)-3-Nonen-1-ol	10340-23-5	C9H18O	35.14	1689	55 41,68,81	2.71E-03	4.32E-03	3.62E-03	4.99E-03	5.19E-03	6.58E-03	9.70E-04	9.68E-03	9.70E-04	1.95E-02	Yes
Aldehyde	(E)-2,4-Nonadienal	5910-87-2	C9H14O	35.69	1701	81 67,41,39	3.94E-02	3.09E-02	4.10E-02	2.27E-02	3.47E-02	4.33E-02	5.48E-03	8.67E-02	7.86E-03	1.23E-01	Yes
Monoterpene	β -Phellandren-8-ol	6529-49-6	C10H16O	38.37	1769	59 94,79	1.28E-02	9.97E-03	1.05E-03	1.29E-04	6.45E-03	4.78E-03	1.29E-04	5.21E-02	1.29E-04	3.20E-02	Yes
Monoterpene	β -Citronellol	106-22-9	C10H20O	38.41	1762	69 41,55,81	6.06E-03	8.35E-03	6.56E-03	2.67E-03	6.65E-03	6.31E-03	2.59E-05	3.11E-02	2.59E-05	3.20E-02	Yes
Monoterpene	γ -Isogeraniol	13066-51-8	C10H18O	39.17	1792	69,1 41,1,11,1,1,93,1	4.18E-03	6.65E-03	1.90E-03	1.66E-03	5.60E-03	5.55E-03	6.28E-04	1.54E-02	6.28E-04	1.04E-02	Yes
Monoterpene	Neol	106-25-2	C10H18O	39.64	1770	69 41,93,53	6.76E-03	2.01E-02	2.05E-03	3.14E-03	5.77E-03	1.03E-02	1.05E-03	2.98E-02	1.89E-03	4.08E-02	Yes
Monoterpene	Geraniol	106-24-1	C10H18O	39.68	1834	69 41,93,53	8.99E-02	1.48E-01	5.01E-02	4.00E-02	1.06E-01	1.12E-01	1.57E-02	2.50E-01	2.10E-02	2.69E-01	Yes
Acid	Hexanoic acid	142-62-1	C6H12O2	41.51	1851	60 41,73,87	3.52E-02	3.66E-02	4.76E-02	4.42E-02	3.99E-02	4.09E-02	2.41E-02	6.64E-02	2.92E-02	8.06E-02	Yes
Aromatic	Benzyl alcohol	100-51-6	C7H8O	43.20	1865	79 108,107,77	1.75E-03	3.89E-03	6.81E-03	1.23E-02	4.67E-03	6.15E-03	1.11E-03	1.38E-02	1.88E-03	1.27E-02	Yes
Aromatic	2-Phenylethanol	60-12-8	C8H10O	43.62	1925	91 92,65,122	1.19E-02	1.66E-02	2.35E-02	3.04E-02	2.01E-02	2.53E-02	9.12E-03	5.53E-02	1.10E-02	6.43E-02	Yes
Acid	(E)-2-Hexenoic acid	13419-69-7	C6H10O2	45.84	1981	73,1 41,1,55,1,42,1	1.62E-02	1.68E-02	1.90E-02	1.50E-02	1.59E-02	1.59E-02	7.81E-03	2.59E-02	8.22E-03	2.43E-02	Yes
Miscellaneous	Octane	111-65-9	C8H18	2.19	860	43,1 85,157,1,41,1	3.23E-02	9.08E-03	1.11E-02	1.95E-02	1.24E-02	2.50E-02	1.10E-03	5.57E-02	1.10E-03	2.51E-01	No
Miscellaneous	Nonane	111-84-2	C9H20	3.22	907	43,1 57,1,41,1,85,1	3.21E-02	3.71E-02	4.47E-02	1.08E-01	5.51E-02	4.75E-02	2.36E-03	1.99E-01	2.69E-03	1.68E-01	No
Ketone	2,3-Butanedione	431-03-8	C4H6O2	5.07	970	43 86,42,1,5	2.67E-02	8.89E-02	6.11E-02	7.90E-02	4.51E-02	3.97E-02	7.42E-03	1.85E-01	5.23E-03	1.31E-01	No
Ketone	4,6-Dimethylheptan-2-one,	19549-80-5	C9H18O	15.52	1243	43 58,84,69	5.30E-03	6.37E-03	8.88E-03	5.48E-03	6.57E-03	6.24E-03	1.10E-03	1.66E-02	1.10E-03	1.33E-02	No
Ketone	Octan-2-one	111-13-7	C8H16O	17.48	1284	43 58,71,59	2.56E-02	2.38E-02	3.09E-02	2.45E-02	2.73E-02	2.64E-02	2.05E-02	3.57E-02	1.93E-02	5.87E-02	No
Furan	Furfural	98-01-1	C5H4O2	25.91	1472	96,1 95,1,39,1,42,1	3.92E-04	3.55E-03	9.73E-02	7.30E-04	1.01E-03	1.93E-02	1.48E-04	5.88E-03	1.48E-04	7.46E-01	No
Aldehyde	Decanal	112-31-2	C10H20O	27.36	1499	57,1 41,1,43,1,55,1	4.54E-03	5.98E-03	5.35E-03	2.64E-03	5.10E-03	4.57E-03	1.16E-03	1.59E-02	1.16E-03	1.05E-02	No
Aromatic	Methyl Benzoate	93-58-3	C8H8O2	32.47	1622	105 77,136,51	3.05E-02	3.65E-02	3.71E-02	3.43E-02	3.21E-02	3.77E-02	1.26E-02	5.64E-02	1.56E-02	7.22E-02	No
Ester	Ethyl Decanoate	110-38-3	C12H24O2	33.34	1646	88,1 101,1,60,1,41,1	1.84E-02	4.40E-02	5.84E-02	3.82E-02	3.40E-02	6.39E-02	9.95E-03	1.88E-01	8.50E-03	4.74E-01	No
Aromatic	Phenol	108-95-2	C6H6O	47.10	2019	94 66,65,40	1.66E-02	1.65E-02	2.04E-02	1.91E-02	1.70E-02	1.79E-02	1.14E-02	2.51E-02	1.21E-02	2.98E-02	No
Aromatic	Benzoic acid	65-85-0	C7H6O2	55.33	2258	105 122,77,151,1	8.66E-03	9.06E-03	1.97E-02	8.86E-03	9.21E-03	1.19E-02	1.34E-02	2.08E-02	2.87E-03	7.39E-02	No
Aromatic	Benzophenone	119-61-9	C13H10O	55.84	2270	105 77,1,182,1,51,1	3.16E-03	5.33E-03	2.69E-03	2.73E-03	3.38E-03	3.75E-03	1.39E-03	7.89E-03	6.95E-04	8.44E-03	No

Supplementary Table S5B: 2021 Monoterpene glycosides measured at pre-harvest (P) and harvest (H) in a subset of the Riesling $\times$ Cabernet Sauvignon population, showing mean concentrations in parental cultivars (Riesling and Cabernet Sauvignon) and progeny (8 genotype) means and ranges at each timepoint. Abundances are shown in decyl- β -D-glucoside equivalents.

Compound	R Mean (P)	R Mean (H)	CS Mean (P)	CS Mean (H)	Progeny Mean (P)	Progeny Mean (H)	Progeny Min (P)	Progeny Min (H)	Progeny Max (P)	Progeny Max (H)
DH1	4.8951	7.2923	0.0063	0.0384	1.7728	3.1431	0.0167	0.0007	9.5819	13.8913
PH1	1.0339	1.1661	0.1998	0.2618	1.0039	1.3494	0.0596	0.0977	3.9414	4.3694
PH2	1.1548	1.0827	0.1500	0.2207	0.8828	0.9815	0.0562	0.0862	3.6616	3.6240
PH3	0.0501	0.0748	0.0163	0.0191	0.0613	0.0970	0.0016	0.0052	0.2129	0.3479
PH4	0.2818	0.4920	0.0044	0.0062	0.1926	0.3841	0.0033	0.0052	1.2202	2.3517
PH5	5.8962	9.5177	0.0213	0.0644	2.9800	4.0666	0.0263	0.0205	18.1561	19.5717
PH6	0.5756	0.9540	0.0001	0.0002	0.2613	0.3493	0.0001	0.0001	1.5629	1.7708
PH7	2.7230	4.0194	0.0016	0.0123	0.8650	1.0108	0.0035	0.0003	4.6999	4.8524
PH8	0.3166	0.4434	0.0599	0.1165	0.2185	0.4859	0.1273	0.2285	0.6067	1.6404
PH9	0.8517	1.3184	0.0004	0.0042	0.3083	0.4060	0.0004	0.0004	1.9280	2.1028
PH10	1.1337	1.2364	0.3534	0.4449	1.1233	1.6266	0.5422	0.5937	1.9133	3.3312
PH11	0.0932	0.1116	0.0301	0.0419	0.1077	0.1631	0.0497	0.0763	0.1983	0.3610
PH12	0.7020	0.5895	0.2425	0.2781	0.8325	0.9502	0.3382	0.3462	2.2418	2.4753

Supplementary Table S6A: Pearson Correlation between shared monoterpene glycosides in 2020–2021. Parental cultivars and 8 progeny were sampled. P-values were adjusted using Benjamini-Hochberg correction.

Compound	r-squared	P value	P-adj
PH9	0.97	3.63E-06	4.14E-05
PH5	0.96	6.37E-06	4.14E-05
PH7	0.96	1.50E-05	6.23E-05
PH2	0.95	2.02E-05	6.23E-05
PH1	0.95	2.40E-05	6.23E-05
DH1	0.92	0.0001	0.0003
PH4	0.91	0.0003	0.0006
PH3	0.87	0.0011	0.0017
PH10	0.87	0.0012	0.0017
PH8	0.85	0.0021	0.0027
PH6	0.83	0.0027	0.0032
PH12	0.64	0.0465	0.0504
PH11	0.41	0.2345	0.2345

Supplementary Table S6B: Pearson Correlation between shared volatile compounds in 2020–2021. Parental cultivars and 8 progeny were sampled. P-values were adjusted using Benjamini-Hochberg correction.

Compound	CAS Number	r-squared	P	P-adj
B-Linalool	78-70-6	0.92	0.00016188	0.0047
(Z)-Thespirane	36431-72-8	0.91	0.00022659	0.0047
Nerol	106-25-2	0.91	0.00026622	0.0047
1-Nonanol	143-08-8	0.90	0.00036204	0.0048
Geraniol	106-24-1	0.76	0.0106555	0.1129
1-Hexanol	111-27-3	0.73	0.01702292	0.1504
1-Octanol	111-87-5	0.70	0.02392483	0.1811
(E,E)-2,4-Nonadienal	5910-87-2	0.63	0.05168506	0.3110
Heptanal	111-71-7	0.62	0.05746459	0.3110
(Z)-3-Nonen-1-ol	10340-23-5	0.61	0.06054024	0.3110
γ -Isogeraniol	13066-51-8	0.60	0.06453956	0.3110
(E)-Hexen-3-ol	4440-65-7	0.58	0.07697654	0.3182
Acetic acid	64-19-7	-0.58	0.07805233	0.3182
(E,E)-2,4-Heptadienal	881395	0.55	0.09783128	0.3704
β -(E)-Ocimene	3779-61-1	0.54	0.11065598	0.3910
(Z)-4-Hexen-1-yl Acetate	42125-17-7	0.51	0.13118315	0.4157
Benzyl alcohol	100-51-6	0.51	0.1364859	0.4157
(E)-3-Hexen-1-ol	928-97-2	0.50	0.1416837	0.4157
(Z)-Hexen-2-ol	505-57-7	0.48	0.16128243	0.4377
Pentanol-1-ol	71-41-0	0.48	0.16515605	0.4377
Hexanal	66-25-1	0.46	0.18602336	0.4660
(E,E)-2,4-Hexadienal	142-83-6	0.43	0.2092014	0.4660
Octan-3-one	106-68-3	0.43	0.21125355	0.4660
2-Phenylethanol	604-12-8	0.42	0.22315266	0.4660
2-Ethylhexan-1-ol	104-76-7	-0.42	0.22625494	0.4660
Hexyl Acetate	142-92-7	0.42	0.22861973	0.4660
3-Methylbutan-1-ol	123-51-3	-0.39	0.25862785	0.5077
(Z)-3-Hexen-1-ol	928-96-1	0.37	0.29768899	0.5635
Toluene	108-88-3	-0.35	0.328658	0.6007
6-Methyl-5-hepten-2-one	110-93-0	-0.29	0.41000739	0.7243
(Z)-Hexen-3-ol	6789-80-6	0.28	0.43912134	0.7508
Cistus cyclohexanone	2408-37-9	-0.25	0.48684715	0.8063
Trichloromethane	67-66-3	0.24	0.50884546	0.8172
Octanal	124-13-0	0.23	0.5247318	0.8180
Decane	124-18-5	-0.20	0.57885051	0.8765
Heptan-1-ol	111-70-6	0.19	0.59853508	0.8812
2-Methylbenzaldehyde	529-20-4	0.18	0.61898929	0.8849
(E)-Hexen-2-ol	6728-26-3	0.17	0.63447762	0.8849
Nonanal	124-19-6	0.16	0.65247443	0.8867
1,4-Cineole	470-67-7	-0.14	0.69604436	0.9223
β -Myrcene	123-35-3	0.12	0.7485312	0.9538
(E,Z)-Hepten-2-ol	18829-55-5	-0.11	0.75586917	0.9538
Ethylbenzene	100-41-4	-0.08	0.8217959	0.9566
1-Octen-3-ol	3391-86-4	0.08	0.82848858	0.9566
Ethyl Acetate	141-78-6	-0.08	0.83237648	0.9566
1,3-Dimethylbenzene	108-38-3	0.07	0.83943446	0.9566
(E)-Nonen-2-ol	18829-56-6	-0.07	0.84829856	0.9566
1,1-Diethoxyhexane	3658-93-3	-0.04	0.90834591	0.9602
Benzaldehyde	100-52-7	0.04	0.91450695	0.9602
2-Pentylfuran	3777-69-3	0.04	0.92295656	0.9602
(E)-2-Hexen-1-ol	928-95-0	0.03	0.92714914	0.9602
4,6-Dimethylheptan-2-one,	19549-80-5	0.03	0.94207649	0.9602
2-Butylfuran	4466-24-4	-0.02	0.96671973	0.9667

Supplementary Table S7: Comparison of volatile compound and monoterpene glycoside abundances between preharvest (P) and harvest (H) in a subset of the RxCS population (n=10).

Compound Class	Compound	estimate	std error	statistic	P value	P-adj	mean (P)	mean (H)	sd (P)	sd (H)	mean (H-P)	sd (H-P)	direction
Acid	Acetic acid	0.389	0.165	2.360	0.022	0.053	-3.490	-3.101	0.801	0.763	0.389	0.252	Higher at H
	Hexanoic acid	0.027	0.069	0.395	0.695	0.763	-4.681	-4.653	0.329	0.299	0.027	0.138	Higher at H
	(E)-2-Hexenoic acid	-0.027	0.077	-0.351	0.727	0.777	-5.989	-6.016	0.324	0.317	-0.027	0.223	Higher at P
Alcohol	1-Heptanol	0.519	0.081	6.441	0.000	0.000	-5.714	-5.195	0.683	0.720	0.519	0.257	Higher at H
	(E)-3-Hexen-1-ol	0.808	0.178	4.531	0.000	0.000	-7.867	-7.060	0.987	0.874	0.808	0.946	Higher at H
	(Z)-3-Hexen-1-ol	-0.509	0.116	-4.388	0.000	0.000	-3.408	-3.917	1.473	1.524	-0.509	0.728	Higher at P
	Hexan-1-ol	0.719	0.171	4.198	0.000	0.001	-1.151	-0.432	1.018	0.994	0.719	0.969	Higher at H
	1-Octen-3-ol	0.253	0.066	3.839	0.000	0.002	-4.218	-3.965	0.349	0.308	0.253	0.289	Higher at H
	Octan-1-ol	0.209	0.071	2.958	0.005	0.015	-4.047	-3.838	0.704	0.609	0.209	0.224	Higher at H
	Pentan-1-ol	0.206	0.076	2.716	0.009	0.025	-6.554	-6.348	0.435	0.482	0.206	0.354	Higher at H
	3-Methylbutan-1-ol	-0.138	0.082	-1.685	0.098	0.174	4.268	4.130	0.537	0.416	-0.138	0.427	Higher at P
	(Z)-3-Nonen-1-ol	0.202	0.146	1.388	0.172	0.282	-7.930	-7.727	0.762	1.104	0.202	0.547	Higher at H
	(E)-2-Hexen-1-ol	0.124	0.167	0.738	0.464	0.594	-1.738	-1.615	1.011	0.806	0.124	0.834	Higher at H
	2-Ethylhexan-1-ol	0.028	0.047	0.594	0.555	0.692	-4.766	-4.738	0.199	0.192	0.028	0.184	Higher at H
	Octan-3-ol	-0.063	0.128	-0.490	0.626	0.741	-7.256	-7.319	0.562	0.452	-0.063	0.517	Higher at P
	Nonan-1-ol	0.009	0.064	0.136	0.892	0.913	-5.050	-5.041	0.633	0.675	0.009	0.175	Higher at H
	(E)-Hexen-3-al	-0.446	0.088	-5.079	0.000	0.000	-1.770	-2.216	0.562	0.428	-0.446	0.533	Higher at P
Aldehyde	(Z)-Hexen-2-al	-0.206	0.078	-2.647	0.011	0.029	-1.809	-2.015	0.519	0.452	-0.206	0.400	Higher at P
	(E,Z)-Hepten-2-al	-0.304	0.138	-2.211	0.032	0.073	-4.592	-4.897	0.524	0.623	-0.304	0.577	Higher at P
	Hexanal	0.084	0.044	1.919	0.061	0.132	4.349	4.433	0.205	0.271	0.084	0.135	Higher at H
	(Z)-Hexen-3-al	-0.226	0.133	-1.692	0.097	0.174	-5.092	-5.318	0.506	0.725	-0.226	0.520	Higher at P
	(E)-Hexen-2-al	-0.130	0.079	-1.632	0.109	0.189	4.335	4.205	0.522	0.403	-0.130	0.418	Higher at P
	(E,E)-2,4-Nonadienal	0.189	0.153	1.233	0.223	0.349	-5.042	-4.853	0.854	0.685	0.189	0.759	Higher at H
	(E,E)-2,4-Hexadienal	-0.102	0.092	-1.113	0.271	0.394	-1.639	-1.741	0.390	0.399	-0.102	0.327	Higher at P
	Nonanal	-0.072	0.076	-0.945	0.349	0.478	-4.641	-4.713	0.369	0.353	-0.072	0.327	Higher at P
	(E)-Nonen-2-al	-0.069	0.118	-0.589	0.559	0.692	-7.288	-7.357	0.829	0.793	-0.069	0.433	Higher at P
	(E,E)-2,4-Heptadienal	-0.111	0.199	-0.557	0.580	0.707	-4.663	-4.774	0.743	0.992	-0.111	0.893	Higher at P
	Octanal	-0.037	0.082	-0.448	0.656	0.742	-5.826	-5.863	0.483	0.459	-0.037	0.450	Higher at P
	Heptanal	-0.017	0.057	-0.298	0.767	0.808	-4.303	-4.320	0.342	0.281	-0.017	0.204	Higher at P
	Pentanal	-0.016	0.129	-0.125	0.901	0.913	-4.158	-4.174	0.615	0.644	-0.016	0.473	Higher at P
	2-Methylbenzaldehyde	1.033	0.221	4.678	0.000	0.000	-4.971	-3.939	1.149	0.824	1.033	0.836	Higher at H
Aromatic	Benzyl alcohol	0.558	0.150	3.714	0.001	0.002	-8.027	-7.469	0.885	0.800	0.558	0.548	Higher at H
	Benzaldehyde	0.223	0.069	3.253	0.002	0.007	-5.254	-5.030	0.395	0.299	0.223	0.275	Higher at H
	2-Phenylethanol	0.334	0.115	2.897	0.006	0.016	-5.815	-5.481	0.607	0.655	0.334	0.369	Higher at H
Ester	Ethyl Acetate	0.742	0.163	4.566	0.000	0.000	-5.315	-4.572	1.025	0.912	0.742	0.835	Higher at H
	(Z)-4-Hexen-1-yl Acetate	-0.534	0.145	-3.677	0.001	0.002	-4.061	-4.595	1.229	1.016	-0.534	0.821	Higher at P
	Hexyl Acetate	0.356	0.240	1.485	0.144	0.244	-7.931	-7.575	1.519	1.273	0.356	1.211	Higher at H
Mono. Glycoside	PH8	0.969	0.091	10.661	0.000	0.000	-2.528	-1.559	0.906	0.981	0.969	0.379	Higher at H
	PH11	0.581	0.099	5.880	0.000	0.000	-3.598	-3.017	0.895	0.839	0.581	0.390	Higher at H
	PH10	0.414	0.088	4.712	0.000	0.000	-0.148	0.267	0.813	0.942	0.414	0.387	Higher at H
	PH4	0.700	0.151	4.631	0.000	0.000	-5.517	-4.817	3.039	3.262	0.700	0.630	Higher at H
	PH5	0.794	0.179	4.439	0.000	0.000	-1.706	-0.912	3.573	3.722	0.794	0.846	Higher at H
	PH3	0.743	0.197	3.776	0.000	0.002	-5.527	-4.784	2.189	1.959	0.743	0.709	Higher at H
	PH1	0.505	0.145	3.491	0.001	0.004	-1.406	-0.901	1.965	1.865	0.505	0.651	Higher at H
	PH9	0.767	0.244	3.139	0.003	0.010	-6.128	-5.361	4.724	4.951	0.767	1.202	Higher at H
	PH2	0.338	0.132	2.570	0.013	0.034	-1.474	-1.136	1.917	1.771	0.338	0.462	Higher at H
	PH6	0.557	0.233	2.393	0.021	0.050	-8.148	-7.591	6.159	6.684	0.557	1.227	Higher at H
	DH1	0.584	0.340	1.718	0.092	0.171	-3.518	-2.933	4.262	4.796	0.584	1.686	Higher at H
	PH7	0.391	0.294	1.328	0.190	0.303	-4.523	-4.132	4.518	4.926	0.391	1.544	Higher at H
	PH12	0.100	0.082	1.212	0.231	0.354	-0.728	-0.628	0.925	1.032	0.100	0.301	Higher at H
	6-Methyl-5-hepten-2-one	0.163	0.086	1.902	0.063	0.133	-6.365	-6.201	0.465	0.401	0.163	0.385	Higher at H
Ketone	1-Octen-3-one	-0.240	0.173	-1.382	0.174	0.282	-5.929	-6.169	0.798	0.612	-0.240	0.790	Higher at P
	4-Methyl-2-heptanone	0.083	0.179	0.463	0.646	0.741	-5.553	-5.470	0.980	0.444	0.083	0.674	Higher at H
	Octan-3-one	0.005	0.120	0.039	0.969	0.969	-6.673	-6.668	0.520	0.455	0.005	0.541	Higher at H
Miscellaneous	Decane	0.400	0.133	3.012	0.004	0.013	-5.919	-5.519	0.716	0.543	0.400	0.478	Higher at H
	2-Butylfuran	0.190	0.109	1.742	0.088	0.171	-6.193	-6.003	0.395	0.571	0.190	0.398	Higher at H
	Trichloromethane	0.111	0.064	1.732	0.090	0.171	-5.852	-5.741	0.312	0.328	0.111	0.235	Higher at H
	Toluene	0.102	0.092	1.109	0.273	0.394	-5.071	-4.969	0.494	0.371	0.102	0.230	Higher at H
	2-Pentylfuran	-0.114	0.106	-1.075	0.288	0.408	-4.371	-4.484	0.447	0.473	-0.114	0.422	Higher at P
	1,3-Dimethylbenzene	0.240	0.253	0.948	0.348	0.478	-8.252	-8.013	1.342	0.783	0.240	1.077	Higher at H
	Ethylbenzene	-0.118	0.143	-0.824	0.414	0.547	-7.745	-7.863	0.825	0.581	-0.118	0.530	Higher at P
	p-Xylene	0.026	0.054	0.482	0.632	0.741	-6.101	-6.075	0.319	0.249	0.026	0.182	Higher at H
	1,1-Diethoxyhexane	-0.014	0.087	-0.164	0.871	0.905	-3.973	-3.987	0.445	0.271	-0.014	0.393	Higher at P
	Nerol	0.881	0.126	7.016	0.000	0.000	-7.896	-7.015	0.980	1.112	0.881	0.335	Higher at H
Monoterpeneoid	β -Myrcene	0.470	0.114	4.114	0.000	0.001	-8.157	-7.687	1.293	1.632	0.470	0.501	Higher at H
	Linalool Oxide	0.696	0.179	3.900	0.000	0.001	-9.806	-9.110	2.237	2.365	0.696	0.847	Higher at H
	(E)-Linalool 3,7-oxide	0.524	0.181	2.892	0.006	0.016	-10.012	-9.488	2.917	2.615	0.524	0.937	Higher at H
	Hotrienol	0.939	0.363	2.585	0.013	0.033	-11.296	-10.357	3.162	3.451	0.939	1.187	Higher at H
	1,4-Cineole	1.578	0.721	2.187	0.034	0.075	-7.266	-5.689	3.612	2.330	1.578	2.732	Higher at H
	(3S)-Linalool	0.346	0.185	1.864	0.068	0.140	-5.795	-5.450	4.020	4.196	0.346	1.115	Higher at H
	Limonene	0.351	0.198	1.775	0.082	0.164	-11.552	-11.200	2.405	2.763	0.351	0.651	Higher at H
	β -Phellandrene	-0.232	0.261	-0.888	0.379	0.509	-10.072	-10.305	3.044	2.805	-0.232	1.178	Higher at P
	Geraniol	0.095	0.123	0.771	0.445	0.578	-3.634	-3.539	0.944	1.016	0.095	0.355	Higher at H
	Isogeraniol	0.060	0.128	0.469	0.641	0.741	-7.947	-7.887	1.028	1.056	0.060	0.377	Higher at H
	β -(E)-Ocimene	0.080	0.201	0.396	0.694	0.763	-9.678	-9.598	2.199	2.563	0.080	0.887	Higher at H
	β -Citronellol	-0.070	0.187	-0.376	0.708	0.767	-8.044	-8.114	1.922	2.030	-0.070	0.467	Higher at P
	(Z)-Theaspirane	-1.035	0.115	-8.966	0.000	0.000	-5.730	-6.766	1.374	1.683	-1.035	0.552	Higher at P
	Cistus Cyclohexanone	-0.372	0.333	-1.118	0.269	0.394	-10.138	-10.510	1.349	1.619	-0.372	1.080	Higher at P

Supplementary Table S8: Spearman correlation analysis between annotated Riesling MEP pathway genes and (3S)-linalool relative abundance at pre-harvest (P) and harvest (H) timepoints, based on 10 genotypes (2 parents and 8 progeny), showing correlation coefficients (ρ), P-values, and adjusted P-values (Benjamini–Hochberg FDR correction).

Gene	Gene ID	ρ (P)	P-adj (P)	ρ (H)	P-adj (H)
DXS	VITVvi_vRiesFPS24_v1.1.hap2.chr07.ver1.0.g428690	0.3	0.22	-0.07	0.89
DXS3	VITVvi_vRiesFPS24_v1.1.hap1.chr04.ver1.0.g051150	0.45	0.06	0.38	0.14
DXS3	VITVvi_vRiesFPS24_v1.1.hap2.chr04.ver1.0.g365270	-0.39	0.1	-0.28	0.35
DXS1	VITVvi_vRiesFPS24_v1.1.hap1.chr05.ver1.0.g067950	0.19	0.45	0.18	0.63
DXR	VITVvi_vRiesFPS24_v1.1.hap2.chr17.ver1.0.g589960	0.05	0.85	-0.07	0.89
DXR	VITVvi_vRiesFPS24_v1.1.hap1.chr17.ver1.0.g270610	0.19	0.45	0.38	0.14
MCT	VITVvi_vRiesFPS24_v1.1.hap2.chr12.ver1.0.g511180	0.19	0.45	-0.02	0.94
MCT	VITVvi_vRiesFPS24_v1.1.hap1.chr12.ver1.0.g196990	-0.05	0.85	-0.04	0.91
CMK	VITVvi_vRiesFPS24_v1.1.hap2.chr06.ver1.0.g409230	0.09	0.74	0.51	0.03
CMK	VITVvi_vRiesFPS24_v1.1.hap1.chr06.ver1.0.g092790	-0.2	0.45	-0.04	0.91
CMS	VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g019180	0.22	0.43	0.11	0.79
HDR	VITVvi_vRiesFPS24_v1.1.hap1.chr03.ver1.0.g037920	0.44	0.06	0.15	0.7
GPPS	VITVvi_vRiesFPS24_v1.1.hap1.chr15.ver1.0.g235820	0.49	0.03	0.46	0.05
GPPS	VITVvi_vRiesFPS24_v1.1.hap2.chr15.ver1.0.g553710	-0.42	0.07	-0.45	0.05

Supplementary Table S9: Spearman correlation analysis between gene expression ($\log_2$ TPM) of genes within the Riesling Hap2 chromosome 10 (3S)-linalool QTL and (3S)-linalool relative abundance ($\log_2$ octan-2-ol equivalents) based on 10 genotypes (2 parents and 8 progeny). Correlation coefficients (ρ), P-values, and adjusted P-values (Benjamini–Hochberg FDR correction) are shown.

Gene ID	Median ($\log_2$ TPM)	Variance ($\log_2$ TPM)	Std. Dev ($\log_2$ TPM)	ρ (Both Stages)	P-value (Both Stages)	P-adj (Both Stages)	ρ (P)	P-value (P)	ρ (H)	P-value (H)	stage_pattern
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47268	0.96	3.63	1.91	0.86	6.00E-19	2.36E-16	0.86	7.76E-10	0.90	1.00E-11	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47080	3.00	9.39	3.06	0.83	1.47E-16	2.90E-14	0.85	2.34E-09	0.84	5.18E-09	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47383	2.25	5.68	2.38	0.83	4.60E-16	5.10E-14	0.84	5.49E-09	0.82	3.35E-08	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47197	1.56	1.42	1.19	0.83	5.17E-16	5.10E-14	0.83	1.47E-08	0.86	1.34E-09	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47244	2.47	6.76	2.60	0.81	2.62E-15	2.06E-13	0.74	3.16E-06	0.89	5.42E-11	Harvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47311	6.42	3.26	1.81	0.81	4.78E-15	3.14E-13	0.85	2.57E-09	0.78	4.64E-07	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47195	2.52	2.35	1.53	0.80	1.01E-14	5.70E-13	0.87	3.38E-10	0.74	3.06E-06	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47128	3.08	1.38	1.18	0.80	1.69E-14	7.15E-13	0.76	9.54E-07	0.80	9.37E-08	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47408	3.82	11.84	3.44	0.80	1.74E-14	7.15E-13	0.79	1.63E-07	0.77	6.55E-07	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47310	3.10	6.17	2.48	0.80	1.82E-14	7.15E-13	0.84	4.37E-09	0.76	1.29E-06	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47254	1.41	2.36	1.54	0.80	2.60E-14	9.30E-13	0.77	5.40E-07	0.83	1.17E-08	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47343	1.47	2.05	1.43	0.79	7.30E-14	2.40E-12	0.77	6.74E-07	0.81	4.45E-08	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47175	1.00	2.02	1.42	0.79	8.42E-14	2.55E-12	0.77	5.54E-07	0.81	4.61E-08	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47273	2.28	2.57	1.60	0.79	9.08E-14	2.55E-12	0.82	2.32E-08	0.78	2.89E-07	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47174	1.35	3.30	1.82	0.78	1.56E-13	4.09E-12	0.77	6.99E-07	0.79	2.67E-07	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47155	2.88	4.39	2.09	0.78	3.06E-13	7.53E-12	0.74	2.47E-06	0.80	9.64E-08	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47177	1.97	0.77	0.88	0.77	7.79E-13	1.61E-11	0.86	7.21E-10	0.74	3.26E-06	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47198	2.36	5.12	2.26	0.76	1.52E-12	2.99E-11	0.73	4.16E-06	0.81	4.61E-08	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47161	2.06	4.30	2.07	0.75	4.16E-12	7.81E-11	0.81	7.22E-08	0.73	3.95E-06	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47313	1.54	3.98	2.00	0.74	1.58E-11	2.71E-10	0.76	1.18E-06	0.72	5.86E-06	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47167	1.82	4.27	2.07	0.73	2.49E-11	3.92E-10	0.76	9.82E-07	0.71	1.18E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47278	2.46	6.42	2.53	0.73	2.61E-11	3.95E-10	0.66	7.05E-05	0.79	2.20E-07	Harvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47376	1.55	3.61	1.90	0.73	2.80E-11	4.09E-10	0.75	1.85E-06	0.75	2.17E-06	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47141	3.30	6.64	2.58	0.73	4.18E-11	5.68E-10	0.71	1.19E-05	0.76	1.10E-06	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47189	1.72	3.51	1.87	0.72	6.18E-11	8.12E-10	0.78	3.71E-07	0.70	1.48E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47093	1.88	1.04	1.02	0.72	6.48E-11	8.24E-10	0.75	2.06E-06	0.73	5.37E-06	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47355	1.75	3.17	1.78	0.72	6.81E-11	8.38E-10	0.73	5.69E-06	0.74	3.47E-06	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47344	1.17	0.23	0.47	0.72	1.08E-10	1.29E-09	0.79	1.64E-07	0.65	1.08E-04	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47395	1.98	4.08	2.02	0.72	1.29E-10	1.50E-09	0.75	1.97E-06	0.70	1.65E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47194	2.23	2.42	1.55	0.71	1.47E-10	1.66E-09	0.79	2.27E-07	0.69	2.18E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47188	4.11	6.44	2.54	0.71	1.94E-10	2.06E-09	0.72	8.92E-06	0.74	3.71E-06	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47384	2.24	5.49	2.34	0.71	2.02E-10	2.10E-09	0.69	2.68E-05	0.71	1.34E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47241	4.93	1.15	1.07	0.71	2.35E-10	2.37E-09	0.78	3.34E-07	0.63	1.74E-04	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47242	4.22	6.60	2.57	0.71	2.90E-10	2.86E-09	0.78	3.02E-07	0.70	1.57E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47201	1.24	2.51	1.58	0.71	3.20E-10	3.07E-09	0.69	2.33E-05	0.74	3.36E-06	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47354	3.80	9.36	3.06	0.70	4.44E-10	4.17E-09	0.74	3.33E-06	0.70	1.69E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47352	1.83	3.63	1.91	0.69	7.39E-10	6.62E-09	0.68	3.14E-05	0.64	1.33E-04	Similar both stages

Supplementary Table S9 (cont.)

Gene ID	Median (log2 TPM)	Variance (log2 TPM)	Std. Dev (log2 TPM)	ρ (Both Stages)	P-value (Both Stages)	P-adj (Both Stages)	ρ (P)	P-value (P)	ρ (H)	P- value (H)	stage_pattern
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47407	2.40	5.75	2.40	0.69	1.50E-09	1.31E-08	0.67	4.74E-05	0.69	2.36E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47385	1.23	1.08	1.04	0.68	1.89E-09	1.61E-08	0.69	2.19E-05	0.68	3.60E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47143	5.61	9.99	3.16	0.68	1.94E-09	1.63E-08	0.70	1.66E-05	0.69	2.27E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47287	1.76	2.96	1.72	0.68	2.50E-09	2.05E-08	0.68	4.02E-05	0.68	3.26E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47196	3.31	1.00	1.00	0.68	2.71E-09	2.18E-08	0.74	3.69E-06	0.70	1.49E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47316	1.90	0.63	0.79	0.67	3.68E-09	2.84E-08	0.62	2.32E-04	0.75	1.64E-06	Harvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47157	2.11	3.87	1.97	0.67	5.54E-09	4.20E-08	0.74	3.44E-06	0.64	1.60E-04	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47312	2.80	6.36	2.52	0.66	7.91E-09	5.88E-08	0.69	2.16E-05	0.67	5.13E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47398	3.39	3.54	1.88	0.66	1.05E-08	7.69E-08	0.66	7.68E-05	0.68	3.95E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47373	1.54	0.97	0.99	0.65	1.66E-08	1.19E-07	0.71	1.24E-05	0.59	6.72E-04	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47236	7.04	1.02	1.01	0.65	2.65E-08	1.77E-07	0.70	1.41E-05	0.64	1.38E-04	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47371	2.66	4.97	2.23	0.63	5.31E-08	3.43E-07	0.63	1.89E-04	0.65	8.65E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47401	3.35	3.07	1.75	0.63	6.11E-08	3.82E-07	0.66	6.38E-05	0.68	4.08E-05	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47087	3.51	0.54	0.73	0.62	1.08E-07	6.47E-07	0.69	2.23E-05	0.54	2.20E-03	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47358	6.14	0.32	0.57	0.61	2.54E-07	1.45E-06	0.69	2.64E-05	0.79	2.38E-07	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47089	2.45	0.38	0.61	0.59	7.59E-07	3.98E-06	0.75	2.23E-06	0.54	2.25E-03	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47106	3.52	0.98	0.99	0.55	5.85E-06	2.59E-05	0.64	1.24E-04	0.47	9.21E-03	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47132	2.81	0.30	0.55	0.55	6.22E-06	2.72E-05	0.69	2.82E-05	0.48	6.75E-03	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47139	3.35	0.80	0.89	0.52	1.69E-05	6.61E-05	0.87	6.90E-10	0.19	3.17E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47340	1.68	2.26	1.50	0.46	2.57E-04	8.22E-04	0.49	6.37E-03	0.47	8.60E-03	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47279	5.39	0.16	0.40	0.44	4.19E-04	1.30E-03	0.62	2.94E-04	0.34	6.44E-02	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47342	2.36	1.99	1.41	0.42	7.30E-04	2.13E-03	0.64	1.56E-04	0.29	1.25E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47179	5.49	0.18	0.43	0.41	1.18E-03	3.32E-03	0.46	1.11E-02	0.40	2.84E-02	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47091	2.67	1.23	1.11	0.40	1.36E-03	3.73E-03	0.45	1.32E-02	0.41	2.53E-02	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47321	4.34	0.33	0.57	0.40	1.76E-03	4.68E-03	0.52	2.91E-03	0.35	6.08E-02	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47180	6.26	0.23	0.48	0.39	1.90E-03	4.97E-03	0.45	1.18E-02	0.47	8.20E-03	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47326	1.31	0.19	0.44	0.37	3.77E-03	9.12E-03	0.54	2.27E-03	0.28	1.28E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47335	1.79	0.40	0.64	0.31	1.46E-02	3.13E-02	0.54	2.14E-03	0.20	2.93E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47131	4.49	0.41	0.64	0.30	1.81E-02	3.79E-02	0.40	3.00E-02	0.29	1.19E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47369	1.97	0.41	0.64	0.28	2.80E-02	5.38E-02	0.03	8.69E-01	0.55	1.70E-03	Harvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47135	3.12	1.99	1.41	0.28	2.86E-02	5.45E-02	0.24	1.93E-01	0.44	1.41E-02	Harvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47351	3.89	0.43	0.66	0.27	3.51E-02	6.50E-02	0.53	2.71E-03	0.14	4.58E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47338	2.48	0.41	0.64	0.25	5.38E-02	9.13E-02	0.29	1.16E-01	0.31	9.07E-02	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47365	3.94	1.00	1.00	0.25	5.56E-02	9.40E-02	0.28	1.32E-01	0.84	7.03E-09	Harvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47191	6.01	0.23	0.48	0.24	6.67E-02	1.09E-01	0.31	1.01E-01	0.20	2.82E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47309	2.43	2.66	1.63	0.22	9.61E-02	1.48E-01	0.28	1.35E-01	0.14	4.51E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47348	1.95	1.19	1.09	0.19	1.39E-01	2.04E-01	0.40	2.71E-02	0.00	9.91E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47357	3.18	0.68	0.83	0.19	1.44E-01	2.08E-01	0.27	1.49E-01	0.08	6.65E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47350	4.23	4.27	2.07	0.14	2.90E-01	3.77E-01	0.40	2.72E-02	0.05	8.09E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47327	4.10	1.13	1.06	0.14	2.96E-01	3.84E-01	0.22	2.47E-01	0.10	5.86E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47366	3.24	5.15	2.27	0.08	5.25E-01	5.99E-01	0.43	1.73E-02	-0.30	1.12E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33820	4.30	5.72	2.39	0.03	7.92E-01	8.19E-01	0.20	2.85E-01	-0.05	7.84E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47088	1.31	0.27	0.52	0.00	9.92E-01	9.92E-01	0.40	2.98E-02	-0.23	2.14E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33798	3.36	1.35	1.16	-0.02	9.09E-01	9.31E-01	-0.09	6.18E-01	0.08	6.75E-01	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47200	2.67	0.73	0.85	-0.03	8.29E-01	8.53E-01	-0.04	8.33E-01	-0.10	6.14E-01	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47308	1.14	0.36	0.60	-0.05	6.96E-01	7.45E-01	-0.19	3.27E-01	0.02	9.28E-01	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33814	2.70	2.48	1.58	-0.09	4.91E-01	5.68E-01	0.02	8.97E-01	-0.21	2.67E-01	Harvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33810	4.04	5.38	2.32	-0.15	2.56E-01	3.40E-01	-0.12	5.11E-01	-0.15	4.22E-01	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33790	1.04	1.60	1.27	-0.18	1.64E-01	2.30E-01	-0.05	7.81E-01	-0.34	6.95E-02	Harvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33796	4.46	0.43	0.66	-0.18	1.63E-01	2.29E-01	-0.11	5.61E-01	-0.18	3.35E-01	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33800	3.65	2.42	1.56	-0.19	1.55E-01	2.23E-01	-0.23	2.24E-01	-0.24	2.07E-01	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33813	5.39	9.29	3.05	-0.21	1.15E-01	1.72E-01	-0.20	2.90E-01	-0.22	2.41E-01	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33812	4.18	4.86	2.21	-0.21	1.12E-01	1.69E-01	-0.25	1.75E-01	-0.28	1.28E-01	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g33821	5.14	7.70	2.78	-0.23	8.35E-02	1.32E-01	-0.30	1.13E-01	-0.26	1.72E-01	Similar both stages
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47329	5.01	0.08	0.28	-0.37	3.85E-03	9.20E-03	-0.53	2.51E-03	-0.33	7.54E-02	Preharvest stronger
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.0.g47203	1.49	1.04	1.02	-0.49	7.15E-05	2.54E-04	-0.66	8.00E-05	-0.50	4.54E-03	Preharvest stronger

Supplementary Table S10: Spearman correlations between gene expression (TPM) within the chromosome 2 QTL and (3S)-linalool relative abundance at pre-harvest (P) and harvest (H). Correlation coefficients (ρ) are shown for each timepoint. P-values are adjusted with Benjamini–Hochberg correction.

Gene ID	ρ (P)	P value (P)	P-adj (P)	ρ (H)	P value (H)	P-adj (H)
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021151	-0.2126038	0.55538653	0.97153736	-0.2000977	0.57939487	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021161	-0.1878788	0.60321761	0.97153736	-0.0424242	0.90736382	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021171	-0.0545455	0.88103618	0.97153736	-0.2363636	0.51088532	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021181	-0.2	0.579584	0.97153736	-0.2727273	0.44583834	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021191	0.06253054	0.86374803	0.97153736	-0.0500244	0.89084498	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021211	-0.3212121	0.36546831	0.97153736	-0.2727273	0.44583834	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021231	-0.3090909	0.38484123	0.97153736	-0.3818182	0.27625533	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021241	0.06666667	0.85481309	0.97153736	-0.2121212	0.55630578	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021271	-0.2492413	0.48741042	0.97153736	0.13981828	0.70005687	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021301	-0.1165732	0.7484333	0.97153736	-0.0425534	0.90708284	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021331	-0.1911808	0.59674515	0.97153736	-0.38029	0.27834319	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021341	-0.0666667	0.85481309	0.97153736	-0.2121212	0.55630578	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021351	-0.300427	0.39898781	0.97153736	-0.2731155	0.44516509	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021361	0.01818182	0.96024042	0.97153736	-0.1878788	0.60321761	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021371	-0.1393939	0.70093188	0.97153736	-0.3090909	0.38484123	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021381	-0.1533858	0.67225191	0.97153736	-0.3454545	0.32822651	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021401	-0.1411149	0.69738507	0.97153736	-0.3586643	0.30879863	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021451	0.11515152	0.75141965	0.97153736	0.17575758	0.62718834	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021461	0.66060606	0.03758838	0.97153736	0.07878788	0.82871739	0.99835165
VITVvi_vCabSauv08_v2.0.hapCF.chr02.ver2.0.g021471	-0.1393939	0.70093188	0.97153736	-0.0909091	0.80277173	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317691	0.29787372	0.40320387	0.97153736	0.20246923	0.57481255	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317701	0.23636364	0.51088532	0.97153736	0	1	1
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317711	0.32121212	0.36546831	0.97153736	0.07878788	0.82871739	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317721	0.41650113	0.23118346	0.97153736	-0.1715034	0.63567787	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317731	0.04242424	0.90736382	0.97153736	-0.2727273	0.44583834	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317741	0.3742613	0.28666319	0.97153736	0.01840629	0.95974987	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317791	0.10430233	0.77430465	0.97153736	0.31290699	0.37868902	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317801	0.33333333	0.34659351	0.97153736	0.109423	0.76348265	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317811	0.09090909	0.80277173	0.97153736	0.46666667	0.1739386	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317821	0.34545455	0.32822651	0.97153736	0.27963655	0.43392584	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317911	0.27272727	0.44583834	0.97153736	0.05454545	0.88103618	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317921	0.49090909	0.14965567	0.97153736	0.38181818	0.27625533	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317941	0.2	0.579584	0.97153736	-0.0545455	0.88103618	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317951	0.2	0.579584	0.97153736	0.2	0.579584	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317961	0.17629261	0.62612342	0.97153736	0.15805544	0.6627623	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317981	0.01818182	0.96024042	0.97153736	-0.1458973	0.68755671	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g317991	0.38181818	0.27625533	0.97153736	0.17021355	0.63825936	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g318001	-0.0424242	0.90736382	0.97153736	0.06666667	0.85481309	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g318021	0.05454545	0.88103618	0.97153736	-0.1533858	0.67225191	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g318041	0.36969697	0.29305008	0.97153736	0.32826899	0.35441833	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g318051	-0.030303	0.93377296	0.97153736	-0.1636364	0.65147734	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024460	0.06666667	0.85481309	0.97153736	0.21212121	0.55630578	0.99835165

Supplementary Table S10 (cont.)

Gene ID	ρ (P)	P value (P)	P-adj (P)	ρ (H)	P value (H)	P-adj (H)
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g31800	-0.0424242	0.90736382	0.97153736	0.06666667	0.85481309	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g31802	0.05454545	0.88103618	0.97153736	-0.1533858	0.67225191	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g31804	0.36969697	0.29305008	0.97153736	0.32826899	0.35441833	0.99835165
VITVvi_vCabSauv08_v2.0.hapSB.chr02.ver2.0.g31805	-0.030303	0.93377296	0.97153736	-0.1636364	0.65147734	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024460	0.06666667	0.85481309	0.97153736	0.21212121	0.55630578	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024470	0.26060606	0.46708905	0.97153736	0.03751832	0.91804409	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024480	-0.0060606	0.98674291	0.98674291	0.24848485	0.4887763	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024490	0.2	0.579584	0.97153736	0.27272727	0.44583834	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024500	0.12727273	0.72605701	0.97153736	0.12727273	0.72605701	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024510	-0.0364743	0.92031846	0.97153736	0.19019837	0.5986683	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024520	-0.0181818	0.96024042	0.97153736	0.23928182	0.50552485	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024530	0.16363636	0.65147734	0.97153736	0.15151515	0.67606518	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024540	0.51064066	0.13150199	0.97153736	-0.0182372	0.96011946	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024550	0.09203147	0.80037764	0.97153736	0.12884406	0.72278608	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024560	-0.0666667	0.85481309	0.97153736	0.29179466	0.4133266	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024590	-0.1636364	0.65147734	0.97153736	0.01823717	0.96011946	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024680	0.38143629	0.2767763	0.97153736	0.01250611	0.97264717	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024690	0.04255339	0.90708284	0.97153736	0.17021355	0.63825936	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024710	0.09090909	0.80277173	0.97153736	0.18787879	0.60321761	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024720	-0.0909091	0.80277173	0.97153736	0.33333333	0.34659351	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024730	0.20060883	0.57840614	0.97153736	0.11657319	0.7484333	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024740	0.3951386	0.25841873	0.97153736	0.24316222	0.49843371	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024760	0.1641345	0.65047314	0.97153736	0.25532033	0.47649345	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024770	0.2	0.579584	0.97153736	0.01818182	0.96024042	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024780	0.13131413	0.71765246	0.97153736	0.31678901	0.37248044	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024800	0.18787879	0.60321761	0.97153736	-0.0060606	0.98674291	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024810	0.03030303	0.93377296	0.97153736	0.23636364	0.51088532	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024830	0.40606061	0.24428229	0.97153736	-0.3575758	0.31037609	0.99835165
VITVvi_vRiesFPS24_v1.1.hap1.chr02.ver1.0.g024850	0.19452977	0.59020678	0.97153736	0.34545455	0.32822651	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337890	0.2	0.579584	0.97153736	-0.4969699	0.14392655	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337900	-0.0909091	0.80277173	0.97153736	-0.4787879	0.16152293	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337920	0.25155268	0.48324689	0.97153736	0.27272727	0.44583834	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337930	-0.2147401	0.55132475	0.97153736	-0.3161109	0.37356133	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337940	-0.1228366	0.7353129	0.97153736	-0.061451	0.86608243	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337950	-0.2363636	0.51088532	0.97153736	0.1030303	0.77699846	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337960	-0.1757576	0.62718834	0.97153736	-0.2606061	0.46708905	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337970	0.02731155	0.94030053	0.97153736	-0.4202303	0.22660334	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337980	-0.1515152	0.67606518	0.97153736	0.04242424	0.90736382	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g337990	-0.0787879	0.82871739	0.97153736	-0.3454545	0.32822651	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338000	-0.1757576	0.62718834	0.97153736	-0.2727273	0.44583834	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338050	-0.1875916	0.60378168	0.97153736	-0.0061354	0.98657925	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338090	0.04525557	0.90120594	0.97153736	-0.522233	0.12150292	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338100	-0.1043023	0.77430465	0.97153736	-0.1625794	0.65360977	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338120	-0.2484848	0.4887763	0.97153736	-0.3333333	0.34659351	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338130	-0.2121212	0.55630578	0.97153736	-0.2553203	0.47649345	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338140	0.07878788	0.82871739	0.97153736	-0.2553203	0.47649345	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338200	0.27963655	0.43392584	0.97153736	-0.0674897	0.8530368	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338210	-0.3212121	0.36546831	0.97153736	-0.2606061	0.46708905	0.99835165
VITVvi_vRiesFPS24_v1.1.hap2.chr02.ver1.0.g338250	-0.1702136	0.63825936	0.97153736	0.0547115	0.8806762	0.99835165

Supplementary Table S11. BLASTP results for the candidate (3S)-linalool/nerolidol synthase gene VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680 against terpene synthase (TPS) proteins from the *Vitis vinifera* PN40024 reference genome, showing percent identity, query and subject coverage, alignment statistics (length, mismatches, gap openings, alignment coordinates), expectation values (E-values), bit scores, and a combined identity–coverage metric ($\text{id} \times \text{cov}$).

VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	PN40024_protein_ID	id (%)	cov query (%)	cov ref (%)	Length h query	Length ref	Alignment length	Mismatches	Gap openings	Alignment Start (Query)	Alignment End (Query)	Alignment Start (Subj.)	Alignment End (Subj.)	Expectation value	Bit score	id*cov*cov
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0385g00020.0	98.23	87.14	99.80	583	509	508	9	0	1	508	1	508	0	1042	854250.48
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0271g00010.0	98.58	84.39	99.80	583	493	492	6	1	92	583	1	491	0	1006	830239.7935
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0266g00020.0	97.56	84.39	100.82	583	488	492	7	2	92	583	1	487	0	987	830067.902
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0266g00070.0	91.12	88.85	100.00	583	518	518	18	3	92	583	1	516	0	954	809608.2333
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0385g00010.0	98.54	82.33	97.76	583	491	480	7	0	1	480	1	480	0	984	793131.1113
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0271g00060.0	77.26	98.80	99.65	583	578	576	130	1	1	575	1	576	0	939	760682.2414
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0372g00020.0	97.28	69.47	99.51	583	407	405	11	0	179	583	1	405	0	820	672466.4849
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0271g00030.0	80.87	82.50	99.59	583	483	481	91	1	96	575	1	481	0	823	664449.395
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0266g00010.0	73.21	86.45	99.21	583	508	504	134	1	72	574	2	505	0	787	627912.6214
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0372g00040.0	73.21	86.45	99.21	583	508	504	134	1	72	574	2	505	0	784	627912.6214
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0372g00020.0	72.66	86.45	99.21	583	508	504	137	1	72	574	2	505	0	783	622852.2683
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0572g00010.0	76.06	76.67	99.11	583	451	447	106	1	129	574	2	448	0	726	577997.9135
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0847g00020.0	73.17	70.33	99.76	583	411	410	109	1	72	480	2	411	0	608	513322.6077
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0372g00070.0	72.89	87.31	78.79	583	646	509	137	1	67	574	135	643	0	787	501420.9116
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0084g00010.0	39.26	88.68	93.66	583	552	517	303	8	65	575	35	546	2.00E-118	365	326079.6896
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00370.0	40.95	86.28	88.71	583	567	503	290	6	78	575	64	564	1.00E-126	387	313428.34
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0014g04930.0	38.52	88.16	92.11	583	558	514	309	5	66	575	46	556	2.00E-123	379	312830.9854
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00370.0	39.65	88.68	87.48	583	591	517	304	7	65	572	76	588	4.00E-120	372	307587.1883
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0019g00060.0	34.73	88.51	86.72	583	595	516	301	9	65	572	76	589	8.00E-118	365	304952.6754
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04050.0	34.93	89.37	97.20	583	536	521	328	4	64	574	15	534	2.00E-105	331	303417.5434
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g03790.0	39.61	88.34	86.70	583	594	515	302	8	65	572	76	588	4.00E-120	371	303364.1807
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04870.0	36.22	89.02	93.35	583	556	519	325	5	57	571	35	551	7.00E-101	320	300981.5091
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0724g00010.0	41.61	72.56	98.83	583	428	423	244	3	91	570	1	423	3.00E-106	330	298377.5384
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0014g02590.0	36.03	89.02	92.35	583	562	519	320	6	67	575	44	560	1.00E-105	332	296206.1747
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05240.0	38.45	86.11	89.32	583	562	502	303	5	78	575	61	560	1.00E-110	345	295732.4002
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0014g01060.0	36.94	87.31	90.89	583	560	509	312	6	74	575	52	558	3.00E-104	329	293140.5336
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0361g00060.0	36.12	88.34	90.83	583	567	515	322	6	65	574	51	563	1.00E-100	319	289808.1443
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0015g02070.0	36.4	87.65	90.76	583	563	511	314	7	72	575	55	561	3.00E-104	329	289578.4468
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s2271g00010.0	37.03	72.73	107.07	583	396	424	229	5	156	575	1	390	2.00E-82	266	288351.1478
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04120.0	38.23	85.25	88.43	583	562	497	302	5	57	551	40	533	3.00E-110	344	288212.0969
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_12s0134g00030.0	36.7	88.34	88.79	583	580	515	319	5	67	575	61	574	7.00E-106	333	287861.7584
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0014g04810.0	35.67	87.99	91.61	583	560	513	319	4	73	575	47	558	4.00E-103	326	287528.7377
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04280.0	37.63	85.25	89.39	583	556	497	306	3	78	571	56	551	1.00E-106	335	286750.1472
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0014g04900.0	38.98	87.14	83.97	583	605	508	304	5	66	571	46	549	6.00E-122	376	285197.2476
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04780.0	37.63	85.25	88.43	583	562	497	305	5	57	551	40	533	2.00E-108	340	283688.7577
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0372g00060.0	98.31	30.36	94.15	583	188	177	94	3	304	480	1	177	1.00E-121	360	281007.4441
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_12s0134g00020.0	37.09	88.34	85.55	583	602	515	317	5	67	575	83	596	4.00E-109	343	280289.1234
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0014g01070.0	36.25	86.11	89.48	583	561	502	314	6	58	556	40	538	3.00E-99	316	279308.4207
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05230.0	35.42	85.25	89.39	583	556	497	312	3	78	571	56	551	5.00E-102	323	277529.6402
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0372g00030.0	97.01	28.64	99.40	583	168	167	5	0	1	167	1	167	6.00E-116	345	276230.4878
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0014g04800.0	35.87	87.99	86.36	583	594	513	318	4	73	575	81	592	1.00E-105	333	272590.7454
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04570.0	39.35	78.90	87.12	583	528	460	276	1	93	551	42	499	5.00E-101	319	270494.1785
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00130.0	39.64	77.02	86.02	583	522	449	265	6	65	509	71	517	4.00E-105	330	262595.4943
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0014g02580.0	34.38	82.33	92.66	583	518	480	275	4	98	575	75	516	6.00E-89	287	262295.0125
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05360.0	33.33	69.47	109.76	583	369	403	224	4	142	545	7	366	7.00E-67	224	254126.7832
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0014g02550.0	37.42	82.50	80.70	583	596	481	297	3	98	575	115	594	2.00E-103	327	249160.4585
VITVvi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04170.0	34.92	79.07	89.34	583	516	461	296	3	117	574	55	514	3.00E-95	304	246693.5697

Supplementary Table S11 (cont.)

VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	PN40024_protein_ID	id (%)	cov query (%)	cov ref (%)	Length query	Length ref	Alignment length	Mismatches	Gap openings	Alignment Start (Query)	Alignment End (Query)	Alignment Start (Subj.)	Alignment End (Subj.)	Expectation value	Bit score	id* cov* cov
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05290.00	38.62	82.16	77.63	583	617	479	292	2	78	555	117	594	4.00E-103	327	246336.9599
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g03740.00	38.19	68.27	94.09	583	423	398	238	6	165	555	2	398	2.00E-83	270	245305.2711
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04080.00	35.27	85.59	79.46	583	628	499	290	6	78	572	155	624	1.00E-87	287	239871.3351
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04220.00	37.01	74.61	85.97	583	506	435	265	6	145	575	75	504	8.00E-88	284	237398.8044
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_06s0372g00100.00	95.07	24.36	99.30	583	143	142	7	0	67	208	1	142	6.00E-91	280	229940.563
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g03770.00	38.73	59.35	99.43	583	348	346	209	2	232	575	1	345	8.00E-83	266	228534.5656
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00090.00	39.02	59.35	99.35	583	351	346	208	2	232	575	1	345	4.00E-81	261	228277.8594
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_06s0372g00080.00	68.85	31.39	96.32	583	190	183	56	1	97	278	7	189	5.00E-81	255	208153.6201
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0001g04080.00	36.52	61.06	92.95	583	383	356	219	3	222	575	31	381	3.00E-75	247	207282.8809
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_06s0392g00030.00	37.78	54.03	99.06	583	318	315	191	4	264	574	1	314	5.00E-66	220	202202.903
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_06s0266g00040.00	98.29	20.07	99.15	583	118	117	2	0	92	208	1	117	1.00E-75	238	195582.7267
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g03830.00	43.07	58.15	76.87	583	441	339	187	6	65	398	76	413	7.00E-79	258	192516.1305
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_12s0134g00140.00	41.73	47.68	94.88	583	293	278	159	1	297	571	1	278	1.00E-81	261	188799.918
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_12s0134g00090.00	41.07	48.03	94.92	583	295	280	160	1	297	571	1	280	1.00E-80	258	187219.1179
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_06s0522g00020.00	98.35	20.75	91.67	583	132	121	2	0	360	480	1	121	1.00E-79	250	187112.4214
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04820.00	30	48.03	109.80	583	255	280	170	2	265	544	1	254	4.00E-40	147	158208.0517
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_12s0059g02720.00	40.55	49.91	74.81	583	291	291	168	2	65	354	77	363	9.00E-70	233	151411.4367
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04710.00	32.42	43.91	103.23	583	248	256	150	7	331	575	3	246	4.00E-27	110	146951.0541
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_12s0059g02710.00	40.87	55.40	62.12	583	323	323	186	2	65	386	77	395	2.00E-75	251	140649.3677
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04510.00	35.21	45.80	86.97	583	307	267	158	6	33	295	49	304	3.00E-39	146	140423.137
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04110.00	32.93	42.71	98.42	583	253	249	164	2	328	574	4	251	2.00E-41	151	138420.7981
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_06s0692g00020.00	26.8	62.09	82.27	583	440	362	246	9	178	527	1	355	7.00E-28	116	136908.5919
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g0450.00	34.21	39.11	100.00	583	228	228	124	3	328	555	4	205	4.00E-29	116	133788.6792
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04530.00	40.61	39.11	90.12	583	253	228	144	1	328	555	4	230	3.00E-38	142	128286.8087
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_12s0134g00040.00	40.61	37.79	87.56	583	225	197	99	1	276	454	12	208	7.00E-43	154	120147.3978
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_10s0116g00750.00	25.32	79.93	56.97	583	818	466	291	15	89	522	254	694	6.00E-28	119	115295.8502
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00300.00	41.94	26.59	97.48	583	159	155	90	0	232	386	1	155	8.00E-37	135	108699.1488
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g0540.00	41.01	37.22	69.77	583	311	217	127	1	78	293	56	272	1.00E-45	164	106507.5251
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05510.00	37.23	32.25	87.44	583	215	188	116	2	364	551	1	186	4.00E-26	107	104978.828
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_08s0007g06860.00	37.21	36.88	74.65	583	288	215	130	2	65	278	77	287	6.00E-37	139	102441.41
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00250.00	37.67	36.88	73.63	583	292	215	129	5	68	278	78	291	5.00E-34	131	102287.1631
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0085g00830.00	27.01	72.38	51.03	583	827	422	280	9	90	486	313	731	5.00E-38	149	99764.36022
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00380.00	39.37	37.91	65.00	583	340	221	129	5	63	279	69	288	7.00E-39	146	97006.8694
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s1319g00010.00	27.64	47.17	69.80	583	394	275	193	4	253	527	8	276	2.00E-26	111	90999.42534
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_06s0450g00010.00	28.47	50.60	54.13	583	545	295	176	8	89	349	251	544	1.00E-17	86.3	77976.98554
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04200.00	37.82	20.41	99.17	583	120	119	73	1	152	269	1	119	6.00E-20	87	76553.60492
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0001g04850.00	28.66	26.93	98.13	583	160	157	109	2	421	575	2	157	3.00E-17	80.5	75733.31261
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05390.00	36.42	25.90	78.24	583	193	151	92	3	404	552	17	165	2.00E-19	87.4	73801.973
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04830.00	40.26	26.42	67.25	583	229	154	86	3	66	213	64	217	8.00E-28	112	71517.3107
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_06s0392g00060.00	34.78	36.88	51.19	583	420	215	136	3	65	277	51	264	8.00E-29	118	66734.20526
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00320.00	34.17	19.73	96.64	583	119	115	72	2	469	581	2	115	1.00E-15	75.1	66299.422
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05520.00	34.17	47.68	39.49	583	704	278	147	3	78	354	453	695	2.00E-43	164	64341.82228
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05430.00	39.9	34.82	42.03	583	483	203	121	1	78	279	56	258	1.00E-39	151	58391.4535
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04090.00	36.59	21.10	70.69	583	174	123	58	3	48	152	35	155	1.00E-10	60.8	54570.11001
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0001g02080.00	38.27	13.89	100.00	583	81	81	49	1	444	524	1	80	5.00E-11	60.5	53171.01201
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05430.00	34.15	35.16	42.44	583	483	203	134	1	351	555	257	460	9.00E-28	116	50966.25756
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_07s0151g01070.00	30.55	47.17	34.77	583	791	275	160	8	90	334	306	579	1.00E-19	92.8	50098.28917
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_07s0151g01040.00	31.64	47.17	33.17	583	829	275	155	9	90	333	330	602	1.00E-21	99.4	49508.38701
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s0001g040920.00	37.5	15.09	84.62	583	104	88	54	1	460	547	15	101	1.00E-12	66.2	47895.50073

Supplementary Table S11 (cont.)

VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	PN40024_protein_ID	id (%)	cov query (%)	cov ref (%)	Length query	Length ref	Alignment length	Mismatches	Gap openings	Alignment Start (Query)	Alignment End (Query)	Alignment Start (Subj.)	Alignment End (Subj.)	Expectation value	Bit score	id* cov* cov
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04430.00	32.8	32.42	42.66	583	443	189	121	3	396	579	15	202	1.00E-24	106	45365.44456
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s00014g04840.00	58.33	10.29	74.07	583	81	60	25	0	328	387	4	63	4.00E-18	80.9	44467.31466
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04490.00	40.74	9.26	98.18	583	55	54	32	0	444	497	1	54	1.00E-05	43.9	37049.06908
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04690.00	28.35	21.78	56.44	583	225	127	87	3	448	572	2	126	4.00E-08	53.9	34858.55918
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g05350.00	31.58	13.04	69.72	583	109	76	47	2	450	525	4	74	1.00E-04	42	28704.12136
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04480.00	57.45	8.06	60.26	583	78	47	20	0	328	374	4	50	3.00E-12	64.3	27907.60654
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s00067g00380.00	34.68	21.27	36.47	583	340	124	61	4	282	397	222	333	2.00E-07	52.8	26901.40652
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0392g00060.00	32.85	23.50	32.62	583	420	137	89	2	440	574	281	416	2.00E-13	72	25180.1703
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04560.00	39.68	10.81	51.64	583	122	63	34	2	78	138	52	112	2.00E-06	47.4	22142.38394
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04690.00	29.17	16.47	42.67	583	225	96	65	2	482	575	129	223	7.00E-08	53.1	20494.05146
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00050.00	40.94	21.78	20.68	583	614	127	72	3	65	189	67	192	2.00E-20	94.7	18446.68596
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_13s0067g00050.00	33.93	19.21	18.24	583	614	112	71	2	466	575	499	609	5.00E-12	68.2	11890.03079
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_18s0001g04080.00	38.2	15.27	14.17	583	628	89	53	2	133	220	54	141	1.00E-09	60.5	8264.473239
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0274g00010.00	60	6.00	18.42	583	190	35	14	0	32	66	9	43	2.00E-05	45.8	6635.370588
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_19s00014g04900.00	35.62	12.52	12.07	583	605	73	46	1	69	140	532	604	2.00E-06	50.1	5381.653176
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.p01	VIT_00s0274g00030.00	63.45	24.87	1.07	583	13571	145	53	0	1	145	36	180	1.00E-52	197	1686.115131

Supplementary Table S12. TargetP 2.0 predictions of subcellular targeting signals for Riesling, Cabernet Sauvignon, and PN40024 homologs of VvTPS54, including probabilities for signal peptides, mitochondrial targeting peptides, chloroplast transit peptides, and thylakoid luminal transfer peptides. The chloroplast-localized (3S)-linalool synthase VvTPS56 is included as a reference.

Gene	Other	Signal peptide	Mitochondrial transfer peptide	Chloroplast transfer peptide	Thylakoid luminal transfer peptide
VITV-vi_vCabSauv08_v2.0.hapCF.chr10.ver2.0.g145220.001	0.9113	0.0002	0.0012	0.0857	0.0017
VITV-vi_vCabSauv08_v2.0.hapSB.chr10.ver2.0.g440600.001	0.9557	0.0003	0.0013	0.0424	0.0003
VITV-vi_vRiesFPS24_v1.1.hap1.chr10.ver1.1.g155480.001	0.9998	0.0002	0	0	0
VITV-vi_vRiesFPS24_v1.1.hap2.chr10.ver1.1.g472680.001	0.8005	0.0003	0.0077	0.1861	0.0053
VIT_00s0385g00020.001	0.9414	0.0002	0.0015	0.055	0.002
VvTPS56/VvRiLinNer	0.524	0.3992	0.0223	0.0086	0.0459

Supplementary Table S13. Count of predicted transcription factor binding sites within the promoter regions (3kb) of VvTPS54 homologs, summarized by haplotype (Riesling Hap1, Riesling Hap2, Cabernet Sauvignon HapCF, and HapSB). Highlighted TF binding sites correspond to those more abundant in Riesling Hap2 VvTPS54 promoter region (yellow) or less abundant (blue). Transcription factors were classified into families based on PlantTFDB annotations.

Transcription Factor	Family	R Hap1	R Hap2	CS hapCF	CS hapSB
AHL12	AHL	78	76	42	78
AHL20	AHL	34	34	20	34
AHL25	AHL	46	46	28	46
RAV1	AP2/ERF	1	1	0	1
ARR11	ARR	1	1	2	1
ARR14	ARR	1	1	0	1
ARR18	ARR	1	1	0	1
LEC2	B3 domain	2	2	2	2
BHLH34	bHLH	1	1	0	1
MYC4	bHLH	1	1	0	1
PHYPADRAFT_1438	bHLH	2	2	0	2
PHYPADRAFT_7248	bHLH	2	2	0	2
PIF5	bHLH	1	1	0	1
bHLH77	bHLH	1	1	0	1
BEE2	bHLH	2	2	0	2
BIM1	bHLH	2	2	0	2
BIM2	bHLH	2	2	0	2
BIM3	bHLH	2	2	0	2
UNE10	bHLH	1	1	0	1
BZR2	BZR/B	1	1	0	1
CCA1	CCA1/	1	1	1	1
LHY1	CCA1/	3	3	2	3
RVE1	CCA1/	2	2	2	2
CDF2	CDF	3	4	5	3
CDF3	CDF	2	4	1	2
AT2G20110	CPP	5	7	3	5
SOL1	CPP	7	8	3	7
TSO1	CXC domain	4	4	1	4
AT1G47655	DOF	3	3	2	3
AT3G52440	DOF	2	3	4	2
DAG2	DOF	2	2	3	2
DOF1.8	DOF	4	5	3	4
DOF2.4	DOF	3	4	3	3
DOF2.5	DOF	4	4	3	4
DOF5.3	DOF	5	5	10	5
DOF5.6	DOF	3	4	2	3
DOF5.7	DOF	3	3	4	3
DOF6	DOF	4	3	5	4
OBP4	DOF-like	0	1	2	0
KAN1	GARP/	1	1	1	1
GATA15	GATA	2	2	0	2
SGR5	GRAS	0	1	0	0
ANL2	HD-Zip	3	3	1	3
ATHB-16	HD-Zip	19	19	9	19
ATHB-51	HD-Zip	19	19	7	19
ATHB-6	HD-Zip	7	7	4	7
ATHB13	HD-Zip	15	15	6	15
ATHB18	HD-Zip	9	9	4	9
ATHB20	HD-Zip	9	9	4	9
ATHB23	HD-Zip	1	1	1	1
ATHB24	HD-Zip	1	1	2	1
ATHB25	HD-Zip	3	4	3	3
ATHB33	HD-Zip	2	3	4	2
ATHB34	HD-Zip	6	7	5	6
ATHB4	HD-Zip	2	2	4	2
ATHB53	HD-Zip	7	7	3	7
EDT1	HD-Zip	4	4	4	4
HAT1	HD-Zip	2	1	3	2
HAT2	HD-Zip	5	5	3	5
HAT22	HD-Zip	1	1	1	1
HDG1	HD-Zip	3	3	1	3
AGL42	MADS	4	4	1	4
AGL55	MADS	2	2	2	2
MYB111	MYB	1	1	3	1
MYB113	MYB	2	2	0	2
MYB24	MYB	1	1	1	1
MYB4	MYB	1	1	2	1
MYB59	MYB	1	1	1	1
MYB81	MYB	1	1	0	1
AT3G10113	MYB-like	1	1	1	1
AT5G61620	MYB-like	1	1	1	1
At1g74840	MYB-like	1	2	2	1
At3g09600	MYB-like	1	1	1	1
At4g01280	MYB-like	1	1	1	1
At5g47390	MYB-like	1	1	1	1
At5g52660	MYB-like	1	1	1	1
LCL1	MYB-like	1	1	1	1
TBP3	MYB-like	1	1	0	1
NAC025	NAC	0	1	2	0
NAC083	NAC	0	1	3	0
Glyma19g26560.1	No Hits	1	1	0	1
MNB1A	No Hits	8	7	12	8
P0510F09.23	No Hits	1	1	0	1
PHYPADRAFT_1407	No Hits	3	4	1	3
PHYPADRAFT_1533	No Hits	3	4	3	3
PHYPADRAFT_3883	No Hits	3	4	2	3
PHYPADRAFT_4826	No Hits	1	1	0	1
SPL9	SPL	1	1	0	1
ARALYDRAFT_4844	TCP	1	1	0	1
ARALYDRAFT_4930	TCP	2	1	0	2
ARALYDRAFT_4952	TCP	1	1	0	1
At1g72010	TCP	1	1	0	1
At5g08330	TCP	1	1	0	1
OJ1581_H09.2	TCP	2	2	0	2
Osl_08196	TCP	2	2	0	2
SBRC1	TCP	1	1	0	1
TCP15	TCP	1	1	0	1
TCP19	TCP	1	1	0	1
TCP20	TCP	1	1	0	1
TCP7	TCP	1	1	0	1
TCX2	TCP-like	4	5	2	4
GT-1	Trichelia	1	1	0	1
GT3a	Trichelia	1	1	0	1

Chapter 3: Allelic variation at a terpene synthase locus shapes wine monoterpene composition and aroma

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Abstract

The genetic basis of wine monoterpene variation and its sensory consequences remain poorly understood. We investigated how allelic variation at a chromosome 10 linalool QTL harboring a terpene synthase cluster, including *VviTPS54*, influences wine aroma in a Riesling × Cabernet Sauvignon mapping population. Twenty-six wines from progeny selected for contrasting genotypes were subjected to HS-SPME-GC-MS profiling and descriptive sensory analysis. Genotypes carrying the high-linalool Riesling allele produced substantially elevated monoterpene levels dominated by (3*S*)-linalool. Berry and wine monoterpene profiles were significantly correlated, and berry monoterpene glycosides showed broad correlations with wine monoterpenes. High-monoterpene genotypes were more closely associated with Floral, Tropical Fruit, and Apricot/Peach attributes, though perception was modulated by matrix effects from berry pigmentation. These results demonstrate that allelic variation at a terpene synthase locus drives coordinated changes in wine monoterpene composition and linked sensory attributes, while underscoring the complexity of translating genetic variation into sensory outcomes.

Introduction

Aroma is a central aspect of the wine experience and strongly influences consumer perception.¹ Many components of wine aroma originate from compounds produced by the grape

berry or are formed from grape-derived precursors during fermentation.^{2,3} Among these compounds, monoterpenes represent an important class of diverse odor-active molecules that contribute floral, citrus, and fruity notes to wine.^{2,4} Compounds such as (3*S*)-linalool, geraniol, and (-)-*cis*-rose oxide are particularly associated with aromatic cultivars such as Muscat, Riesling, and Gewürztraminer,^{5–7} though even in neutral cultivars, their low odor thresholds can influence sensory perception⁸. Variation in monoterpene composition contributes substantially to differences in wine aroma profiles across cultivars.^{4,9–11}

Despite advances in identifying genetic loci associated with monoterpene accumulation in grape berries,^{12–14} the extent to which this variation translates into differences in wine aroma and sensory perception remains poorly understood. Fermentation and aging substantially alter volatile composition, complicating efforts to link berry chemistry to the sensory attributes of finished wine.^{1,15–17} While several studies have characterized berry volatile composition in intraspecific and interspecific wine grape populations, none have extended this analysis to winemaking and descriptive sensory evaluation.^{18–21}

In earlier work,¹³ we profiled the berry metabolite composition of an F₁ population derived from a cross between Riesling and Cabernet Sauvignon, two commercially important wine cultivars with distinct aroma profiles.²² Through quantitative trait locus (QTL) mapping and transcriptomic analyses, we identified a major QTL on chromosome 10 of the Riesling genome containing *VviTPS54*, a functionally characterized (3*S*)-linalool synthase whose expression closely tracked (3*S*)-linalool abundance across genotypes.^{13,23} A single nucleotide polymorphism (SNP) marker, CHR10_5431663, was identified as a reliable predictor of (3*S*)-linalool content in berry tissue.

Here, we investigate how allelic variation at the chromosome 10 QTL influences wine aroma chemistry and sensory perception. A subset of the RxCS population segregating for this locus was selected for experimental winemaking. Wine volatile compounds were quantified by HS-SPME-GC-MS, and the resulting wines were evaluated by descriptive analysis with a trained sensory panel. By integrating chemical and sensory data, this study asks whether genetic differences driving monoterpene accumulation in grape berries translate into perceptible differences in wine aroma.

Materials and Methods

Plant Materials and Sampling

This study utilized a subset of genotypes from a mapping population resulting from a cross between *Vitis vinifera* ssp. *vinifera* cv. Riesling clone FPS 24/GM 110 and *V. v. vinifera* cv. Cabernet Sauvignon clone FPS 08 (RxCS). This population was planted at the Viticulture and Enology Department Experimental Station (Oakville, Napa, CA) on *V. rupestris* × *riparia* Couderc 3309 rootstock in 2018 as three randomized complete blocks with 9 replicates of each genotype. A border consisting of a minimum of 6 vines of Cabernet Sauvignon clone 08 surrounded the experimental vines in order to minimize edge effects. The 140 genotypes (Riesling clone 24, Cabernet Sauvignon clone 08, and 138 progeny) in this vineyard were trained to bilateral cordons and pruned to two bud spurs. The canopy was trained in a vertical-shoot-position system modified with a crossarm with rows in a northwest-southeast orientation. Vine spacing was 1.8 meters with rows 2.4 meters apart. This population has been previously used for identifying quantitative trait loci (QTLs) associated with metabolite production, cluster architecture, and microbiome composition.^{13,24,25} A major QTL located on chromosome 10 linked to (3*S*)-linalool production was identified from this population. The marker CHR10_5431663 located in the QTL was used

for the selection of genotypes for this study. In 2021, 24 RxCS genotypes were chosen for winemaking and descriptive analysis alongside the parent cultivars. Genotypes were selected using the following criteria: sufficient yield to produce enough wine for all replicates and training samples for descriptive analysis, equal numbers of pigmented and unpigmented genotypes, and equal numbers of TT and CT genotypes at marker CHR10_5431663. Berry sampling was initiated 12 weeks after flowering. A composite sample was taken from the three replicates of each experimental genotype in one block of the trial. Due to the limited amount of fruit, each sample consisted of 10-20 berries collected from different locations on multiple clusters. Samples were then crushed by hand. Juice Brix was measured weekly using a refractometer until 14 weeks post-bloom, increasing to biweekly thereafter until harvest.

Winemaking

Grapes were hand harvested for winemaking when sugar levels reached 21 Brix or stabilized for three consecutive sampling dates. Due to low yield, fruit from all nine biological replicates in all three blocks was combined for each genotype. Following mechanical destemming and crushing, the must was adjusted if needed to a titratable acidity (TA) of 6 g/L and yeast assimilable nitrogen (YAN) of 250 mg/L using tartaric acid and diammonium phosphate, respectively. A 15% potassium metabisulfite solution was added at crush to achieve 50 mg/L of SO₂. All fermentations were conducted in 22-liter plastic buckets at 18 °C and inoculated with 0.2 g/L of *Saccharomyces cerevisiae* strain EC1118 (Lallemand, Montreal, Canada) according to manufacturer directions. Manual punch downs were performed twice daily until fermentation was complete. Completion of fermentation was determined by a residual sugar of less than 2 g/L. The wines were then hand-pressed into 4- or 8-liter glass carboys and allowed to settle for three days. Wines were then racked off lees, sulfite adjusted to 50 mg/L SO₂, and stored at 4 °C until bottling.

Any headspace was sparged with N₂ gas. The wines were bottled by hand after five months into 375 mL clear glass bottles and stored at 14 °C until sensory and chemical analysis.

HS-SPME-GC-MS Analysis of Wine Samples

Head-space solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS) was used to analyze volatile compounds in the wines following the protocol from Hendrickson et al.²⁶ Berry volatile compound and monoterpene glycoside data for a subset of genotypes were taken from a previous study.¹³ Wine samples for volatile analysis were collected at the time of sensory evaluation and stored at 4 °C for no more than one month prior to analysis. Samples (10 mL) were placed in 20 mL amber glass headspace vials (Agilent Technologies) with 3 g NaCl and 50 µL of a 10 mg/L undecan-2-one solution (internal standard; Sigma-Aldrich, St. Louis, MO, USA), sealed with PTFE/silicone septa, and vortexed prior to analysis. Extraction was performed using a 1 cm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (Supelco, St. Louis, MO, USA). Samples were equilibrated at 30 °C for 5 min with agitation (500 rpm), followed by fiber exposure for 45 min at 30 °C with agitation (250 rpm). The fiber was desorbed in the GC inlet at 260 °C in split mode (10:1). Chromatographic separation was carried out on an Agilent 7890A GC system coupled to a 5975C inert XL MSD and equipped with a Gerstel MPS2 autosampler. Separation was achieved using a DB-WaxETR capillary column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies) under constant pressure with helium as the carrier gas and the GC method was retention time locked based on undecan-2-one.²⁶ The oven temperature program was as follows: initial temperature of 40 °C held for 5 min, ramped at 3 °C/min to 180 °C, then at 30 °C/min to 260 °C and held for 7.67 min (total run time 62 min). The MS operated in scan/SIM mode over an *m/z* range of 40–300 with a solvent delay of 2 min. Peak areas were normalized to the undecan-2-one internal standard, and results are therefore reported

as semiquantitative relative abundances. Due to limited fruit yield, all biological replicates were combined into a single fermentation per genotype; consequently, analytical replication reflects technical rather than biological variation. Each wine was analyzed in triplicate from a single bottle. For the parental cultivars (Riesling and Cabernet Sauvignon), bottle-to-bottle variation was assessed by analyzing three bottles in triplicate each ($n = 9$); variation among bottles was low (average coefficient of variance $< 10\%$).

Descriptive Sensory Analysis

To evaluate the sensory impact of the CHR10_5431663 alleles, 26 wines (24 progeny and the two parents) were subjected to descriptive sensory analysis (DA) of wine aroma approximately three months after bottling, following the methodology outlined by Lawless and Heymann.²⁷ Analysis was conducted in the J. Lohr Wine Sensory Room at the University of California, Davis, CA, with 11 panelists (5 females and 6 males between the ages of 21 and 70) recruited from the university and Davis, CA community. Approval from the university Institutional Review Board (UCD IRB; IRB project number 1867764-1) and informed consent from the panelists were obtained prior to the onset of the study. Panelists were not cognizant of the purpose of the study. The panelists were trained over ten one-hour training sessions through which a consensus list of attributes was generated. Panelists were asked to assess the aroma of the research wines blindly and generate a list of aroma attributes. This list was narrowed through group discussion. Aroma standards were prepared for each attribute on the consensus list and used to calibrate the panelists prior to each evaluation session (**Table 1**). The wines were evaluated over a two-week period and presented as a randomized complete block design with three replicates. Assessment of the wines occurred in isolated booths with positive airflow. 20 mL of wine was served in black glasses (Riedel, Sommeliers Blind Tasting glass) were used to prevent visual bias from wine color. The

research wines were evaluated by rating each attribute on an unstructured line scale anchored from ‘not present’ to ‘very intense’ using Compusense® software (Compusense Inc., Guelph, ON, Canada).

Table 1. Aroma reference standards used for sensory evaluation of Riesling × Cabernet Sauvignon wines.

Aroma	Reference Standard
Alcohol	20 mL vodka (Ketel One Vodka)
Apple	1 wedge of sliced Granny Smith apple
Apple Cider	20 mL apple cider (Martinelli's)
Apricot/Peach	10 mL each apricot and peach juice (Kerns)
Asparagus	2 mL brine from canned asparagus (Green Giant)
Banana	1 slice of banana
Bubble Gum	1/12 th of a piece of Hubba Bubba gum
Caramel	1 tsp caramel topping (Smucker's)
Cheesy	1 tsp grated Parmigiano Reggiano cheese
Citrus	¼ wedge of lemon
Dark Chocolate	½ tsp 100% cocoa powder (Ghirardelli)
Dark Fruit	½ tsp blackberry syrup (Monin), 1 tsp blackcurrant juice (Ribena), 1 tbsp cherry juice (Knudsen)
Earthy/Musty	1 tbsp soil (Miracle-Gro Potting Mix) and 10 mL water
Floral	1% (3S)-linalool in neutral white wine (Bota box, Chardonnay)
Grape Juice	15 mL Concord grape juice (Knudsen, Organic Grape)
Grass	½ tbsp grass
Green Bell Pepper	2 x1 cm piece of green bell pepper
Green Olives	1 green olive with 3 mL brine (Mezzetta)
Leather	6 1 cm strips of leather (Kiwi Brand shoelaces) with 2 mL water
Molasses	1 tsp molasses (Brer Rabbit)
Red Fruit	½ tsp strawberry jam, 1 tsp raspberry jam (A natural fruit spread, Mountain Fruit Company)
Rubber	10 thin slices rubber (ACE rubber gasket)
Solvent/Acetone	10% acetone in water
Tropical Fruit	1/6 piece each of pineapple, mango, and kiwi candy (HiChew)
Violet	1.5 mL water with 1.5 mL violet syrup (Monin)
Wax/Crayon	2 1 cm pieces of crayon (Crayola)

Statistical Analysis

All statistical analyses were conducted in R (R Foundation for Statistical Computing, Vienna, Austria). Data handling and visualization were performed using the tidyverse v. 2.0 suite of packages, and multivariate and univariate statistical analyses were carried out using base R

(stats v. 3.6.2) and supporting packages as listed below. The potential influence of wine matrix parameters was assessed to determine whether they should be included as covariates in subsequent analyses. The effects of wine alcohol content and pH on volatile compound abundance and sensory attributes were evaluated using Spearman rank correlations (`cor.test, method = "spearman"`), linear models (`lm`), and multivariate analysis of variance (MANOVA) applied to genotype-averaged data. For MANOVA, all sensory attributes were included as a multivariate response, with alcohol content and pH treated as continuous predictors and significance assessed using Wilks' λ . P-values from univariate tests were adjusted for multiple testing using the false discovery rate (FDR) method (`p.adjust, method = "fdr"`). As no significant associations were detected ($P\text{-adj} > 0.05$), alcohol and pH were not included as covariates in subsequent analyses.

For chemical and sensory datasets, multivariate analysis of variance (MANOVA) was then used to assess overall product effects. Models for descriptive analysis (DA) data included product, judge, and replicate as fixed effects to account for panelist variability and repeated measurements. Wilks' λ was used as the test statistic, with approximate F-tests used to determine significance. For volatile compound datasets, MANOVA models included product and replicate effects. Following MANOVA, univariate analyses of variance (ANOVA) were performed for individual variables using the same model structure. For DA data, ANOVA models included product, judge, and replicate effects, whereas chemical data models included product and replicate. P-values were adjusted for multiple testing using the false discovery rate (FDR) method (`p.adjust, method = "fdr"`). Attributes or compounds showing significant product effects ($\alpha = 0.05$) were retained for subsequent multivariate analyses.

Permutational multivariate analysis of variance (PERMANOVA) was used to evaluate multivariate differences in wine volatile profiles and assess the potential influence of wine matrix

variables. Analyses were conducted using the `adonis2` function in the `vegan` package (v. 2.7-2)²⁸. To test whether volatile composition differed among genetic marker classes, a Euclidean distance matrix was constructed from the scaled compound data and modeled as a function of marker class using 999 permutations. Separately, the effects of wine alcohol and pH on volatile composition were evaluated with alcohol and pH as explanatory variables. Principal component analysis (PCA) was performed on mean-centered, scaled chemical data using the *FactoMineR* v. 2.13 package²⁹ to visualize patterns of variation among wines.

Canonical variate analysis (CVA) was conducted using the package `candisc` v 1.10³⁰ on significant sensory attributes to maximize separation among products relative to within-group variation. For CVA, replicate observations were averaged within each judge $\times$ product combination to reduce within-panel noise while preserving judge-level variation. Wine centroids were calculated as the mean position across judges, and score and loading plots were used to interpret multivariate structure. Relationships between chemical composition and sensory attributes were explored using multiple factor analysis (MFA) with the package *FactoMineR* v. 2.13 to identify associations between volatile compounds and descriptive sensory attributes and to visualize their joint contribution to sample differentiation. Significance for all tests was determined at $\alpha = 0.05$ unless otherwise stated

Results

Genotype selection and winemaking

Metabolite profiling of berries revealed significant variation in monoterpene content between genotypes with differing alleles at the CHR10_5431663 marker (**Fig. 1**). As both parent cultivars are grown for commercial wine production, we selected a subset of genotypes for winemaking and descriptive analysis to assess the sensory impact of allelic variation at this locus. Genotype selection was constrained by the need for adequate fruit yield. Alongside both parents, 24 progeny were selected: 12 genotypes heterozygous (CT) at the CHR10_5431663 locus (6 red and 6 white), which produced moderate to high levels of (3*S*)-linalool, and 12 homozygous (TT) (7 red and 5 white), with low (3*S*)-linalool production.

Due to variation in phenological stages, harvest of the RxCS genotypes spanned two weeks (**Table 2**). In nine genotypes, sugar accumulation slowed or stopped before the target of 21 °Brix was reached, and these were harvested early to avoid dehydration, sour rot, and bird damage. Consequently, °Brix at crushing ranged from 19.1 to 24.0, with white genotypes averaging slightly higher than red genotypes (22.1 vs 21.1 °Brix). Fruit yield ranged from 4.28 kg to 13.77 kg with a mean of 7.45 kg. All fermentations proceeded to dryness without detectable issues. Despite substantial variation in alcohol content and pH among finished wines, neither parameter significantly influenced volatile composition or sensory attributes (MANOVA, $P\text{-adj} > 0.05$), indicating that differences in wine chemistry and aroma were not driven by these matrix variables and were therefore excluded from subsequent analyses.

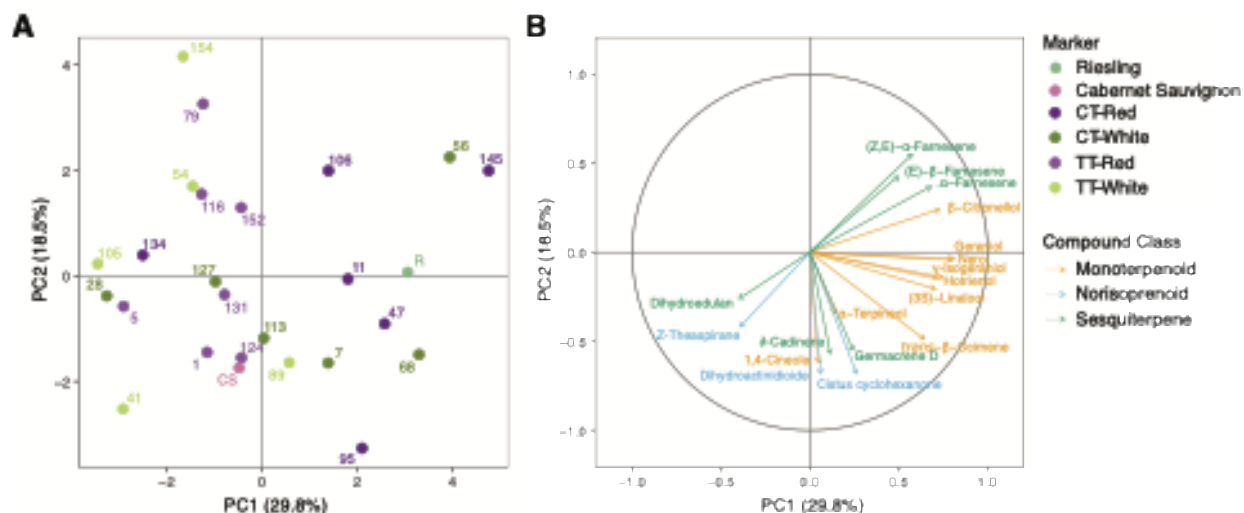


Figure 1. Principal component analysis (PCA) of selected RxCS genotypes used for winemaking based on berry terpene profiles, including monoterpenes, sesquiterpenes, and norisoprenoids. (A) PCA scores plot showing the distribution of genotypes colored by marker genotype class, with parental cultivars (Cabernet Sauvignon and Riesling) shown separately. (B) Corresponding loading plot showing compound contributions to PC1 and PC2, colored by chemical class.

Wine volatile composition varies with marker genotype

Genotypes showed substantial differences in volatile composition after fermentation (Fig. 2 and Fig. 3). A total of 47 compounds were identified from the wines (Supplementary Table S1). PCA of all volatile compounds (Fig. 2A) showed that the first two dimensions explained 23.3% and 18.6% of the variance, respectively. Separation between Cabernet Sauvignon and Riesling wines was driven mainly by esters such as ethyl butanoate and phenylethyl acetate, the norisoprenoids 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and β -cyclocitral, and monoterpenoids including (3S)-linalool. Progeny wines were distributed between the parents, with nonpigmented CT genotypes largely occupying the upper right quadrant.

Table 2. Harvest parameters and resulting wine composition for selected genotypes of the RxCs population, including parental cultivars (Cabernet Sauvignon [CS] and Riesling [R]). Measured variables include fruit metrics at harvest (fruit weight, °Brix, pH, titratable acidity [TA], malic acid, and yeast assimilable nitrogen [YAN]) and finished wine properties (residual sugar, final pH, TA, alcohol content, and final volume). Genotypes are categorized by marker genotype (CT, TT) and berry color (red, white).

Genotype	Marker Genotype	Color	Harvest Date	Fruit Weight (kg)	Brix	Initial pH	Initial TA (g/L)	Initial Malic (mg/L)	Initial YAN (mg/L)	Residual Sugar (g/L)	Final pH	Final TA (g/L)	Alcohol %	Final Volume (L)
1	TT	Red	8-Sep	6.69	21.70	3.12	7.05	1741	38	0.00	3.49	6.9	12.4	3.79
5	TT	Red	27-Aug	5.36	21.1	3.06	8.37	1862	122	0.01	3.31	7.5	11.6	3.79
7	CT	White	2-Sep	5.71	21.6	3.16	6.36	1214	50	0.02	3.16	8.1	11.2	3.03
11	CT	Red	8-Sep	5.81	20.50	3.23	5.02	1001	35	0.01	3.30	7.5	11.7	3.41
28	CT	White	2-Sep	6.00	23.9	2.75	10.42	1913	65	0.00	3.44	7.0	12.1	3.41
41	TT	White	27-Aug	6.37	22.4	3.03	9.06	3690	106	0.00	3.54	6.9	12.1	3.79
47	CT	Red	2-Sep	8.23	20.6	3.45	5.25	2320	45	0.00	3.53	6.7	11.7	4.43
54	TT	White	9-Sep	9.63	22.90	3.19	5.53	1418	98	0.00	3.26	7.9	12.3	5.68
56	CT	White	8-Sep	8.44	20.60	3.30	5.15	1608	92	0.01	3.08	9.4	13.1	5.07
68	CT	White	27-Aug	9.16	23	3.09	8.76	3904	168	0.02	3.24	8.5	12.8	5.37
79	TT	Red	8-Sep	7.20	20.10	3.02	7.95	1648	146	0.02	3.41	7.5	12.8	5.07
89	TT	White	2-Sep	5.85	21.4	3.00	7.27	1775	58	0.01	3.49	6.9	12.5	3.41
95	CT	Red	9-Sep	6.30	20.60	3.38	4.87	1171	87	0.00	3.52	6.5	12.1	3.79
105	TT	White	27-Aug	8.07	22.3	3.17	6.73	2130	101	0.01	3.39	6.3	12.5	5.07
106	CT	Red	2-Sep	11.04	20.6	3.11	6.43	1498	64	0.02	3.28	6.2	12.8	5.56
113	CT	White	27-Aug	7.71	23	3.19	6.19	1360	211	0.02	3.27	6.3	12.4	5.07
116	TT	Red	8-Sep	10.15	22.20	3.32	5.68	2159	128	0.02	3.38	6.0	12.3	6.62
124	TT	Red	9-Sep	7.58	21.30	3.29	5.31	1302	98	0.01	3.41	6.8	12.7	5.68
127	CT	White	2-Sep	4.80	21.4	3.08	6.98	1362	51	0.00	3.33	8.1	13.2	3.03
131	TT	Red	9-Sep	8.72	19.10	3.30	5.32	1306	67	0.00	3.28	7.6	12.4	5.68
134	CT	Red	9-Sep	5.74	20.50	3.33	5.57	1756	97	0.00	3.23	7.0	11.3	4.16
145	CT	Red	8-Sep	4.28	21.50	3.36	5.52	1963	77	0.01	3.27	7.1	11.5	3.79
152	TT	Red	2-Sep	5.77	21.7	3.34	5.52	1956	139	0.01	3.30	7.2	12.0	3.41
154	TT	White	8-Sep	6.84	21.60	3.39	4.94	1956	130	0.01	3.49	6.4	11.9	5.07
CS	TT	Red	9-Sep	13.77	24.00	3.27	5.47	2257	116	0.01	3.59	5.8	11.6	7.57
R	CT	White	27-Aug	8.48	20.7	3.05	7.1	1719	69	0.01	3.46	6.4	12.1	5.68

Restricting the analysis to monoterpenes (**Fig. 2B**) increased the variance explained by PC1 to 61.5% and sharpened the separation between marker genotypes. Genotypes were distributed along PC1 according to (3*S*)-linalool content and that of correlated monoterpenoids. PC2 captured stratification associated with the relative abundance of β -citronellol versus the monocyclic terpenes *p*-cymene and α -terpinene, which were predominant in genotypes 28 and 11, whereas β -citronellol was more abundant in genotypes 95 and 106. PERMANOVA confirmed that the effect of marker genotype on volatile composition was specific to monoterpenes, which showed strong differentiation between TT and CT genotypes ($R^2 = 0.46$, $P\text{-adj} = 0.008$; **Fig. 2C**).

Hierarchical clustering (**Fig. 3**) reflected this differentiation, though imperfectly: two TT individuals clustered with CT genotypes and vice-versa. While marker effects were significant only for monoterpenes (**Fig. 2C**), norisoprenoids showed notable variation among genotypes, with β -cyclocitral, TDN, β -ionone, and β -damascenone occurring at substantially higher levels in some specific individuals. TDN was most abundant in wine from Riesling, genotype 28, and genotype 54, but was detectable in all wines including Cabernet Sauvignon. TDN and β -cyclocitral were significantly positively correlated with each other and with most monoterpenes.

Berry and wine monoterpene profiles are correlated

Berry volatile compounds and monoterpene glycosides of a subset of 10 genotypes analyzed in a previous study were compared with volatile compounds measured in the corresponding wines.¹³ Correlations between berry and wine compounds are shown in **Fig. 4**. Wine monoterpenes, including (3*S*)-linalool, β -myrcene, geraniol, limonene, and nerol were significantly correlated with a broad range of berry monoterpenes and monoterpene glycosides ($r > 0.7$, $P\text{-adj} < 0.05$), indicating that berry monoterpene composition is a reliable predictor of wine monoterpene content. Correlations between the same compound in berry and wine were significant

for (3*S*)-linalool and nerol, suggesting that these compounds are transferred relatively directly from berry to wine with limited transformation. Most monoterpene glycosides showed broad correlations with many wine monoterpenes, with the exception of PH1 and PH2, which were only significantly correlated with α -terpinene (P -adj <0.05) and showed a trend toward correlation with *p*-cymene (P -adj <0.1), consistent with their potential role as precursors to these monocyclic terpenes during fermentation.

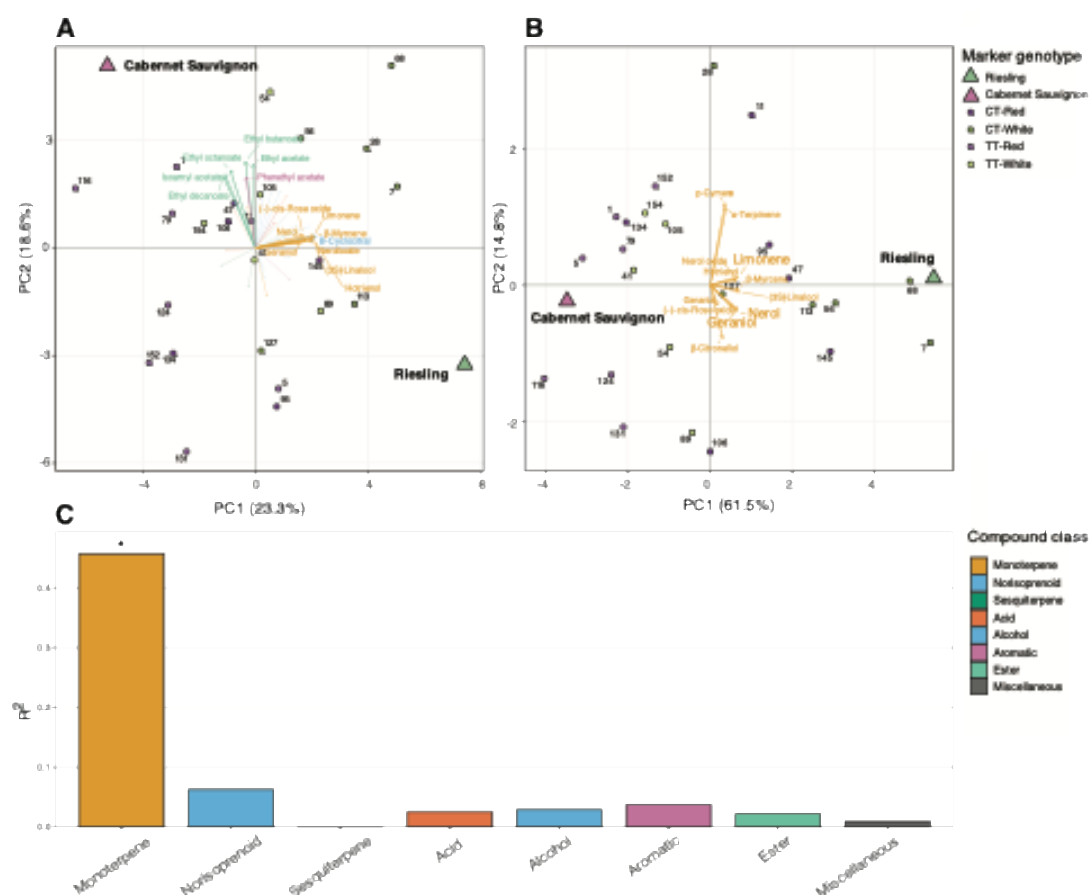


Figure 2. Multivariate analysis of wine volatile composition in the RxCS population. (A) PCA biplot of all detected volatile compounds. (B) PCA biplot of monoterpenes only. In both panels, points represent individual genotypes colored by marker genotype class, with parental cultivars shown separately. Vectors represent compound loadings colored by chemical class. (C) Proportion of variance in each volatile compound class explained by marker genotype (R^2), as determined by PERMANOVA. The bar marked with an asterisk (*) indicates significance at P -adj < 0.05.

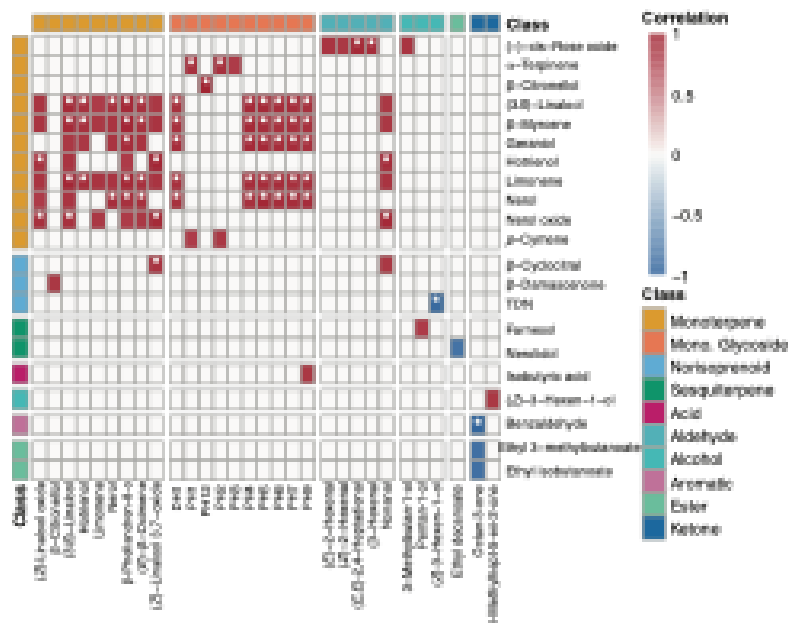


Figure 4. Correlation heatmap between berry-derived volatile compounds and monoterpene glycosides (columns) and wine volatile compounds (rows) in the RxCS population. Only pairs with correlation coefficients $r > 0.7$ and adjusted P-value < 0.1 are displayed; all other cells are blank. Color indicates the direction and strength of correlation (red = positive; blue = negative). Asterisks denote pairs significant at $P\text{-adj} < 0.05$. Compounds are grouped by chemical class, indicated by color-coded row and column annotations, with breaks separating major classes.

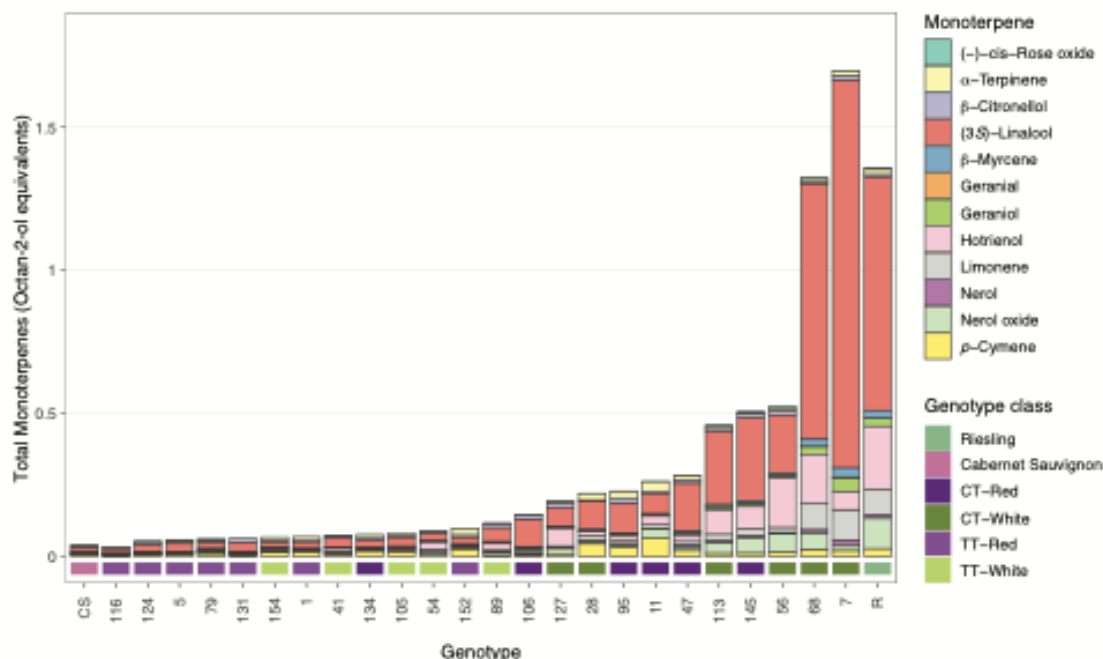


Figure 5. Genotype-dependent variation in wine monoterpene composition across the RxCS population. Stacked bars show the relative abundance of individual monoterpenes in wines from each genotype, expressed in undecan-2-one equivalents. Genotypes are ordered from lowest to highest total monoterpene abundance. Parental cultivars (CS, Cabernet Sauvignon; R, Riesling) are labeled separately. Genotype class is indicated by the color-coded bar below each genotype label.

Monoterpene composition varies continuously across the population

Wine monoterpene composition varied widely across the RxCS population, with total relative abundances spanning more than an order of magnitude among genotypes (**Fig. 5**). Monoterpene levels formed a continuous gradient, with some individuals reaching levels approaching or exceeding those of Riesling. With one exception, CT genotypes produced higher total monoterpene levels than TT genotypes; genotype 134 (CT) produced lower levels than four TT genotypes. (3*S*)-Linalool was the predominant monoterpene across all genotypes (**Table 3**), contributing 22.6-79.7% of the total, with the highest-abundance genotypes also exhibiting the greatest proportion of (3*S*)-linalool. The second most abundant wine monoterpene varied among genotypes, with β -citronellol, *p*-cymene, and hotrienol most frequently observed. Geranial was the second most abundant monoterpene in only a single genotype.

Descriptive analysis reveals genotype-dependent aroma profiles

The DA panelists generated 26 aroma descriptors by consensus (**Table 1**). MANOVA indicated significant product effects on aroma profiles in the full dataset after accounting for judge and replicate effects (Wilks' $\lambda = 0.13$, $p < 0.05$, **Supplementary Table S2**). Subsequent ANOVA identified 14 attributes that were statistically significant at a P-adj of <0.05 . CVA of these attributes is shown in **Fig. 6A** and **6B**. The first canonical variate (Can1) accounted for 35.2% of the variation among products, and Can2 for an additional 16.2%. Red and white genotypes were clearly separated along Can1. White genotypes showed a close association with five descriptors: Asparagus, Green Olives, Floral, Tropical Fruit, and Apricot/Peach, with genotypes 7, 28, 56, 68, 113, and Riesling showing a particularly strong positive correlation with Floral and Asparagus. Red genotypes were distinguished by three loose groupings: Dark Chocolate, Dark Fruit, Acetone, and Banana; Caramel and Cheesy; and Green, Grape Juice, and Apple. Can2 mainly separated red

genotypes between the Caramel and Cheesy grouping and the other two. The aroma profiles of Cabernet Sauvignon and genotype 106 were highly driven by the Cheesy attribute. White genotypes showed less variation along Can2 than red genotypes. Within each color grouping, CT genotypes were generally located to the left of TT genotypes in canonical space.

Table 3. Monoterpene composition of wines from selected RxCS genotypes. Total monoterpene relative abundance is expressed in undecan-2-one equivalents. The percentage contributions of (3*S*)-linalool and the second most abundant monoterpene to the total monoterpene pool are shown for each genotype. Genotypes are ordered by increasing total monoterpene abundance.

Genotype	Marker Genotype	Berry Color	Total Monoterpenes (Relative Abundance)	(3 <i>S</i>)-Linalool % Total	2nd Major Monoterpene	% Total
CS	TT	Red	0.038	32.3	β-Citronellol	17.8
116	TT	Red	0.033	40.7	β-Citronellol	19.6
124	TT	Red	0.053	40.4	β-Citronellol	21.3
5	TT	Red	0.056	49.5	β-Citronellol	13.6
79	TT	Red	0.06	32.3	<i>p</i> -Cymene	16.4
131	TT	Red	0.066	43.5	β-Citronellol	26.1
154	TT	White	0.069	23	<i>p</i> -Cymene	21.1
1	TT	Red	0.07	31.8	<i>p</i> -Cymene	24.4
41	TT	White	0.075	42.8	Hotrienol	14.1
134	CT	Red	0.078	31.8	<i>p</i> -Cymene	24.4
105	TT	White	0.085	35.1	<i>p</i> -Cymene	19.7
54	TT	White	0.092	26.4	Hotrienol	28.9
152	TT	Red	0.098	22.6	<i>p</i> -Cymene	26.4
89	TT	White	0.117	41.1	Hotrienol	19.4
106	CT	Red	0.147	63.5	β-Citronellol	11.1
127	CT	White	0.193	34.5	Hotrienol	31.4
28	CT	White	0.22	44.4	<i>p</i> -Cymene	20.1
95	CT	Red	0.228	45	<i>p</i> -Cymene	13.5
11	CT	Red	0.262	26.8	<i>p</i> -Cymene	24.3
47	CT	Red	0.283	58.7	Hotrienol	7.2
113	CT	White	0.458	55.9	Hotrienol	18.5
145	CT	Red	0.506	57.1	Hotrienol	15.2
56	CT	White	0.525	38.8	Hotrienol	32.8
68	CT	White	1.322	67.4	Hotrienol	13
7	CT	White	1.696	79.7	Geranial	6.4
R	CT	White	1.357	60	Hotrienol	16.2

Because berry color dominated separation in the full dataset, MANOVA, ANOVA, and CVA were repeated separately on red and white wines (**Fig. 6C-F**). Product effects were significant for both subsets (Wilks' λ = 0.20 and 0.18 for reds and whites, respectively; $p < 0.05$, **Supplementary Table S2**). For red wines, Can1 accounted for 40.6% of the variation (**Fig. 6C**

and **D**), with wines distributed along a gradient from fruity attributes such as Banana and Apricot/Peach to Cheesy and Caramel. CT genotypes tended to score higher in Apple, Apricot/Peach, and Green than TT genotypes, though substantial overlap was present, indicating only moderate differentiation. One CT individual, 106, was an exception, showing a negative association with Apple and Apricot/Peach.

Analysis of white wines showed weaker structure (**Fig. 6E and F**). Can1 and Can2 explained 39.8% and 31.3% of the variation, respectively, but marker genotype classes showed substantial overlap. Nevertheless, the Floral descriptor was much more closely associated with CT individuals. The Waxy/Crayon descriptor, not significant in the full or red wine dataset, was a strong contributor to Can1 separation and positively associated with genotypes 54 and 56. Overall, marker genotype effects on aroma perception were more discernible among red wines than white wines, where genotype classes were less distinctly separated in canonical space.

Integration of chemical and sensory data

To assess the relationship between sensory and volatile profiles, multiple factor analysis (MFA) was performed, integrating DA attributes with wine volatile compound data (**Fig. 7A-F**). As with the CVA, MFA was conducted on all wines and on subsets by berry color. When all wines were considered, the first two dimensions explained 34.8% of the variance. Dimension 1 (22.3%) distinguished genotypes primarily by berry color and secondarily by marker genotype, with white genotypes clustering on the positive side of the plot, consistent with the CVA. Within each color grouping, CT individuals were located to the right of TT genotypes, corresponding to positive loadings of monoterpenes including (3S)-linalool, limonene, and β -myrcene, and the sensory attributes Floral, Tropical Fruit, and Apricot/Peach (**Fig. 7B**). Interestingly, genotype 145, despite

having the fourth highest monoterpene level, scored lower in monoterpene-associated descriptors than several white genotypes with substantially lower monoterpene content.

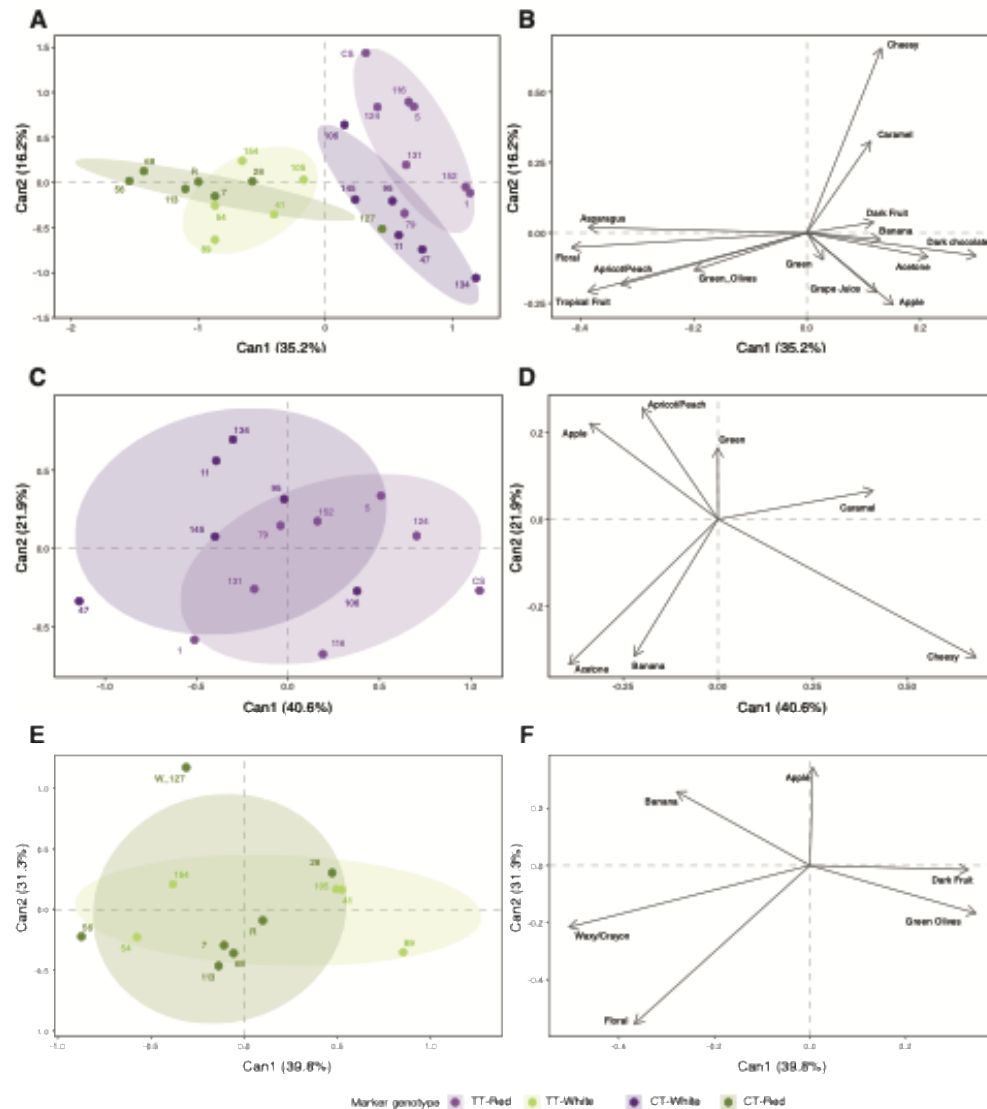


Figure 6. Canonical variate analysis (CVA) of descriptive analysis data from RxCS wines. (A, C, E) Score plots of the first two canonical variates for all wines, red wines only, and white wines only, respectively. Points represent individual wine genotypes colored by marker genotype class. Shaded ellipses indicate the dispersion of wines within each marker genotype class. (B, D, F) Corresponding loading plots displaying canonical structure coefficients for sensory attributes significant under ANOVA ($P\text{-adj} < 0.05$) within each dataset. Percent variance explained by each canonical variate is indicated on the axes. Eigenvalues are provided in **Supplementary Table S3**.

Genotypes on the left side of Dimension 1 were associated with fermentation-derived descriptors such as Cheesy, Dark Fruit, and Banana. Except for a moderate positive correlation between hexyl acetate and Cheesy, no strong associations between sensory attributes and volatile compounds were identified in this region. Dimension 2 (12.5%) was driven by the relative abundance of yeast-derived esters including isoamyl acetate, phenylethyl acetate, and ethyl hexanoate, none of which were significantly associated with any DA attributes. Of note, the fruit descriptors Banana, Dark Fruit, and Grape Juice showed little to no correlation with these esters, despite their association with fruity aromas ^{17,31}.

Restricting the analysis to red genotypes (**Fig. 7C and D**) preserved the separation of markers along Dimension 1 (24.2%), though with reduced dispersion. Compound-descriptor associations not apparent in the full dataset emerged here: Cheesy was highly correlated with isoamyl alcohol, and Caramel with ethyl isobutanoate. Among white genotypes (**Fig. 7E and F**), CT genotypes largely occupied the upper-right quadrant of the plot in association with monoterpenes and the Floral descriptor, though separation from TT genotypes was incomplete. Two additional associations emerged in this subset: Waxy/Crayon, not significant in the other datasets, was correlated with ethyl decanoate and octanoic acid, both known for fatty or waxy odor character ^{31,32}; and Green Olives was highly correlated with nerolidol, a sesquiterpene, and ethyl cinnamate, a phenylpropanoid-derived ester.

The association between Floral and Apricot/Peach and most monoterpenes was consistent across all three datasets (**Fig. 7B, D, F**), with increased loading separation within color subsets suggesting additional compound contributions once major between-group differences are removed. Collectively, these results indicate that genotype-dependent variation in wine aroma is driven primarily by coordinated changes in monoterpene composition, with monoterpene-linked

sensory attributes providing the strongest and most consistent chemical-sensory associations within the RxCS population.

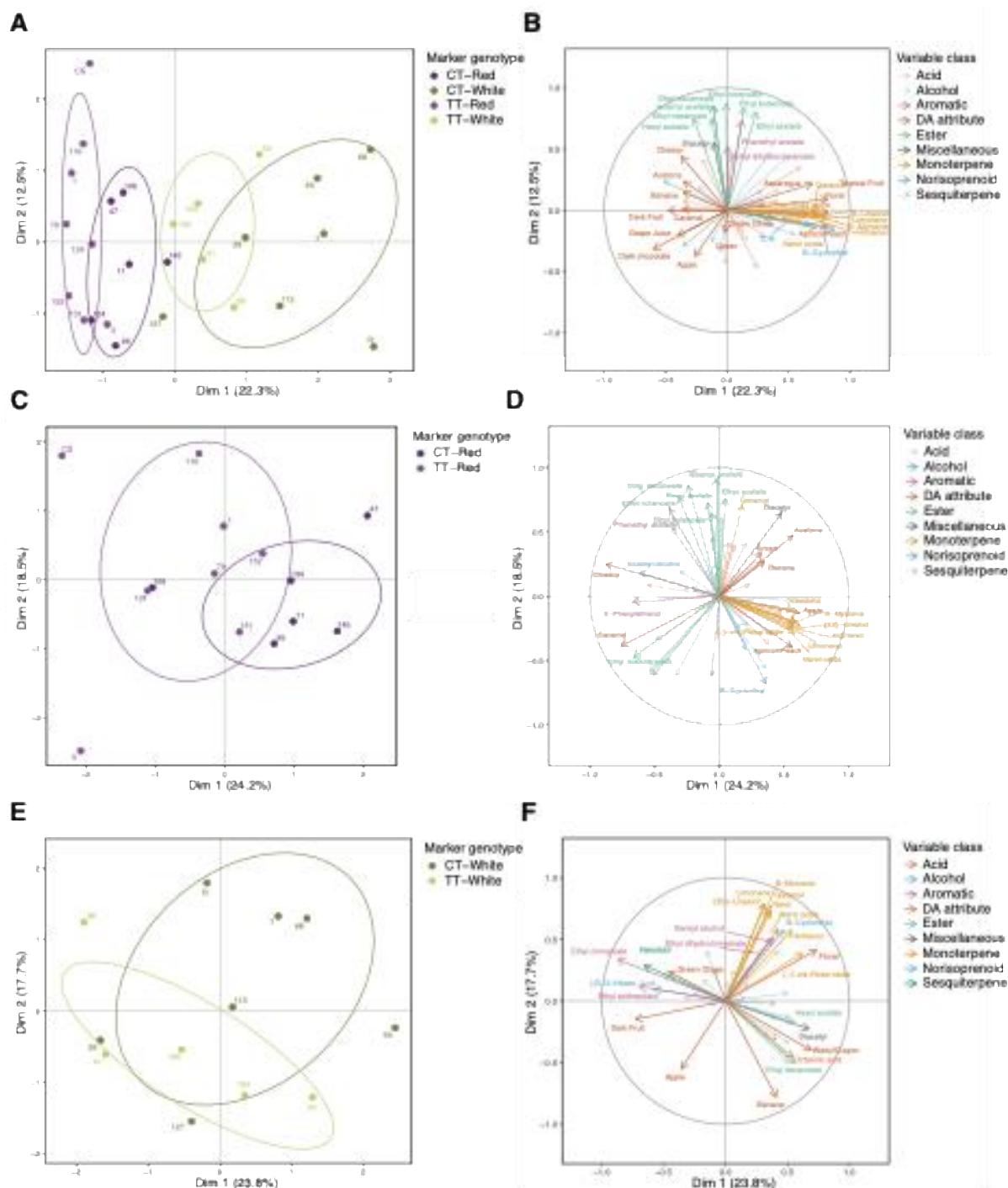


Figure 7. Multiple factor analysis (MFA) of wine descriptive analysis (DA) attributes and SPME-GC-MS volatile compounds across the Riesling $\times$ Cabernet Sauvignon population. Panels A, C, and E: MFA scores plots showing genotype clustering based on combined sensory and chemical profiles for wines produced from (A) all genotypes (n=26), (C) red genotypes (n=14), and (E) white genotypes (n=12). Points represent genotype means,

colored by marker genotype class, with ellipses indicating group dispersion within one standard deviation (68% confidence interval). **Panels B, D, and F:** Corresponding variable correlation circles displaying loadings of significant DA attributes and GC–MS compounds. Arrows indicate the direction and strength of variable contributions to the first two MFA dimensions, with colors denoting compound class. DA attributes are highlighted in orange. For clarity, only the top 10 contributors to Dimensions 1 and 2 among GC–MS variables are labeled, along with all significant DA attributes. Percent variance explained by each dimension is shown on the axes. Eigenvalues are provided in **Supplementary Table S4**.

Discussion

This study demonstrates that genetic variation at the *CHR10_5431663* locus exerts a strong influence on monoterpene composition and relative abundance in wines produced from the Riesling × Cabernet Sauvignon population. This chemical variation translated, albeit imperfectly, into detectable differences in wine aroma. While monoterpenes were the primary drivers of genotype-dependent variation in volatile composition, their effects on sensory perception were modulated by additional matrix factors, resulting in continuous and partially overlapping phenotypes rather than discrete sensory classes.

in wine volatile composition were most pronounced for monoterpenes, as indicated by both multivariate analyses and PERMANOVA results. Separation of wines along the primary axis of monoterpene PCA space was largely driven by (3*S*)-linalool and related compounds, underscoring the dominant role of (3*S*)-linalool in shaping wine monoterpene composition⁹ and consistent with the known role of monoterpene synthase genes located within the Chr. 10 genomic region.^{13,33} The differentiation observed between marker groups was expressed as a continuous gradient in monoterpene abundance rather than discrete classes, a pattern observed in previous studies.^{12,21,34,35} This suggests that variation in monoterpene composition reflects quantitative differences in biosynthesis, glycoside hydrolysis, and interconversion during fermentation, likely influenced by precursor availability and molecular rearrangement.^{36–40}

In addition to total monoterpene abundance, substantial variation was observed in monoterpene composition among genotypes in this study. A meta-analysis of 19 studies identified 22 distinct monoterpenes occurring in grape and wine with (3*S*)-linalool and its derivatives constituting half of the compounds.⁹ Consistent with this finding, (3*S*)-linalool was the predominant compound across all RxCS wines regardless of monoterpene abundance, contributing between 22.6% and 79.7% of the total monoterpene pool. The identity and relative abundance of secondary monoterpenes varied considerably with compounds such as β -citronellol, *p*-cymene, and hotrienol frequently occurring as the second most abundant. Moreover, (3*S*)-linalool contributed a greater proportion of total monoterpenes in high-abundance genotypes. This pattern is consistent with the Chr. 10 locus acting primarily on (3*S*)-linalool biosynthesis rather than on downstream interconversion pathways.

In contrast to monoterpenes, other volatile compound classes exhibited weaker and less structured patterns of variation across wines. Esters, alcohols, and acids showed more diffuse distributions. Consistent with previous studies, phenylpropanoid pathway products such as 2-phenylethanol and phenylethyl acetate were considerably more abundant in Cabernet Sauvignon and a few progeny genotypes.^{17,22,41} Similarly, norisoprenoid content varied across genotypes, with Riesling and genotypes 28 and 54 exhibiting elevated levels of the characteristic Riesling aroma compound 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), consistent with genetic regulation of norisoprenoid accumulation in this population.

Comparison of berry and wine metabolite profiles revealed direct relationships primarily among monoterpenes. Although berry monoterpenes broadly correlated with those in wine, only (3*S*)-linalool and nerol showed significant berry-to-wine correlations, indicating that their abundance in wine is directly related to berry composition. Most berry monoterpene glycosides

were highly correlated with a range of wine monoterpenes, yet *in vitro* enzymatic hydrolysis of Riesling monoterpene glycosides yielded a single monoterpene aglycone per glycoside,⁴² suggesting that glycoside hydrolysis alone does not account for the diversity of wine monoterpenes observed. Isomerization, cyclization, and other mechanisms of molecular rearrangement could explain the lack of direct correspondence between berry compounds and wine monoterpenes.⁴³ Notably, two monoterpene glycosides (PH1 and PH2) correlated specifically with α -terpinene in wine, a compound that could not be linked to any berry monoterpene in our previous study.¹³ Surprisingly, no significant correlations between berry C6 alcohols and hexyl acetate were detected in the wines of this study despite previous documentation of acetate esters increasing in proportion with their corresponding precursor.⁴⁴ While berry composition provides an important baseline, it is not solely determinative of wine volatile profiles.

The sensory consequences of genotype-dependent variation in monoterpenes were evident but not absolute. Descriptive analysis revealed significant product effects, with wines varying along gradients defined by attributes such as Floral, Tropical Fruit, and Apricot/Peach. These were strongly associated with monoterpene abundance in MFA. The association between (3*S*)-linalool and floral/fruit descriptors is well-established.^{2,45–47} Wines produced from CT genotypes tended to score higher for these descriptors, consistent with their generally elevated monoterpene levels. However, substantial overlap among genotypes was observed, and not all high-monoterpene wines exhibited correspondingly high sensory scores. For example, genotype 145, despite possessing relatively high monoterpene levels, was not strongly associated with monoterpene-derived sensory attributes. This decoupling highlights the complexity of aroma perception, which depends not only on compound concentration but also on odor thresholds, interactions among volatiles, and matrix effects.

Despite identical fermentation conditions and obscuration of wine color during evaluation, the phenolic matrix associated with berry pigmentation accounted for much of the variation among wines in this study. Regardless of monoterpene level, pigmented genotypes grouped more closely with each other than with unpigmented individuals of comparable monoterpene abundance, indicating that matrix composition strongly influences aroma expression. Differences in genotype-dependent separation between red and white wines further support this interpretation: clearer gradients were observed among red wines, whereas white wines exhibited weaker structure and greater overlap among marker classes. Although wine color was not visible to panelists, odor alone has been shown to be sufficient to distinguish red from white wines,⁴⁸ suggesting that color-associated aroma differences may have contributed to separation in the sensory data independent of monoterpene content. However, the white wines in this study were atypical in that they were fermented on the skins, and matrix effects from elevated phenolic content likely modulated aroma expression in both color groups. Further work involving a larger number of genotypes within a single color group could help clarify the contribution of (3*S*)-linalool to wine aroma perception.

Matrix effects are known to modulate the perception of volatile compounds in wine.^{49–53} Ethanol suppresses the volatilization of polar aroma compounds while enhancing that of nonpolar ones, and has been shown to alter the perception of aroma descriptors.^{54–56} Phenolic compounds such as gallic acid, catechin, and quercetin can inhibit both the release of monoterpenes from glycosidic precursors and their subsequent volatilization through intermolecular interactions, including hydrogen bonding and dispersive forces.^{57,58} (3*S*)-Linalool, in particular, has been shown to interact strongly with phenolic acids, which may reduce its volatility and perceived intensity. In the context of this study, these interactions may have attenuated the perceived intensity of (3*S*)-linalool-associated aromas in wines produced from pigmented genotypes without completely

eliminating relative differences among genotypes, resulting in lower sensory scores relative to white wines while preserving genotype-dependent separation. Additionally, our decision to ferment all genotypes on their skins may have contributed to the lack of differentiation between white genotypes by increasing phenolic content, thereby partially obscuring genotype-dependent differences in monoterpene-associated aroma.

In addition to chemical interactions, aroma perception is influenced by the mode of sensory evaluation. Wine aroma is perceived through both orthonasal and retronasal pathways, which can differ in their sensitivity to matrix effects.⁵⁹ While this study focused on orthonasal evaluation, retronasal perception plays a critical role in wine aroma perception, and interactions between aroma compounds, the wine matrix, and oral components such as mucosal surfaces can further alter volatility, persistence, and perception,^{60,61} Together, these factors suggest that matrix-mediated modulation of aroma release and perception may partially mask genotype-dependent chemical differences.

Integration of chemical and sensory data through MFA further highlighted the complexity of relationships between volatile compounds and aroma attributes. While strong associations were observed between monoterpenes and floral and fruity descriptors, many expected relationships, particularly between esters and red- or dark-fruit-related attributes, were weak or absent.^{8,62,63} This finding is consistent with previous work showing that the sensory impact of individual compounds in wine cannot be reliably inferred from their concentrations alone.³¹ Instead, aroma perception emerges from complex interactions among multiple volatiles, including additive, synergistic, and suppressive effects.^{4,55,64} For example, the presence of (3*S*)-linalool has been found to reduce the sensory impact of acetate esters and alter the perception of limonene.^{10,47} The lack of strong associations between yeast-derived esters and fruit descriptors suggests that these compounds may

contribute to background aroma or interact with other volatiles in ways not captured by simple correlation analyses. The absence of "petrol" or "diesel" from the consensus descriptor list is notable, given that TDN is present in all wines. Although TDN concentration increases during wine aging, peri- or suprathreshold levels are common in Riesling wines less than two years old,⁶⁵ and concentrations in the RxCS wines may still have been insufficient to surpass the individual identification threshold.

In this study, we evaluated only wine aroma characteristics; however, the results have broader implications for wine grape breeding. The strong and consistent effect of the CHR10 locus on monoterpene composition indicates that CHR10_5431663 could serve as an effective marker for selecting enhanced floral and fruity aroma potential in breeding programs. At the same time, the incomplete translation of chemical differences into sensory outcomes highlights the need to consider the broader metabolic and matrix context when selecting for aroma traits. Selection based solely on a single compound or locus may not yield predictable sensory profiles, particularly in complex matrices such as wine. Even within the limited set of 24 genotypes examined, substantial variation was observed in ripening time, pH, titratable acidity, yield, and Brix. These traits are of clear importance for the development of new cultivars adapted to current and future viticultural challenges.⁶⁶ For example, warmer temperatures, particularly elevated nighttime temperatures, can accelerate the loss of acidity and anthocyanins in grapes.^{67–69} Understanding the sensory consequences of variation in aroma-related pathways is therefore critical, as alleles associated with desirable agronomic traits may also carry linked effects on aroma composition, ultimately influencing the sensory expression of newly developed cultivars.

In conclusion, this study demonstrates that genotype-dependent variation in wine aroma arises from coordinated changes in monoterpene composition driven by allelic variation at a key

genomic locus, with additional modulation by other volatile compounds and matrix effects. The integration of genetic, chemical, and sensory data reveals a clear but non-linear relationship between genotype and phenotype, underscoring the complexity of aroma formation in wine and the importance of a systems-level approach to its study.

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Supplementary Tables

Supplementary Table S1. Volatile compounds identified in wines by HS-SPME-GC-MS. Compounds are listed with retention time (RT), CAS number, compound class, and monitored ions used for identification. Abundances are expressed as relative peak area normalized to the internal standard (undecan-2-one). Mean values for Cabernet Sauvignon (CS) and Riesling (R) are presented, along with population mean, minimum, and maximum values calculated from progeny genotypes only. Odor descriptors adapted from FlavorNet (<http://www.flavornet.org/index.html>)

Compound	CAS number	Compound class	Odor descriptor	R mean	CS mean	Progeny Mean	Progeny Min	Progeny Max	RT (min)	Qualifier ions (m/z)	Scan type
Ethyl acetate	141-78-6	Ester	fruity	2.8798	4.3541	3.1913	2.2728	4.6777	3.113	43,61.0, 88.0	MSI SIM
Ethyl isobutanoate	97-62-1	Ester	fruity	0.0898	0.0793	0.0786	0.0424	0.1529	4.5	43,71.0, 86.0, 116.0	MSI SIM
Diethyl	431-03-8	Miscellaneous	butter	0.0148	0.0290	0.0243	0.0109	0.0419	4.796	43,86.0, 116.0, 71.0	MSI SIM
Ethyl butanoate	105-54-4	Ester	apple	0.1513	0.2355	0.1918	0.1558	0.2414	6.621	71,88.0, 116.0	MSI SIM
Ethyl 2-methylbutanoate	7452-79-1	Ester	apple	0.0407	0.0322	0.0363	0.0184	0.0958	7.164	57,102.0, 130.0	MSI SIM
Ethyl 3-methylbutanoate	108-64-5	Ester	apple	0.0496	0.0373	0.0416	0.0217	0.1046	7.714	55,88.0, 130.0	MSI SIM
Isobutanol	78-83-1	Alcohol	solvent	0.3308	0.4105	0.3580	0.2798	0.4551	8.761	43,55.0, 74.0	MSI SIM
Isomyl acetate	123-92-2	Ester	banana	1.8600	5.3299	3.1119	1.6456	4.7976	10.036	55,87.0, 130.0	MSI SIM
β-Myrcene	123-35-3	Monoterpene	balsamic	0.0262	0.0003	0.0042	0.0005	0.0323	11.9	121,93.0, 136.0	MSI SIM
α-Terpinene	99-86-5	Monoterpene	lemon	0.0172	0.0023	0.0099	0.0007	0.0328	12.224	93,121.0, 136.0	MSI SIM
Limonene	138-86-3	Monoterpene	lemon	0.0896	0.0020	0.0159	0.0018	0.1090	13.089	68,93.0, 136.0	MSI SIM
Isomyl alcohol	123-51-3	Alcohol	whiskey	5.7301	10.1592	7.0926	5.1932	9.2109	14.1	70,57.0, 88.0	MSI SIM
Ethyl hexanoate	123-66-0	Ester	apple	3.0200	4.3505	3.9521	3.1607	4.6220	15.171	88,115.0, 144.0	MSI SIM
p-Cymene	99-87-6	Monoterpene	citrus	0.0265	0.0063	0.0161	0.0029	0.0636	16.602	91,119.0, 134.0	MSI SIM
Hexyl acetate	142-92-7	Ester	fruit	1.0586	3.0889	1.9692	0.9927	3.9621	17	43,56.0, 84.0, 45.0, 88.0, 101.0	MSI SIM
(-)-cis-Rose oxide	16409-43-1	Monoterpene	rose	0.0055	0.0006	0.0020	0.0005	0.0069	20.523	69,139.0, 154.0	MSI SIM
Hexanol	111-27-3	Alcohol	green	0.1250	0.1892	0.1866	0.0893	0.2605	20.982	69,84.0, 101.0	MSI SIM
(E)-3-Hexen-1-ol	928-97-2	Alcohol	green	0.0078	0.0122	0.0111	0.0045	0.0174	21.383	41,67.0, 82.0, 57.0, 100.0	MSI SIM
(Z)-3-Hexen-1-ol	928-96-1	Alcohol	green	0.0207	0.0169	0.0411	0.0093	0.1710	22.424	67,82.0, 100.0, 41.0, 57.0	MSI SIM
Ethyl octanoate	106-32-1	Ester	fruity	16.4859	25.5033	20.3959	16.6523	27.4993	24.629	88,101.0, 172.0	MSI SIM
1-Octen-3-ol	3391-86-4	Alcohol	mushroom	0.0090	0.0011	0.0039	0.0006	0.0103	25.488	57,72.0, 127.0	MSI SIM
Nerol oxide	1786-08-9	Monoterpene	floral	0.1073	0.0008	0.0157	0.0066	0.0651	25.967	68,83.0, 152.0	MSI SIM
Acetic acid	64-19-7	Acid	sour	0.4433	0.4723	0.4147	0.2703	0.8671	25.983	43,45.0, 60.0	MSI Scan
Benzaldehyde	100-52-7	Aromatic	almond	0.0272	0.0147	0.0193	0.0051	0.0626	28.375	106,105.0, 77.0	MSI SIM
(3S)-Linalool	78-70-6	Monoterpene	flower	0.8137	0.0123	0.1633	0.0134	1.3525	29.742	71,93.0, 154.0	MSI SIM
Isobutyric acid	79-31-2	Acid	rancid	0.0006	0.0004	0.0005	0.0003	0.0009	30.473	73.88	MSI SIM
β-Cyclohexal	432-25-7	Norisoprenoid	mint	0.0044	0.0014	0.0025	0.0013	0.0043	32.308	137,152.0, 123.0	MSI SIM
Hotrienol	29957-43-5	Monoterpene	hyacinth	0.2206	0.0007	0.0331	0.0002	0.1723	32.36	82.67	MSI Scan
Ethyl decanoate	110-38-3	Ester	waxy	3.7364	6.6710	4.7198	3.6336	6.9847	33.307	88,200.0, 105.0	MSI SIM
Geraniol	141-27-5	Monoterpene	lemon	0.0008	0.0011	0.0007	0.0002	0.0019	36.883	69,152.0, 94.0	MSI SIM
TDN	30364-38-6	Norisoprenoid	petrol	0.1022	0.0048	0.0229	0.0044	0.0973	37.3	142,172	MSI Scan
β-Citronellol	106-22-9	Monoterpene	rose	0.0108	0.0068	0.0108	0.0045	0.0198	38.494	69,82.0, 156.0	MSI SIM
Nerol	106-25-2	Monoterpene	sweet	0.0101	0.0009	0.0032	0.0008	0.0174	39.693	68,83.0, 152.0	MSI SIM
β-Damascenone	23696-85-7	Norisoprenoid	apple	0.0857	0.0777	0.1044	0.0437	0.1562	40.19	69,121.0, 190.0	MSI SIM
Phenethyl acetate	103-45-7	Aromatic	rose	2.2185	5.4980	2.9880	1.0999	5.2748	40.27	104.91	MSI Scan
Geraniol	106-24-1	Monoterpene	rose	0.0292	0.0040	0.0083	0.0020	0.0500	41.551	69,93.0, 154.0	MSI SIM
Hexanoic acid	142-62-1	Acid	sweat	0.2593	0.3320	0.3304	0.2496	0.4246	41.744	73.87	MSI Scan
Benzyl alcohol	100-51-6	Aromatic	floral	0.0142	0.0333	0.0205	0.0132	0.0342	42.556	79,108.0, 107.0, 91.0	MSI SIM
Ethyl dihydrocinnamate	2021-28-5	Aromatic	floral	0.0139	0.0396	0.0205	0.0132	0.0342	42.635	108,79.0, 107.0, 91.0, 178.0, 10	MSI SIM
2-Phenylkethan-1-ol	60-12-8	Alcohol	rose	7.5805	15.6603	10.2883	5.5874	17.5282	43.772	91,122.0, 65.0	MSI SIM
β-Ionone	79-77-6	Norisoprenoid	violet	0.0007	0.0005	0.0007	0.0004	0.0010	44.44	177,135.0, 192.0	MSI SIM
γ-Nonalactone	104-61-0	Miscellaneous	coconut	0.0040	0.0023	0.0025	0.0014	0.0048	47.744	85,99.0, 156.0	MSI SIM
Nerolidol	7212-44-4	Sesquiterpene	woody	0.0044	0.0062	0.0055	0.0009	0.0100	48.232	69,93.0, 222.0	MSI SIM
Octanoic acid	124-07-2	Acid	fatty	2.0990	2.1913	2.5079	1.8867	3.2362	49.196	60,73.0, 115.0	MSI Scan
Ethyl cinnamate	103-36-6	Aromatic	honey	0.0027	0.0006	0.0019	0.0009	0.0043	51.204	131,103.0, 176.0	MSI SIM
Ethyl anthranilate	87-25-2	Aromatic	grape	0.0020	0.0014	0.0016	0.0008	0.0029	53.918	119,165.0, 92.0	MSI SIM
Farnesol	4602-84-0	Sesquiterpene	flower	0.0004	0.0043	0.0014	0.0002	0.0022	54.606	69,93.0, 222.0	MSI SIM

Supplementary Table S2: Multivariate analysis of variance (MANOVA) results for descriptive analysis (DA) data across all wines and subsets (red and white). Fixed effects included product, judge, replicate, and the judge $\times$ product interaction. Wilks' λ is reported as the multivariate test statistic, with approximate F-values, degrees of freedom, and associated p-values.

Set	Effect	Wilks_lambda	F_value	df1	df2	p_value
All wines	Judge	0.00026	30.49	240	5092	<0.001
All wines	Replicate	0.81	2.53	48	1094	<0.001
All wines	Product	0.127	2.02	600	9590	<0.001
All wines	Judge x Product	3.07E-05	1.21	6000	13307	<0.001
Red wines	Judge	0.00016	17.29	240	2647	<0.001
Red wines	Replicate	0.794	1.44	48	566	0.031
Red wines	Product	0.206	1.56	312	3286	<0.001
Red wines	Judge x Product	8.24E-05	1.08	3120	6903	0.004
White wines	Judge	5.18E-05	17.73	240	2239	<0.001
White wines	Replicate	0.7	1.95	48	478	<0.001
White wines	Product	0.18	1.71	264	2428	<0.001
White wines	Judge x Product	6.53E-05	1.12	2640	5822	<0.001

Supplementary Table S3: Eigenvalues and proportion of between-product variance explained by each canonical variate from canonical variate analysis (CVA) of descriptive analysis data, shown for all wines and for red and white subsets.

Set	Axis	Eigenvalue	% Variance	Cumulative
All wines	Can1	0.72787186	35.1929529	35.1929529
All wines	Can2	0.33450898	16.1736695	51.3666224
All wines	Can3	0.25321265	12.2429527	63.609575
All wines	Can4	0.21098249	10.2011046	73.8106796
All wines	Can5	0.1434523	6.93598721	80.7466668
All wines	Can6	0.10747883	5.19665273	85.9433195
All wines	Can7	0.09591251	4.6374159	90.5807354
All wines	Can8	0.07463672	3.60872125	94.1894567
All wines	Can9	0.03360811	1.62496797	95.8144247
All wines	Can10	0.0324359	1.56829123	97.3827159
All wines	Can11	0.02451468	1.1852963	98.5680122
All wines	Can12	0.01452664	0.7023698	99.270382
All wines	Can13	0.01066807	0.51580624	99.7861883
All wines	Can14	0.00442212	0.21381175	100
Red wines	Can1	0.31926793	40.5627712	40.5627712
Red wines	Can2	0.17274909	21.947653	62.5104242
Red wines	Can3	0.13493182	17.142995	79.6534191
Red wines	Can4	0.08336071	10.5909212	90.2443403
Red wines	Can5	0.04938232	6.27398955	96.5183299
Red wines	Can6	0.02275092	2.89048839	99.4088183
Red wines	Can7	0.00465317	0.59118174	100
White wines	Can1	0.26071353	39.8356786	39.8356786
White wines	Can2	0.2046646	31.2716925	71.1073711
White wines	Can3	0.09949239	15.2019229	86.3092939
White wines	Can4	0.05635475	8.61071442	94.9200084
White wines	Can5	0.03069911	4.69066501	99.6106734
White wines	Can6	0.00254804	0.38932662	100

Supplementary Table S4: Eigenvalues and proportion of total variance explained by each dimension of the multiple factor analysis (MFA) performed on descriptive and chemical datasets, shown for all wines and for red and white subsets. Cumulative variance indicates the total variance explained up to each dimension.

Set	Dimension	Eigenvalue	% Variance	Cumulative
All wines	Dim. 1	1.77	22.26	22.26
All wines	Dim. 2	0.99	12.47	34.73
All wines	Dim. 3	0.90	11.39	46.12
All wines	Dim. 4	0.66	8.26	54.38
All wines	Dim. 5	0.59	7.41	61.79
All wines	Dim. 6	0.48	6.10	67.89
All wines	Dim. 7	0.41	5.22	73.11
All wines	Dim. 8	0.32	3.98	77.09
All wines	Dim. 9	0.28	3.48	80.57
All wines	Dim. 10	0.26	3.25	83.82
All wines	Dim. 11	0.24	2.97	86.79
All wines	Dim. 12	0.16	2.08	88.87
All wines	Dim. 13	0.14	1.72	90.58
All wines	Dim. 14	0.12	1.56	92.15
All wines	Dim. 15	0.12	1.52	93.67
All wines	Dim. 16	0.11	1.34	95.01
All wines	Dim. 17	0.08	0.97	95.97
All wines	Dim. 18	0.07	0.92	96.89
All wines	Dim. 19	0.06	0.80	97.69
All wines	Dim. 20	0.06	0.75	98.44
All wines	Dim. 21	0.04	0.48	98.92
All wines	Dim. 22	0.03	0.39	99.31
All wines	Dim. 23	0.02	0.30	99.61
All wines	Dim. 24	0.02	0.20	99.81
All wines	Dim. 25	0.01	0.19	100.00
Red wines	Dim. 1	1.56	24.23	24.23
Red wines	Dim. 2	1.19	18.47	42.70
Red wines	Dim. 3	0.89	13.81	56.51
Red wines	Dim. 4	0.65	10.01	66.52
Red wines	Dim. 5	0.52	8.00	74.51
Red wines	Dim. 6	0.44	6.81	81.32
Red wines	Dim. 7	0.35	5.40	86.72
Red wines	Dim. 8	0.27	4.11	90.83
Red wines	Dim. 9	0.20	3.13	93.96
Red wines	Dim. 10	0.18	2.79	96.75
Red wines	Dim. 11	0.11	1.69	98.44
Red wines	Dim. 12	0.06	0.88	99.32
Red wines	Dim. 13	0.04	0.68	100.00
White wines	Dim. 1	1.61	23.78	23.78
White wines	Dim. 2	1.20	17.65	41.43
White wines	Dim. 3	1.10	16.30	57.73
White wines	Dim. 4	0.81	12.02	69.76
White wines	Dim. 5	0.56	8.28	78.04
White wines	Dim. 6	0.46	6.79	84.83
White wines	Dim. 7	0.33	4.92	89.75
White wines	Dim. 8	0.29	4.24	93.99
White wines	Dim. 9	0.19	2.74	96.73
White wines	Dim. 10	0.14	2.01	98.75
White wines	Dim. 11	0.08	1.25	100.00

Conclusion

This dissertation focused on integrating genetic, metabolic, and sensory approaches in order to fully understand grape and wine aroma variation. Chapter 1 established the biological framework for this work by reviewing the major pathways and genes involved in grape aroma biosynthesis and modification. The review highlighted that several aroma-related genes have been functionally characterized, particularly for terpenoids and methoxypyrazines, but also showed that many pathways remain only partially resolved. What work has been done has shown that variation in aroma profile may arise from structural differences among gene families, regulatory differences among alleles, precursor availability, and downstream modification during berry development and winemaking.

In Chapter 2, we applied this integrated approach to berry aroma variation in a Riesling x Cabernet Sauvignon population. We found that variation in many volatile compounds and monoterpene glycosides was controlled by multiple genomic regions, including two major QTLs associated with (3*S*)-linalool accumulation located on chromosomes 2 and 10. These QTLs had been recorded noted before, but no candidate genes were previously identified. Integration of QTL mapping with haplotype-resolved transcriptomics identified *VviTPS54* as the strongest candidate gene at the chromosome 10 locus. The association between haplotype-specific *VviTPS54* expression and (3*S*)-linalool accumulation supported a regulatory model in which haplotype-specific expression, rather than coding sequence divergence alone, contributes to monoterpene profile differences.

Chapter 3 extended these findings from berries to wine. Wines produced from progeny carrying the high-linalool Riesling-associated allele at the chromosome 10 locus had significantly altered monoterpene composition, with (3*S*)-linalool representing a major

component of the monoterpene profile. Berry and wine monoterpenes were significantly related, indicating that genetic differences in berry aroma potential can persist through fermentation. Sensory analysis further showed that wines with increased monoterpene content were associated with floral and fruit attributes. However, these relationships were not absolute. Wine color, matrix effects, and broader volatile composition influenced aroma perception, demonstrating that the sensory impact of a genetic locus depends on the chemical context in which aroma compounds are expressed.

These chapters demonstrate that allelic variation at a terpene synthase locus can shape monoterpene accumulation in berries, influence wine volatile composition, and contribute to sensory differences among wines. However, the results also show that aroma traits are not determined by single compounds or loci alone. Rather, they reflect interactions among genes, fermentation processes, wine matrix composition, and human perception. Although we focused mainly on (3*S*)-linalool, its genetic regulation in the RxCS population, and resulting impacts on wine volatile profile and aroma, another 39 volatile compounds were associated with significant QTLs, providing a foundation for identifying genes and pathways involved in other aroma traits.

Further study of the chromosome 10 terpene synthase cluster is also needed to determine how expression of the *VviTPS54* candidate gene is regulated. Comparative analysis of promoter regions, transcription factor binding, chromatin accessibility, and allele-specific expression across developmental stages could help resolve the regulatory mechanisms underlying this QTL. In addition, the unresolved chromosome 2 QTL for (3*S*)-linalool warrants further investigation, particularly because it may point to indirect regulation of monoterpene accumulation. Additional wine-scale experiments would also help clarify the sensory consequences of monoterpene variation. Repeating the descriptive analysis with wines produced from a larger subset of

genotypes, preferably within a single berry color class, would reduce confounding effects from wine matrix differences. This would strengthen the connection between genotype, metabolite accumulation, wine chemistry, and sensory perception.

Overall, this work supports the use of genomic and metabolomic approaches for aroma trait selection in wine grape breeding. At the same time, it emphasizes that breeding for aroma cannot rely solely on individual compounds or marker effects. Sensory outcomes must be evaluated within the broader wine matrix, where genetic effects interact with fermentation, compound release, and perceptual context to shape the final aroma profile