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Impact of CPPU and combined CPPU-thidiazuron treatments on fruit quality and volatile compounds in 'Jinling Xiangxin' grapes

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Abstract

(2-Chloro-4-pyridyl)-N′-phenylurea (CPPU) is a widely used plant growth regulator (PGR) in table grape production to promote seedlessness and increase berry size. However, the mechanisms by which PGRs influence aroma quality remain poorly understood. In this study, the effects of different CPPU and thidiazuron (TDZ) treatments on the exterior and nutritional quality of 'Jinling Xiangxin' grapes were evaluated, and a non-targeted volatile metabolomics analysis was conducted using gas chromatography-mass spectrometry (GC–MS). Results showed that the 5C–5C treatment (20 mg·L^{-1} GA_3 + 5 mg·L^{-1} CPPU at 3 d after bloom and 25 mg·L^{-1} GA_3 + 5 mg·L^{-1} CPPU at 15 d after bloom) significantly improved the fruit exterior and nutritional traits, whereas the 5C–0C treatment (20 mg·L^{-1} GA_3 + 5 mg·L^{-1} CPPU at 3 d after bloom and 25 mg·L^{-1} GA_3 at 15 d after bloom) enhanced aroma quality. A total of 113 volatile compounds were identified, with terpenoids (35 compounds) and esters (46 compounds) being predominant. Aldehydes and esters were confirmed as the primary contributors to grape aroma. Radar analysis revealed that grapes under the 5C–0C treatment exhibited stronger herbaceous, citrus, and caramelized flavors. K-means clustering further indicated that esters and terpenoids were mainly regulated by CPPU concentration, with β-terpinol and ethyl 3-oxohexanoate consistently affected. Moreover, 13 and eight differential compounds were identified as characteristic markers in CPPU-only and CPPU + TDZ treatments, respectively. Comprehensive evaluation of fruit exterior, nutritional, and aroma traits demonstrated that the 5C–0C treatment was optimal, enhancing marketability while minimizing negative impacts on aroma.

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Introduction

Aroma is an essential aspect of the fruit quality and serves as a key factor in consumer preference. 'Jinling Xiangxin' is a newly developed diploid grape variety with a pronounced rose-like aroma, selected from the seedlings progeny of 'Shine Muscat' by Nanjing Agricultural University. Recent studies have identified several volatile compounds that contribute to grape aroma, including esters, alcohols, aldehydes, terpenoids[1–3], C6 compounds, and C13-norisoprenoids[4,5]. Among these, esters and terpenoids are mainly responsible for fruity and floral notes, whereas C6 compounds impart green flavors[6,7]. Terpenoids, particularly monoterpenoids, represent the dominant contributors to the floral and fruity aromas of Muscat-type grapes[8–12].

Plant growth regulators (PGRs), such as thidiazuron (TDZ) and (2-chloro-4-pyridyl)-N′-phenylurea (CPPU), are widely applied in table grape cultivation to increase berry size and promote seedlessness. However, these compounds often exert adverse effects on aroma quality. TDZ has been reported to influence terpenoid biosynthesis by modulating the methylerythritol phosphate pathway. Application of TDZ increases the concentrations of hexanal and (E)-2-hexenal while reducing terpenoid levels, resulting in lighter grassy notes and stronger rose-like aromas[13]. CPPU markedly alters the volatile composition of 'Shine Muscat', Sable Seedless, and Sangiovese grapes[1,14,15]. Wang et al.[16] reported that high CPPU concentrations are unfavorable for aroma development, leading to reduced diversity of volatile compounds and decreased terpenoid content. Combined GA_3, TDZ, and CPPU treatment has been shown to reduce the activity values of characteristic aroma compounds[17]. In addition, CPPU interferes with amino acid metabolism and influences the formation of aromatic esters in melon fruit[18].

The development of new grape varieties and the study of PGR applications hold significant scientific importance for improving quality, yield, stress resistance, and sustainable development. Therefore, using the newly developed variety 'Jinling Xiangxin' as the material, the present study evaluated the effects of different PGR concentrations on fruit quality parameters, including color, total soluble solids (TSS), titratable acidity (TA), berry size and aroma, The findings aim to provide a foundation for improving fruit quality, advancing the cultivation and dissemination of new varieties, and expanding consumer choices.

Materials and methods

Plant material and field conditions

The present study was conducted in 2023 at the experimental vineyard of Nanjing Agricultural University, Nanjing, China. Three-year-old 'Jinling Xiangxin' grapevines were selected as experimental material. Trees were trained in a 'T' shape and grown under sheltered tunnels in sandy soil, with a planting distance of 3 m within rows and 6 m between rows.

Treatment with CPPU and TDZ

To synchronize flowering time, the shoulder and middle parts of the inflorescences were removed before bloom, leaving only 3 cm at the tip. All treatment details are provided in Table 1. Each treatment

group included 45 biological replicates (inflorescences). A randomized complete block design was applied.

Sampling and measurement of fruit exterior and nutritional quality

Berry ripening was completed on September 13, 2023, when harvest was performed. For quality assessment, 90 berries per plant were randomly collected to analyze polyphenol content, berry weight, diameter, seedlessness rate, and color, as well as total soluble solids (TSS), titratable acidity (TA), and the maturation index (TSS/TA). The remaining samples were stored at −80 °C for aroma analysis.

Titratable acidity was measured following the method described by Shahab et al.[19]. Berry color was determined with a CR-10 colorimeter (Minolta®, Tokyo, Japan), recording L* (lightness), a* (red/green), b* (yellow/blue), and C* (chroma) values as described by Koyama et al.[20]. The total polyphenol concentration in berry skins was quantified using the Folin–Ciocalteu method[21].

Extraction of aroma compounds

A total of 100 g of berries were deseeded, ground, and centrifuged at 4,000 rpm for 5 min at 4 °C. The supernatant was filtered and subjected to direct extraction of free aroma compounds using solid-phase microextraction (SPME). Extraction conditions followed the protocol described by Wang et al.[16]. Each sample container was equilibrated in a water bath at 50 °C for 30 min, and volatiles were adsorbed onto an SPME fiber (Supelco, USA) using a magnetic stirrer (Corning, USA) at 50 °C for 40 min. The extraction fiber was then inserted into the GC injector at 220 °C and desorbed in splitless mode for 2 min. Analyses were performed on a trace GC–MS instrument (Finnigam, USA) equipped with an Agilent 66890N (Agilent, Palo Alto, CA, USA) and a 30 m × 0.25 mm × 0.25 μm column (J&W122–4732DB–17 ms, USA). The oven temperature program was set as follows: initial temperature of 40 °C for 5 min, increased at 2 °C·min^{-1} up to 70 °C and held for 2 min, then 3 °C·min^{-1} to 120 °C, 5 °C·min^{-1} to 150 °C, and finally 10 °C·min^{-1} to 220 °C with a 2 min hold. The ion source temperature was maintained at 220 °C, and mass spectra were recorded over a range of m/z 29–540 with a purge flow rate of 1.0 ml·min^{-1}.

Aroma component metabolomics analysis

Volatile compounds were identified using authentic chemical standards, retention indices, or the NIST MS Search 2.0 library. Tentative identifications were assigned when standards were not available, based on comparison with the NIST MS Search 2.0, the NIST Chemistry WebBook, and retention indices reported in the literature. Quantification was performed using octan-3-ol as the internal standard.

The K-means clustering was conducted in R software (version 3.6.1) using the 'K-means' function in the 'stats' package, with the optimal cluster number determined by the 'fviz_nbclust' function in the 'factoextra' package (https://CRAN.R-project.org/package=factoextra).

Principal component analysis (PCA) and fuzzy mathematics were performed using SPSS 19.0 to calculate the comprehensive score[22]. Differences among treatments under different dehydration stages and temperatures were evaluated using one-way ANOVA followed by Duncan's multiple comparison test at $p < 0.05$. Differential metabolites (DMs) were screened primarily based on variable importance in projection (VIP) and fold change (FC). VIP values were calculated from the first principal component of the OPLS-DA model. The thresholds were set at VIP > 1.0 and FC > 1.5 or FC < 0.667[23]. Radar maps and aroma wheels were generated in Origin 9.1. All measurements were performed in triplicate.

Table 1. Treatment of 'Jinling Xiangxin' clusters with CPPU and TDZ.

Treatments	3 d post-bloom (DPB)	15 d post-bloom (DPB)
5C–0C	20 mg·L^{-1} GA_3 + 5 mg·L^{-1} CPPU	25 mg·L^{-1} GA_3
5C–2.5C	20 mg·L^{-1} GA_3 + 5 mg·L^{-1} CPPU	25 mg·L^{-1} GA_3 + 2.5 mg·L^{-1} CPPU
5C–5C	20 mg·L^{-1} GA_3 + 5 mg·L^{-1} CPPU	25 mg·L^{-1} GA_3 + 5 mg·L^{-1} CPPU
3C+2T–0C	20 mg·L^{-1} GA_3 + 3 mg·L^{-1} CPPU + 2 mg·L^{-1} TDZ	25 mg·L^{-1} GA_3
3C+2T–2.5C	20 mg·L^{-1} GA_3 + 3 mg·L^{-1} CPPU + 2 mg·L^{-1} TDZ	25 mg·L^{-1} GA_3 + 2.5 mg·L^{-1} CPPU
3C+2T–5C	20 mg·L^{-1} GA_3 + 3 mg·L^{-1} CPPU + 2 mg·L^{-1} TDZ	25 mg·L^{-1} GA_3 + 5 mg·L^{-1} CPPU

Calculating relative odor activity value (ROAV)

The key flavor substances in 'Jinling Xiangxin' were determined by calculating the relative odor activity value (rOAV) according to the method described by Xie et al.[8].

Results

Impact of different treatments on phenotypic characteristics, exterior, and fruit nutrition quality

All grape berries were harvested on the same day to ensure comparability. The longitudinal diameter of the berries was greatest under the 5C–5C treatment, while the transverse diameter reached its maximum under the 3C+2T–2.5C treatment. Due to a higher berry shape index, berries from the 5C–2.5C and 5C–5C treatments exhibited a long elliptical shape. The lowest berry weight was observed in the 5C–0C treatment, whereas the 3C+2T–5C treatment produced the heaviest berries. In addition, the 5C–5C treatment yielded the highest a* value and the lowest L* value, indicating a deeper green skin color (Fig. 1, Table 2).

The seedless ratio, TSS, TSS/TA, soluble protein, and soluble sugar content were significantly higher in the 5C–5C treatment compared to other treatments. In contrast, the phenolic content was higher in CPPU + TDZ-treated grapes than in grapes treated with CPPU alone (Table 3).

PCA was performed on 13 quality indices. Three primary components were extracted, explaining 44.16%, 25.14%, and 12.18% of the total variance, respectively. The cumulative variance contribution of the first three principal components reached 81.97% (Table 4), indicating that these components could serve as primary indicators for evaluating the effects of different treatments on fruit exterior and nutritional quality. Based on the comprehensive index, the ranking of treatments was as follows: 5C–5C > 5C–0C > 3C+2T–5C > 3C+2T–2.5C > 3C+2T–0C > 5C–2.5C (Table 5).

Effect of different treatments on the aroma composition of grapes

Qualitative and quantitative analyses of volatile compounds in grape berries were performed using gas chromatography–mass spectrometry (GC–MS). As shown in Supplementary Table S1, a total of 113 volatile compounds were identified, including 12 alcohols, 15

Fig. 1 The grape clusters of 'Jinling Xiangxin' during the ripening period under CPPU and TDZ treatments. Letters indicate different plant growth regulator treatments; specific concentrations are listed in Table 1.

Table 2. Phenotypic characteristics of grape berries in CPPU and TDZ treatments.

Phenotypic characteristics	5C–0C	5C–2.5C	5C–5C	3C+2T–0C	3C+2T–2.5C	3C+2T–5C
Longitudinal diameter (mm)	35.58 ± 2.55 bc	37.17 ± 1.57 a	37.55 ± 1.65 a	35.01 ± 1.27 c	35.77 ± 1.24 bc	36.15 ± 1.29 b
Transverse diameter (mm)	24.81 ± 1.36 c	24.94 ± 0.91 bc	25.37 ± 1.06 bc	25.53 ± 1.28 b	27.26 ± 1.31 a	26.73 ± 1.02 a
Berry shape index	1.44 ± 0.11 b	1.49 ± 0.08 a	1.48 ± 0.05 a	1.37 ± 0.07 c	1.31 ± 0.06 d	1.35 ± 0.05 c
Single Berry weight (g)	11.23 ± 1.40 e	12.80 ± 1.10 cd	13.75 ± 1.21 b	12.48 ± 1.22 d	13.28 ± 1.12 bc	14.78 ± 0.99 a
L	50.39 ± 0.42 a	49.60 ± 0.20 b	44.30 ± 0.30 f	47.88 ± 0.42 c	46.76 ± 0.54 d	45.47 ± 0.36 e
a	−6.80 ± 0.69 ab	−7.28 ± 0.41 bc	−6.50 ± 0.49 a	−7.84 ± 0.83 c	−7.46 ± 0.18 c	−7.68 ± 0.26 c
b	15.89 ± 0.44 a	14.47 ± 0.21 b	12.62 ± 0.52 c	15.31 ± 0.98 a	13.78 ± 0.66 b	13.89 ± 0.21 b
C	25.57 ± 1.55 a	23.52 ± 0.50 b	21.31 ± 1.13 c	25.77 ± 2.11 a	23.18 ± 1.05 b	23.85 ± 0.92 b

Note: Different letters within the same row mean significant differences according to a Duncan test ($p < 0.05$). The same below.

Table 3. The fruit quality of 'Jinling Xiangxin' grape in CPPU and CPPU + TDZ treatments.

Fruit quality	5C–0C	5C–2.5C	5C-5C	3C+2T–0C	3C+2T–2.5C	3C+2T–5C
Seedless ratio (%)	37.01	57.50	60.00	26.67	33.33	46.77
TSS (°Brix)	17.48 ± 0.15 b	16.95 ± 0.23 c	18.25 ± 0.18 a	16.35 ± 0.21 d	16.88 ± 0.19 c	17.68 ± 0.19 b
TA	0.18 ± 0.01 d	0.27 ± 0.01 a	0.16 ± 0.01 e	0.21 ± 0.02 c	0.24 ± 0.01 b	0.19 ± 0.01 d
TSS/TA	97.46 ± 6.53 b	63.70 ± 1.65 d	114.87 ± 6.88 a	77.04 ± 7.99 c	69.40 ± 3.57 cd	91.61 ± 3.39 b
Soluble sugar ($mg \cdot g^{-1}$)	140.18 ± 2.38 bc	134.7 ± 3.20 c	154.09 ± 3.11 a	128.60 ± 2.93 d	136.04 ± 2.44 c	142.50 ± 4.07 b
Soluble protein ($mg \cdot g^{-1}$)	0.25 ± 0.02 e	0.40 ± 0.04 bc	0.52 ± 0.05 a	0.31 ± 0.01 d	0.36 ± 0.02 c	0.44 ± 0.01 b
Phenol ($mg \cdot g^{-1}$)	11.81 ± 0.33 c	11.16 ± 0.19 d	10.43 ± 0.31 e	13.14 ± 0.19 a	12.30 ± 0.28 b	11.71 ± 0.15 c

Table 4. Score matrix, Eigenvalue and contribution ratio of principal components.

Index	Principal component		
	PC1	PC2	PC3
Longitudinal diameter	0.37	0.11	−0.78
Transverse diameter	0.08	−0.71	0.51
Single berry quality	0.52	−0.69	0.17
L	−0.39	0.86	0.01
a	0.65	0.17	−0.20
b	−0.82	0.51	0.01
C	−0.79	0.41	0.19
TSS	0.77	0.50	0.12
TA	−0.55	−0.55	−0.54
TSS/TA	0.70	0.55	0.39
Soluble protein content	0.84	0.37	0.11
Soluble sugar content	0.88	−0.30	−0.00
Total phenol content	−0.77	−0.30	0.38
Variance contribution rate (%)	44.16	25.64	12.18
Cumulative variance contribution rate (%)	44.16	69.79	81.97

Table 5. Comprehensive score and ranking of grape berry quality under different treatments.

Treatments	F1	F2	F3	Comprehensive score	Rank
5C–0C	−0.80	2.17	0.25	0.54	2
5C–2.5C	−0.76	−0.16	−1.10	0.24	6
5C–5C	4.14	−0.11	−0.01	0.68	1
3C+2T–0C	−2.73	0.17	0.26	0.24	5
3C+2T–2.5C	−0.82	−1.37	0.17	0.25	4
3C+2T–5C	0.96	−0.71	0.43	0.45	3

aldehydes, 35 terpenoids, five ketones, and 46 esters. In alcohols, the concentration of (E)-2-decenol was significantly higher in the 5C–5C treatment compared with other groups. Among aldehydes, (E)-2-decenal and tridecanal were significantly enriched in the 5C–5C treatment, whereas (E)-2-heptenal was markedly reduced in the 5C–2.5C treatment. For esters, cis-3-hexenyl butyrate and 3-mercaptohexyl acetate were significantly elevated in the 5C–5C treatment. Regarding terpenoids, the 3C+2T–0C and 3C+2T–2.5C treatments significantly decreased the levels of β-bisabolene and α-pinene oxide, respectively (Table 6).

As summarized in Table 7, terpenoids represented the most abundant class of volatiles across all treatments, followed by esters. The 5C–2.5C treatment reduced the relative proportions of alcohols and aldehydes, while increasing the proportions of esters and terpenoids.

ROAV analysis of aroma compounds in 'Jinling Xiangxin' grapes with different treatments

Fruit aroma results from the combined influence of compound concentration and odor threshold. Generally, volatile compounds with a relative odor activity value (rOAV) ≥ 1 are considered to contribute perceptibly to the overall aroma of grapes, and higher rOAV values indicate stronger contributions.

Compounds with rOAV ≥ 1 across all treatments were selected for analysis. The main aroma-active compounds are summarized in Table 8. In total, 40 aroma compounds were quantified in this study, among which 37, 36, 37, 38, 38, and 36 aroma-active compounds were detected in the 5C–0C, 5C–2.5C, 5C–5C, 3C+2T–0C, 3C+2T–2.5C, and 3C+2T–5C treatments, respectively.

Aldehydes and esters emerged as the predominant contributors to grape aroma (Table 9). Key aldehydes included (Z)-6-nonenal, (Z, Z)-3, 6-nonadienal, and 2-nonenal, while major esters comprised phenylethyl isovalerate, methyl benzoate, 3-mercaptohexyl acetate, and γ-decalactone (Table 8). Among the aldehydes, the rOAVs of 1-heptanol and 2-hexenal were significantly lower in the 3C+2T–5C treatment, whereas the rOAV of (E)-2-decenal was significantly higher in the 3C+2T–2.5C treatment (Table 8).

Aroma radar analysis in different treatments

Based on the ROAVs of volatile compounds, aroma radar charts were constructed to visualize the overall flavor profiles of 'Jinling Xiangxin' grapes subjected to different treatments (Fig. 2). The 5C–0C treatment exhibited the largest radar area, characterized by stronger herbaceous, citrus, and caramelized notes. In contrast, the 5C–2.5C and 5C–5C treatments enhanced tropical flavors while reducing herbaceous and tree-fruit notes compared with 5C–0C.

Table 6. Concentrations (μg/kg) of volatile compounds determined in 'Jinling Xiangxin' grapes.

Compounds (μg/kg)		5C–0C	5C–2.5C	5C–5C	3C+2T–0C	3C+2T–2.5C	3C+2T–5C
Alcohol	1-Dodecanol	26.09 ± 3.37ab	21.52 ± 0.79b	29.79 ± 2.77a	25.99 ± 3.53ab	29.94 ± 3.51a	25.02 ± 1.38ab
	1-Heptanol	5.87 ± 0.60a	4.96 ± 0.60ab	5.33 ± 0.59ab	6.31 ± 1.37a	6.06 ± 1.14a	3.83 ± 0.42b
	1-Octanol	0.98 ± 0.17ab	0.72 ± 0.09b	1.01 ± 0.11ab	0.98 ± 0.19ab	1.17 ± 0.19a	0.76 ± 0.10b
	(E)-2-Decenol	11.42 ± 0.90c	12.74 ± 0.19bc	15.84 ± 3.05a	14.43 ± 1.79ab	15.27 ± 0.78ab	13.37 ± 0.95abc
Aldehyde	(E)-2-Decenal	1.06 ± 0.10c	1.2 ± 0.20bc	2.02 ± 0.45a	1.13 ± 0.21bc	1.58 ± 0.32ab	0.98 ± 0.09c
	(E)-2-Heptenal	0.8 ± 0.24b	0.31 ± 0.02c	1.1 ± 0.18ab	0.94 ± 0.32ab	1.39 ± 0.13a	0.63 ± 0.45bc
	2-Hexenal	31.12 ± 0.74a	34.12 ± 6.16a	33.08 ± 2.63a	29.63 ± 2.96a	32.71 ± 2.30a	22.45 ± 1.51b
	Veratraldehyde	9.82 ± 0.87a	7.46 ± 1.30b	12.02 ± 1.42a	9.97 ± 1.37a	12.02 ± 1.53a	10.06 ± 0.52a
	Tridecanal	4.25 ± 0.29bc	2.93 ± 0.78c	7.14 ± 2.97a	4.15 ± 1.00bc	6.56 ± 1.41ab	4.12 ± 0.24bc
Ester	Ethyl sorbate	2.91 ± 0.11bc	3.22 ± 0.06b	3.29 ± 0.5ab	2.7 ± 0.24c	3.74 ± 0.31a	2.45 ± 0.18c
	cis-3-Hexenyl butyrate	1.59 ± 0.34bc	1.43 ± 0.07c	2.13 ± 0.17a	1.52 ± 0.22c	1.98 ± 0.05ab	1.54 ± 0.33c
	Heptyl acetate	1.52 ± 0.42b	1.1 ± 0.16b	3.59 ± 1.12a	2.01 ± 0.73b	3.83 ± 1.38a	1.75 ± 0.47b
	3-Mercaptohexyl acetate	1.21 ± 0.25bc	0.5 ± 0.08d	1.89 ± 0.53a	1.06 ± 0.42cd	1.74 ± 0.39ab	0.89 ± 0.09cd
Ketone	Apocynin	1.38 ± 0.12b	0.96 ± 0.08c	2.04 ± 0.37a	1.51 ± 0.18b	2 ± 0.14a	1.65 ± 0.09b
Terpenoid	Dihydro-β-ionol	0.52 ± 0.06ab	0.46 ± 0.09b	0.5 ± 0.09b	0.55 ± 0.03ab	0.46 ± 0.03b	0.63 ± 0.05a
	β-Bisabolene	1.11 ± 0.19bc	0.88 ± 0.07bc	1.53 ± 0.31a	0.8 ± 0.18c	1.23 ± 0.12ab	0.92 ± 0.17bc
	α-Pinene oxide	0.46 ± 0.17bc	0.88 ± 0.09a	0.41 ± 0.13bc	0.48 ± 0.09bc	0.31 ± 0.02c	0.58 ± 0.05b

Note: Data are means (n = 3). The aroma compounds were listed on the left of the concentration arrays. Different superscript letters within a row indicate significant differences ($p < 0.05$).

Table 7. Concentrations (%) of volatile compounds determined in 'Jinling Xiangxin' grapes.

Compounds (%)	5C–0C	5C–2.5C	5C–5C	3C+2T–0C	3C+2T–2.5C	3C+2T–5C
Alcohol	10.33%	9.45%	11.72%	9.95%	10.98%	10.39%
Aldehyde	21.31%	18.47%	20.24%	18.49%	21.66%	19.07%
Ester	23.60%	26.35%	23.42%	25.73%	22.72%	23.00%
Ketone	4.10%	3.70%	3.67%	3.96%	3.78%	5.01%
Terpenoids	40.67%	42.04%	40.95%	41.87%	40.86%	42.53%

The 3C+2T–5C treatment showed a general reduction in overall flavor intensity. The 3C+2T–0C and 3C+2T–2.5C treatments displayed similar flavor patterns, with notable contributions from caramelized, herbaceous, tree-fruit, and tropical notes. However, the 3C+2T–2.5C treatment lacked citrus flavor (Fig. 2).

Effect of CPPU concentration on aroma composition

To identify volatile compounds influenced by CPPU concentration, all volatiles from CPPU and CPPU + TDZ treatments were subjected to K-means cluster analysis based on their expression patterns, which yielded seven distinct clusters (Fig. 3). Clusters one and seven displayed similar trends between CPPU and CPPU + TDZ treatments, with most compounds in these clusters identified as esters and terpenoids (Supplementary Table S2). These results suggest that esters and terpenoids were the main groups affected by changes in CPPU concentration.

A Venn diagram of DMs identified by VIP and FC analysis is shown in Fig. 4. Six overlapping metabolites (tridecanal, (Z)-3-hexenyl crotonate, ethyl 3-oxohexanoate, apocynin, citronellal, β-terpinol) were shared between the comparisons of 5C–2.5C vs 5C–0C and 5C–5C vs 5C–0C. Seven overlapping metabolites (2-nonanone, ethyl 3-oxohexanoate, 3-mercaptohexyl acetate, α-ionol, β-terpinol, (E)-2-heptenal, (E)-benzyl 2-methylbut-2-enoate) were detected between 3C+2T–2.5C vs 3C+2T–0C and 3C+2T–5C vs 3C+2T–0C (Figs 4, 5 and 6). Two compounds, β-terpinol (woody aroma) and ethyl 3-oxohexanoate (herbaceous aroma), were common across all treatments, indicating that CPPU concentration exerted a stronger influence on these metabolites than TDZ (Figs 4 and 6).

The discriminatory biomarkers identified in different treatments are presented in Fig. 5. In CPPU-only treatments, apocynin contributed to caramelized notes in the 5C–0C group. In the 5C–5C group, (E)-2-heptenal, 3-mercaptohexyl acetate, and 3-oxo-α-ionol were associated with herbaceous and caramelized notes. In the 5C–2.5C group, 3,5,5-trimethyl-1-hexanol, (E)-2-decenal, tridecanal, (Z)-3-hexenyl crotonate, and citronellal were linked to herbaceous, fruity, and floral aromas. In CPPU + TDZ treatments, 2-hexenal and (E)-benzyl 2-methylbut-2-enoate contributed to herbaceous and floral notes in the 3C+2T–0C group. α-Ionol contributed to caramelized notes in the 3C+2T–5C group. In the 3C+2T–2.5C group, (E)-2-heptenal, ethyl 3-oxohexanoate, 2-nonanone, and β-terpinol contributed to herbaceous, fruity, and woody aromas.

The aroma wheel provided a visualization of the flavor distribution of differential metabolites (Fig. 6). CPPU treatments were characterized by fruity, herbaceous, woody, and caramelized flavors, whereas CPPU + TDZ treatments lacked caramelized notes but exhibited additional nutty and floral notes. In the comparison between 5C–0C and 5C–2.5C treatments, fruity flavors dominated the wheel, accounting for more than half of the area, suggesting that CPPU application 15 d after bloom substantially altered the composition of aroma compounds.

Calculation of the comprehensive membership function value

In fuzzy mathematics, the membership function method integrates the membership values of each evaluated index and calculates the mean value to provide a comprehensive assessment. In this study, only compounds with rOAV > 1 across all treatments were included in the calculation. As shown in Table 10, larger mean values of the membership function indicate a stronger overall flavor contribution under the corresponding treatment. The ranking of treatments based on membership function values was as follows: 5C–0C > 3C+2T–2.5C > 5C–2.5C > 5C–5C > 3C+2T–0C > 3C+2T–5C.

Table 8. Relative odor activity values (rOAVs) of active volatile compounds.

Compounds	5C–0C	5C–2.5C	5C–5C	3C+2T–0C	3C+2T–2.5C	3C+2T–5C
Alcohol						
1-Decanol	2.93 ± 0.23a	2.63 ± 0.20a	3.01 ± 0.59a	2.65 ± 0.11a	2.58 ± 0.34a	2.42 ± 0.22a
1-Heptanol*	19.58 ± 2.00a	21.05 ± 4.57a	16.52 ± 2.00ab	20.19 ± 3.78a	17.76 ± 1.97ab	12.75 ± 1.38b
1-Nonanol	107.85 ± 13.42a	104.69 ± 21.62a	94.55 ± 9.44a	111.29 ± 14.00a	126.24 ± 29.61a	95.34 ± 11.84a
cis-3-Hexenol	6.8 ± 0.32a	7.36 ± 0.98a	7.45 ± 0.26a	7.38 ± 0.15a	8.35 ± 1.00a	7.3 ± 0.10a
Aldehyde						
(E)-2-Decenal*	2.12 ± 0.21c	2.27 ± 0.42bc	2.41 ± 0.39bc	3.17 ± 0.63ab	4.03 ± 0.90a	1.96 ± 0.17c
(E)-2-Heptenal	< 1	< 1	< 1	1.07 ± 0.10	< 1	< 1
(Z,Z)-3,6-Nonadienal	6,958.62 ± 1,584.57a	5,172.79 ± 1,046.83a	5,731.27 ± 1,779.45a	5,586.22 ± 1,550.26a	5,522.15 ± 466.04a	4,696.66 ± 78a
(Z)-2-Decenal	1.94 ± 0.19a	1.93 ± 0.39a	1.77 ± 0.05a	2.19 ± 0.22a	2.02 ± 0.14a	1.74 ± 0.10a
(E)-2-Dodecenal	1.2 ± 0.33a	1.21 ± 0.17a	1.56 ± 0.51a	< 1	1.15 ± 0.73a	1.33 ± 0.62a
2-Hexenal*	18.3 ± 0.44a	17.43 ± 1.74a	20.07 ± 3.62a	19.24 ± 1.35a	19.46 ± 1.55a	13.21 ± 0.89b
2-Nonenal	3,318.74 ± 415.65a	3,364.87 ± 579.68a	2,994.82 ± 229.00a	3,072.69 ± 269.00a	3,871.55 ± 1,195.27a	3,093.93 ± 305.02a
(E)-4-Nonenal	34.43 ± 7.94a	25.3 ± 6.69a	26.76 ± 7.17a	28.31 ± 9.52a	26.27 ± 4.27a	25.34 ± 5.47a
(Z)-6-Nonenal	9,059.03 ± 1,363.81a	7,871.97 ± 1,895.74a	7,654.85 ± 1,617.99a	8,510.44 ± 2,850.93a	7,726.05 ± 472.40a	6,940.68 ± 1183.23a
Decanal	163.13 ± 12.82a	167.42 ± 13.94a	170.55 ± 27.07a	173.72 ± 5.50a	172.02 ± 16.18a	158.27 ± 13.89a
Hexanal	52.06 ± 9.69a	46.86 ± 1.88a	51.42 ± 17.45a	52.64 ± 4.71a	43.98 ± 4.84a	35.59 ± 4.18a
Nonanal	388.65 ± 26.36a	396.84 ± 46.30a	369.43 ± 33.52a	437.3 ± 67.22a	464.32 ± 82.78a	360.56 ± 14.38a
Tridecanal	< 1	< 1	< 1	< 1	1.02 ± 0.42	< 1
Ester						
Ethyl (2E,4Z)-decadienoate	5.03 ± 0.80a	4.55 ± 0.64a	4.35 ± 0.14a	5.4 ± 0.72a	5.41 ± 0.62a	4.56 ± 0.26a
γ-Octalactone	9.61 ± 0.71a	9.53 ± 1.47a	9.63 ± 1.84a	8.85 ± 0.47a	9.27 ± 1.98a	8.96 ± 2.01a
γ-Decalactone	132.2 ± 12.20a	134.83 ± 12.53a	136.24 ± 37.37a	141.39 ± 14.55a	133.82 ± 32.17a	137.19 ± 18.15a
Methyl benzoate	189.24 ± 87.67a	102.67 ± 55.96a	147.76 ± 90.77a	123.05 ± 75.08a	103.97 ± 20.04a	90.75 ± 41.03a
Hexyl propionate	11.1 ± 0.77a	11.76 ± 1.18a	10.55 ± 0.12a	10.18 ± 1.07a	13.52 ± 2.33a	12.55 ± 2.66a
2-Methylbutyl isobutyrate	4.96 ± 0.10a	6.49 ± 0.73a	6.73 ± 0.37a	6.41 ± 0.27a	6.34 ± 0.27a	5.39 ± 1.65a
2-Methylbutyl hexanoate	1.75 ± 0.20a	1.57 ± 0.23a	1.57 ± 0.05a	1.71 ± 0.18a	2.31 ± 0.63a	1.69 ± 0.36a
Phenylethyl isovalerate	4,939.68 ± 751.48a	4,475.38 ± 645.28a	4,334.68 ± 114.62	5,175.7 ± 630.70a	5,205.61 ± 575.74a	4,447.44 ± 262.49a
Methyl caprate	10.11 ± 2.51a	10.18 ± 4.25a	11.15 ± 2.30a	18.48 ± 16.32a	10.1 ± 3.05a	10.89 ± 3.25a
Butyl butyrate	1.04 ± 0.04ca	< 1	1.15 ± 0.02ba	1.34 ± 0.11aa	1.17 ± 0.18aba	< 1
Ethyl heptanoate	4.08 ± 0.3a	3.85 ± 0.51a	4.15 ± 0.34a	4.31 ± 0.51a	4.62 ± 0.78a	4.24 ± 0.75a
3-Mercaptohexyl acetate	173.3 ± 21.39a	168.53 ± 24.47a	177.21 ± 31.24a	401.06 ± 315.41a	188.57 ± 18.67a	163.65 ± 20.67a
Ketone						
(E,E)-3,5-Octadien-2-one	1,217.65 ± 231.00a	948.28 ± 121.36a	1,068.41 ± 188.01a	1,055.86 ± 270.81a	933.75 ± 45.11a	979.68 ± 138.92a
4-Undecanone	2.68 ± 1.47a	2.15 ± 0.25a	1.85 ± 0.28a	1.59 ± 0.07a	1.62 ± 0.20a	1.99 ± 0.16a
Terpenoids						
Safranal	257.18 ± 30.49a	238.55 ± 35.69a	216.35 ± 7.70a	268.58 ± 25.92a	273.98 ± 26.46a	225.19 ± 22.38a
β-Ionone epoxide	1.16 ± 0.19a	0.94 ± 0.13a	1.14 ± 0.08a	1.06 ± 0.12a	1.11 ± 0.11a	< 1
L(−)-Borneol	12.98 ± 4.48a	19.38 ± 3.62a	16.88 ± 2.76a	17.59 ± 1.00a	19.06 ± 3.81a	17.16 ± 1.45a
Carvone	2.02 ± 0.20a	1.91 ± 0.28a	1.98 ± 0.04a	2.01 ± 0.18a	2.18 ± 0.29a	1.83 ± 0.05a
Isoterpinene	6.83 ± 3.23a	3.57 ± 2.08a	5.07 ± 3.43a	4.34 ± 2.98a	3.6 ± 0.73a	3.19 ± 1.47a
Linalool	92.65 ± 40.99a	54.6 ± 20.82a	66.22 ± 45.06a	62.79 ± 40.79a	53.37 ± 6.86a	45.25 ± 17.49a
δ-Cadinene	3.76 ± 1.60a	4.03 ± 4.40a	6.3 ± 3.52a	5.4 ± 3.08a	4.79 ± 3.36a	3.33 ± 2.22a
Endo-Borneol	3.46 ± 1.19a	5.17 ± 0.96a	4.5 ± 0.74a	4.69 ± 0.27a	5.08 ± 1.02a	4.58 ± 0.39a

Note: compounds with rOAV values less than 1 are not shown in the table. * Represents compounds with significant differences ($p < 0.05$).

Discussion

Effect of PGRs on phenotypic characteristics and fruit nutrition quality

The present study examined the influence of PGRs on the phenotypic characteristics and nutritional quality of 'Jinling Xiangxin' grapes. Compared with the control group, PGR-treated groups showed significant increases in fruit size (longitudinal and transverse diameters) and berry weight (Table 2), consistent with previous studies[24–26]. Both CPPU and TDZ are known to alter fruit morphology in crops such as grapes, apples, and kiwifruit[27–29]. In this study, CPPU treatments increased longitudinal diameter, whereas CPPU + TDZ treatments increased transverse diameter. Grapes from CPPU treatments exhibited a higher berry shape index and a long elliptical form, while CPPU + TDZ treatments produced grapes with a lower a* value and deeper green skin, in contrast to the yellowish skin observed in CPPU treatments (Table 2).

Table 9. rOAVs (%) of volatile compounds determined in 'Jinling Xiangxin' grapes.

Compounds (%)	5C–0C	5C–2.5C	5C–5C	3C+2T–0C	5C–5C	3C+2T–5C
Alcohol	0.50%	0.58%	0.52%	0.56%	0.62%	0.54%
Aldehyde	73.47%	72.90%	72.81%	70.55%	71.43%	70.90%
Ester	20.14%	21.05%	20.72%	23.26%	22.75%	22.61%
Ketone	4.48%	4.06%	4.58%	4.17%	3.74%	4.54%
Terpenoids	1.40%	1.41%	1.37%	1.46%	1.46%	1.41%

In terms of nutritional quality, CPPU + TDZ treatments decreased TSS (Table 3), consistent with Patil et al.[30]. However, in contrast to

previous findings that reported decreasing TSS with increasing CPPU concentration[31–33], the high CPPU concentration (5C–5C treatment) in the study increased TSS. Moreover, increasing CPPU concentration enhanced soluble protein content while reducing phenol levels (Table 3), consistent with previous findings[34,35]. PCA indicated that the 5C–5C treatment had the highest comprehensive index for fruit exterior and nutritional quality, suggesting that this treatment was most favorable (Table 5).

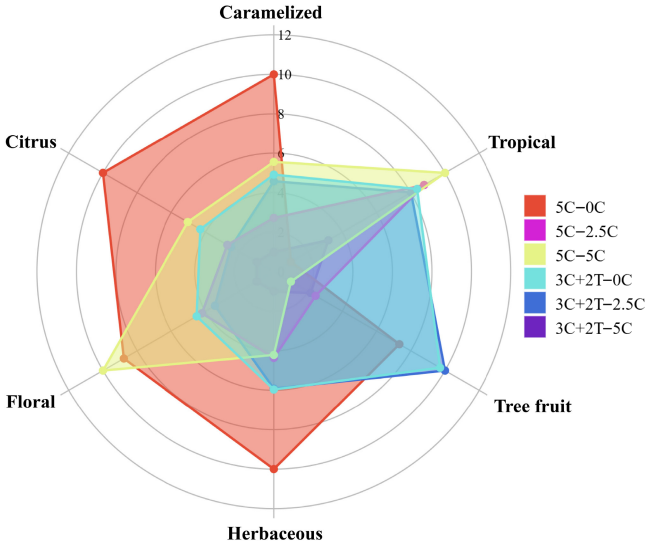

Fig. 2 Aroma radar map of 'Jinling Xiangxin' grapes with different treatments.

Effect of exogenous PGRs treatments on aroma quality

The present study demonstrated that terpenoids and esters were the dominant volatile compounds in 'Jinling Xiangxin' grapes under different treatments (Table 7). Terpenoids, synthesized from glucose via the isoprenoid pathway, are abundant in both Muscat and non-Muscat aromatic cultivars and play a central role in floral and fruity aromas[36]. Based on free monoterpenoid content, grape cultivars are classified as Muscat/floral cultivars (≥ 6 mg·L^{-1}), non-Muscat aromatic cultivars (1–4 mg·L^{-1}), and neutral cultivars (< 1 mg·L^{-1})[37]. As a seedling progeny of 'Shine Muscat,' 'Jinling Xiangxin' belongs to the Muscat/floral category. Previous studies reported that monoterpenes decreased following TDZ application[17]. In this study, only α-pinene oxide content was significantly reduced in the 3C+2T–2.5C treatment compared with the 5C–2.5C treatment (Table 6). Aldehydes and esters were also identified as major contributors to aroma (Table 9). Terpenoids and aldehydes have been reported as characteristic volatiles in 'Shine Muscat'[38], while esters are particularly important for fruity and floral attributes in grapes[11], especially in fox grapes (*V. labrusca*)[39] and strawberry-scented grapes[40]. In this

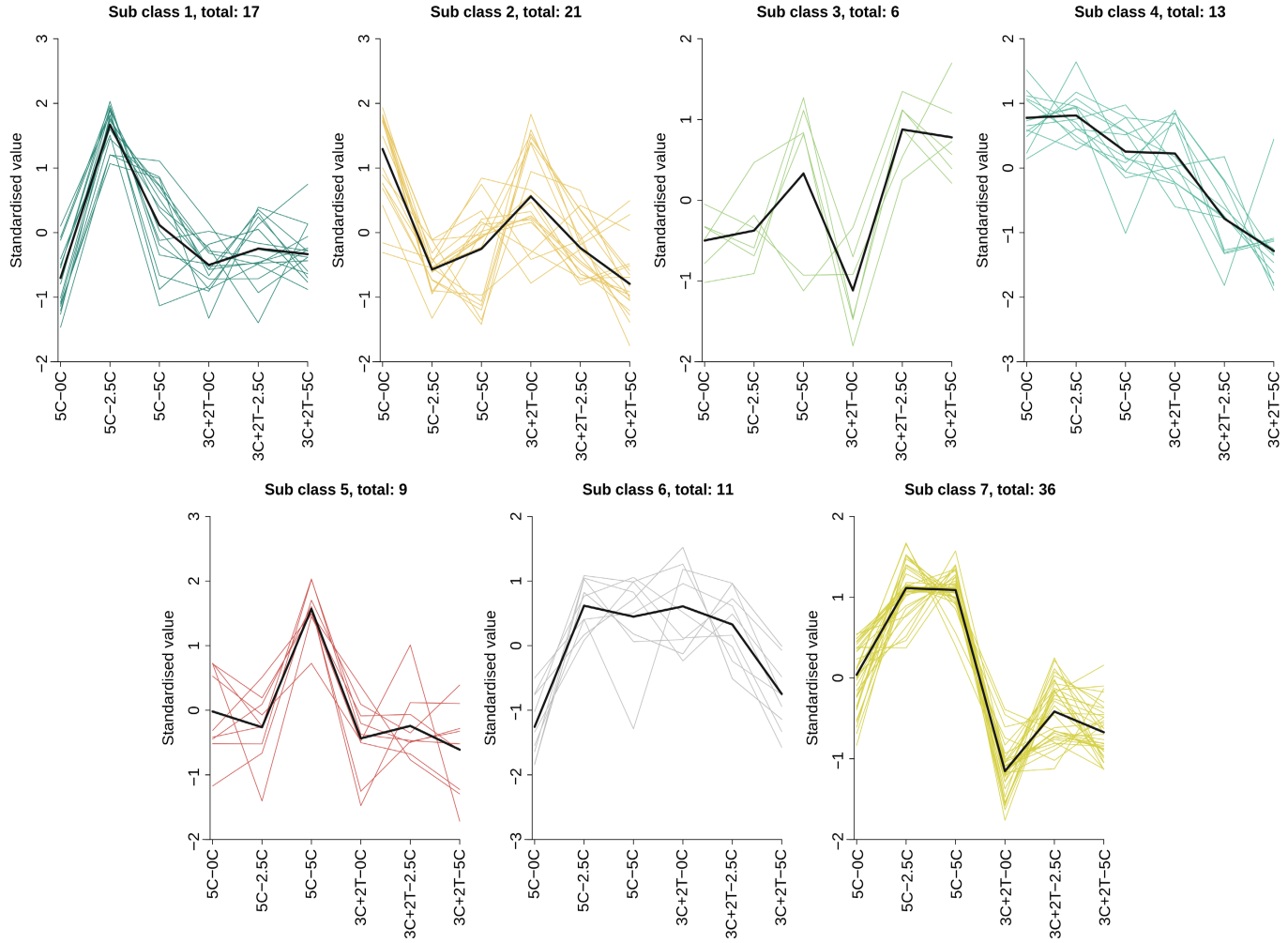

Fig. 3 Expression trend analysis of 113 metabolites from CPPU and CPPU + TDZ treatments data sets.

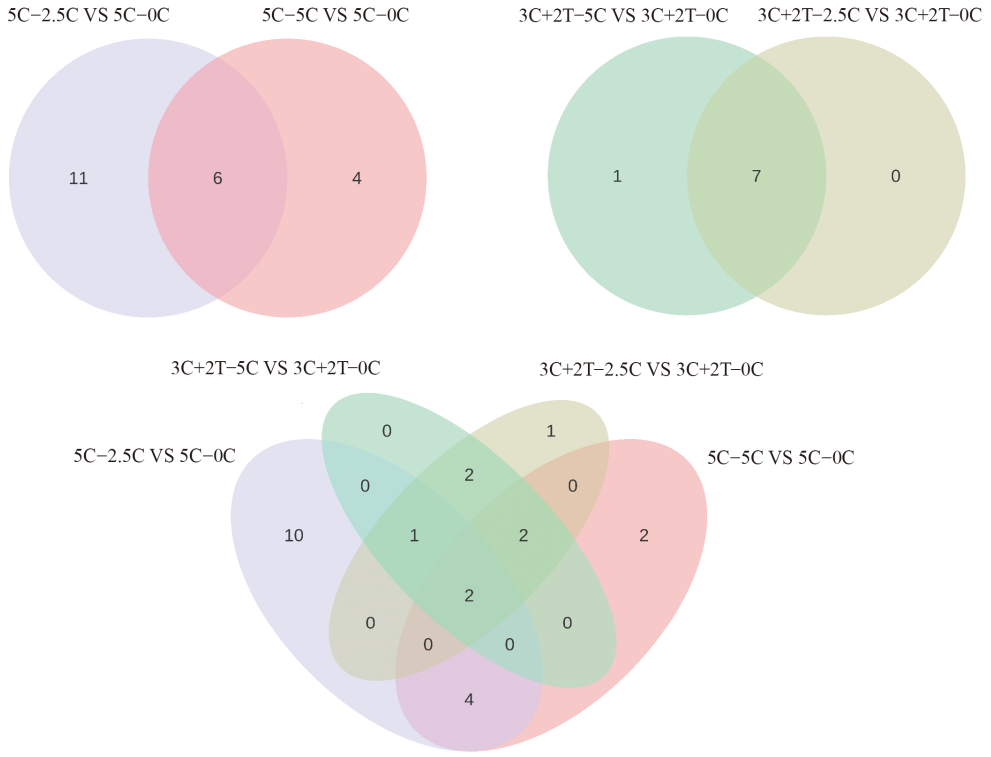

Fig. 4 Venn diagram depicting the shared and unique differentially expressed metabolites (DMs) identified by VIP and FC values across treatments.

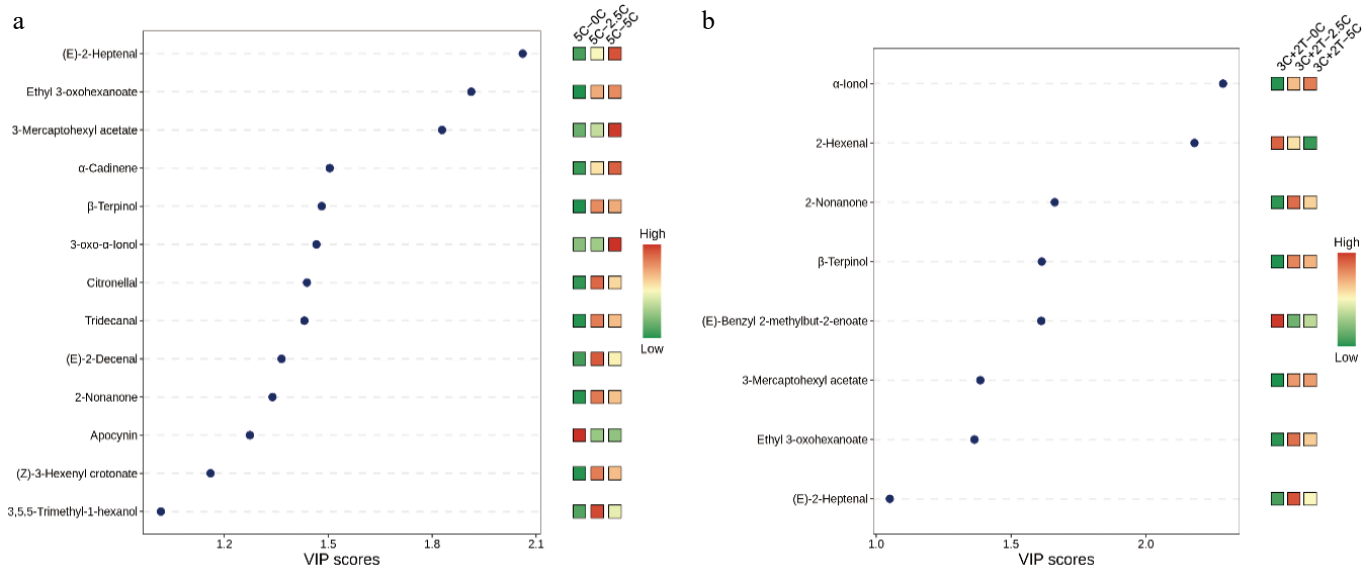

Fig. 5 Differential metabolites identified by VIP and FC values. (a) FC values for 5C–0C vs 5C–2.5C, 5C–0C vs 5C–5C. (b) FC values for 3C+2T–0C vs 3C+2T–2.5C, 3C+2T vs 5C–5C.

study, the rOAVs of 1-heptanol, (E)-2-decenal, and 2-hexenal were significantly lower in the 3C+2T–5C treatment, and the rOAV of 2-hexenal was also significantly decreased compared with the 5C–5C treatment (Table 8).

Aroma radar charts further revealed treatment-specific differences (Fig. 2). Grapes in the 5C–0C treatment exhibited the largest radar area, characterized by stronger herbaceous, citrus, and caramelized notes. In contrast, 5C–5C treatment enhanced tropical flavors but reduced herbaceous and tree fruit notes relative 5C–0C. The 3C+2T–0C and 3C+2T–2.5C treatments produced similar profiles with contributions from caramelized, herbaceous, tree fruit, and tropical notes. However, the 3C+2T–2.5C treatment lacked citrus flavor.

Comprehensive evaluation of aroma quality based on the membership function method indicated that the 5C–0C treatment achieved the highest overall score (Table 10). Overall, 5C–0C treatment enhanced herbaceous, citrus, and caramelized aromas and simultaneously increased total soluble solids while reducing titratable acidity, thereby improving both aroma quality and taste (Tables 5 and 10).

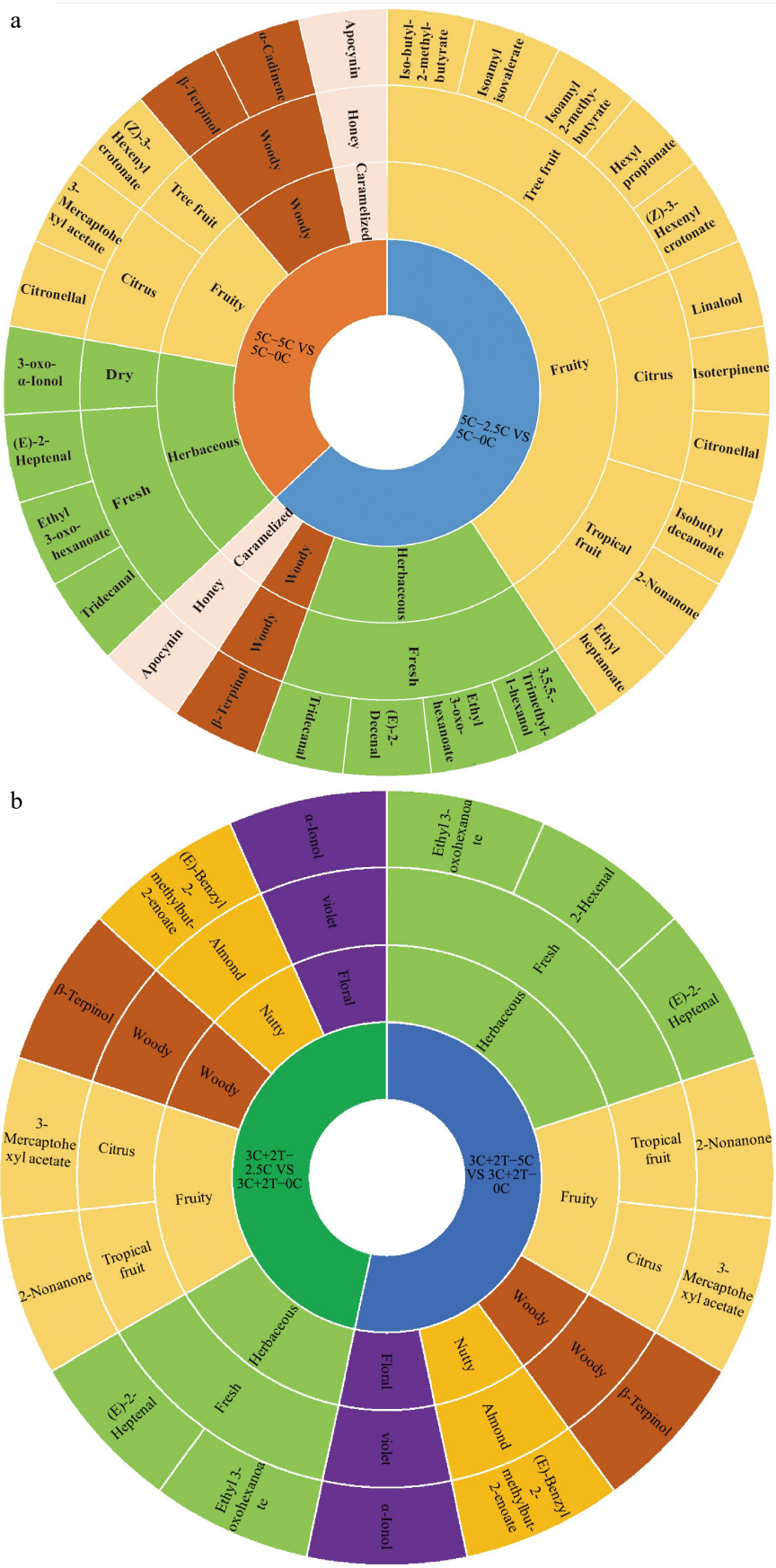

Fig. 6 Aroma wheel of DMs in 5C–2.5C vs 5C–0C, 5C–5C vs 5C–0C, 3C+2T–2.5C vs 3C+2T–0C, and 3C+2T–5C vs 3C+2T–0C. Different colors represent treatment groups and correspond to the flavor characteristics and specific metabolites.

Table 10. Membership function values of flavor in grapes with different treatments.

Treatment	5C–0C	5C–2.5C	5C–5C	3C+2T–0C	3C+2T–2.5C	3C+2T–5C
Comprehensive score	0.63	0.55	0.53	0.36	0.58	0.10
Order	1	3	4	5	2	6

Effect of different CPPU concentrations on aroma quality

CPPU, a synthetic cytokinin, is a strong promoter of cell division and sink activity. At high concentration (5C–5C), CPPU stimulated excessive berry enlargement, primarily through enhanced cell division and water uptake. This enlargement diluted the fixed pools of primary precursors (e.g., fatty acids and amino acids) and secondary metabolites (e.g., aroma volatiles) within a larger fruit volume, leading to lower concentrations of key compounds despite a potential increase in their absolute content per berry[41]. Conversely, the modest fruit expansion observed in the low-concentration treatment (5C–0C) avoided such dilution, thereby concentrating synthesized volatiles and resulting in the most intense aroma profile. Beyond dilution effects, cytokinins such as CPPU also participate in hormonal crosstalk. Evidence suggests that the high exogenous cytokinin levels may interfere with the jasmonic acid (JA) signaling pathway, a key regulator of defense responses and secondary metabolite biosynthesis. Suppression of JA signaling by high-dose CPPU provides a plausible mechanism for the observed reduction of volatile esters and terpenes in the 5C–5C group[16].

K-means cluster analysis further clarified the influence of CPPU concentration on volatile compounds (Fig. 3). Previous studies reported that post-bloom applications of GA_3 and CPPU enhanced terpene biosynthesis[15], whereas high CPPU concentrations reduced the abundance of ethyl esters and acetate esters[1,42]. In the present study, esters and terpenoids emerged as the compound classes most affected by CPPU concentration. Notably, two metabolites (β-terpinol, ethyl 3-oxohexanoate) were consistently identified across all treatments, indicating that they may serve as markers predominantly influenced by CPPU dosage (Figs 3, 4, and 6).

Using VIP > 1 and FC > 1.5 or < 0.667 as screening criteria, 13 differential compounds were identified in CPPU-only treatments and eight in CPPU + TDZ treatments (Fig. 5). In CPPU-only groups (5C–0C, 5C–2.5C, 5C–5C), apocynin, (E)-2-heptenal, 3-mercaptohexyl acetate, 3-oxo-α-ionol, 3,5,5-trimethyl-1-hexanol, (E)-2-decenal, tridecanal, (Z)-3-hexenyl crotonate, and citronellal served as discriminatory biomarkers. In CPPU + TDZ groups (3C+2T–0C, 3C+2T–2.5C, 3C+2T–5C), 2-hexenal, (E)-benzyl 2-methylbut-2-enoate, α-ionol, (E)-2-heptenal, ethyl 3-oxohexanoate, 2-nonanone and β-terpinol distinguished among treatments. These metabolites effectively differentiated treatment groups and highlighted the compound classes most responsive to CPPU concentration and its combination with TDZ.

Conclusions

In this study, the 5C–0C treatment (20 mg·L^{-1} GA_3 + 5 mg·L^{-1} CPPU applied 3 d after bloom and 25 mg·L^{-1} GA_3 applied 15 d after bloom) was found to be the most effective strategy for enhancing the marketability of 'Jinling Xiangxin' grapes while minimizing adverse effects on aroma. Aroma radar analysis revealed that grapes under the 5C–0C treatment exhibited a larger overall area characterized by stronger herbaceous, citrus, and caramelized notes. Multivariate analysis further identified 13 and eight volatile compounds as characteristic markers distinguishing CPPU-only and CPPU + TDZ treatments, respectively. Among these, β-terpinol and ethyl 3-oxohexanoate were consistently influenced by CPPU concentration, indicating their potential role as key biomarkers for aroma modulation.

Author contributions

The authors confirm contributions to the paper as follows: study conception and design: Zheng H, Tao J; data collection: Wang Y, Ren M, Chen J; analysis and interpretation of results: Huang L, Li H, and Du K; draft manuscript preparation: Wang Y, Zheng H; supervision: Wen Z and Tao J. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article.

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Conflict of interest

The authors declare that they have no conflict of interest.

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