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The Effect of Pre-Harvest Methyl Jasmonate Treatment on the Selected Volatile Compounds and Endogenous Hormones Contends in the Pulp of Grape Berries

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Abstract

Grapevine is one of the most valued and widely cultivated fruit crop worldwide, with their pleasant flavor and valuable health effects. During the consequent ripening at ambient temperature, the volatile compounds of table grape often decreased, to affect their sensory evaluation. The development of new and effective methods to increase the volatile compounds of berries is necessary. Present study was carried out to investigate the pre-harvest methyl jasmonate (MeJA) treatment on the selected volatile compounds and endogenous hormones content from 'Shine Muscat' berries pulp. The results indicated that, pre-harvest application of MeJA (0.1 mM or 0.01 mM) on grape berries generally enhanced the production of terpenes, like nerol, linalool, alpha-terpineol; While some C6 compounds were reduced, such as (E)-2-hexenol, hexanol, (Z)-3-hexenol, hexanal and (E)-2-hexenal. The endogenous hormones like IAA (indole acetic acid), ABA (abscisic acid) and JA (jasmonate acid) content were also changed after MeJA treatment. We also observed that MeJA palys a key role in fruit endogenous hormones level and volatile compounds by increasing the expression level of several related genes, such as aroma-related genes Vvter, Vv-syn and hormone-related genes VvOPR3, VvAuI, VvEth, VvNCED1. We hypothesize that, MeJA as an effective elicitor affects the volatile compounds by altering endogenous hormones level in berries pulp of 'Shine Muscat'.

Keywords: Methyl jasmonate (MeJA); Grape; Volatiles; Hormone; Gene expression

Introduction

Grape (Vitis Vinifera L) considered as an important fruit crop in the world and consumed as fresh as well in the form of several values added products. 'Shine Muscat', a Japanese table grape cultivar is currently popular among consumers, due to seedlessness, high brix, attractive bunches and large berry size especially the pleasant muscat flavor [1]. Recent studies have reported that in several grape cultivars, the high ambient temperature in summer and the moisture during rainy season, storage of grape berries at low temperature, currently being used on commercially always reduces flavor and generate an off-flavor. All these practices are considered to reduce the market quality of grapes [2-4]. Volatile compounds in grape berries have a considerable economic impact for human beings, as parameters of food quality and consumer preference, likely to determine the perception and acceptability of berries and its products by end users.

Grape produces a variety of significant volatile compounds which are important in determining the sensory quality and health promoting properties of horticultural food products including taste and aroma. The volatiles include large number of compounds, like moneterpenes, C6 alcohols, alcohols, esters, acids, aldehydes and terpenes [5]. Among them, terpenes are known to contribute in floral and fruiting characters [6]. Although an overwhelming number of chemical compounds have been detected as volatile compounds in fresh berries, but only a fraction of them were identified as important components of fruit flavor, based on their quantitative abundance and olfactory thresholds. Previous studies have found that terpenes like linalool, nerol, alpha-terpienol and C6 alcohols like (E)-2-Hexenol, Hexanol, (Z)-3-Hexenol, Hexanal, (E)-2-Hexenal were considered as major compounds that contribute to the aromatic composition of muscat grape cultivars [7,8]. In present study, we mainly measured the content of these eight aroma compounds, as well as analysed the expression pattern of Vvter and Vv-syn genes, which involved in the terpenes synthesis and metabolism of grape [9,10].

Methyl jasmonate (MeJA), which is derived from α-linolenic acid in the octadecanoid pathway [11], is already classified by the U.S. Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS) substances, involved in various functions from the morphological to the molecular level in fruits. The pre-harvest applications of MeJA in fruit cultivation have used extensively. Hyunjin et al. [11] reported a significant increase in the total phenolic content of sweet basil after 0.1 and 0.5 mM MeJA treatments compared with the control. Rudell et al. [12] also found that 0.5 mM MeJA application on apples enhanced β-carotene biosynthesis through adaptation to cold temperatures, which reduces orchard temperature fluctuations and confers photoprotection on the fruit. Recently, MeJA application at pre-harvest stage in raspberry plants resulted significant increase in relevant health promoting compounds such as ellagic acid, quercetin and myricetin [13]. The pleiotropic effect of MeJA improve a variety of plant processes including fruit ripening as well as response to abiotic or biotic stresses [14-16]. There are also some studies having shown that MeJA affect the volatile compounds of plants, and its application on tea leaves could increase its volatile compounds level, especially the geraniol, linalool and its oxids [17]. Reporters also evaluated the effect of (2)- and (+)-methyl jasmonate on the bioformation of aroma-active

esters in strawberry fruits, the application of methyl jasmonate enantiomers is proposed as a possible mean to minimize strawberry aroma alterations or losses during post-harvest and storage [18].

Apart from the volatile compounds, MeJA also reported to influence the endogenous hormones content or alter the hormone related genes expression level of plants. Maciejewska et al. [19] have reported that the effect of MeJA pre-harvest treatments on pharbitis nil is similar to abscisic acid applications, reduces the growth of leaves, roots, buds and shoots. Studies revealed that MeJA treatment on tomato influence the flavonoids content and which modulate the polar transport of IAA, ultimately accumulated the IAA content in tomato [20]. Rudell et al. [12] observed that pre-harvest MeJA treatments (10 mM) has shown a positive response in 'Fuji' apple, increased the fruit pigmentation and ripening by enhancing the biosynthetic enzymes 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and ACC synthase in fruits. Limited studies were conducted on MeJA application and its effect on volatile compounds during berry development.

In present study the volatile compounds like linalool, nerol, alpha-terpienol, (E)-2-Hexenol, Hexanol, (Z)-3-Hexenol, Hexanal, (E)-2-Hexenal of MeJA treated 'Shine Muscat' berries flesh was investigated by headspace automatic solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS). The objective of present study was to explore the effect of pre-harvest MeJA treatment on the endogenous hormones in the pulp of grape berries, qRT-PCR analysis of selected genes Vvter, Vv-syn and VvOPR3, VvAuI, VvEth, VvNCED1 was performed for subsequent annotation. This study will provide new insights that may helpful to highlight the harvest timing after exogenous application of MeJA to increase volatiles level in grape berries and ultimately improve fruit quality and uplifting of table grape industry.

Materials and Methods

Chemicals

Methanol (HPLC grade), phosphate-buffered saline (PBS), (Na_2HPO_4: 1.427% (w/v), KH_2PO_4: 0.907(w/v), ratio 8:2, pH: 7.2), and chloroform was purchased from Scigene Co (Nanjing, Jiangsu, China). NaCl (analytical grade) was collected from Sinopharm Chemical Reagent Co (Beijing, China); and pure water was obtained from the Milli-Q purification system (Millipore, Bedford, MA). MeJA, 3-octanol was supplied by Sigma–Aldrich (Steinheim, Germany). RNA extraction kits were bought from Foregene (Fujian, China). Enzyme-linked immunosorbent assay (ELISA) kits (Rapid test) were got from USA.

Plant materials and treatments

In the present study, five-year-old 'Shine Muscat' grape plants, spaced at 3 m apart within rows and 5 m apart between rows under a rain-shelter of polyvinyl film, were planted in a sandy soil and supplemented with drip irrigation. The vineyard was located in Tangshan Valley, Nanjing, Jiangsu province, China. Samples were treated on July 5, 2016 (60 days after full bloom). Clusters of fruit on three separate grape vines were selected at random and divided into three groups. First and second group of clusters were sprayed @ 0.1 and 0.01 mM MeJA respectively. The remaining group of clusters was sprayed with water as control. For gene expression analysis, the samples were collected at 0, 6, 12, 24, 48, 96 hours post first treatment (hpt), for endogenous hormones and volatile compounds determination, the fruits were sampled at 0, 2, 4, 6, 8 10 weeks post first treatment (wpt). 3 berries per bunch were picked from the top, the middle, and the tip of a bunch, mixed. Berries were peeled manually, and the seeds were separated from the flesh. The final sample collection date was recorded to optimize the balance between sweetness, acidity and flavor. Flesh of grape were immediately frozen in liquid nitrogen and stored at -80°C until use.

Determination of TSS and TA content in the pulp of berries

Fresh grape berries were used for the determination of TSS and TA. Berries pulp was homogenized with a laboratory blender (Grindomix GM 200; Retsch, Haan, Germany) at 8000 rpm for 30 s. Total soluble solids (TSS) were determined using an Abbé refractometer (type Rx-5000; Atago, Tokyo, Japan). Titratable acidity (TA) expressed as citric acid in grams per 100 g of fresh weight was determined by titration with 0.1 N NaOH to a final pH of 8.1 using an automatic titration system (Titrino 702 SM; Metrohm, Herisau, Switzerland). TSS and TA were analyzed in triplicate for each berry.

Determination of endogenous hormones content

Frozen pulp of grape samples was dissolved in 2.0 mL of phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween-20 and 0.1% (w/v) gelatin (pH 7.5) to quantify free abscisic acid (ABA), jasmonic acid (JA), indole acetic acid (IAA) by ELISA following the protocol described by Fernández et al. [21]. The mouse monoclonal antigen and antibodies against free ABA, JA and IAA were produced at phyto-hormones research institute, China Agricultural University, Beijing China. Calculations of the ELISA data were performed as described by Raghava et al. [22]. The recovery percentage obtained by using internal standards during extraction and analysis was all >90%.

Determination of volatile compounds by Headspace Automatic Solid-phase Microextraction (HS-SPME) combined with Gas Chromatography-Mass Spectrometry (GC-MS)

Each berry sample was finely cleaned with distilled water and the seeds were manually removed. Juice from the samples was extracted. After centrifugation at 4000 rpm for 5 minutes, 8 mL of supernatant was placed into a headspace bottle with NaCl (3.0 g) and 3-octanol (818 $mg.L^{-1}$, 5 µL) as an internal standard. Each container was placed in a 50°C water bath for 30 minutes. After adsorption on SPME fiber (Supelco, USA) on a magnetic stirrer (Corning, USA) at 50°C for 40 minutes, the extraction fiber was loaded into a GC set at 220°C and analyzed without splitting the stream for 2 minutes. A trace GC-MS instrument (Finnigam, USA) equipped with an Agilent 66890 N (Agilent, PaloAlto, CA, USA) was used with a 30 m × 0.25 mm × 0.25 µm column (J&W122-4732DB-17ms, USA). The temperature program was as follows: 40°C, held for 5 minutes, followed by 2°C/min up to 70°C, held for 2 min, 3°C /min up to 120°C and 5°C /min up to 150°C, and finally 10°C /min up to 220°C, held for 2 minutes. The temperature of the ion source was set at 220°C and the MS range was collected from 29 m/z to 540 m/z. The purge flow rate was 1.0 mL/min. Eight aroma volatiles (linalool, nerol, alpha-terpienol, (E)-2-hexenol, hexanol, (Z)-3-hexenol, hexanal, (E)-2-hexenal) were quantified by comparing their peak areas with those of authentic standards using an internal

standard (3-octanol) method. The NIST/WILEY spectrum library and related literature were used to identify the mass spectra. The corresponding peak areas were calculated using an extracted ion chromatogram. This method was partly reference to other's paper.

RNA extraction and quantitative real-time PCR analysis

Frozen flesh of berries of different ripening stages were ground in mortar, the resulting powder was used for RNA extraction. Contaminating DNA was eliminated using an RNase-free DNase I Kit (Takara, Japan) according to the manufacturer's instructions. Then the agarose gel electrophoresis was used to assess the quality of every RNA sample. The complementary DNA (cDNA) was synthesized from total RNA using the Takara Prime Script TMRT-PCR Kit (Takara, Japan). Primers in this experiment were designed using Primer 5 software (http: //www. premierbiosoft.com/), which were used for real-time PCR, are listed in Table S1. Gene expression analysis was determined by qRT-PCR (ABI7300, Italy), using SYBR green chemistry. Dissociation curve was performed to check the absence of primer dimers and other amplification by-products. amplification procedure was conducted using the following thermocycling program: 1 cycle of 95°C for 2 minutes, and 40 cycles of template denaturation at 94°C for 20 s, primer annealing at 56°C for 20 s, and primer extension at 72°C for 30 s, and 71 cycles increasing from 60 to 95°C at 0.5°C per cycle for 30 s. The gene expression level was presented as log fold changes of up-regulated or down-regulated to compare with untreated control. Relative transcription levels were calculated using the 2-ΔΔCt method [23].

Results and Discussion

Effect of MeJA treatment on TA and TSS of grape berries

The relative standard deviation (RSD) value obtained for the TA and TSS (Figure 1) from three replicates of the overall analytical step. The different row of the figure means the various harvest time after MeJA treatment. After analysing the TA, TSS contents, we observed that, 4 weeks post treatment (wpt), the TA and TSS content of MeJA treated berries were significantly changed. The decreasing trend of TA contents were observed in all three treatments. (Figure 1). TA contents in CK ranged from 1.86 g/100 g FW to 0.72 g/100 g FW, while decreasing trend of TA contents 0.51 g/100 g FW and 0.53 g/100 g were observed in 0.1 and 0.01 mM MeJA treated berries respectively. The TSS contents of berries recorded among the CK, 0.1 mM and 0.01 mM MeJA treatments in 4 wpt were 13.9, 16.1 and 15.8 g/100 g FW respectively. From 0 wpt to 8 wpt, the TSS contents of 0.1 and 0.01 mM MeJA treated berries were increased while the TA content of treated berries were decreased.

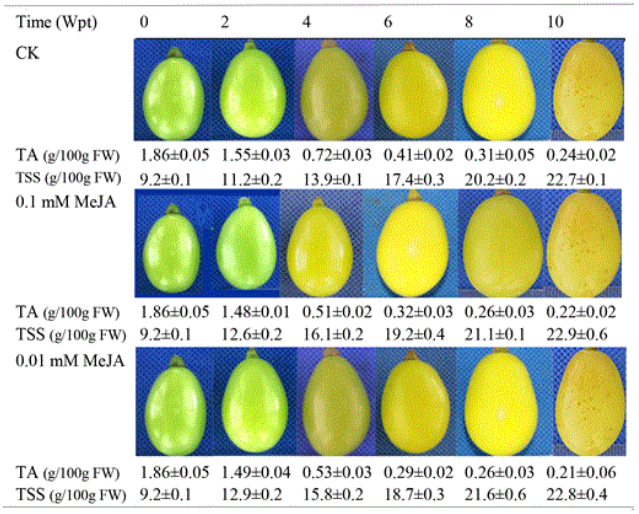


Figure 1: Total acidity (TA, g/100 g FW) and total soluble solids (TSS, g/100 g FW) of MeJA treated and untreated berries in different weeks post first treatment (wpt). Datas are shown as the means ± standard error (n=3).

Recent studies revealed that 10 and 100 μM MeJA both induced grape coloring, fruit softening also accelerated the grape-ripening progress and affect the TA or TSS content of berries. Our studies were in consensus with previous report by Jia et al. [14]. For some non-aromatic fruit like eggplant, the role of MeJA is reversed to compare with grape, which delays postharvest ripening and senescence in the eggplant by decreasing enzymes association with phenolics metabolism [24]. The effect of MeJA on fruit ripening, TA and TSS contents is depend on the fruit species and concentration of MeJA applied [24,25].

Effects of MeJA treatment on the endogenous IAA, ABA and JA content in the pulp of grape berries

The variation trends of IAA (A), ABA (B), and JA (C) contents in 'Shine Muscat' berries pulp among the three treatments during different sample collection time points were presented in Figure 2. For IAA contents, from 0 wpt to 8 wpt the control and MeJA treated samples did display the same decreasing trend. But MeJA treatment retarding decline of IAA in berries during 0 wpt to 4 wpt, the IAA contents in the control, MeJA0.1, and MeJA0.01 were decreased to 101 ng/g FW, 132 ng/g FW and 129 ng/g FW from 0 wpt (197 ng/g FW) respectively. In 6 wpt, IAA contents were recorded higher in CK than MeJA0.1 and MeJA0.01. No significant differences in IAA contents were observed in 8 wpt and 10 wpt among the three treatments. ABA contents was increased from 0 wpt to 4 wpt while from 4 wpt to 8 wpt was decreased. In 2 wpt the ABA contents of berries in control was higher than MeJA treated, but from 4 wpt the ABA content in control was lowest compared with treatments MeJA0.1 and MeJA0.01. In 8 wpt the ABA contents among the three treatments were approximately same, 36 ng/g FW, 32 ng/g FW and 36 ng/g FW respectively. Totally, MeJA induced a marked change in ABA content, after both 0.1 and 0.01 mM MeJA treated in 0 wpt. To compare with IAA and ABA, JA content in pulp of berries was lowest. The maximum value was 7.23 ng/g FW. There were two peaks of JA content, from 0 wpt to 2 wpt and

4 wpt to 6 wpt the JA content was increased, while from 2 wpt to 4 wpt and 6 wpt to 10 wpt the value was decreased. The JA content in MeJA treated berries was notably higher than control during all the samples collection time points. The results of individual hormone presented herein indicated that MeJA remarkably affect the endogenous hormones contents, we postulated that MeJA play an important role in affecting volatile compounds of grapes, to some extent, by modulating the endogenous hormones.

Grape ripening corresponds to a physiological period that begins at the onset of fruit coloring (veraison) and lasts until the fruit attained fully maturity [26]. Studies revealed that endogenous hormones content affected by external factors like temperature, hormone, and photoperiod [27]. Present study was conducted to check the effect of MeJA on the endogenous hormones content of IAA, ABA and JA, interestingly, these three categories of hormone contents in our study were all augment by MeJA treatment. Ziosi et al. [28] indicated that Abscisic acid (ABA) content was affected by exogenous MeJA treatments of 1 mM on Cucurbita pepo and 0.2 mM on peach. IAA and IAA response transcription factors have been shown to control the expression of the JAZ1 gene, enabling molecular interplay between IAA and JA signaling [29]. We did not find any study about the exogenous MeJA treatment that change or affect endogenous JA content. Present study was first conducted to observe the effect of exogenous MeJA on the endogenous JA content of grape berries.

Identification and characterization of the volatile compounds in 'Shine Muscat' grapes

A total of 40 volatile compounds were identified in 'Shine Muscat' berries pulp from first treatment (0 wpt) to over- maturity stage (10 wpt) (Figure 3). These compounds included 6 C6 alcohol and aldehydes, 4 alcohols, 6 esters, 3 acids, 7 aldehydes, 5 alkanes and alkenes and 9 terpenes respectively. In pulp of 'Shine Muscat' berries, C6 alcohol and aldehydes and terpenes were the predominant volatile compounds during samples collection period. In particular, the dominating terpenes from 'Shine Muscat' berries were nerol (peak2), linalool (peak5), alpha-terpineol (peak6). The concentration of C6 alcohols and aldehydes like (E)-2-hexenol (peak3), hexanol (peak4), (Z)-3-hexenol (peak7), hexanal (peak8), and (E)-2-hexenal (peak9) were highest among the volatiles (Figure 4).

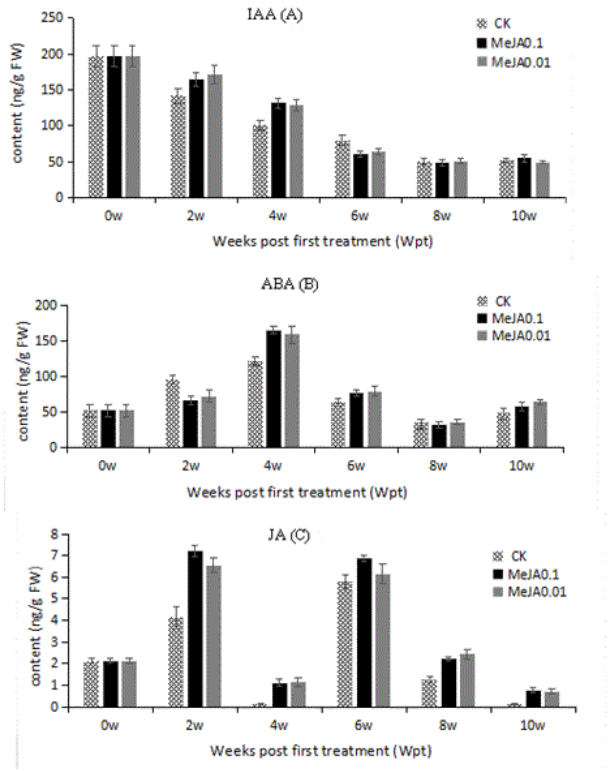
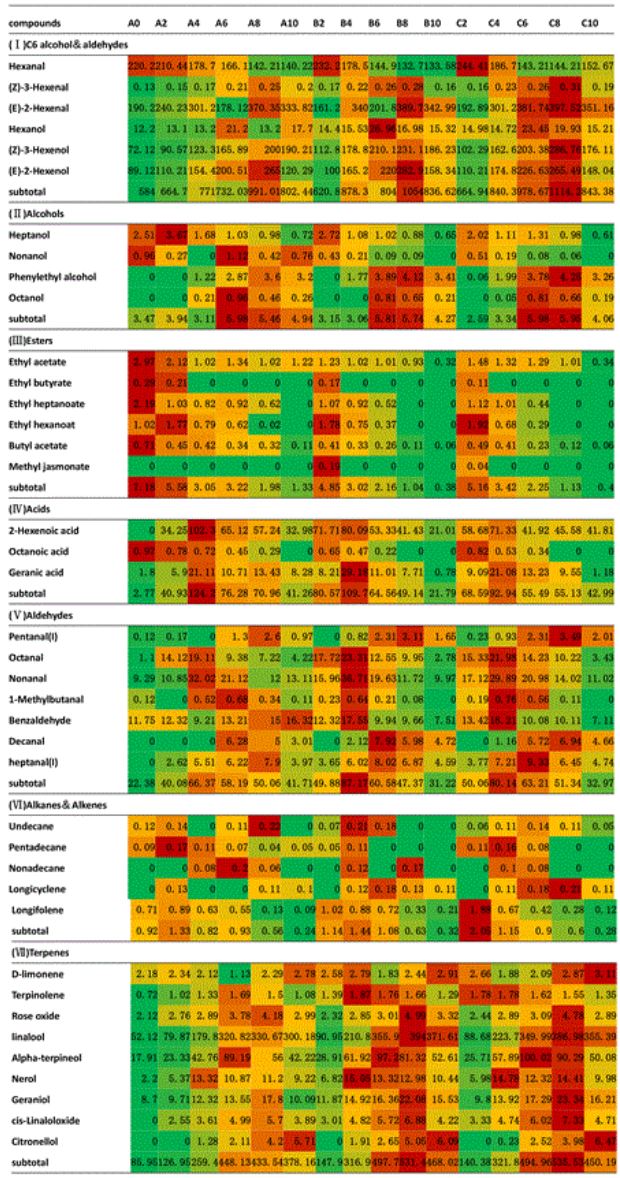


Figure 2: The variation trend of IAA (A), ABA (B), JA (C) content in MeJA treated and untreated berri es. Values are means+SD of three biological replicates.



compounds	A0	A2	A4	A6	A8	A10	B2	B4	B6	B8	B10	C2	C4	C6	C8	C10
(Ⅰ)C6 alcohol& aldehydes																
Hexanal	220.	2210.	44178.	7 166.	1142.	21140.	22232.	2178.	5144.	9132.	7133.	58244.	41186.	7143.	21144.	21152. 67
(Z)-3-Hexenal	0. 13	0. 15	0. 17	0. 21	0. 25	0. 2	0. 17	0. 22	0. 26	0. 29	0. 16	0. 16	0. 23	0. 26	0. 31	0. 19
(E)-2-Hexenal	190.	2240.	23301.	2178.	12376.	35333.	82161.	2	340	201.	6389.	7342.	99192.	8930	1. 2361.	74397. 52351. 16
Hexanol	12. 2	13. 1	13. 2	21. 2	13. 2	17. 7	14. 4	15. 5	326. 9	616. 98	15. 32	14. 98	14. 72	23. 48	19. 93	15. 21
(Z)-3-Hexenol	72. 12	90. 57	123. 3	165. 89	200	190. 21	112. 8	178. 8	210. 1	231. 1	186. 2	3102. 29	162. 6	203. 38	286. 76	176. 11
(E)-2-Hexenol	89. 12	110. 21	154. 4	200. 51	265	120. 29	100	165. 2	220	282. 9	158. 34	110. 21	174. 8	226. 63	265. 49	148. 04
subtotal	584	666. 7	771	732. 03	991. 01	802. 44	620. 8	878. 3	804	1054	836. 62	664. 94	840. 3	978. 67	1114. 2	843. 38
(Ⅱ)Alcohols																
Heptanol	2. 51	3. 67	1. 68	1. 03	0. 98	0. 72	2. 72	1. 08	1. 02	0. 88	0. 65	2. 02	1. 11	1. 31	0. 98	0. 61
Nonanol	0. 96	0. 27	0	1. 12	0. 42	0. 76	0. 43	0. 21	0. 09	0. 09	0	0. 51	0. 19	0. 08	0. 06	0
Phenylethyl alcohol	0	0	1. 22	2. 87	3. 6	3. 2	0	1. 77	3. 89	4. 12	3. 41	0. 66	1. 99	3. 78	4. 28	3. 26
Octanol	0	0	0. 21	0. 98	0. 46	0. 26	0	0	0. 81	0. 65	0. 21	0	0. 05	0. 81	0. 66	0. 19
subtotal	3. 47	3. 94	3. 11	5. 98	5. 46	4. 94	3. 15	3. 06	5. 81	5. 74	4. 27	2. 69	3. 34	5. 98	5. 96	4. 06
(Ⅲ)Esters																
Ethyl acetate	2. 97	2. 12	1. 02	1. 34	1. 02	1. 22	1. 23	1. 02	1. 01	0. 93	0. 32	1. 48	1. 32	1. 29	1. 01	0. 34
Ethyl butyrate	0. 29	0. 21	0	0	0	0	0. 17	0	0	0	0	0. 11	0	0	0	0
Ethyl heptanoate	2. 19	1. 03	0. 82	0. 92	0. 62	0	1. 07	0. 92	0. 52	0	0	1. 12	1. 01	0. 44	0	0
Ethyl hexanoat	1. 02	1. 77	0. 79	0. 62	0. 02	0	1. 78	0. 75	0. 37	0	0	1. 92	0. 68	0. 29	0	0
Butyl acetate	0. 71	0. 45	0. 42	0. 34	0. 32	0. 11	0. 41	0. 33	0. 26	0. 11	0. 06	0. 49	0. 41	0. 23	0. 12	0. 06
Methyl jasmonate	0	0	0	0	0	0	0. 19	0	0	0	0	0. 04	0	0	0	0
subtotal	7. 18	5. 58	3. 05	3. 22	1. 98	1. 33	4. 85	3. 02	2. 16	1. 04	0. 38	5. 16	3. 42	2. 25	1. 13	0. 4
(Ⅳ)Acids																
2-Hexenoic acid	0	34. 25	102. 3	65. 12	57. 24	32. 98	71. 7	180. 09	53. 33	41. 43	21. 01	58. 68	71. 33	41. 92	45. 58	41. 81
Octanoic acid	0. 97	0. 78	0. 72	0. 45	0. 29	0	0. 65	0. 47	0. 22	0	0	0. 82	0. 53	0. 34	0	0
Geranic acid	1. 8	5. 9	21. 11	10. 71	13. 43	8. 28	8. 21	29. 18	11. 01	7. 71	0. 78	9. 09	21. 08	13. 23	9. 55	1. 18
subtotal	2. 77	40. 93	124. 2	76. 28	70. 96	41. 26	80. 57	209. 76	54. 56	49. 14	21. 79	68. 59	92. 94	55. 49	55. 13	42. 99
(Ⅴ)Aldehydes																
Pentanal(I)	0. 12	0. 17	0	1. 3	2. 6	0. 97	0	0. 82	2. 31	3. 11	1. 65	0. 23	0. 93	2. 31	3. 49	2. 01
Octanal	1. 1	14. 12	19. 11	9. 38	7. 22	4. 22	17. 72	23. 31	12. 55	9. 96	2. 78	15. 33	21. 98	14. 23	10. 22	3. 43
Nonanal	9. 29	10. 85	32. 02	21. 12	12	13. 11	15. 96	36. 71	19. 63	11. 72	9. 97	17. 12	29. 89	20. 98	14. 02	11. 02
1-Methylbutanal	0. 12	0	0. 52	0. 68	0. 34	0. 11	0. 23	0. 64	0. 21	0. 08	0	0. 19	0. 76	0. 56	0. 11	0
Benzaldehyde	11. 75	12. 32	9. 21	13. 21	15	16. 32	12. 32	17. 55	9. 94	9. 66	7. 51	13. 42	18. 21	10. 08	10. 11	7. 11
Decanal	0	0	0	6. 28	5	3. 01	0	2. 12	7. 92	5. 98	4. 72	0	1. 16	5. 72	6. 94	4. 66
heptanal(I)	0	2. 62	5. 51	6. 22	7. 9	3. 97	3. 65	6. 02	8. 02	6. 87	4. 59	3. 77	7. 21	9. 33	6. 45	4. 74
subtotal	22. 38	40. 08	66. 37	58. 19	50. 06	41. 71	49. 88	87. 17	60. 58	47. 37	31. 22	50. 06	80. 14	63. 21	51. 34	32. 97
(Ⅵ)Alkanes& Alkenes																
Undecane	0. 12	0. 14	0	0. 11	0. 22	0	0. 07	0. 21	0. 18	0	0	0. 06	0. 11	0. 14	0. 11	0. 05
Pentadecane	0. 09	0. 17	0. 11	0. 07	0. 04	0. 05	0. 05	0. 11	0	0	0	0. 11	0. 16	0. 08	0	0
Nonadecane	0	0	0. 08	0. 2	0. 06	0	0	0. 12	0	0. 17	0	0	0. 1	0. 08	0	0
Longicyclene	0	0. 13	0	0	0. 11	0. 1	0	0. 12	0. 18	0. 13	0. 11	0	0. 11	0. 18	0. 21	0. 11
Longifolene	0. 71	0. 89	0. 63	0. 55	0. 13	0. 09	1. 02	0. 88	0. 72	0. 33	0. 21	1. 88	0. 67	0. 42	0. 28	0. 12
subtotal	0. 92	1. 33	0. 82	0. 93	0. 56	0. 24	1. 14	1. 44	1. 08	0. 63	0. 32	2. 05	1. 15	0. 9	0. 6	0. 28
(Ⅶ)Terpenes																
D-limonene	2. 18	2. 34	2. 12	1. 13	2. 29	2. 78	2. 58	2. 79	1. 83	2. 44	2. 91	2. 66	1. 88	2. 09	2. 87	3. 11
Terpinolene	0. 72	1. 02	1. 33	1. 69	1. 5	1. 08	1. 39	1. 87	1. 76	1. 66	1. 29	1. 78	1. 78	1. 62	1. 55	1. 35
Rose oxide	2. 12	2. 76	2. 89	3. 78	4. 18	2. 99	2. 32	2. 85	3. 01	4. 99	3. 32	2. 44	2. 89	3. 09	4. 78	2. 89
linalool	52. 12	79. 87	179. 8	320. 8	2330. 6	7300. 1	890. 9	5210. 8	355. 9	394	371. 61	88. 68	223. 7	349. 9	9286. 9	8355. 39
Alpha-terpineol	17. 91	23. 33	42. 76	89. 19	56	42. 22	28. 9	161. 92	97. 28	1. 32	52. 61	25. 71	67. 89	100. 02	90. 29	50. 08
Nerol	2. 2	5. 37	13. 32	10. 87	11. 2	9. 22	6. 82	15. 68	13. 32	12. 98	10. 44	5. 96	14. 78	12. 32	14. 41	9. 98
Geraniol	8. 7	9. 71	12. 32	13. 55	17. 8	10. 09	11. 87	14. 92	16. 36	22. 08	15. 53	9. 8	13. 92	17. 29	23. 3	16. 21
cis-Linaloloxide	0	2. 55	3. 61	4. 99	5. 7	3. 89	3. 01	4. 82	5. 72	6. 88	4. 22	3. 33	4. 74	6. 02	7. 33	4. 71
Citronellol	0	0	1. 28	2. 11	4. 2	5. 71	0	1. 91	2. 65	5. 05	6. 09	0	0. 23	2. 52	3. 98	6. 47
subtotal	85. 95	126. 9	269. 4	448. 1	3433. 5	4378. 1	947. 9	316. 9	497. 7	831. 4	468. 02	140. 38	321. 8	494. 9	6535. 5	3450. 19

Figure 3: Concentrations (ng/g) of volatile compounds determined in the pulp of CK (A), 0.1 mM MeJA (B), and 0.01 mM MeJA (C) treated grapes. Grape berries were collected fortnightly from the first treated time (A0) to over-maturity stage (A10, B10, and C10). Data are means (n=3). The capital letters refer to the sample collection time with different MeJA concentration treatment listed in Figure 1. The aroma compounds were listed on the left of the concentration arrays, color scale was shown at the bottom. The higher concentration for each compound was presented in red, the lower concentration for each compound was presented in green. While the yellow color means the concentration of the volatile compounds were in medium. 0 Indicated that the compound was not detected.

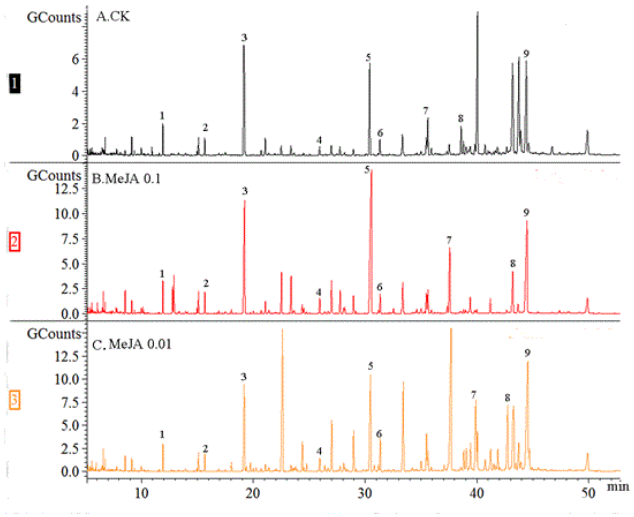


Figure 4: Chromatograms showing the differences in common and major volatile compounds in CK (A), 0.1 mM MeJA treated berries (B), 0.01 mM MeJA treated berries (C) during the fully maturity period (8 wpt). Peaks: (1) 3-Octanol; (2) Nerol; (3) (E)-2-Hexenol; (4) Hexanol; (5) Linalool; (6) Alpha-terpineol; (7) (Z)-3-Hexenol; (8) Hexanal; (9) (E)-2-Hexenal.

Impact of MeJA treatment on the selected volatile compounds

The absolute values of the studied volatile compounds in different weeks after treatment were presented in Figure 3. We need to bear in mind that the data obtained on week 0 correspond to the same sample for CK, 0.1 mM and 0.01 mM MeJA treated berries. Aromatic compounds of berries were influenced by MeJA and the compounds most affected by MeJA were hexanal, (E)-2-hexenal, hexanol, (Z)-3-hexenol, (E)-2-hexenol, linalool, alpha-terpineol and nerol (Figure 5). These compounds showed higher level in treated berries than in control samples. These compounds identified and described by another researchers Wu and Matsumoto. The compounds not mentioned in Figure 5 probably due to their scarce concentration or their occurrence below the detection limit in 'Shine Muscat' berries.

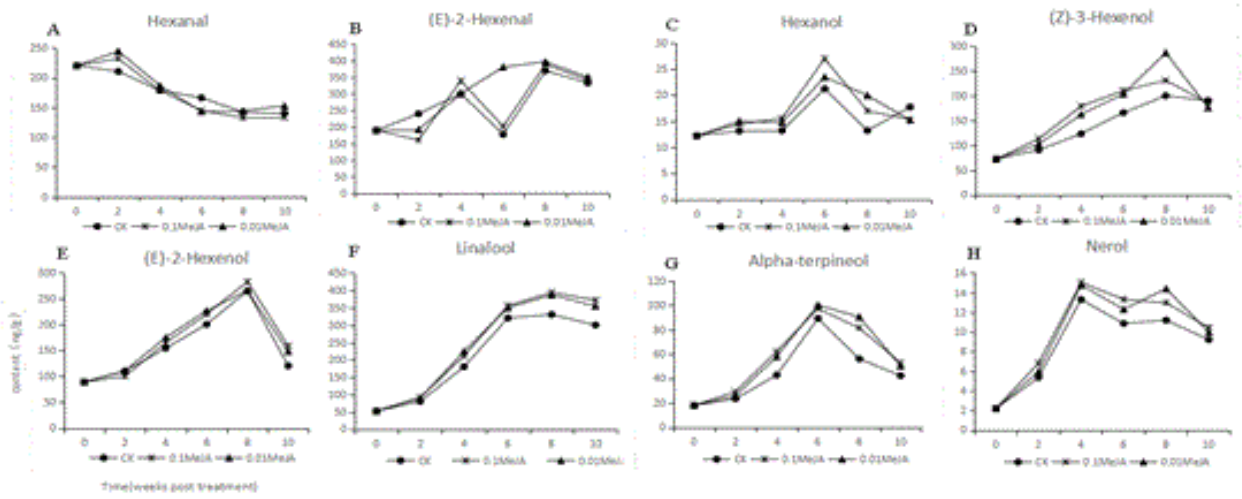


Figure 5: Concentrations (ng/g) of major volatile compounds determined in the pulp of CK, 0.1 mM MeJA, and 0.01 mM MeJA treated grapes. Grape berries were collected fortnightly from the first treated time (A0) to over maturity stage (A10, B10, and C10). These volatiles were (A) Hexanal; (B) (E)-2-Hexenal; (C) Hexanol; (D) (Z)-3-Hexenol; (E) (E)-2-Hexenol; (F) Linalool; (G) Alpha-terpineol; (H) Nerol.

In Figures 3 and 5, after the first treatment of MeJA, the major compounds in berries pulp were (E)-2-hexenal (161.21-397.52 ng.g^{-1}) and linalool (52-399.99 ng.g^{-1}). For (E)-2-hexenal, in 6 weeks after treatment, the contents were higher in 0.1 MeJA and 0.01 MeJA than control, especially in 0.01 mM MeJA treated berries, the value almost reach to the peak (381.74). For linalool, which is one of the contributory agents of muscat berries, an increase of linalool content was observed in 8 wpt, the time for berries attained the commercial maturity [30]. For nerol and alpha-terpineol, in 2 wpt, the concentration of these two compounds among all the treatment without a visible change, until 4 wpt the concentration of these two substances attained a rapid growth peak. In MeJA treated berries the alpha-terpineol and nerol contents remained higher compare with control. These two volatile compounds and linalool belonged to terpenes, which contribute in muscat flavor of muscat grape berries [7]. The level of several specific key aroma compounds in the C6 alcohol and aldehydes such as hexanal, hexanol, (Z)-3-hexenol and (E)-2-hexenol were significantly increased in treated berries with respect to untreated samples (Figure 5).

To best of our knowledge, fewer studies were carried out for evaluation of aromatic series and aroma profiles of table grapes. Studies have found that MeJA plays an important role in volatile compounds in strawberry and mango [31]. We observed that during grape ripening, in 2 wpt, the major content of volatiles in 'Shine Muscat' were C6 aldehydes and alcohols, from 2 wpt to 6 wpt, these compounds were increased. 6 wpt these compounds in berries began decreased because the fruit were close to full-maturity. When berries reached commercial maturity stage (8 wpt), the major compounds in berries were terpenes, like linalool, alpha-terpineol and nerol. At over-maturity stage (10 wpt), decreasing trend of volatile compounds in berries among all the treatments were observed. The changing of volatile compounds in berries during ripen stage were also reported in other studies [32].

Transcript levels of selected genes affected by MeJA

The expression patterns of 6 genes were observed in grape berries by qRT-PCR using an actin gene (LOC100246825) as internal standard (Figure 6). Genes involved in terpene biosynthesis were (A) terpene synthase(Vvter); (B) (3S,6E)-nerolidol synthase 1(Vv-syn); (C) 12-oxophytodienoate reductase 3 (VvOPR3); Genes related to hormones were (D) IAA-induced protein 22D (VvAuI); (E) ethylene-responsive transcription factor 2 (VvEth); (F) 9-cis-epoxycarotenoid dioxygenase 1 (VvNCED1). The genes Vvter and Vv-syn, which account for linalool and alpha-terpineol synthesis, the same expression trend of thses genes were observed, these two genes showed a strong (3-fold and 9-fold respectively) increment after 6 h treatment. For VvOPR3, which devoted to the jasmonate acid synthesis, a high-gene expression level of VvOPR3 in MeJA treated berries was recorded at the same time point (6 h), in 12 hpt, there was a slight augment in expression level of VvOPR3 gene than control. For VvAuI, an IAA response transcription factor, the most marked effect of gene expression level in 0.1 MeJA and 0.01 MeJA treated berries were recorded after 12 h with an 11- and 16-fold increase in transcript level. A 6-fold and 10-fold increase of VvEth (ethylene-responsive transcription factor) in 0.1 mM and 0.01 mM MeJA treated samples were observed after 24 hpt. The VvNCED1, a key gene for ABA biosynthesis, also up-regulated in 12 h and 24 h after 0.1 mM or 0.01 mM MeJA treatment. The Vvter and Vv-syn considered as key genes for terepenes synthesis, showed same expression pattern after MeJA treatment during the fruit ripening stage. Higher expression levels of these two genes were observed in MeJA treated berries compared with control, while the content of linalool and alpha-terpenol were more abundant in MeJA treated berries (Figure 3). The change in expression levels of hormone-related genes were observed and these represent that MeJA not act independently but function in complex networks with other phyto-hormonal signaling pathways, like IAA, ethylene, ABA, to control the reprogramming of plant volatile metabolism. A significant cross talk was found in previously reported studies about MeJA and other hormone signaling pathways, including MeJA-IAA, MeJA-ethylene, MeJA-ABA [33-35]. Furthermore, genes

related to hormones were previously reported to associate with volatile compounds in fruits [36-38]. Thus, MeJA is principally important for its prominent and universal role in the regulation of plant metabolism, which is typically manifested as the elicitation of volatile metabolite biosynthesis. In conclusion, MeJA could be an efficient tool to improve the volatile profile of grape berries.

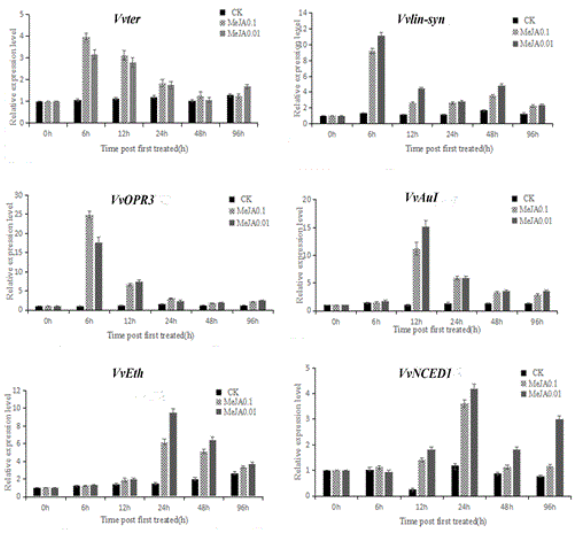


Figure 6: QRT-PCR analysis of transcript accumulation of aroma related genes and hormone related genes induced by MeJA. Gene name and descriptions are follows: (A) terpene synthase (Vvter); (B) (3S, 6E)-nerolidol synthase 1 (Vv-syn); (C) 12-oxophytodienoate reductase 3 (VvOPR3); (D) IAA-induced protein 22D (VvAuI); (E) ethylene-responsive transcription factor 2 (VvEth); (F) 9-cis-epoxycarotenoid dioxygenase 1 (VvNCED1).

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Conflict of Interest

The authors declare that they have no conflict of interest.

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