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葡萄果实中绿叶气味组分(GLVs)合成与调控的研究进展

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摘 要:绿叶气味组分(GLVs)是葡萄果实中含量最高的挥发性物质,主要以游离态形式存在于葡萄果实及葡萄酒中。能为白葡萄酒带来"清新"的香气,也是红葡萄酒中果香气味组分乙酸己酯的直接前体物,其组成与含量对葡萄及葡萄酒香气品质具有重要的影响。笔者综述了葡萄果实中主要绿叶气味组分(GLVs)的种类、香气特点、生物合成途径及关键酶、生物合成影响因素等方面的研究进展,并提出相关转录因子的鉴定及其调控机制是今后主要的研究方向。

关键词:葡萄;果实;绿叶气味组分(GLVs);生物合成;调控

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Research advance on biosynthesis and regulation of Green leaf volatiles (GLVs) in grape berry

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Abstract: Aroma is an important sensory quality of grape berries and wines. There are hundreds of aroma compounds in grape berries. According to the source of aroma compounds, they are mainly divided into C6/C9 compounds derived from fatty acids, terpenes and norisoprenoids derived from isoprene, aromatics compounds and methoxypyrazines derived from amino acids. Green leaf volatiles (GLVs) are short-chain alcohols, aldehydes and esters formed through the oxylipin pathway, belonging to C6/C9 compounds. They are called GLVs because of their "fresh grass" and "crushed leaf" aroma. GLVs can not only contribute "fresh" aroma to white wines, but also provide a direct precursor for the synthesis of the fruity compound hexyl acetate in red wines. In addition, GLVs may be involved in the formation of precursors of thiols, volatile thiols can provide "grapefruit" and "passion fruit" aroma, some studies have found that volatile thiols contribute significantly to the varietal aroma of Sauvignon Blanc wines. Therefore, the composition and concentration of GLVs have a significant impact on the aroma quality of grape berries and wines. In addition to its effects on aromas, GLVs play a key role in plant defense, plant-plant interactions and plant-insect interactions to participate in plant defense mechanisms to cope with biotic and abiotic stresses. The first step in the synthesis of GLVs is that lipoxygenases (LOXs) enzyme catalyze the hydroperoxidation of linolenic acid and linoleic acid which contain *Z,Z*-1,4-pentadiene moieties to form 9-or 13-hydroperoxides. LOXs can be divided into 9-LOXs and 13-LOXs respectively depending on the oxygenation position (either carbon 9- or carbon 13-) on fatty acid backbone. There was a study reported that 18 LOX genes were cloned in Sauvignon Blanc berries, *VvLOXA* was the most abundant lipoxygenases (LOXs) which belong to 13-LOXs across the whole developmental

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stages. Then, hydroperoxide lyases(HPLs) catalyze the hydroperoxide into aldehydes, according to the substrates, HPLs can be divided into 13-HPLs, 9-HPLs and 9/13-HPLs, 13-HPLs, 9-HPLs can catalyze 13-hydroperoxides and 9-hydroperoxides into C6 aldehydes and C9 aldehydes respectively, 9/13-HPLs can use both 13- and 9-hydroperoxides as substrates. The oxylipin pathway in grape berries is mainly catalyzed by 13- HPLs. The C6/C9 aldehydes is further catalyzed by the alcohol dehydrogenases (ADHs) and the alcohol-acyltransferases (AATs) to form the corresponding C6/C9 alcohols and esters. The aroma compounds mostly exist as free forms and the bound forms, GLVs mainly present as free forms in grape berries. The C6 compounds in the grape berries mainly contain hexanal, hexanol, hexyl acetate, *Z*-3-hexenal, *Z*-3-hexenol, *E*-2-hexenal, *E*-2-hexenol, etc. The C9 compounds include *E*-2-nonenal and *E,Z*-2,6-decadienal, etc. Currently, most studies have focused on C6 compounds, C9 compounds are rarely studied. GLVs, especially hexanal, *E*-2-hexenal and hexanol are the most abundant volatile compounds in grape berries. C6 compounds accumulate as esters, aldehydes and alcohols in the early, middle and late stages of ‘Cabernet Sauvignon’ grape growth respectively. *E*-2-nonenal and *E,Z*-2,6-nonadienal were detected only in small amounts in grape berries. The accumulation of GLVs from different fatty acids (either linolenic acid or linoleic acid) is related to grape varieties. In ‘Frontenac’ ‘Cabernet Sauvignon’ and ‘Riesling’ grape berries, C6 compounds are mainly derived from linolenic acid metabolism during the ripening stage. Most C6 compounds are derived from both the linolenic acid pathway and linoleic acid pathway during the entire developmental period of ‘Marquette’ grapes. Environmental factors such as water and area where grapes grow also influence the accumulation of GLVs a lot. Regulated irrigation and rain-shelter cultivation are common measures to control water. Grapes grown in areas with less rainfall can produce wines with more esters, the content of aldehydes and C9 compounds was negatively correlated with rainfall. A certain degree of water deficiency could increase the content of GLVs in grape berries, especially aldehydes and esters. However, the simple rain-shelter cultivation of ‘Cabernet Sauvignon’ was carried out in Miyun, Beijing by our laboratory, found that the content of C6 esters was increased, but unlike other results, the content of *E*-2-hexenal was significantly lowered. The cultivation techniques of grapes also affect the GLVs of grape berries, such as training systems, cluster thinning, bunch shading, defoliation, etc. In general, the type of training systems that can provide the vines with more sunlight is more conducive to the formation of C6 esters in corresponding wines. But a study found that the training system Ballerinas which exposed more can provide fewer esters to ‘Cabernet Sauvignon’ grape berries than vertical shoot positioned (VSP) training system, which may due to different regions and climate the grapes grew. Our laboratory found that the wine treated with cluster thinning has no significant changes in the ethyl hexanoate content of the berry, while the 1-hexanol content is lower, so the ‘green leaf’ odor in the wine can be reduced. The defoliation for two years in three years has increased the concentration of hexyl acetate in wines, which is beneficial to provide fruitier aroma to red wines. The bunch shading generally reduces the content of C6 alcohol and aldehyde. This paper reviews the aroma characteristics of GLVs, the research progress of biosynthetic pathways and key structural enzymes, as well as the factors affecting the production of GLVs in developing grape berry. Finally, it is suggested that the future researches could be focused on the identification and regulatory mechanism of the transcriptional factors controlling the biosynthesis of GLVs.

Key words: Grape; Berry; Green leaf volatiles (GLVs); Biosynthesis; Regulation

绿叶气味组分(Green Leaf Volatiles，简称GLVs)是不饱和脂肪酸通过脂氧合途径生成的C6/C9短链脂肪醛、醇和酯[1]。由于具有新鲜割草的独特气味，被称为绿叶气味组分(GLVs)[2]。葡萄果实中的香气物质主要以游离态和糖苷结合态两种形式存在[3]，其中绿叶气味组分(GLVs)主要以游离态形式存在，能给白葡萄酒带来“清新”的香气，也是红葡萄酒中果香成分之一乙酸己酯的直接前体物[1,4]，对于酿酒葡萄和葡萄酒的香气品质有重要贡献，但绿叶气味组分(GLVs)也有可能给红葡萄酒带来“不成熟”的感官印象[5]。此外，绿叶气味组分(GLVs)是植物防御系统的重要组成部分[6]，是植物间信号传递的主要化合物，能在胁迫条件下释放[7]，也可对食草动物、细菌和真菌病原体产生防御作用[8]。研究绿叶气味组分(GLVs)的合成与调控，能为葡萄及葡萄酒香气品质的改善提供科学依据。笔者对近年来国内外在葡萄果实绿叶气味组分(GLVs)生物合成与调控方面的主要研究成果进行了综述，以期为绿叶气味组分(GLVs)的深入研究提供参考。

1　葡萄果实中的绿叶气味组分及其呈香特点

1.1　种类及其呈香特点

绿叶气味组分(GLVs)是指亚麻酸和亚油酸通过酶代谢产生的C6/9醛、醇及其酯类化合物，其中C6化合物主要包括己醛、己醇、乙酸己酯、*Z*-3-己烯醛、*Z*-3-己烯醇、*E*-2-己烯醛、*E*-2-己烯醇等，C9化合物主要包括*E*-2-壬烯醛及*E*，*Z*-2，6-壬二烯醛等（图1）。‘赤霞珠（Cabernet Sauvignon）’果实中绿叶气味组分(GLVs)主要以游离态形式存在，其含量占总量的99%以上[1]。目前研究最多的是葡萄果实中的C6化合物，C9化合物鲜有人进行研究，其中一些重要

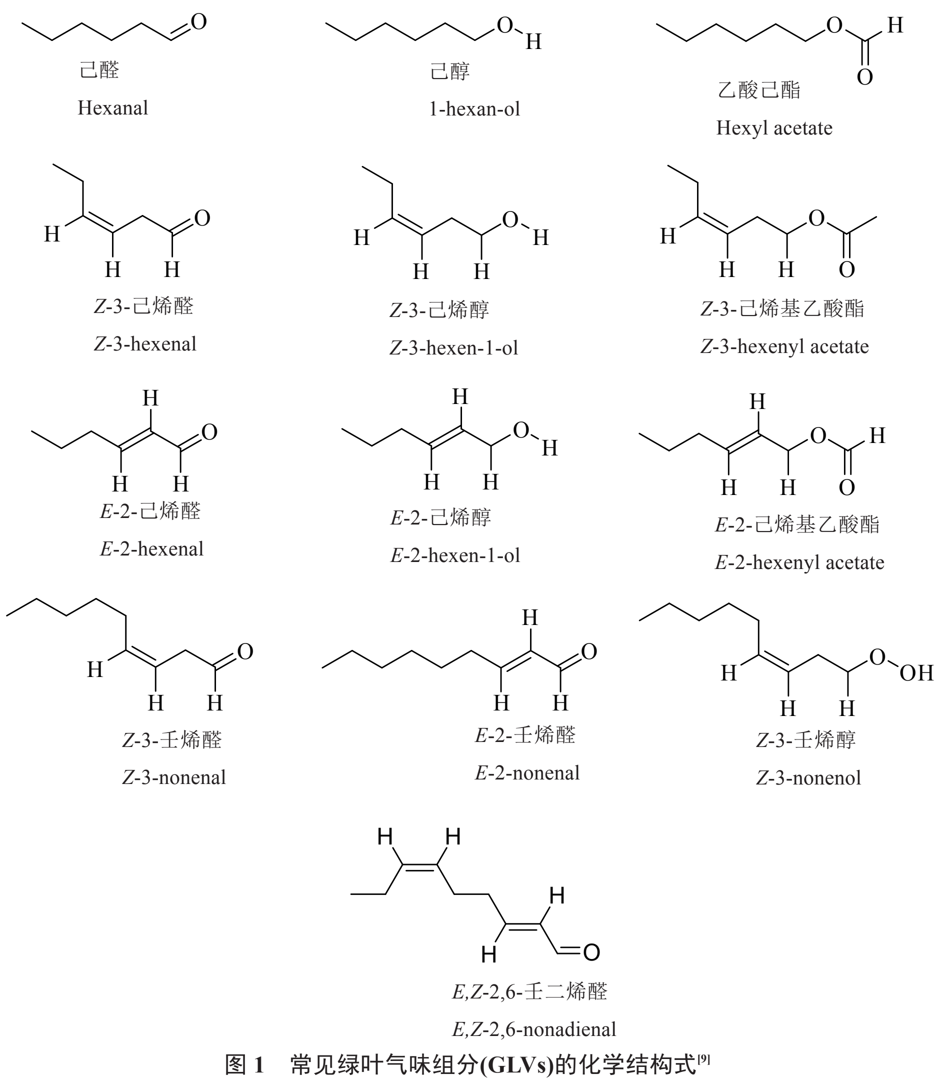

图1　常见绿叶气味组分(GLVs)的化学结构式[9]

Fig. 1 The chemical structure of GLVs[9]

的绿叶气味组分(GLVs)及其感官特性如表1所示。

对于白葡萄酒来说，绿叶气味组分(GLVs)能给白葡萄酒带来植物气味，给人一种清新感，C6化合物也被认为是新西兰‘长相思（Sauvigon Blanc）’葡萄酒独特香气来源硫醇3-巯基己-1-醇(3MH)的前体物质[9-12]。

对于红葡萄酒来说，己醇、己醛、*E*-2-己烯醇及*E*-2-己烯醛是红葡萄酒中乙酸己酯的直接前体物质，在模拟葡萄汁中增加这些C6化合物的浓度，乙酸己酯的浓度成比例增加[4]。乙酸己酯能为红葡萄酒提供“红色浆果”以及令人愉快的“梨果实”香气，是红葡萄酒重要的发酵香气[13-14]。但另一方面，绿叶气味组分(GLVs)所呈现的“新鲜割草”、“绿叶”、“新鲜植物”气味[1-2]，会带给红葡萄酒“不成熟”的感官感觉。其中C6醛的“青草”味阈值比C6醇低，其不良影响会更加明显；但随着葡萄成熟，C6醇/C6醛比例不断提高，且在发酵过程中，大部分C6醛会转化为醇和酯[5]，因此，也有学者认为，C6化合物给葡萄果实和葡萄酒带来的这种不良影响十分有限[15]。

表1　葡萄果实或葡萄酒中主要的绿叶气味组分（GLVs）及其感官特性

Table 1 Odour and content of some important green leaf volatiles in grape and wine

种类 Type	名称 Compounds	葡萄果实或葡萄酒中的含量 Content/(μg·g⁻¹)	阈值 Threshold/(μg·L⁻¹)	气味特征 Odour description	参考文献 Reference
C6	己醛 Hexanal	tr○	5a	低浓度时具有果香，苦味 Fruity aroma, bitter taste at low concentration	[14-16] http://www.leffingwell.com/odorthre.htm
	1-己醇 1-hexan-1-ol	0.25-25000○△	2 500a; 8 000b	青草、吐司味 Grass, toast	[14,16] http://www.leffingwell.com/odorthre.htm
	乙酸己酯 Hexyl acetate	20-1000☆	1500c	令人愉快的水果香气，梨果实风味 Pleasant fruity aroma, pear aroma	[14]
	E-2-己烯醛 *E*-2-hexenal	0.9-3.7○	17a		[15] http://www.leffingwell.com/odorthre.htm
	E-2-己烯醇 *E*-2-hexen-1-ol	nd○	400a		[15] http://www.leffingwell.com/odorthre.htm
	Z-3-己烯醛 *Z*-3-hexenal	nd○	0.25a		http://www.leffingwell.com/odorthre.htm
	Z-3-己烯醇 *Z*-3-hexen-1-ol	0.032-0.08○	70a; 400b		[15-16] http://www.leffingwell.com/odorthre.htm
C9	*E*-2-壬烯醛 *E*-2-nonenal	tr○	0.08-0.1a	烹饪过的蔬菜味 Cooked vegetables	[17-18] http://www.leffingwell.com/odorthre.htm
	E,Z-2,6-壬二烯醛 *E,Z*-2,6-nonadienal	tr○	0.09a	黄瓜味 Cucumber	[9, 14, 18] http://www.leffingwell.com/odorthre.htm

注：○. 葡萄果实；☆. 红葡萄酒；△. 红葡萄酒或白葡萄酒；a. 水；b. 10%乙醇水溶液；c. 9.4%乙醇水溶液；nd；未检出；tr. 少量检出。

Note: ○. Grape berry；☆. Red wine；△. Red or white wine; a. Water；b. 10% Water/ethanol 90+10 w/w；c. 9.4% Water/ethanol 90.6+9.4 V/V; nd. Not detect; tr. Trace.

1.2 在果实发育过程中的积累规律

C6化合物，尤其是己醛，*E*-2-己烯醛和己醇是葡萄浆果中最丰富的挥发性化合物。C6化合物在‘赤霞珠’葡萄发育的早、中、后期分别主要以酯、醛、醇为主的形式积累[1,19]。有研究提出，*Z*-3-己烯醇和*E*-2-己烯醛的比可以用来从香气的角度判断葡萄浆果的成熟度，成熟前该比例持续下降[20]。在‘赤霞珠’浆果中没有检出*Z*-3-己烯醛，而*E*-2-己烯醛和己醛的含量随着葡萄浆果的成熟先升高后下降，在成熟果实中源于亚麻酸代谢的*E*-2-己烯醛含量比源于亚油酸代谢的己醛含量更高[19,21]。

随着‘赤霞珠’和‘雷司令（Riesling）’葡萄果实的成熟，己醇和*Z*-3-己烯醇的含量先升高，转色前略微下降，转色后己醇含量显著升高，而*Z*-3-己烯醇几乎没有显著的改变，且转色后‘赤霞珠’中的*Z*-3-己烯醇显著高于‘雷司令’[19,21]。当葡萄浆果受伤或破碎时，会迅速生成并释放大量己醇、*Z*-3-己烯醇和*E*-2-己烯醇[22]，这是由于受伤组织中脂氧合酶基因被大量上调，从而对C18脂肪酸降解增强[4]。

‘赤霞珠’中，源于亚油酸代谢的*Z*-3-己烯基乙酸酯的含量远远高于源于亚麻酸代谢的乙酸己酯，是浆果中最丰富的C6酯类。*Z*-3-己烯基乙酸酯在葡萄浆果发育早期显著增加，转色期则迅速下降，而乙酸己酯只在坐果后有所检出[19]。

葡萄浆果中C6化合物的积累与品种有关，在‘马凯特（Marquette）’葡萄中，转色和成熟时期主要的C6化合物既来源于亚麻酸途径，也来源于亚油酸途径，而在‘芳堤娜（Frontenac）’、‘赤霞珠’及‘雷司令’中，整个成熟时期C6化合物主要来源于亚麻酸代谢[20-21]。对4个鲜食葡萄比较发现，‘香妃（Xiangfei）’果实中C6醛最为丰富，‘达米娜（Tamina）’和‘摩尔多瓦（Moldova）’中C6醇含量较高，‘亚历山大（Muscat of Alexandria）’中C6酯最为主要[23]。

关于葡萄果实中的C9化合物目前鲜有报道，*E*-2-壬烯醛和*E*，*Z*-2，6-壬二烯醛在葡萄果实中只有少量检出[14]，其积累容易受到年份气候变化的影响[9]。有研究发现，*E*-2-壬烯醛和*E*，*Z*-2，6-壬二烯醛在‘赤霞珠’葡萄酒酒精发酵开始一天后迅速下降至0[24]。

2　葡萄果实中C6/C9化合物的生物合成

2.1　C6/C9化合物的合成路径

绿叶气味组分(GLVs)由亚麻酸或亚油酸经脂氧合途径降解产生，而底物亚麻酸和亚油酸的合成则依赖于糖酵解的最终产物——乙酰辅酶A，后者先转化为丙二烯辅酶A，经过一系列反应生成亚麻酸或亚油酸，脂氧合反应主要包括以下几个步骤。

2.1.1　亚麻酸、亚油酸的脂氢过氧化反应　如图2所示，脂氧合酶(LOX)首先催化亚麻酸和亚油酸发生氢过氧化反应，根据脂肪酸被氧化位置的不同，亚麻酸分别被9-脂氧合酶(9-LOX)和13-脂氧合酶(13-LOX)氧化为9-亚麻酸氢过氧化物(9-HPOT)和13-亚麻酸氢过氧化物(13-HPOT)，同样的，亚油酸通过脂氧合酶(LOX)分别被催化为9-亚油酸氢过氧化物(9-HPOD)和13-亚油酸氢过氧化物(13-HPOD)[25-26]。

2.1.2　脂氢过氧化物的裂解　亚麻酸氢过氧化物分别在9-氢过氧化物裂解酶(9-HPL)和13-氢过氧化物裂解酶(13-HPL)的催化下形成C9醛—*Z*，*Z*-3，6-壬二烯醛和C6醛—*Z*-3-己烯醛，这两种醛会通过酶促或非酶促转化为相应的更加稳定的同分异构体：*E*，*Z*-2，6-壬二烯醛和*E*-2-己烯醛；亚油酸氢过氧化物在9-HPL和13-HPL的作用下分别形成C9醛—*Z*-3-壬烯醛和C6醛—己烯醛[2,27]。

接下来，这些C6/C9醛依次在对应醇脱氢酶(ADH)、醇-酰基转移酶(AAT)的作用下进一步生成相应的C6/C9醇和酯[27-28]。

2.2　C6/C9化合物合成的关键酶基因

2.2.1　脂氧合酶(LOX)基因　脂氧合酶(LOX，EC 1.13.11)是脂氧合途径的关键酶，是一组非血红素铁脂肪酸双加氧酶，在脂氧合途径的第一步中，催化亚麻酸和亚油酸等含有*Z*,*Z*-1,4-戊二烯基团的脂肪酸的氢过氧化反应[29]。LOXs存在于很多植物的叶片、根、茎、花、果实和种子中[9,30-31]，根据脂肪酸链上氧合位置(C-9和C-13)的不同，LOX主要分为两大类：9-LOXs和13-LOXs，对葡萄果实而言，相对于9-LOXs，13-LOXs路径的脂氧合途径更为活跃[19]。13-LOXs多出现在绿色植物组织中，且主要定位于叶绿体中[6,32]，而9-LOXs主要定位于植物非绿色器官中[9,33]。

Podolyan等[34]首次从‘长相思’葡萄果实中克隆到了18条LOX基因，并对其中主要的4条LOX基因(*VvLOXA*、*VvLOXO*、*VvLOXC*、*VvLOXD*)进行了功能鉴定和转录表达的分析。其中*VvLOXA*和*VvLOXO*属于13-LOXs，*VvLOXC*属于9-LOXs；‘长相思’果实中的*VvLOXA*转录水平最为丰富，是葡萄果实中主要的13-LOX，主要在葡萄果皮中表达，*VvLOXC*和*VvLOXD*在种子、果肉和果皮中均匀表达，而*VvLOXO*主要在种子中表达。在‘赤霞珠’浆果发育的早期阶段VvLOXA的表达水平增加，转色后下降，而*VvLOXO*(13-LOX)和*VvLOC*(9-LOX)的表达量在浆果发育期间不断下降[22]。机械伤害及灰霉菌(Botrytis cinerea)侵染会使*VvLOXC*和*VvLOXO*的转录水平迅速升高，染病葡萄浆果中*VvLOXA*的表达量下降[34]。

2.2.2　CYP74酶家族和脂氢过氧化物裂解酶(HPL)基因　脂氢过氧化物裂解酶(HPL)能催化脂肪酸氢过氧化物裂解为C6或C9醛，属于细胞色素P450酶家族中CYP74亚家族的成员，该亚家族包含丙二烯氧化酶(AOS)、脂氢过氧化物裂解酶(HPL)、联乙烯醚合酶(DES)[2]，AOS路径可合成茉莉酸，后者不仅参与植物发育的生理过程，还在植物防御中起到重要作用[6]，DES路径生成的联乙烯醚，具有杀菌作用[35]。

根据底物的不同，可以将HPL分为三类：13-HPLs、9-HPLs和9/13-HPLs[36]，13-HPLs催化13-氢过氧化物生成C6醛，9-HPLs催化9-氢过氧化物生成C9醛，9/13-HPLs既能以9-氢过氧化物为底物，又能以13-氢过氧化物为底物[2]。植物中大部分HPLs

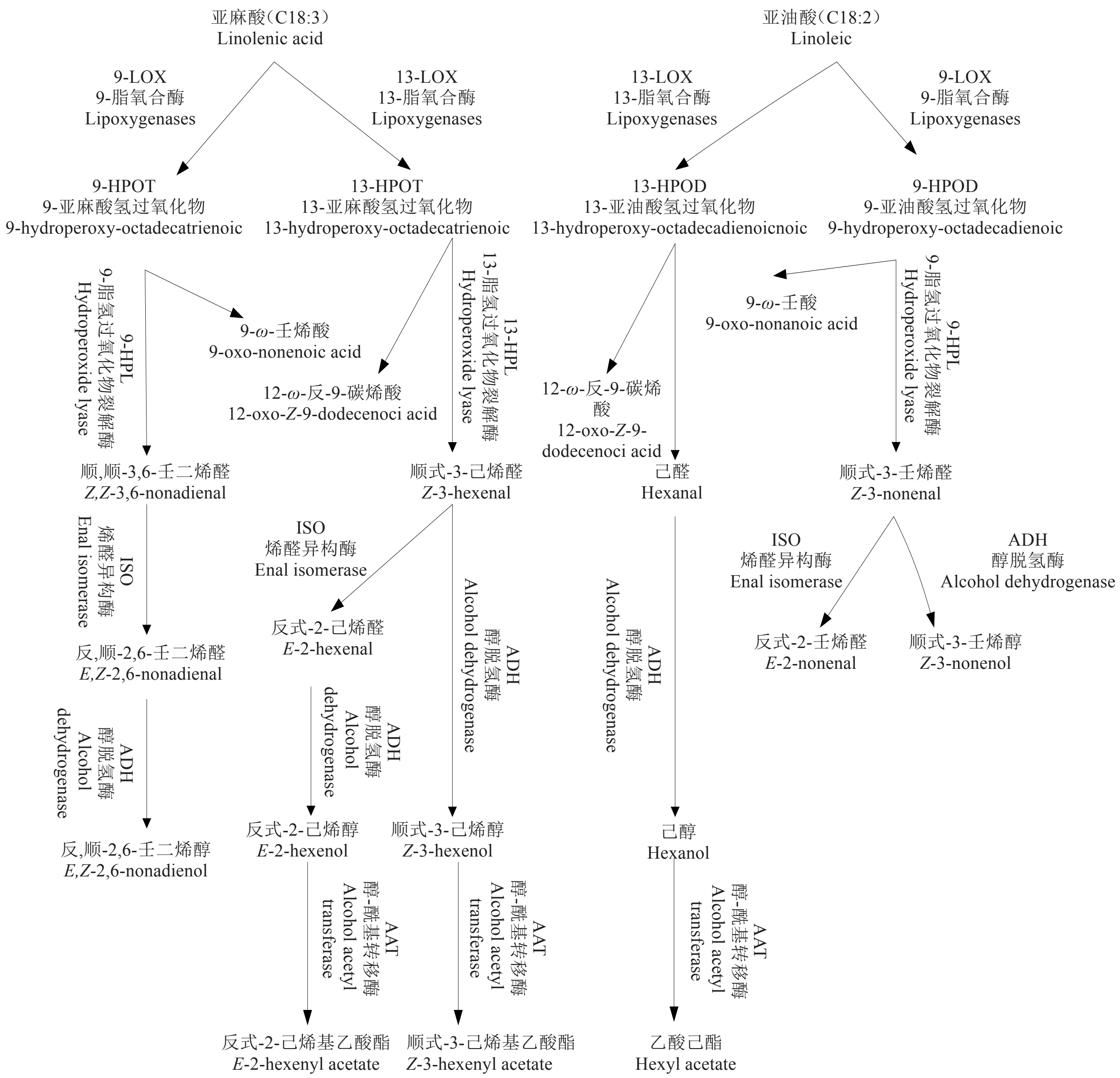

图2 绿叶气味组分(GLVs)的生物合成途径[27]

Fig. 2 The biosynthetic pathway of green leaf volatiles[27]

都以13-氢过氧化物为底物[37]，不同植物中同工酶类型有所差异，9/13-HPLs在葡萄[22]、黄瓜[38]及水稻[39]中都有所发现[6]，而茶叶叶片中只存在13-HPLs[40]。葡萄果实中脂氧合途径主要是13-LOXs和13-HPLs的催化，而不是9-LOXs和9-HPLs[22]。

目前为止，已经从拟南芥叶[41]、番茄果实[42]和葡萄等植物中鉴定了30多条基因[22]。本实验室在'赤霞珠'浆果中克隆获得了两条HPLs：*VvHPL1*(13-HPL)和*VvHPL2*(9-/13-HPLs)，HPLs的表达随着发育期有很大的变化，且存在组织特异性，VvHPL2在葡萄的叶片及花中高表达，而在浆果、卷须和茎中较低，*VvHPL1*在所有检测的组织中都有高表达，在葡萄果实发育期间，两个基因的表达呈现出了同样的趋势[22]。水分缺失上调了VvLOX和VvHPL基因的表达[43]。

2.2.3 醇脱氢酶(ADH)基因 醇脱氢酶(ADH，EC 1.1.1.1)是植物中一个含锌酶，负责醛和醇的转化。ADH是中等长度脱氢酶/还原酶(MDR)蛋白超家族的一员，植物中ADH基因的表达受到果实成熟的严格调控，在桃[44]、杏[45]中，ADH活性和醇的量在成熟早期最高，成熟时果实中以酯类为主[9]，而对于葡萄果实来说，ADH在成熟后期表现出更高的转录水平

和酶活性[46]，成熟果实中醇类含量较高[19]。

在葡萄果实中，共检测到三个ADH基因的转录本：*VvADH1*、*VvADH2*、*VvADH3*，其中*VvADH1*和*VvADH3*主要在葡萄果实发育早期表达，随着葡萄浆果成熟其表达量下降；*VvADH2*随浆果成熟表达量增加，且ADH酶活性与*VvADH2*表达量呈正相关，因此，认为*VvADH2*是影响葡萄果实醛向醇转化的关键基因[46-47]。*VvADH1*和*VvADH2*的表达没有组织特异性，在葡萄的整个发育期中，枝条、成熟的卷须及花序中都能发现*VvADH1*的转录本，而在其他器官和胚性愈伤组织中的表达水平却非常低，'西拉（Shiraz）'葡萄皮和果肉中*VvADH2*表达水平相当[47]。

此外，有报道指出，ADH基因表达与植物响应环境刺激和栽培方式有关，在缺水条件下，'赤霞珠'果实中*VvADH*表达上调[43,48]；"厂字形"(M-VSP)整形的'赤霞珠'果实中*VvADH2*与其他*VvADH*相比有较高的表达水平，但不同年份存在差别[49]。

2.2.4　转录水平调控　关于调控葡萄果实绿叶气味组分(GLVs)生物合成的转录因子，目前尚未见报道。本实验室利用基因共表达网络分析发现，与LOX路径基因具有较强正相关的转录因子中，以MYB家族居多，LOX主要与WRKY家族转录因子具有较强正相关，WRKY22、WRKY33和*VvNAC*是潜在作用于LOX的NAC转录因子，可能参与到茉莉酸代谢的调控[9]。

在番茄中的研究成果可以为葡萄LOX途径的转录调控提供参考，MADS box RIN转录因子是果实成熟过程中的一种全局调控因子，能在多个点直接调节LOX途径并改变番茄果实成熟期间的香气组成。在RIN突变型果实中，C6化合物含量非常低。*TomloxC*和*ADH2*在果实成熟时表达，*TomloxC*影响着香气的形成，用ChIP和EMSA分析都表明，RIN在植物体内可与*TomloxC*和*ADH2*的启动子结合，推测可能参与这两个基因的表达调控[50]。

3　环境因素对葡萄果实中C6/C9化合物生物合成的影响

3.1　水分

适度水分缺失能提高绿叶气味组分(GLVs)的含量，尤其是醛类和酯类。水分缺失可能提高LOX和HPL的转录水平，从而影响了绿叶气味物质的代谢[43,51]。

本实验室前期对甘肃高台及河北昌黎的'赤霞珠'香气进行对比时，发现降雨量较小的地区生长出来的葡萄能赋予葡萄酒更多的酯类和醛类，且降雨量小的高台产区的葡萄浆果中*E*-2-己烯醛、己醛、*Z*-3-己烯基乙酸酯的含量都高于昌黎产区，C9化合物的含量与降雨量呈现负相关[52]。Ju等[51]所做的研究也表明，一定程度的调亏灌溉提高了葡萄浆果中的香气含量，尤其是C6和C9醛。

除了调亏灌溉，避雨栽培也能有效调整水分。避雨栽培增加了鲜食葡萄'金手指（Gold Finger）'的游离态醛含量，尤其是具有清香味的2-己烯醛[53]，'赤霞珠'中的游离态酯类、醛类在避雨栽培下都有所提高[54]。本实验室前期在北京密云对'赤霞珠'进行简易避雨栽培，提高了C6酯的含量，但与其他结果有所不同的是，*E*-2-己烯醛的含量显著降低，但整体来说，避雨栽培能够减弱葡萄的绿叶气味，对果实香气品质有潜在的积极贡献[1]。

3.2　栽培方式

3.2.1　整形方式　能给葡萄树带来优良光照的叶幕类型更有利于葡萄酒中C6酯的生成。本实验室在2011、2012年采用"厂字形"(M-VSP)、双主蔓形(F-TT)及多主蔓形(F-MT)三种整形方式对'赤霞珠'葡萄进行修剪，多主蔓形使叶片具有相对较低的光合有效辐射[55]，葡萄酒中C6酯更低，不饱和C6醇更高，可能提供给葡萄酒更为强烈的青草香气。"厂字形"和双主蔓形能增强果际光合有效辐射，在一定程度上提高了果实中C6醛的含量，降低了C9化合物的含量，并提高了葡萄酒中C6酯的含量，香气品质更好[49]。另一个研究中，斯马特-戴森形(Smart-Dyson，SD)与新梢直立分布形(Vertical shoot-positioned，VSP)相比具有更好的光合有效辐射，该叶幕形生产的葡萄酒中含有更高含量的己酸乙酯和1-己醇[56]。但也有研究表明曝光较多的芭蕾舞女形(Ballerina)和新梢直立分布形(VSP)栽培的'赤霞珠'葡萄果实相比，酯类更少[57]，这可能是产区及气候不同带来的影响。

在新梢直立分布形(VSP)，斯科特·亨利架形(Scott Henry)，斯马特-戴森形(SD)，高龙干形(High cordon)，和日内瓦双帘形(Geneva double curtain)五种整形处理中，新梢直立分布形(VSP)和斯马特-戴森(SD)整形的'塔明内（Traminette）'葡萄果实中分

别有最高和最低含量的C6醛[58]。Fragasso等[59]报道了对意大利的'普里米蒂沃(Primitivo)'葡萄进行四长枝形(Four Rays，FR)、小灌木形(Little trees，LT)和双龙干形(Bilateral Guyot，BG)整形处理，四长枝(FR)整形的葡萄C6醛更高，C6醇更少。

3.2.2 疏穗处理 疏穗处理是葡萄园常用的一种栽培手段，这种技术能够改善和平衡葡萄树的库源关系，从而调节葡萄果实品质[60]。本实验室对新疆两个不同葡萄园的'赤霞珠'进行留穗量为73.3%和50%的疏穗处理，疏穗处理在一定程度上提高了葡萄果实中的己醛含量，降低了C6醇和C9醛的含量，且经过疏穗处理的葡萄酒贡献愉悦"浆果香"的己酸乙酯含量没有显著变化，而1-己醇含量更低，因此疏穗处理能降低葡萄酒中的'绿叶'气味，提高葡萄酒的香气品质[9]。

3.3 光照

3.3.1 曝光处理 对葡萄进行摘、挪叶及遮光处理可以有效改变果际的光照环境[61-62]。早期摘叶能够改良'丹魄(Tempranillo)'葡萄酒中的C6醇和C6乙酸酯而不对其他挥发性化合物产生影响[63]。本实验室前期在膨大期、转色期及转色结束时分别对'赤霞珠'葡萄果穗进行全摘叶、半摘叶及挪叶处理，转色期全摘叶和全程未进行曝光处理有利于果实中游离态C6醛的积累，转色期挪叶降低了果实中的游离态C6醛，但提高了游离态C6醇和乙酸酯，同时提高了葡萄酒中乙酸己酯的含量。多数曝光处理不影响果实中C9醛的含量，只有2011年膨大期全摘叶、转色期挪叶及全摘叶提高了果实中C9醛的含量。总的来说，曝光处理在三年中的两年都提高了葡萄酒中乙酸己酯的含量，有利于给干红葡萄酒提供更多的"红色浆果香"[9]。

3.3.2 遮光处理 葡萄浆果中1-己醇、*E*-2-己烯醇的含量与日照时长呈正相关[52]，采用聚酯薄膜吸收太阳光中的紫外线，以减弱果实周围的紫外线辐射时，葡萄浆果中的*E*-2-己烯醛及*Z*-3-己烯基乙酸酯的含量显著下降[64]。本实验室对新疆天山北麓的'赤霞珠'葡萄果实进行不同的遮光处理，发现遮光处理总体上降低了C6醇和醛的含量。坐果到采收全程遮光提高了采收期果实中C9醛的含量，各个遮光处理中，转色开始到采收遮光的葡萄果实酿造的葡萄酒中具有更高含量的GLVs[9]。也有不同结果，在Bureau等[65]的研究中，遮光处理能提高'西拉'果实中C6醛的含量，游离C6醇含量降低。Scafidi等[66]从坐果到采收进行全部遮光(聚丙烯盒)或者部分遮光(高密度聚乙烯网袋)，能提高'格雷罗(Grillo)'葡萄果实中游离态己醛，*E*-2-己烯醛和*Z*-3-己烯醇的含量。

3.4 外源激素处理

茉莉酸与GLVs同来源于脂氧合途径。茉莉酸是一种植物间交流的信号分子，对植物的生长发育存在一定的调控作用[6]，茉莉酸甲酯能调节LOX的酶活性[2]。在转色初期对'赤霞珠'果实喷施不同浓度的外源脱落酸(ABA)及茉莉酸甲酯时，能够显著提高LOX的酶活性，其中喷施中等浓度600 $mg \cdot L^{-1}$脱落酸和最低浓度50 $\mu mol \cdot L^{-1}$茉莉酸甲酯两个处理的葡萄果实中，LOX酶活性最高；而且脱落酸处理后，总C6香气化合物含量为406.47~474.00 $\mu g \cdot L^{-1}$，高于对照组的329.99 $\mu g \cdot L^{-1}$，茉莉酸甲酯处理也显著提高C6化合物含量，并随着处理浓度增加而增加，就组分而言，外源脱落酸和茉莉酸甲酯处理后，增加最多的是己醇、己醛[67]。

3.5 外源矿质元素处理

本实验室应用微量元素混合液(包括硼B、锰Mn、铁Fe、锌Zn、铜Cu和钼Mo等6种微量元素)或稀土元素混合液(包括钇Y、镧La、铈Ce、镨Pr、铌Nb、钐Sm等15种元素)，分别在花前7 d，花后28、56、84 d对'赤霞珠'与'霞多丽(Chardonnay)'进行叶面喷施。结果表明，喷施微量元素提高了采收期果实中(*E*)-2-己烯醛和己醛的含量，对'霞多丽'葡萄果实采收期果实中的C6化合物则没有显著影响，可能微量元素对LOX和HPL酶有激活作用，但是存在一定的品种依赖性。喷施稀土元素促进了两个品种成熟采收期果实中己醛的积累[68]。

4 结论及展望

迄今，关于葡萄果实中GLVs生物合成与调控的研究，主要涉及合成途径中结构基因的鉴定及其生化功能、GLVs组分的定性定量及其在葡萄果实发育过程中的积累规律、环境因素(如光照、水分、产区)以及栽培方式(整形、疏穗、摘叶、遮光)对GLVs积累及合成途径相关基因表达的影响。而调控葡萄果实GLVs生物合成的转录因子是什么？影响不同GLVs组分之间转化(如醛醇转化)的关键因子又是什么？这些转录因子又受到哪些因素影响？这些问

题仍有待探明。了解并揭示葡萄果实GLVs组分的积累及调控机制，对于从栽培及工艺上改善葡萄及葡萄酒的香气具有极其重要的意义。

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