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Profiling of Volatile Compounds and Associated Gene Expression in Two Anthurium Cultivars and Their F1 Hybrid Progenies

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Abstract: Anthurium is an important ornamental crop in the world market and its floral scent can enhance its ornamental value. To date, studies of the components and formation mechanism of the floral scent of Anthurium are relatively few. In this study, the scent profiles of two Anthurium varieties were measured by gas chromatograph-mass spectrometer (GC-MS). There were 32 volatile organic compounds (VOCs) identified in Anthurium ‘Mystral’, and the most abundant compound was eucalyptol (57.5%). Extremely small amounts of VOCs were detected in Anthurium ‘Alabama’. Compared with A. ‘Alabama’, most genes related to floral scent synthesis exhibited a higher expression in A. ‘Mystral’, including AaDXS, AaDXR, AaMDS, AaHDS, AaTPS, AaDAHPS, AaADT2, AaPAL1, and AaPAL2. In order to produce new varieties of Anthurium with fragrance, 454 progenies of two crossbred combinations of A. ‘Mystral’ and A. ‘Alabama’ were obtained. Four F1 generation plants with different floral scent intensities were selected for further study. The major components of floral scent in the progenies were similar to that of the parental A. ‘Mystral’ plant. The expression patterns of genes related to floral scent synthesis were consistent with the relative contents of different types of VOCs. This study revealed the profiles of volatile compounds and associated gene expression in two Anthurium cultivars and their F1 hybrids, which provided a basis for the floral scent inheritance of Anthurium andraeanum.

Keywords: Anthurium andraeanum; hybrid progenies; floral scent; VOCs; gene expression

1. Introduction

The floral scent is an important trait of ornamental plants and plays a crucial role in attracting pollinators [1] and pathogen resistance [2]. In addition, flower scent can attract customers and improve the commercial potential of an ornamental plant [3]. The floral scent is composed of a series of low-molecular-weight volatile organic compounds (VOCs). To date, VOC profiles have been analyzed in many species, including Dianthus caryophyllus L. [4], Rosa × hybrida [5], Osmanthus fragrans Lour. [6], Freesia [7], Lycoris [8], Gelsemium sempervirens [9], Lilium [10], Chimonanthus praecox [11], Polianthes tuberosa L. [12], Prunus mume [3], and Freesia hybrid [13], amongst others. Although the VOCs emitted by flowers vary greatly among different species, they can be divided into three major groups according to their biosynthesis origins: terpenoids, phenylpropanoids/benzenoids, and fatty acid derivatives [14].

Terpenoids are the largest class of plant VOCs and their metabolic pathways have been well characterized in the plant kingdom (Figure 1). Terpenoids are synthesized via the...
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cytosolic mevalonate acid (MVA) pathway and the plastidial methylerythritol phosphate (MEP) pathway [15]. The MVA pathway begins with the condensation of three molecules of acetyl-CoA, whereas the MEP pathway starts with the condensation of D-glyceraldehyde 3-phosphate and pyruvate. Both pathways generate isopentenyl diphosphate (IPP) and its homologous isomer dimethylallyl pyrophosphate (DMAPP) through a series of enzymatic reactions [16]. The sequential head-to-tail condensation of IPP and DMAPP leads to the formation of FPP in the cytosol, as well as pyrophosphate (GPP) and geranylgeranyl diposphate (GGPP) in plastids [17]. Subsequently, different terpene synthases (TPSs) convert GPP, GGPP, and FPP into structurally diverse monoterpenes, diterpenes, and sesquiterpenes, respectively [18]. The monoterpenes are mainly synthesized by the MEP pathway, in which nine key enzymes catalyze a series of successive reactions, including 1-deoxy-D-xylulose 5-phosphate synthase (DXS), 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), D-erythritol 4-phosphate cytidylyltransferase (MCT), 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase (CMK), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS), 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (HDS), 4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase (HDR), geranyl pyrophosphate synthase (GPPS), and TPS [19]. Phenylpropanoids and benzenoids, the second largest class of plant VOCs, are divided into three subclasses depending on their carbon skeleton: phenylpropanoids (with a C6–C3 backbone), benzenoids (with a C6–C1 backbone), and phenylpropanoid-related compounds (with a C6–C2 backbone) [14]. All three subclasses are derived from the aromatic amino acid (AAAS) phenylalanine (Phe). Aromatic amino acid aminotransferase (AAAT) and phenylacetaldehyde synthase (PAAS) are key enzymes in phenylpropanoid-related compound biosynthesis, and phenylalanine ammonia-lyase (PAL) is the key enzyme in benzenoid and phenylpropanoid biosynthesis, which deaminates Phe to trans-cinnamic acid (CA) and competes with PAAS for Phe utilization. Phe is derived from chorismate, the final product of the shikimate pathway [20]. Many studies have demonstrated that the level of key enzymes in the shikimate and arogenate pathways (Figure 1), such as 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHPS), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), chorismate mutase (CM), prephenate dehydratase (PDT), arogenate dehydratase (ADT), and S-adenosylmethionine synthetase (SAMS) [21,22]. Fatty acid derivatives, the third class of plant VOCs, are derived from unsaturated C18 fatty acids, namely linolenic or linoleic acids [23]. Lipoygenase (LOX) directly leads to the formation of 9- and 13-hydroperoxy intermediates via two branches: the allene oxide synthase (AOS) branch, which gives rise to jasmonic acid (JA), and the hydroperoxide lyase branch, which leads to the formation of volatile alcohols and their esters [24]. Many transcription factor (TF) families are involved in regulating the biosynthesis of VOCs. The TPS gene family, responsible for the formation of terpenes, has been characterized in many plants—however, the regulatory network that controls TPS expression is still unclear. In Arabidopsis thaliana (Arabidopsis), myeloblastosis protein 21 (MYB21) and myelocytomatosis protein 2 (MYC2) regulate the expression of TPS11 and TPS21 via the gibberellic and JA pathways [25,26]. In Arabidopsis, auxin response factors 6 and 8 (ARF6 and ARF8) can bind to the promoters of TPS11 and TPS21 to regulate the synthesis of sesquiterpenes [25]. In addition, MYB TFs can interact with other base helix–loop–helix (bHLH) TFs to form an MYB–bHLH complex to participate in the regulation of sesquiterpene biosynthesis [27,28]. WRKY TFs, NaWRKY3, and NaWRKY6 participate in the regulation of the defensive terpene emission in Nicotiana attenuata [29]. In Petunia × hybrida, R2R3-type MYB TFs, ODORANT1 (ODO1), EMISSION OF BENZENOIDS I (EBOI), and EMISSION OF BENZENOIDS II (EBOII) control the biosynthesis of phenylpropanoids/benzenoids by regulation of the shikimate pathway [30,31]. Suppression of PhODO1 and PhEBOII expression leads to a reduced amount of floral volatiles, through decreasing transcript level of many phenylpropanoid/benzenoid genes such as PhDAHPS, PhEPSPS, PhPAL, PhCM, and PhSAMS [32]. In contrast, the MYB4 TF is a repressor of cinnamate-4-hydroxylase from the phenylpropanoid pathway in petunias [33]. For the biosynthetic pathway of the...
volatiles derived from fatty acids, studies on their transcriptional regulation have primarily focused on JA [34,35]. Many TFs, including MYC, MYB, GAI, RGA, EIN3, EIL, ERF, and RGL1, have been demonstrated to be involved in the regulation of JA biosynthesis [23,36].

Figure 1. Overview of the main volatile organic compounds’ biosynthetic pathways: terpenoids (pink), phenylpropanoids/benzenoids (blue), and fatty acid derivatives (yellow). In this study, the components of floral scents and the expression levels of floral scent biosynthesis-related genes were identified in A. ‘Mystral’ (with strong fragrance) and A. ‘Alabama’ (with no fragrance). In addition, the floral scent biosynthesis characteristics were further explored in the F1 hybrids of A. ‘Mystral’ and A. ‘Alabama’, including the presence or absence of floral scent, the types and contents of VOCs, and the expression patterns of VOC synthesis-related genes. In this study, the hybrid progenies of Anthurium andraeanum with aroma and Anthurium andraeanum without aroma were established and the inheritance of aroma was preliminarily explored, providing a theoretical basis for the inheritance of floral scent in A. andraeanum and laying a foundation for the creation of new A. andraeanum varieties with fragrance.

Anthurium andraeanum is an important tropical and subtropical ornamental crop in the world market. The popularity of A. andraeanum is largely due to the exotic shapes and colors of the spathe, and the remarkable longevity of the plant’s flowering period. The floral scent varies greatly among Anthurium cultivars. A survey of floral scent in 147 Anthurium species and hybrids showed that most plants emitted scents ranging from pleasant to unpleasant and from very weak to very strong [37]. The majority of Anthurium species that emit fragrance and volatile compounds do so at the pistillate stage of flower development, with the primary emitted compounds consisting of 1,8-cineole, α,β -pinene, sabinene, myrcene, and limonene, as well as some benzenoids [37]. To date, studies characterizing the components and formation mechanism of Anthurium floral scent are scarce.

2. Results
2.1. Floral Scent Compounds in the Spadix of A. ‘Mystral’ and A. ‘Alabama’

A. ‘Mystral’ (with strong fragrance) and A. ‘Alabama’ (with no fragrance) were the best-selling varieties of Anthurium, and were selected for floral scent study (Figure 2). To identify the components of the floral scent of Anthurium, total VOCs from the spadix in fully expended stage (S2) were analyzed (Figure 2) by gas chromatograph-mass spectrometer (GC-MS). In A. ‘Mystral’, 32 VOCs were identified (Table 1), including terpenes (70%) and phenylpropanoid/benzenoids (28.5%). The total amount of VOCs was 85.567 µg h⁻¹·g⁻¹, with eucalyptol (49.2 µg h⁻¹·g⁻¹) and acetic acid, phenylmethyl ester (19.835 µg h⁻¹·g⁻¹) being the predominant components, accounting for 57.5% and 23.2% of the total VOCs, respectively. Compared with A. ‘Mystral’, only 1.376 µg h⁻¹·g⁻¹ VOCs were detected in A. ‘Alabama’, and no terpenes or phenylpropanoid/benzenoids were identified. These results were consistent with the sensory judgment, and indicated eucalyptol was the major component of the floral scent of A. ‘Mystral’.
2.2. Analysis of the Key Genes Involved in Volatile Organic Compound (VOC) Biosynthetic Pathways

In most flowering plants, VOCs are divided into several classes, including terpenoids, benzenes/phenylpropanes, and fatty acid derivatives. In the 32 VOCs identified in *A. ‘Mystral’*, 13 of these were monoterpenes while six were benzenes/phenylpropanes. To further characterize the regulation of VOCs at the molecular level, the transcript levels of key genes involved in monoterpene and phenylpropane biosynthesis were assessed (Figure 3). In total, 10 related genes were retrieved from NCBI and our transcriptome database (unpublished), including six key genes in the MEP pathway (*AaDXS, AaDXR, AaCMK, AaMDS, AaHDS, and AaTPS*) and five key genes in the phenylpropane biosynthesis or shikimate pathways (*AaDAHPS, AaEPSPS, AaADT2, AaPAL1, and AaPAL2*). In *A. ‘Alabama’*, most VOC biosynthesis-related genes showed similar expression patterns in all three parts of the inflorescence, except for *AaPAL1*. The expression level of *AaPAL1* in the middle part of the spadix (MS) was ten times higher than in the top part of the spadix (TS) and the base part of the spadix (BS). Unlike in *A. ‘Alabama’*, most VOC biosynthesis-related genes showed higher expression in the TS of *A. ‘Mystral’*. Compared with the TS of *A. ‘Alabama’*, the expression level of *AaDXS*—the first enzyme in the MEP pathway—increased at least 80-fold in all three parts of the *A. ‘Mystral’* spadix. *AaTPS*, the key enzyme responsible for production of the major monoterpenes, showed a 52-fold increase in the BS of *A. ‘Mystral’*. The expression levels of *AaDXR* and *AaHDS* were much higher in all three parts of the *A. ‘Mystral’* spadix. Compared with the TS of *A. ‘Alabama’*, *AaCMK* and *AaMDS* exhibited higher expression both in the TS and in the MS of *A. ‘Mystral’*. *AaDAHPS* and *AaEPSPS* were the key enzymes in the shikimate pathway assessed in this study, and the expression of *AaDAHPS* was higher in *A. ‘Mystral’* spadix than in *A. ‘Alabama’* spadix, while the expression level of *AaEPSPS* was not significantly different between the two cultivars. Both *AaPAL* and *AaADT*, the key enzymes in the phenylpropane biosynthesis pathway, showed higher expression in *A. ‘Mystral’* than in *A. ‘Alabama’* spadix. These results indicated that the expression levels of VOC biosynthetic pathway-related genes were significantly different between *A. ‘Mystral’* and *A. ‘Alabama’*, and *AaDXS, AaTPS*, and *AaPAL* might be the key functional genes in the biosynthesis of VOCs.

![Figure 2. Phenotype of *A. ‘Alabama’* and *A. ‘Mystral’*. S1, spathe folding stage; S2, pistillate emerge stage; S3, spadix fully extended stage.](image-url)
Table 1. Relative amounts of volatile compounds identified in *A. ‘Mystral’* and *A. ‘Alabama’*.

| No. | Compounds                                                                 | Molecular Formula | RT (min) | Content (µg gFW h⁻¹) ± SD |
|-----|---------------------------------------------------------------------------|-------------------|----------|---------------------------|
|     |                                                                           |                   | A. ‘Mystral’ | A. ‘Alabama’ |
|     |                                                                           |                   |           |               |
| monoterpenes                          |                                                                           |                   |           |               |
| 1   | Eucalyptol                                                                | C₁₀H₁₆O            | 10.211   | 49.2 ± 2.8     | -             |
| 2   | α,α-4-trimethyl-3-cyclohexene-1-methanol                                  | C₁₀H₁₆O            | 14.182   | 7.07 ± 0.6     | -             |
| 3   | β-Pinene                                                                  | C₁₀H₁₆              | 8.62     | 1.31 ± 0.4     | -             |
| 4   | β-Phellandrene                                                            | C₁₀H₁₆              | 8.551    | 0.56 ± 0.0     | -             |
| 5   | 1-methyl-4-(1-methylhexylidene)-cyclohexene                              | C₁₀H₁₆              | 11.876   | 0.41 ± 0.1     | -             |
| 6   | (E)-1,3,6-Octatriene, 3,7-dimethyl-                                       |                   |          |               |               |
| 7   | α-Pinene                                                                  | C₁₀H₁₆              | 7.436    | 2.12 ± 0.0     | -             |
| 8   | β-Myrcene                                                                 | C₁₀H₁₆              | 9.026    | 0.20 ± 0.1     | -             |
| 9   | 4-methyl-1-(1-methylpropyl)-3-cyclohexene-1-ol                            | C₁₂H₂₀O₂            | 14.474   | 0.15 ± 0.2     | -             |
| 10  | cis-2-Cyclohexene-1-ol,2-methyl-3-(1-methylene), acetate                  | C₁₂H₁₉O₂            | 18.874   | 0.12 ± 0.0     | 1.00 ± 0.0    |
| 11  | Thujone                                                                   | C₁₅H₂₆O             | 12.711   | 0.06 ± 0.0     | -             |
| 12  | 3-methyl-6-(1-methylhexylidene)-cyclohexene                              | C₁₃H₁₆              | 13.592   | 0.05 ± 0.0     | -             |
| 13  | 2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester                        | C₁₁H₁₈O₂            | 18.49    | 0.03 ± 0.0     | -             |
|     |                                                                           |                   |           |               |
| sesquiterpenes                         |                                                                           |                   |           |               |
| 14  | 1H-Cyclopental[n]napthalene,1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl | C₁₃H₂₄              | 21.454   | 0.17 ± 0.0     | -             |
| 15  | γ-Himachalene                                                             | C₁₅H₂₄              | 22.576   | 0.12 ± 0.0     | -             |
|     |                                                                           |                   |           |               |
| phenylpropanoid/benzenoids             |                                                                           |                   |           |               |
| 16  | Acetic acid, phenylmethyl ester                                          | C₆H₅O₂              | 14.19    | 19.83 ± 1.5    | -             |
| 17  | Benzaldehyde                                                               | C₈H₇O                | 12.059   | 3.84 ± 0.5     | -             |
| 18  | Benzaldehyde                                                              | C₅H₉O                | 8.242    | 0.21 ± 0.1     | -             |
| 19  | 2-Propenoic acid, 3-phenyl-, methyl ester                                | C₆H₄O₂              | 20.07    | 0.13 ± 0.0     | -             |
| 20  | Indole                                                                     | C₈H₇N                | 17.672   | 0.13 ± 0.2     | -             |
| 21  | Butylated Hydroxytoluene                                                  | C₁₅H₂₀O             | 23.337   | 0.09 ± 0.0     | -             |
| 22  | 1-ethyl-2,4,5-trimethyl-, Benzene                                        | C₁₁H₁₈                | 18.227   | 0.08 ± 0.0     | -             |
|     |                                                                           |                   |           |               |
| Others                                   |                                                                           |                   |           |               |
| 23  | Tetradecene                                                               | C₁₃H₃₀               | 20.453   | 0.24 ± 0.2     | 0.226 ± 0.2   |
| 24  | Heptadecane,2,6,10,14-tetramethyl                                       | C₁₂H₄₄               | 22.015   | 0.24 ± 0.2     | 0.183 ± 0.2   |
| 25  | Pentadecane                                                               | C₁₅H₃₂               | 22.948   | 0.24 ± 0.2     | 0.279 ± 0.2   |
| 26  | 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester                    | C₁₆H₂₂O₄             | 32.011   | 0.23 ± 0.1     | -             |
| 27  | 2,6,10-trimethyl-Dodecane                                                | C₁₃H₃₂               | 19.652   | 0.1 ± 0.0      | 0.082 ± 0.0   |
| 28  | Cyclohexaloxoic acid, dodecymethyl-                                      | C₁₂H₂₀O₆S₆            | 18.628   | 0.081 ± 0.0    | 0.059 ± 0.0   |
| 29  | Decamethyl-cyclopentasiloxane                                            | C₂₀H₃₈O₂S₆           | 13.844   | 0.05I ± 0.0    | -             |
| 30  | Tridecane                                                                 | C₁₃H₂₈               | 17.832   | 0.05I ± 0.0    | -             |
| 31  | 10-Methylnonadecane                                                       | C₂₀H₄₂               | 19.692   | 0.04 ± 0.0     | -             |
| 32  | 2,6,11,15-tetramethyl-Hexadecane                                         | C₂₀H₄₂               | 26.415   | 0.04 ± 0.0     | 0.054 ± 0.0   |
| 33  | 3,5-dimethyl-Undecane                                                    | C₁₃H₂₈               | 26.518   | 0.05 ± 0.0     | -             |
| 34  | Hexadecane                                                                | C₂₁H₄₄               | 27.777   | -              | 0.103 ± 0.1   |

1 RT, retention time; 2 the mass of compound (µg gFW⁻¹ h⁻¹) = mass of internal standard × area under the peak of a compound/area under peak of internal standard/fresh weight of sample; 3 all data are presented as mean ± standard error (n = 3); 4 indicates not detected.
Figure 3. The relative expression levels of VOC biosynthesis-related genes in the inflorescences of *A. 'Mystral'* and *A. 'Alabama'*. (a) The relative expression levels of monoterpene biosynthesis-related genes; (b) The relative expression levels of key genes in the phenylpropane biosynthesis or shikimate pathways. TS, the top part of the inflorescences; MS, the middle part of the inflorescences; BS, the base part of the inflorescences.

2.3. Segregation of Floral Scent Traits in Hybrid Progenies of *A. 'Mystral' × A. 'Alabama'*

In order to determine whether floral scent traits can be inherited or not, the floral scents of F1 hybrids resulting from the genetic crossing of *A. 'Mystral'* and *A. 'Alabama'* were studied. The olfactory tests were performed by treated individuals [13]. The results showed that the proportion of fragrant plants of F1 generation from the cross (*A. 'Mystral' ♀ × A. 'Alabama' ♂*) or the reciprocal cross (*A. 'Mystral' ♂ × A. 'Alabama' ♀*) were 74.38% and 59.34%, respectively (Table 2). The fragrant plants were classified according to floral
scent intensity. There were 60 plants with strong fragrance and 210 plants with weak fragrance in F1 populations from cross combination, and the ratio of fragrant plants to fragrance-free plants was 3:1. In F1 populations from reciprocal cross combination, six plants were identified as strong fragrant plants, 48 plants were weak fragrant plants, and the ratio was 3:2. These data indicate that the floral scent is a complex trait and is likely to have many genes of influence.

Table 2. Olfactory test of F1 hybrids from two-hybrid combinations.

| Hybrid Combinations | No. of Plants (Strong Floral Scent) | No. of Plants (Weak Floral Scent) | No. of Plants (Fragrance Free) | Ratio (No. Strong/No. Fragrance Free) |
|---------------------|-----------------------------------|----------------------------------|-------------------------------|--------------------------------------|
| A. 'Mystral' ♀ × A. 'Alabama' ♂ (08-377) | 60 | 210 | 93 | 3:1 |
| A. 'Alabama' ♀ × A. 'Mystral' ♂ (08-382) | 6 | 48 | 37 | 3:2 |

2.4. The Compounds of Floral Scent in Hybrid Progenies of A. 'Mystral' × A. 'Alabama'

Several individual plants with different floral scent intensities in the hybrid progenies were selected to test the VOCs (Table 3). Progeny plants 08-377-09 and 08-382-20 were strong fragrant plants, while progeny plants 08-377-03 and 08-382-48 were weak fragrant plants. In total, 27 VOCs were identified in plant 08-377-09, and the relative total amount of VOCs was 109.811 µg·h⁻¹·g⁻¹, including terpenes (10.8%), benzenoids (84.5%), and fatty acid derivatives (4.2%). Unlike the fragrant parental A. 'Mystral', acetic acid, phenylmethyl ester was the most abundant component, and the content of eucalyptol was not as high as that of A. 'Mystral'. In plant 08-382-20, the total amount of VOCs was 20 with a relative total amount of 113.137 µg·h⁻¹·g⁻¹, including 15 terpenes that accounted for 90% of the total VOCs. Eucalyptol (33%) was the major compound in plant 08-382-20, followed by 1,3,6-Octatriene, 3,7-dimethyl- (24%), trans-Limonene oxide (10%), trans-Carvone oxide (9%), and trans-2-methyl-5-(1-methylethenyl)-Cyclohexanone (9%). For the plants 08-377-03 and 08-382-48, the total amounts of VOCs were relatively less, and the most abundant components were acetic acid, phenylmethyl ester, and eucalyptol, respectively. These results indicated that the floral scent traits of Anthurium can be inherited, and the major floral scent components were similar to the fragrant parental plants.

Table 3. Relative amounts of volatile compounds identified in F1 hybrids.

| No. | Compounds | Molecular Formula | RT ¹ (min) | Relative Amount (µg gFW h⁻¹)² ± SD ³ |
|-----|-----------|-------------------|-----------|-------------------------------------|
|     |           |                   | 08-377-9 | 08-377-3 | 08-382-20 | 08-382-48 |
|     | Monoterpenes |                   | Strong | Weak | Strong | Weak | Strong | Weak |
| 1   | α-Pinene   | C₁₀H₁₆             | 7.436    | 0.342 ± 0.0 - | 1.424 ± 0.1 | 1.048 ± 0.1 |
| 2   | β-Pinene   | C₁₀H₁₆             | 8.62     | 0.467 ± 0.1 - | 0.937 ± 0.1 - |
| 3   | β-Phellandrene | C₁₀H₁₆ | 8.694   | - - | 2.13 ± 0.2 0.382 ± 0.1 |
| 4   | 4-methylene-1-(1-methylethyl)-cyclohexene | C₁₀H₁₆ | 8.78 | - - | - - | 1.21 ± 0.1 |
| 5   | Eucalyptol | C₁₀H₁₅O            | 10.199   | 4.215 ± 0.3 - | 37.497 ± 1.3 11.49 ± 0.6 |
| 6   | 3,7-dimethyl-1,6-Octadien-3-ol | C₁₀H₁₅O | 12.225 | - - | 27.09 ± 11.0 - |
| 7   | (E)-1,3,6-Octatriene, 3,7-dimethyl- | C₁₀H₁₈ | 10.691 | 0.023 ± 0.0 - | 0.109 ± 0.1 - |
| 8   | cis-Linalool oxide | C₁₀H₁₅O₂ | 11.441 | - - | 0.803 ± 0.1 - |
| 9   | 1-methyl-4-(1-methylethylidene)-cyclohexene | C₁₀H₁₆ | 11.882 | 0.023 ± 0.0 - | - - - |
| 10  | cis-Limonene oxide | C₁₀H₁₇O | 13.198 | 0.023 ± 0.0 - | 0.56 ± 0.0 0.077 ± 0.0 |
| 11  | trans-Limonene oxide | C₁₀H₁₇O | 13.329 | 2.154 ± 0.2 0.013 ± 0.0 | 11.124 ± 0.6 1.265 ± 0.1 |
| 12  | 4-methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol | C₁₀H₁₇O | 14.485 | - - | - - - |
| 13  | α,α-4-trimethyl-3-Cyclohexene-1-methanol | C₁₀H₁₅O | 14.868 | 0.296 ± 0.0 - | 5.72 ± 0.6 - |
| 14  | 4-methyl-1-(1-methylethenyl)-Cyclohexene | C₁₀H₁₆ | 14.971 | 0.308 ± 0.0 - | 1.4 ± 0.1 - |

² ± SD: ± Standard Deviation
| No. | Compounds | Molecular Formula | RT 1 (min) | Relative Amount (µg·gFW·h⁻¹) ² ± SD ³ |
|-----|-----------|------------------|------------|---------------------------------------|
| 15  | trans-2-methyl-5-(1-methylene)-Cyclohexanone | C₁₅H₁₆O₂ | 23.234 | 0.011 ± 0.0 | - | - | - |
| 16  | 2-methyl-5-(1-methylene)-2-Cyclohexen-1-ol | C₁₅H₁₆O₂ | 12.082 | 0.148 ± 0.0 | - | - | - |
| 17  | (S)-2-methyl-5-(1-methylene)-2-Cyclohexen-1-one | C₁₅H₁₆O₂ | 14.285 | 0.014 ± 0.0 | - | - | - |
| 18  | trans-Carvone oxide | C₁₀H₁₄O | 16.934 | 4.125 ± 0.1 | - | 10.126 ± 0.6 | 0.703 ± 0.0 |
| 19  | cis-2-Cyclohexen-1-ol,2-methyl-5-(1-methylene)-acetate | C₁₃H₁₉O₂ | 15.967 | - | - | - | 0.112 ± 0.0 |
| 20  | (S)-1-methyl-4-(5-methylene-4-hexenyl)-Cyclohexone | C₁₃H₂₄ | 23.234 | 0.011 ± 0.0 | - | - | - |
| 21  | Benzyl Alcohol | C₇H₈O | 10.308 | - | 0.035 ± 0.0 | - | - |
| 22  | Benzoic acid, methyl ester | C₆H₅CO₂H | 12.082 | 0.148 ± 0.0 | - | - | - |
| 23  | Acetic acid, phenylethyl ester | C₈H₁₄O₂ | 14.273 | 87.442 ± 1.9 | 6.956 ± 0.5 | - | 0.294 ± 0.0 |
| 24  | Benzoic acid, ethyl ester | C₆H₅CO₂H | 14.285 | - | 0.014 ± 0.0 | - | - |
| 25  | Propionic acid, phenylethyl ester | C₈H₁₄O₂ | 16.278 | 0.011 ± 0.0 | - | - | - |
| 26  | Butylated Hydroxytoluene | C₁₃H₁₄O | 23.354 | - | 0.03 ± 0.0 | - | - |
| 27  | Benzyl Benzoate | C₁₂H₁₄O₂ | 29.133 | 4.809 ± 0.8 | - | 0.082 ± 0.0 |
| 28  | Benzoic acid, 2-hydroxy-, phenylethyl ester | C₁₃H₁₈O₃ | 32.074 | 0.547 ± 0.0 | - | - | - |
| 29  | 1-Butanol, 3-methyl-, acetate | C₅H₁₀O₂ | 6.028 | - | 0.2066 ± 0.0 | - | - |
| 30  | 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester | C₁₈H₂₂O₄ | 32.967 | - | - | 0.316 ± 0.0 | - |
| 31  | Phthalic acid, isobutyl octyl ester | C₁₉H₂₆O₄ | 32.028 | - | 0.025 ± 0.0 | - | 0.233 ± 0.0 |
| 32  | Hexanedioic acid, bis(2-ethylhexyl) ester | C₂₂H₄₄O₄ | 34.958 | 4.627 ± 0.2 | - | - | - |
| 33  | 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-Butanone | C₁₃H₂₅O₂ | 21.569 | - | 0.195 ± 0.0 | - | - |

**Benzene Derivatives**

| No. | Compounds | Molecular Formula |
|-----|-----------|------------------|
| 34  | Tridecane | C₁₃H₂₈ |
| 35  | Tetradecane | C₁₄H₃₀ |
| 36  | Pentadecane | C₁₅H₃₂ |
| 37  | Heptacosane | C₂₇H₅₆ |
| 38  | Hexadecane | C₁₆H₃₄ |
| 39  | 2,6,10-trimethyl-Pentadecane | C₁₆H₃₈ |
| 40  | 3-methyl-Tetradecane | C₁₅H₃₂ |
| 41  | 5-methyl-3-Octyne | C₁₅H₃₄ |
| 42  | Cyclohexasioxane, dodecamethyl- | C₁₃H₉O₃Si₆ |
| 43  | 10-Methylnonadecane | C₂₀H₄₂ |
| 44  | 3-methyl-Tridecane | C₁₄H₂₀ |
| 45  | 2,6,10-trimethyl-Dodecane | C₁₃H₃₂ |
| 46  | 3-cyclohexyl-Deacye | C₁₅H₃₂ |
| 47  | Heptadecane, 2,6,10,14-tetramethyl- | C₂₁H₄₄ |

| Relative Amount (µg·gFW·h⁻¹) ² ± SD ³ | 08-377-9 Strong | 08-377-3 Weak | 08-382-20 Strong | 08-382-48 Weak |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|
| 15 | 2.37 ± 0.2 | - | 10.309 ± 0.9 | 0.226 ± 0.0 |
| 16 | 0.057 ± 0.0 | - | 0.596 ± 0.1 | - |
| 17 | 0.103 ± 0.0 | 0.011 ± 0.0 | 1.789 ± 0.5 | 1.2980 ± 1.0 |
| 18 | 1.425 ± 0.1 | - | 10.126 ± 0.6 | 0.703 ± 0.0 |

1 RT, retention time; ² the mass of compound (µg·gFW·h⁻¹) = mass of internal standard × area under peak of a compound/area under peak of internal standard/fresh weight of sample; ³ all data are presented as mean ± standard error (n = 3); ⁴ indicates not detected.
2.5. Analysis of Volatile Organic Compound Biosynthetic Pathway-Related Genes in Hybrid Progenies

To explore the relationship between floral scent compounds and VOC biosynthesis-related genes in these hybrid progenies, the expression levels of a series of genes involved in VOC biosynthesis were detected (Figure 4). Compared with the fragrant parenteral *A. ‘Mystral’*, the expression of *AaDXS*, *AaDXR*, and *AaCMK* were increased in the plant 08-380-20, which might lead to the production of terpenoid compounds. Other genes involved in the MEP, phenylpropane biosynthesis, or shikimate pathways showed similar expression patterns in plant 08-380-20 and *A. ‘Mystral’*. Compared with *A. ‘Mystral’*, *AaEPSPS* and *AaPAL2* demonstrated increased expression in plant 08-377-9, which was related to the high amount of benzenoid compounds detected. On the other hand, the decreased expression levels of genes involved in the MEP pathway, including *AaDXS*, *AaDXR*, *AaMDS*, *AaHDS*, and *AaTPS*, may have led to the reduced content of terpenoids in plant 08-377-9. In plants 08-377-3 and 08-382-48, the expression levels of most VOC biosynthesis-related genes were decreased, which was consistent with the lower amount of floral scent compounds. These results indicate that the different content of floral scent compounds in the progeny plants of the performed cross may be the result of different expression levels of VOC biosynthetic pathway-related genes.

![Figure 4](image-url)

**Figure 4.** The relative expression levels of VOC biosynthesis-related genes in the top part of the inflorescences of progenies and *A. ‘Mystral’*. (a) The relative expression levels of monoterpene biosynthesis-related genes; (b) The relative expression levels of key genes in phenylpropane biosynthesis or shikimate pathways. The relative bar color intensities represent the total amount of VOCs.
3. Discussion

The floral scent is a significant ornamental characteristic that improves the commercial value of ornamental plants [18]. *Anthurium* is an important commodity flower worldwide, however the odors of *Anthurium* range from pleasant to unpleasant. Therefore, it is of great significance to explore the mechanism of *Anthurium* floral scent production and to cultivate *Anthurium* cultivars with a pleasant fragrance.

In most plants, floral scent is produced by a series of chemical compounds, including terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives, carotenoid derivatives, and sulfur- or nitrogen-containing compounds. In this study, the scents of *A. ‘Mystral’* were found to be composed of two major compounds: terpenoids (70%) and benzenoids (28.5%). Among these compounds, eucalyptol and acetic acid, phenylmethyl ester were the predominant VOCs, accounting for 57.5% and 23.2% of total VOCs, respectively. In the F1 hybrids of *A. ‘Mystral’* and *A. ‘Alabama’*, two plants (08-377-09 and 08-382-20) with strong fragrance and two plants (08-377-09 and 08-382-20) with weak fragrance were chosen to identify different VOC contents in these two groups of plants. In plant 08-377-09, acetic acid, phenylmethyl ester accounted for 79.6% of the total VOCs and the percentage of eucalyptol was 3.8%. In plant 08-382-20, eucalyptol was the major volatile compound and no acetic acid, phenylmethyl ester was detected. Interestingly, the total amounts of VOCs in plants 08-377-09 and 08-382-20 were very low, but acetic acid, phenylmethyl ester (80.5%) and eucalyptol (60.5%) were still the major volatile compounds. These results indicated the major volatile compounds of the F1 hybrid progeny were consistent with the fragrant parent.

A previous survey of floral scent in 147 *Anthurium* species and hybrids showed that most plants emitted scent only in the morning (45%) and at the pistillate stage (77%) of floral development [37]. For *A. ‘Mystral’*, the floral scent lasted from morning to afternoon and spadix samples at the pistillate stage of development, between 10:00 a.m. and 12:00 p.m., were taken for GC-MS analysis. For qPCR analysis, the expression levels of floral scent biosynthesis-related genes were compared across the top, middle, and base parts of the spadix. The qPCR results showed that most genes exhibited their highest level of expression in the top of the spadix (Figure 3). Thus, the top part was chosen to test the expression level of floral scent biosynthesis-related genes in the F1 hybrids (Figure 4).

The inheritance of floral scent was complex, and was not simply controlled by nuclear inheritance or cytoplasmic inheritance. Our results demonstrated that having a fragrance-free parent can lead to progeny with strong fragrance, which indicates that cytoplasmic inheritance is not essential for the fragrance trait but may increase the frequency of scented progeny. In addition, several studies have demonstrated that the relationship between flower color and scent are partly linked by inheritance, as the two traits rely on shared biosynthetic pathways [38]. To explore the relationship between spathe color and spadix scents in *Anthurium andraeanum*, hybridization experiments were carried out using *A. ‘Mystral’* (with red spathes, white spadix, and strong fragrance) and *A. ‘Alabama’* (with white spathes, pink spadix, and no fragrance) as parents. Among 226 F1 hybrid individuals from the cross combination (*A. ‘Mystral’* (♀) × *A. ‘Alabama’* (♂)), 170 of 220 plants with red spathes emitted floral scent at different times of the day, and 135 of the 170 plants had white spadix. For the remaining six plants with white spathes, three individuals emitted floral scent with white or pink spadix. A similar phenomenon was also observed in the F1 hybrids from the reciprocal cross combination (*A. ‘Alabama’* (♀) × *A. ‘Mystral’* (♂)). The results suggested that there was no obvious relationship between the floral scent and flower color in *Anthurium andraeanum*.

In recent years, terpenoid and phenylpropanoid/benzenoid biosynthetic pathways have been well characterized, but less was known about the biosynthesis of fatty acid derivatives [23]. In *A. ‘Mystral’* and the F1 hybrids, monoterpenes and benzenoids were the major floral scent compounds and the transcript levels of key enzyme genes that are involved in the biosynthetic pathways of these two compounds were identified. In the existing database of *Anthurium*, we found six key enzymes in the MEP pathway,
including DXR, CMK, MDS, HDS, and TPS. Our results revealed that all six genes exhibited extremely higher expression in A. ‘Mystral’ than in A. ‘Alabama’. This extreme difference was consistent with the great difference in floral scent content. Compared with A. ‘Mystral’, the content of terpenoids was relatively higher in plant 08-382-20, and three of six key enzyme genes (AaDXS, AaDXR, and AaCMK) were upregulated. These results indicated that AaDXS, AaDXR, and AaCMK were much more important in terpenoid synthesis, but these results might be influenced by other factors such as different development stages and different inflorescence parts. By comparing the content of benzenes and the expression of related genes in A. ‘Mystral’ and the progenies, AaEPSPS and AaPAL2 were identified as more important enzymes in the biosynthesis of benzenoids. In conclusion, the expression patterns of genes related to floral scent synthesis were consistent with the relative contents of different types of floral scent components.

4. Materials and Methods

4.1. Plant Materials and Growth Conditions

A. ‘Mystral’ (with strong fragrance) and A. ‘Alabama’ (with no fragrance), were obtained from Guangzhou Flower Research Center (Guangzhou, China) (Figure 4). Plant group 08-377 were the F1 generation of individual plants from the cross of A. ‘Mystral’ (♀) × A. ‘Alabama’ (♂), while 08-382 were the F1 generation of individual plants from the reciprocal cross of A. ‘Alabama’ (♀) × A. ‘Mystral’ (♂). All plants were grown in the greenhouse at 23–28 °C and 80% relative humidity, under a natural photoperiod. To investigate the formation and regulation of floral scent during different reproductive phases, the development of spadices were divided into three stages: S1, spathe folding stage; S2, pistillate emerge stage; and S3, spadix fully extended stage. The spadices at S2 stage, which were collected between 10:00 a.m. and 12:00 p.m., were used for the analysis of volatile compounds.

4.2. Extraction and Determination of Volatile Compounds

In Anthurium, floral scents are mainly emitted at stage S2. Thus, the spadices at stage S2 were harvested for volatile constituent analysis, according to Yue et al. [39]. The spadix was enclosed in a 500 milliliter (mL) glass bottle with the addition of 1.728 micrograms (µg) ethyl caprate, which served as an internal standard. After waiting 30 min to achieve equilibrium, a polydimethylsiloxane fiber (PDMS, with 50/30 micrometer (µm) divinylbenzene/Carboxen) fiber (Supelco) was used to collect volatiles. Then, the collected volatiles were detected by GC-MS using an Agilent 7890A GC and Agilent 5975C MSD. The instrument was equipped with an Agilent HP-5MS capillary column (30 m × 0.25 mm) and helium was used as a carrier gas at a constant flow of 1.0 mL/min. The oven temperature was initially maintained at 45 °C for 2 min, followed by an increase of 5 °C/min until it was finally maintained at 250 °C for 5 min. Identification of individual compounds was performed by the comparison of mass spectra and retention times with authentic standards, or with the NIST 08 mass spectra library. Quantification was based on peak areas and the quantity of internal standard using the Agilent ChemStation Data Analysis Application. The relative content of aroma components was calculated as follows: the mass of compound (µg·gFW⁻¹·h⁻¹) = mass of internal standard × area under the peak of compound/area under peak of internal standard/fresh weight of sample.

4.3. Analysis of Quantitative Real-Time PCR (qRT-PCR)

To evaluate the transcript profiles of floral scent-related genes, the spadices at stage S2 were harvested for RNA extraction. Total RNA was extracted from the above samples using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s protocol. First-strand cDNAs were synthesized from 1.0 µg total RNA using the HiScript II RT-PCR system (Vazyme, Nanjing, China), according to the manufacturer’s instructions. Q-PCR reactions (20 microliter (µL) volume containing 1.0 µL cDNA as the template) were performed using the CFX connect Real-Time PCR System (Bio-Rad, Hercules, CA, USA)
in standard mode with the KAPA SYBR FAST Universal qRT-PCR Kit (Kapa Biosystems, Wilmington, MA, USA). Anthurium Actin was used as the internal reference gene to quantify the relative expression level of target genes [40]. Primer sequences for qPCR were designed using NCBI Primer-BLAST, and are listed in Supplementary Table S1. The relative expression levels of target genes were calculated by the $2^{-\Delta\Delta CT}$ method. Three biological replicates were performed per experiment.

4.4. The Olfactory Tests of the Hybrid Progenies

The olfactory tests of hybrid progenies were carried out in the greenhouse of the Guangzhou Flower Research Center. Inflorescences with two or three mature pistils were selected as the test materials. The experiment was performed by treated individuals between 10:00 a.m. and 12:00 p.m. in the morning and was repeated six times. “Strong fragrant” indicated the experimenters could easily smell a strong fragrance, and the results of six repetitions were consistent; “weak fragrant” indicated the experimenters could smell a faint fragrance and the results of four repetitions were consistent at least; “no fragrant” indicated the experimenters could not smell any fragrance, and the results of six repetitions were consistent.

5. Conclusions

The floral scent profile of A. ‘Mystral’ was found to be dominated by terpenes (70%), mostly eucalyptol. The scent profile also contained smaller quantities of phenylpropanoid/benzenoids (28.5%), mostly phenylmethyl ester. The main components of the progenies with aroma, which were similar to those of A. ‘Mystral’, were eucalyptol and phenylmethyl ester. By integrating volatile profiles with gene expression analysis, we could infer that variations in the relative proportions of DXS, EPSPS, and PAL genes and volatile compounds may alter the floral scent profile in Anthurium. Further studies will aim to analyze the location, sequences, and upstream sequences of these VOC biosynthesis-related genes, which may provide better understanding of the genetic underpinnings of floral scent and inheritance in Anthurium.

Supplementary Materials: Table S1: Primer Sequences for qPCR.

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