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Feature Review **Open Access**

Integrated Transcriptome and Metabolome Analysis Reveals the Genetic Regulation of Aroma Biosynthesis in *Citrus*

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Abstract This study mainly aims to clarify how the aroma of *Citrus* is produced. By using a combined analysis method of transcriptomics and metabolomics, it identifies the genes and pathways related to aroma synthesis, sorts out the main volatile components in the aroma of *Citrus*, and then introduces how these components are synthesized step by step. It was found that the expression changes of key enzyme genes such as *TPS*, *LOX* and *ADH* are related to the accumulation of aroma substances. This study also summarized some important regulatory factors and signaling pathways from different studies, all of which are related to the regulation of aroma synthesis. It emphasized the significance of multi-omics joint analysis, believing that it can help better improve the aroma traits of *Citrus* and also provide useful theoretical support and methodological ideas for breeding new varieties with better aroma.

Keywords *Citrus* aroma; Transcriptomic analysis; Metabolomics; Aroma biosynthetic pathway; Molecular breeding

1 Introduction

Citrus fruits have always been popular among global consumers because of their good aroma and taste (Wang et al., 2024b). These aromas come from some particular compounds, such as volatile terpenoids, aldehydes, alcohols and esters, which determine the flavor and quality of *Citrus* (Lu et al., 2024). These substances not only affect the taste of fruits when eaten, but also help plants resist pests and diseases and adapt to the environment. Among them, terpene components such as valencene and nootkatone are particularly important. They can make *Citrus* more fragrant and more likely to be favored by consumers (Sharon-Asa et al., 2003; Alquézar et al., 2017).

At present, scientists do not know much about the key enzymes, transcription factors that regulate the synthesis of these aroma substances and the relationships among them (Sharon-Asa et al., 2003; Lu et al., 2024). It is still unclear which genes control the synthesis of aroma, how the expression of these genes is regulated, how the oil glands develop, and what the relationship is between these processes and aroma accumulation (Alquézar et al., 2017; Wang et al., 2024a). The genetic basis for the differences in aroma among various *Citrus* varieties has not yet been fully understood.

This study combined transcriptome and metabolome data to identify several key genes and metabolites related to *Citrus* aroma. It also analyzed how these genes are regulated, explored how oil glands develop, and investigated the connection between them and aroma accumulation. These results can provide some scientific basis for improving the flavor of *Citrus* and enhancing the quality of varieties in the future.

2 *Citrus* Aroma: Composition and Biological Significance

2.1 Major aroma volatiles in *Citrus*: terpenoids, aldehydes, alcohols, esters

The fragrance of *Citrus* mainly comes from many kinds of volatile organic compounds (VOCs). Among them, the most important ones are terpenoids, aldehydes, alcohols and esters. Terpenoids, such as limonene, β -farnene, β -caryophyllene and β -humulene, are particularly abundant in the essential oils of *Citrus* peels and leaves. Limonene is particularly abundant, accounting for more than 90% in the essential oil of sweet orange peel, and it is the main component of *Citrus* fragrance (Baccati et al., 2021; Ferrer et al., 2021; Ferrer et al., 2023). Aldehydes, such as octanal and decal, make fruits smell fresher and more fruity. Alcohols, such as linalool and α -terpineol, and esters, such as linalool acetate and ethyl hexanoate, make the fragrance milder, more floral and sweet (Goh et al., 2022; Pan et al., 2023; Fan et al., 2024). Among different *Citrus* varieties, the types and proportions of these fragrance components vary greatly, which is also an important basis for us to distinguish varieties and create fragrances (Yu et al., 2018; Hu et al., 2024).

2.2 Physiological and ecological roles of volatile organic compounds (VOCs)

The VOCs in *Citrus* fruits can not only make the fruits more fragrant but also help plants protect themselves. Volatile substances like terpenoids can prevent insect bites and reduce bacterial infections (Alquézar et al., 2017). Limonene can attract or drive away specific insects and may also affect the relationship between fruits and pathogens (Rodríguez et al., 2011). These volatile substances may also be involved in processes such as fruit ripening and seed propagation (Sharon-Asa et al., 2003). The variety of VOCs also enables *Citrus* to adapt to different environments and better cope with various challenges (Baccati et al., 2021).

2.3 Consumer preferences and market value of aromatic *Citrus* varieties

Citrus varieties with rich and unique aromas are more popular and sell better. Sensory evaluation and metabolomics studies have found that special aroma components such as farnesene, octanal, and linalool are key to enhancing the aroma of *Citrus* and attracting consumers (Pan et al., 2023; Fan et al., 2024; Hu et al., 2024). The differences in aroma among different varieties not only affect people’s taste experience, but also provide a scientific basis for breeding and the development of high-quality products (Yu et al., 2018; Goh et al., 2022).

3 Biosynthetic Pathways of Aroma Compounds

3.1 Terpenoid biosynthesis: MVA and MEP pathways

Among the aromas of *Citrus*, terpenoids are the most important type of substances. These compounds are mainly synthesized through two pathways. One is the mevalaronate pathway (MVA), which mainly occurs in the cytoplasm. The other one is the MEP pathway, which occurs in plastids. The MVA pathway is responsible for synthesizing sesquiterpenes, such as valencene, while the MEP pathway mainly synthesizes monoterpenes and diterpenes. The formation of valencene in *Citrus* fruits is closely related to a gene called *Cstps1*, which is expressed higher when the fruit is close to ripening, exactly in line with the accumulation time of aroma substances (Sharon-Asa et al., 2003; Alquézar et al., 2017). In addition, there are many kinds of terpene synthases (TPS) in *Citrus* fruits, which can synthesize various terpene substances, making the aroma of *Citrus* fruits more rich (Alquézar et al., 2017).

3.2 Fatty acid and amino acid-derived volatile pathways

Citrus fruits produce a large number of volatile organic compounds derived from the metabolism of fatty acids and amino acids, which are synthesized through several different biological pathways. Fatty acids are converted into peroxides under the action of lipoxygenase (LOX), and then processed by alcohol dehydrogenase (ADH) and aldoesterase (AAT), they are transformed into alcohols and esters with more distinct aromas. These compounds make *Citrus* fruits smell rich and fresh (Figure 1) (Lu et al., 2024). Amino acids can be converted into some fragrant molecules through the action of enzymes such as transaminase, enriching the aroma of *Citrus*.

3.3 Enzyme families: TPS, LOX, ADH, AAT, and others

Terpene synthase (TPS) can synthesize various terpenoids and is the basis of the source of *Citrus* aroma (Alquézar et al., 2017). Lipoxygenase (LOX) is the starting point of the fatty acid pathway, while ADH and AAT are the main enzymes for generating alcohols and esters (Aragüez and Fernández, 2013; Lu et al., 2024). Modifying enzymes such as UGT (glycosyltransferase) and OMT (O-methyltransferase) can modify aroma precursors, making aroma substances more stable and easier to release (Liao et al., 2023) The activity and expression of these enzymes can be affected by the fruit development stage, hormonal changes, and even gene regulation, ultimately determining the intensity and type of *Citrus* aroma (Sharon-Asa et al., 2003; Wang et al., 2024a).

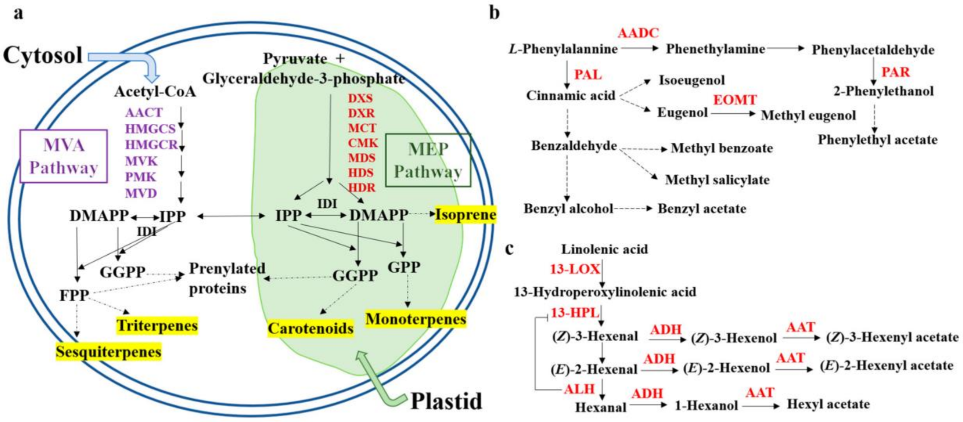

Figure 1 Synthetic pathways of terpenoids (a), phenylpropane (b), and fatty acid derivatives (c) related to the formation of aroma components (Adopted from Lu et al., 2024)

Image caption: AACT, acetyl-CoA C-acetyltransferase; HMGCS, hydroxymethylglutaryl-CoA synthase; HMGCR, hydroxymethylglutaryl-CoA reductase; MVK, mevalonate kinase; PMK, phosphomevalonate kinase; MVD, mevalonate diphosphomevalonate decarboxylase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; HDS, 4-hydroxy-3-methylbut-2-enyl-diphosphate synthase; HDR, 4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase; IDI, isopentenyl-diphosphate delta-isomerase; GPP, geranyl diphosphate synthase; GGPP, geranylgeranyl diphosphate synthase; DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; AADC, aromatic L-amino acid decarboxylase; PAL, phenylalaninammo-nialyase; EOMT, eugenol O-methyltransferase; PAR, phenylacetaldehyde reductase; 13-LOX, 13-lipoxygenase; 13-HPL, 13-hydroperoxidase lyase; ADH, ethanol dehydrogenase; AAT, alcohol acyltransferase (Adopted from Lu et al., 2024)

4 Genetic Regulation of Aroma Formation in *Citrus*

4.1 Key structural genes identified in *Citrus* genomes

The fragrance of *Citrus* fruits mainly comes from some secondary metabolites called volatile terpenoids, which are synthesized within plants. Scientists have identified many genes related to terpene synthase (TPS) in the genome of *Citrus* fruits. In sweet oranges (*Citrus sinensis*), the TPS family has 95 loci, among which 55 can encode potentially functional TPS proteins. These proteins belong to several different subclasses, such as TPS-a, TPS-b, TPS-c, TPS-e/f and TPS-g (Alquézar et al., 2017). Early research by Sharon-Asa et al. (2003) found that the *Cstps1* gene produces a terpene called valencene, which is a key component in the aroma of sweet oranges. This gene is particularly active when the fruit ripens and is influenced by ethylene. The strength of these gene expressions directly determines whether the aroma components of *Citrus* fruits are abundant or scarce and what types they are.

4.2 Transcriptional regulators: MYB, bHLH, and ERF families

The synthesis process of the aroma of *Citrus* also cannot do without the regulation of transcription factors. Transcription factor families such as MYB, bHLH and ERF play a significant role throughout the regulatory process. The latest research shows that MYC5 in bHLH can regulate the formation of oil glands, which are important places for the accumulation of aroma components. Transcription factors such as LMI1 and DRNL can affect the expression of MYC5, thereby promoting the structural development of oil glands, such as sheath cell differentiation and cavity formation, so that flavor substances can accumulate (Wang et al., 2024a). In addition to these, MYB and ERF are also involved in the synthesis of many flavor precursor substances, such as terpenoids and propane phenols (Lu et al., 2024).

4.3 Epigenetic modifications and non-coding RNAs in aroma regulation

In addition to genes and transcription factors, epigenetic mechanisms can also affect the aroma. Regulatory means such as DNA methylation and histone modification can alter the activity levels of certain genes, thereby affecting the synthesis of flavor substances (Lu et al., 2024). In addition, there are some non-coding RNAs, such as miRNAs and lncRNAs, which can affect the expression of transcription factors, sometimes by degrading these mrnas and sometimes by preventing them from being translated. This will also indirectly affect the expression levels of genes related to fragrance.

5 Transcriptome Analysis in *Citrus* Aroma Studies

5.1 RNA-seq approaches to identify differentially expressed genes

RNA-Seq is now the most commonly used method for studying the expression of genes related to *Citrus* fragrance. By conducting high-throughput sequencing on different varieties, tissues or developmental stages of *Citrus* fruits, scientists can identify which genes are related to the accumulation of aroma substances. Zhang et al. (2020) combined transcriptomic and metabolomic studies and found that 31 terpenoids accumulated in large quantities in the leaves of wild or semi-wild *Citrus*, which was closely related to the high expression levels of some key genes in the MVA and MEP pathways. Some terpene synthase genes have also been verified to indeed affect the synthesis of specific fragrance components (Hu et al., 2024).

5.2 Tissue-specific and developmental stage expression patterns

The structure of *Citrus* fruits is complex. Different tissues, such as the peel, flesh and vascular bundles, as well as different developmental stages, all have different gene expressions. High-resolution transcriptome analysis revealed that these differences were important causes of aroma changes (Figure 2) (Feng et al., 2021). For instance, some genes involved in the synthesis of terpene and aldehyde aromas have increased expression levels in specific stages and tissues of fruit development (Hu et al., 2024). Recent studies have also used laser microdissection to separate different cells of *Citrus* peels and then conducted RNA sequencing, which can more clearly observe the expression differences among different cell types (Mei et al., 2024).

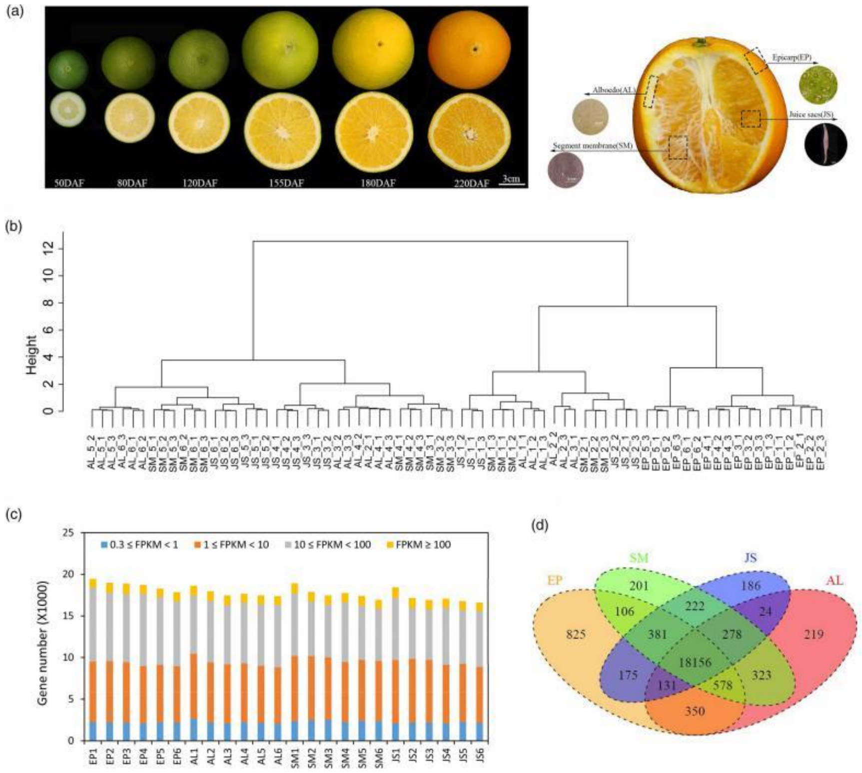

Figure 2 Fruit tissue collection and global analysis of the fruit transcriptomes (Adopted from Feng et al., 2021)

Image caption: (a) Six stages of *C. sinensis* fruit development and four fruit tissue types, epicarp, albedo, segment membrane and juice sac, collected for RNA-seq. (b) Cluster dendrogram showing the global relationships between biological replicates and among different stages and tissues. The y-axis is the degree of variance. In the following figures, samples are named 'Tissue_stage_replicate' or 'Tissue_stage'; 'EP_2_1' represent 'Epicarp_Stage 2_Replicate 1'. (c) Number of genes expressed in each tissue with an average FPKM higher than 0.3. (d) Venn diagram showing the number of shared and uniquely expressed genes among the four fruit tissues (Adopted from Feng et al., 2021)

5.3 Gene co-expression network analysis for aroma-related modules

Co-expression network analysis (such as WGCNA) can help scientists identify a group of genes closely related to aroma synthesis and also identify key regulatory factors (Feng et al., 2021). Hu et al. (2024) found in their comparison of 'Zong Cheng' navel oranges with common varieties that Cs5g12900 and six transcription factors were particularly closely related to the accumulation of valencene (an aroma component), suggesting that they might be the key to regulating the aroma. Some genes related to plant hormones have also been found to be associated with the formation of fragrance (Feng et al., 2021).

6 Metabolomics Approaches in *Citrus* Aroma Research

6.1 Analytical methods: GC-MS, LC-MS, and SPME techniques

Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and solid-phase microextraction (SPME) are often used to study the aroma components of *Citrus*. LC-MS can well separate and analyze various polar and non-polar substances and is suitable for studying various aroma metabolites in *Citrus*. GC-MS is mainly used to detect volatile aroma components, while SPME “concentrates” these low-content volatiles during the sample preparation stage, making the analysis more sensitive (Miao et al., 2022; Wang et al., 2023).

6.2 Quantification and classification of aroma compounds

Researchers can measure the content of each aroma substance in *Citrus* fruits by using metabolomics methods and also classify them. In 2022, Miao et al. used the derivatization -LC-MS method to identify 28 sugars and 18 carboxylic acids in limes, and classified 147 metabolites, among which 92 were discovered for the first time. The combined analysis of metabolomics and genomic data can also identify which genes control the synthesis of these aroma components (Wang et al., 2023).

6.3 Metabolite profiling in different *Citrus* species and tissues

These techniques can also compare the fragrance substances in different varieties and different parts. Traband et al. (2025) recently analyzed data from over 200 *Citrus* leaves and fruits and found a clear metabolic connection between them, indicating that substances in different parts are flowing and mutually regulating. Metabolomics can also identify certain “local characteristic” metabolites when analyzing *Citrus* samples from different regions, which is very helpful for improving aroma quality and differentiating varieties (Miao et al., 2022; Wang et al., 2023).

7 Integration of Transcriptomic and Metabolomic Data

7.1 Correlation analysis between metabolites and gene expression

By combining the transcriptome and metabolome data of different tissues or varieties of *Citrus*, researchers have discovered the relationship between some specific metabolites (such as aroma terpenes and flavonoids) and their related genes. For instance, in the leaves and flowers of *Citrus*, 31 major terpenes are highly present in wild or semi-wild varieties. Moreover, the accumulation of these compounds is highly consistent with the high expression of key genes in the two synthetic pathways of MVA and MEP, indicating that the higher the gene expression, the more of these flavor substances there are (Zhang et al., 2020). In addition, the antioxidant capacity in *Citrus* fruits is also related to the distribution of compounds such as flavonoids and coumarin, and the generation of these substances is closely related to the expression of genes such as *CHS*, *CHI*, and *FNS* (Liang et al., 2024).

7.2 Network-based multi-omics integration methods

Network analysis methods like “WGCNA” can link genes and metabolites and draw their “circles of friends”. Liang et al. (2024) identified some gene modules related to the accumulation of flavonoids and coumarin in their research on different tissues of *Citrus* fruits, and also determined the *OMT* genes involved in coumarin synthesis and the related regulatory factors. Xiong et al. (2024) found in their study on the grafting of ‘Newhall’ fruit peels that transcription factors like AP2/ERF and MYB may have a significant impact on the synthesis of phenolic acids after combining KEGG and GO analyses.

7.3 Discovery of candidate genes through co-expression-metabolite mapping

This method can also help us identify the key genes that regulate the aroma components. For example, in the study of *Citrus* flowers, scientists discovered a gene called sabinene synthase, whose expression level was consistent with the changes of a kind of aroma terpene (Zhang et al., 2020). Also, in the grafted peels of ‘Newhall’, important MYB and ERF transcription factors that may regulate phenolic acid synthesis were identified (Xiong et al., 2024). This method of WGCNA can also discover which structural genes and regulatory factors are related to the accumulation of coumarin, which is very helpful for further research (Liang et al., 2024).

8 Case Study: Aroma Regulation in ‘Newhall’ Navel Orange vs. Wild Relatives

8.1 Experimental design and sampling strategy

This study selected the ‘Newhall’ navel orange (*Citrus sinensis* ‘Newhall’) and its wild relatives as research materials, and conducted a comparative analysis of the accumulation of aroma substances and gene expression during their fruit development. A combined transcriptomic and metabolomic analysis method was adopted, and samples were taken at multiple stages before and after fruit ripening to comprehensively capture the changes in aroma. Gene expression information was obtained by high-throughput sequencing, and aroma components were detected by gas chromatography-mass spectrometry (GC-MS) technology. Combined with bioinformatics analysis, the main differential metabolites and the key genes that may be involved in regulation were screened out (Alquézar et al., 2017; Wang et al., 2024a).

8.2 Key differential metabolites and candidate genes identified

The results show that there are significant differences in the accumulation of some terpene aroma substances (such as valencene, β -caryophyllene and β -humulene) between ‘Newhall’ navel oranges and their wild relatives. Further analysis revealed that some terpene synthase (TPS) genes related to terpene synthesis were expressed more highly in ‘Newhall’, such as *Cstps1*, β -caryophyllene synthase and β -humulene synthase. These genes may directly determine its aroma characteristics (Alquézar et al., 2017). Meanwhile, transcription factors such as LMI1, DRNL and MYC5 were also identified, which may be related to the development of oil glands and the accumulation of aroma precursors (Wang et al., 2024a).

8.3 Functional verification of a key terpene synthase gene

In vitro expression experiments and enzyme activity tests indicated that Cstps1 (valencene synthase) could efficiently convert farnesyl diphosphate (FPP) into valencene, a compound that is the main component of the aroma of ‘Newhall’ fruit. The expression of Cstps1 significantly increased in the later stage of fruit ripening, which was consistent with the increasing trend of valencene. Moreover, the expression of this gene is also regulated by ethylene, suggesting that it may play an important role in the process of fruit ripening and aroma formation (Sharon-Asa et al., 2003). These findings provide a useful theoretical basis for improving the aroma quality of *Citrus* through breeding or genetic engineering in the future.

9 Breeding Applications and Biotechnology

9.1 Marker-assisted selection based on aroma-related loci

The aroma of *Citrus* fruits is related to the genetic inheritance of the variety. Researchers have conducted extensive explorations on the genetic basis of *Citrus* aroma using methods such as QTL mapping and molecular markers. They identified multiple SNP markers and QTLS related to aroma in the hybrid offspring of 'Fortune' and ‘Murcott’, which are closely associated with aroma substances such as monoterpenes and sesquiterpenes. These candidate genes such as QTL and terpene synthase provide a basis for molecular marker-assisted breeding, which is beneficial for breeders to select varieties with better aroma more quickly (Yu et al., 2017; Gill et al., 2022). Yu et al. (2018) demonstrated that the method of GC-MS analysis combined with molecular markers has been able to distinguish different *Citrus* varieties well and can support breeding decisions.

9.2 Genome editing tools for aroma trait improvement

Nowadays, gene editing technologies such as CRISPR-Cas9 have also begun to be used to regulate the aroma of *Citrus* fruits. By this method, some key genes, such as terpene synthase or aromatic synthase, can be precisely knocked out or altered to affect the composition of aroma (Mansoor and Kim, 2024). Although gene editing is still mostly used to improve disease resistance at present, with the increasing understanding of aroma synthesis genes, it is very likely that such technologies will be used in the future to enhance the aroma performance of *Citrus* (Salonia et al., 2020; Conti et al., 2021). Moreover, gene editing can also be combined with molecular marker breeding to improve the efficiency and accuracy of breeding (Gill et al., 2022).

9.3 Synthetic biology approaches for customized *Citrus* aroma profiles

In addition to gene editing, synthetic biology is also bringing about new ideas. By integrating transcriptomic and metabolomic data, researchers can identify which genes and enzymes are most important for aroma synthesis, and then recombine them to make aroma components synthesized faster or more (Maoz et al., 2022). For instance, terpene synthases like Cstps1 are one of the key targets. The regulation or engineered expression of these genes can be used for metabolic engineering and aroma optimization (Sharon-Asa et al., 2003; Aragüez and Fernández, 2013). In the future, synthetic biology may enable modular design of *Citrus* aromas, that is, different aromas can be "customized" to meet different market demands (Conti et al., 2021).

10 Concluding Remarks

By analyzing transcriptome and metabolome data together, researchers have identified the main gene regulatory mechanisms behind the formation of *Citrus* aroma. The aroma of *Citrus* fruits mostly comes from volatile organic compounds (VOCs) such as terpenoids, which are synthesized through metabolic pathways including terpenoids, phenylalanine and fatty acids. Among them, enzyme-coding genes like *Cstps1* and transcription factors such as MYC5 play a very important role in the synthesis of aroma substances and the formation of oil glands. *Cstps1* is expressed more strongly after the fruit ripens and is affected by ethylene signaling, which directly influences the accumulation of aroma components like valencene. In addition, a regulatory pathway composed of LMI1, DRNL and MYC5 has also been confirmed to be involved in the development process of oil glands, which helps to enrich aroma substances in specific tissues.

Although many important genes and regulatory factors related to aroma have been identified, the genetic mechanism of *Citrus* aroma remains very complex. There are many types of terpene synthases, and sometimes they have similar functions. Coupled with the fact that the expression of genes varies at different times or sites, this makes functional verification more difficult. In addition, climate, cultivation methods, and the interaction between genes and the environment can also affect the stability of aroma. The aroma performance of different varieties may vary greatly under different conditions. It is still a considerable challenge to achieve precise control and stable inheritance of aroma characteristics.

Future research can combine high-throughput omics technologies and gene editing tools to gain a deeper understanding of the networks and regulatory patterns of aroma synthesis. CRISPR can be used to precisely regulate key genes like *Cstps1* and *MYC5*, making the aroma more controllable. It is also necessary to continue studying which transcription factors, regulatory elements or epigenetic mechanisms affect aroma. In the future, if a platform can be built that combines multiple omics data and is equipped with the ability to accurately measure aroma, it will be possible to improve the quality of aroma more quickly and accurately, and cultivate new *Citrus* varieties with better fragrance and greater popularity.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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