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The Enhancing Effect of Jasmonic Acid on Fragrance of Kam Sweet Rice

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Abstract This study aimed to characterize the effect of jasmonic acid (JA) on volatile compounds in grains of Kam sweet rice. Using GC-O and SPME-GC-MS, nonanal displayed the highest odor activity value (OAV) in the filling grains and increased after applicaton of jasmonic acid in the rice seedlings. The relative expression of rice *OsLOX3* (rice lipoxygenase 3) and *OsHPL1* (rice hydroperoxide lyase 1) was assessed by RT-PCR in Kam sweet rice Gou Cengao and the non-aromatic Kam rice Lailong rice after rice pollination. Our data showed that *OsLOX3* was elevated in Gou Cengao compared with the non-aromatic rice. In agreement, lipoxygenase (*OsLOX3* gene product) levels and activity were elevated in aromatic rice samples. The positive Pearson correlation (0.715) was found between JA and lipoxygenase activity ($p < 0.01$). Interestingly, a significant positive Pearson correlation (0.936) was found between the concentrations of endogenous JA and the relative expression of rice *OsLOX3* ($p < 0.01$). The results suggest that the enhancing effect of JA on the biosynthetic pathway of nonanal.

Keywords: *Kam sweet rice, Nonanal, 2-Acetyl-1-pyrroline, Lipoxygenase activity, Jasmonic acid, OsLOX3*

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1. Introduction

The Asian cultivated rice (*Oryza sativa L.*) is one of the most important crops and a major food source for more than half of the global human population [1]. Among all sticky rice varieties cultivated worldwide, Kam sweet rice is known for its pleasant fragrance and cultivated for generations without using chemical pesticides or fertilizers [2]. Kam sweet rice is a major type of cultivated rice with a long history and culinary importance in China [3]. Indigenous aromatic Kam sweet rice types cultivated in China include Gou Cengao, Gou Gaoqian and Congjiang, found especially in southwest China where Kam sweet rice is traditional foods. However, major aromatic components found in the grains of Kam sweet rice have not been characterized.

Multiple studies aimed to explore factors that regulate the volatile components from aromatic rice. For example, trace amounts of gibberellic acid result in decreased 2-acetyl-1-pyrroline levels in aromatic rice [4], and many aromatic components have been markedly noticed in aromatic rice after treatment with abscisic acid (ABA) [5]. The aroma quality of rice is affected by environmental factors: rice cropped in rain-fed paddy fields shows higher content of aromatic compounds [6]; the yield of aromatic volatile compounds is affected by salinity [7,8,9]. In addition, enzyme activity is an important factor in volatile compounds synthesis of aromatic rice. Indeed, 2-acetyl-1-pyrroline synthesis was regulated by the expression level of Δ^1-pyrroline-5-carboxylixc acid synthetase *OsP5CS* [10] and it has been shown that volatile aldehydes are regulated by peroxidation and lipid oxidation [11]. Finally, Tomio et al. reported that 2-acetyl-1-pyrroline content in rice was higher when ripened at low temperature (day: 25°C/night: 20°C) compared with high temperature (day: 35°C /night: 30°C) (Itani et al. 2004). Likewise, variation of temperature was suggested as an alternative way to affect aroma changes [12].

In this study, JA amounts and the volatile components of Kam sweet rice Gou Cengao during filling stage were analyzed to determine the correlation between hormone and nonanal, the key volatile compound.

2. Materials and Methods

Plant Materials. The Kam sweet rice (Gou Cengao) and Kam rice (Lailong) are Chinese japonica rice found in the Guizhou Plateau, which is located in southwest China. The samples were milled to remove bran layers, and samples sealed in zip lock bages were kept at -80°C until use. Fifteen-day-old seedlings with three leaves were either treated with water supplemented with 120 mg/L JA or water alone for 48 h. The green leaves were harvested

and samples of equal weight (1.0 g) were homogenized in liquid nitrogen for detetion of volatile components.

SPME Sampling. The equilibration time 20 mins has been enough for most volatile compounds in headspace solid-phase microextraction (SPME). Hence, SPME was carried out by a DVB fiber (50/30 μm, 1 cm) attached to a manual holder from Supelco (Bellefonte, PA, USA). Then, the DVB fiber was desorbed for 5 min on an Agilent 5975C/6890 GC equipped with a HP 5973 mass-selective detector (Agilent Technologies, Palo Alto, CA, USA) and a 30 m× 0.25 mm× 0.25 μm Zebron ZB-5MSI fused silica capillary column (5% Phenyl-95% Dimethylpolysiloxane) from Zebron (USA). The injector temperature was kept at 250°C throughout the separation.

Gas chromatography-mass spectrometry (GC-MS) and Gas chromatography–olfactometry (GC-O). GC-MS experiments were carried out on an Agilent 6890/HP 5973 instrument. Samples were injected in split mode. The column temperature started at 40°C with a 1 min hold followed by increase at incremental rates of 4°C /min to 150°C. The interface temperature was programed at 280°C and the flow rate of the carrier gas-helium was 1.0 mL/min. Volatiles were split between the mass spectrometer and the olfactory detection port (Gersterl ODP2, Germany) for description and intensity assessment. The mass spectrometer was operated in an electron impact (EI) ionization mode with electron energy of 70eV and electron multiplier voltage of 1052V. The temperature of the ion source was 230°C and mass spectra were obtained by scanning from m/z 20-450. For olfactory detection, three independent assessors trained for odor description of volatiles evaluated the samples. Odor intensity was classified as nd (not detected), 1 (very weak), 2 (weak), 3 (intermediate), 4 (strong), and 5 (very strong). The volatiles perceived by all assessors were considered odor active components.

Extraction of JA. Samples (1g each) were freeze-dried and ground in 10 mL of methanol mixture (80%) in weak light. The homogenates were stored at 4°C for 24 h and submitted to centrifugation (4°C, 5000 r/m, 10 min). After methanol removed (vacuum at 35-40°C), the aqueous phase were mixed with 100 μL ammonia. Then, samples were filtered on polyvinylpyrrolidone (PVPP) and were mixed with HCl (2 mol/L) to reduce pH to 3.0. The samples were extracted by equal volume ethyl acetate and mixed with 100 μL ammonia. After desiccating with rotary evaporator (40°C), the samples were dissolved with acetic acid (0.1 mol/L, 5mL). The samples collected by Sep-Pak C18 (1 cm × 15 cm, 30 mL) were eluted with 40% methanol. The resulting samples desiccated with rotary evaporator (40°C) were dissolved with water: acetonitrile (85: 15, 0.05% acetic acid) for HPLC analysis.

Identification and Quantification of Volatile compounds and JA. The following standards were used for the identification of aromatic compounds in cooked rice samples: pentanol, hexanal, heptanal, (*E*)-2-hexenal, 1-octen-3-ol, octanal, (*E*)-2-octenal, octanol, nonanal and decanal were purchased from Aladdin-reagent (China); benzaldehyde was provided by Xiyashiji (China) and 2-acetyl-1-pyrroline by J&K Chemical (China). The n-alkanes C_6-C_{19} (AccuStandard, USA) were used to derive the retention indices. JA was provided by Aladdin-reagent (China).

All aromatic compounds were identified by matching with mass spectra in the NIST and WILEY libraries. Confirmation was carried out by comparing retention times with those authentic standards. For quantification of the compounds, standard curves were generated by analyzing GC-MS data after injection of different concentrations (ppm) of the compounds diluted in hexane (Table 1).

Table 1. Linearity, Sensitivity and Precision of Major Odor-Active Compounds detected in Kam Sweet Rice Sample after pollination

Compound	Standard Curve	R^2	Validation Range	RSD(%)
pentanol	$y = 3\times10^{-6}x+0.5893$	0.9999	0.3-199	1.01
hexanal	$y = 9\times10^{-6}x+1.3659$	0.9997	0.3-202	2.50
(*E*)-2-hexenal	$y = 5\times10^{-6}x+0.9504$	0.9997	0.5-199	0.99
heptanal	$y = 5\times10^{-6}x+0.2959$	0.9994	0.3-198	3.83
2-acetyl-1-pyrroline	$y = 9\times10^{-7}x+0.0402$	0.9997	0.04-4.9	3.48
benzaldehyde	$y = 2\times10^{-5}x–0.8293$	0.9997	0.3-188	0.37
1-octen-3-ol	$y = 9\times10^{-6}x+0.5793$	0.9999	2.9-180	3.19
octanal	$y = 6\times10^{-6}x–1.8464$	0.9993	1.0-150	1.91
(*E*)-2-octenal	$y = 5\times10^{-6}x+3.7986$	0.9997	2.9-218	2.31
octanol	$y = 5\times10^{-6}x+4.3261$	0.9996	3.3-195	2.21
nonanal	$y = 1\times10^{-5}x+18.384$	0.9992	24-199	0.29
decanal	$y = 7\times10^{-6}x–0.5105$	0.9999	1.0-200	2.18

For HPLC, acetonitrile and a mixed solution containing 99.95% water and 0.05% acetic acid were used as mobile phase. The flow rate was 300 μL/min, with a column maintained at 30°C. A total of 10 μL sample were injected. The gradient elution: 1-3 min, 15% acetonitrile; 3-5 min, 15%-100% acetonitrile; 5-6 min, 100% acetonitrile; 6-7 min 100%-15% acetonitrile, 7-8 min, 15% acetonitrile.The equation was obtained for JA standard curve of JA: y = 25342.6x-1929.14, with r^2=0.9992, with a validation range comprised between 3.1 and 102 ng/g and RSD of 3.32%.

RNA isolation and semi-quantitative RT-PCR. Total RNA was isolated from rice grain samples obtained from the aromatic Kam sweet rice Gou Cengao and non-aromatic Kam rice Lailong. RNA extraction from ten independent samples was carried out at four distinct stages of the rice grain development (6, 8, 10, and 15 days after rice flowering), using the E.Z.N.A™ Plant RNA Kit (OMEGA, USA) and following manufacturer's instructions:

1. Collect frozen ground plant tissue(up to 100 mg) in a microfuge tube and immediately add 500 μL Buffer RCL/2-mercaptoethanol. We recommend starting with 50 mg tissues at first. If results obtained are satisfactory increase amount of starting material. Samples should not be allowed to thaw before Buffer RCL/2-mercaptoethanol is added. Vortex vigorously to make sure that all clumps are dispersed. RNA cannot be effectively extracted from clumped tissue.

2. Incubate at 55°C for 1-3 minutes. Centrifuge at maxi speed (14000 × g) for 5 min at room temperature.

3. Transfer the supernatant directly into a gDNA Filter Column in 2 mL collection tube and centrifuge at 14000 × g for 2 min at room temperature.

4. Add eqaul volume Buffer RCB to the flow-through and mix well by pipetting up and down 5-10 times.

5. Apply one half of the mixture from step 4 to a HiBind@ RNA Mini column assembled in a clean 2 mL collection tube (supplied). Centrifuge at 10000 × g for 1 min at room temperature. Discard the flow-through liquid and place the column back into the collection tube.

6. Apply the regaining of the mixture from step 4 to the column. Centrifuge at 10000 × g for 1 min at room temperature. Discard the flow-through liquid and place the column back into the collection tube.

7. Add 400 μL RWC Wash Buffer and centrifuge as above. Discard both flow-through liquid and collection tube.

8. Place column in a clean 2 mL collection tube (supplied), and add 500 μL RNA Wash Buffer Ⅱ diluted with ethanol. Centrifuge as above and discard flow-through. Re-use the collection tube in step 9.

9. Wash column with a second 500 μL RNA Wash Buffer Ⅱ by repeating step 8. Centrifuge as above and discard flow-through. Then with the collection tube empty, centrifuge the column for 2 min at 10000 × g to completely dry the column matrix.

10. Elution of RNA: Transfer the column to a clean 1.5 mL microfuge tube (not suplied) and elute the RNA with 30-50 μL of DEPC water (suplied). Make sure to add water directly onto column matrix. Incubate at room temperature for 2 min. Centrifuge for 1 min at 10000 × g. A second elution into the same tube may be necessary if the expected yield of RNA >30 μg.

Reverse transcription was carried out separately for 10 min (25°C), 120 min (37°C) and 5 min (85°C), using the high-capacity cDNA reverse transcription kit (Applied Biosystems, USA). To prepare the 2 × RT master mix on ice (per 20 μL reaction): 10 × RT Buffer 2.0 μL; 25 × dNTP Mix (100 mM) 0.8 μL; 10 × RT Random Primers 2.0 μL; Multiscribe™ Reverst Transcriptase 1.0 μL; Rnase Inhibitor 1.0 μL; Nuclease-free H_2O 3.2 μL. To prepare the cDNA Reverse Transcription reactions: 1. Pipette 10 μL of 2 × RT master mix into each well of a 96-well reaction plate or individual tube. 2. Pipette 10 μL of RNA sample into each well, pipetting up and down two times to mix. 3. Seal the plates or tubes. 4. Briefly centrifuge the plate or tubes to spin down the contents and to eliminate any air bubbles. 5. Place the plate or tubes on ice until you are ready to load the thermal cycler.

Real-Time PCR was carried out on an ABI 7500 Fast Real Time PCR instrument with the 7500 v2.0.1 software (Applied Biosystems, USA). RT-PCR conditions included an initial denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 15s and 60°C for 1 min. The melting curves were obtained at 95 °C for 15s, 60°C for 1min; and 95°C for 15s. The following primers were used: *OsLOX3* Forward, 5'-CAACAGGCTCTACATTCT-3'; *OsLOX3* Reverse, 5'-GTGGCATAGGTGAAGATA-3' (Reference sequence: GenBank FJ660622.1); *OsHPL1* Forward, 5'-AGCTCCTCCACAACCTCG-3'; *OsHPL1* Reverse, 5'-CGATGTGTGGCAGGAAGAT-3' (Reference sequence: GenBank AK105964.1). The ubiquitin-conjugating enzyme E2 gene was employed as internal control: Forward primer 5'-CCGTTTGTAGAGCCATAATTGCA-3'; Reverse primer 5'-AGGTTGCCTGAGTCACAGTTAAGTG-3' (Reference sequence: GenBank AK059694.1). mRNA levels were expressed relatively to the ubiquitin-conjugating enzyme E2 gene in each sample.

Lipoxygenase Extraction and Enzyme Activity. Lipoxygenase was extracted as previously described [10]. Rice grains were frozen and ground in liquid nitrogen. The resulting powder was stored at -20°C until use. For extraction, 1g powder was added to 10 mL phosphate buffer (1% PVP, pH 6.5, 0.2 M) and the suspension centrifuged at 10000 × g for 20 min (4°C). Ammonium sulfate was added to supernatants to achieve 35% saturation and the mixture was centrifuged as described above. The resulting supernatants were precipitated with 70% $(NH_4)_2SO_4$ for 1h and the samples submitted to centrifugation (4°C, 10000 × g, 20 min). The pellet containing the enzyme was dissolved in 2 mL of 0.01 M phosphate buffer (pH6 .5) containing 10% glycerol. The samples were desalinated by Sephadex G-25 column (1 cm× 15 cm, 30 mL) and stored at 4°C. Enzyme activity was determined as previously described [13]. Briefly, quartz color dishes were filled with 0.2 mL substrate, 2.75 mL phosphate buffer (pH 6.5, 0.2 M) and 50 μL lipoxygenase extract and activity was assessed spectrophotometrically by monitoring the formation of conjugated dienes at 234 nm. The enzyme activity unit was calculated as ΔA_{234} $min^{-1}g^{-1}FW$. The substrate solution was prepared as proposed by Engeseth et al. [14]. Briefly, 0.1 mL linoleic acid stock solution was mixed with 2 mL boric acid (pH 9.0, 0.2 M) and 0.1 mL Tween-20. Then, 0.2 mL NaOH (1 M) was added and mixed until a clear solution was obtained. Finally, boric acid was added to increase the solution volume to 40 mL. The substrate solution was stored at 20°C until use.

Statistical Analysis. Data were analyzed by SPSS version 16.0 (SPSS Inc., Chicago, Il) and Pearson correlation. The Duncan's multiple-range test was used to compare OAVs of the odor-active compounds from rice samples.

3. Results and Discussion

The relative intensities and descriptors of odor-active compounds emanating from aromatic Kam sweet rice Gou Cengao rice after pollination, as determined by trained assessors, are summarized in Table 2. Aldehydes constituted the most abundant group among the 12 odor-active compounds detected in Kam sweet rice Gou Cengao. Indeed, 7 aldehydes were found, including hexanal, (*E*)-2-hexenal, heptanal, octanal, (*E*)-2-octenal, nonanal and decanal. The remaining constituents is a aromatic compound (benzaldehyde), two alcohols (pentanol, 1-octen-3-ol and 1-octanol) and a nitrogen-containing compound (2-acetyl-1-pyrroline). Buttery et al. [15] reported 2-acetyl-1-pyrroline and (*E*, *E*)-2, 4-decadienal as key aroma components in cooked rice, although these compounds were not found cooked Gou Cengao as shown above. This is likely due to the difference in the developmental stages of rice studied. Based on odor intensity, nonanal contributed most to the flavor of Gou Cengao, with odor intensity values after

pollination of 1.6 at sixth day, 2.1 at eighty day. and 2.4 at fifteenth day after , respectively. The popcorn-like 2-acetyl-1-pyrroline contributed the most odor intensity (2.1) at tenth day after pollination.

Table 2. Odor Intensity and Description of Odor-Active Compounds in Kam Sweet Rice after pollination:6 days, 8 days, 10 days, 15 days

RI[b]	Odorant	Odor intensity[a]				Odor description[c]	Identification[d]
		6 days	8 days	10 days	15 days		
627	pentanol	nd	1.0	nd	nd	plastic	MS,STD
643	hexanal	1.2	1.3	1.2	1.2	green	MS.STD,RI
711	(E)-2-hexenal	nd	1.2	1	nd	green	MS,STD,RI
744	heptanal	1.4	1.7	1.1	1.1	floral	MS,STD,RI
793	2-acetyl-1-pyrroline	nd	1.6	2.1	1.4	popcorn	MS,STD,RI
826	benzaldehyde	nd	1.3	nd	nd	almond	MS,STD,RI
849	1-octen-3-ol	nd	1.0	nd	nd	mushroom	MS,STD,RI
869	octanal	1.1	1.7	1.3	1.4	green citrus	MS,STD,RI
906	(E)-2-octenal	nd	1.3	nd	nd	nutty	MS,STD,RI
920	octanol	nd	1.4	1.2	1.1	citrus	MS,STD,RI
968	nonanal	1.6	2.1	1.9	2.4	citrus fatty	MS,STD,RI
1041	decanal	1.1	1.0	1.2	1.3	citrus	MS,STD,RI

[a] Average intensity of compounds that were detected by all three assessors.nd=not detected.[b] Retention indexs were counted using a series of n-alkanes C_6-C_{19}.[c] Odorants were described by assessors during GC-O.[d] MS, by comparison of the mass spectrum with the NIST/Wiley mass spectral library;RI, by comparison of RI with those from the literature;STD, by comparison of retention time, spectrum, and odor description of an identified compound with those of an authentic compound.

To assess the contribution of individual odor active component to the overall aroma, the compounds detected by the assessors (i.e., odor intensity > 0) were considered potentially contributors [16,17]. OAVs, obtained by dividing each component's concentration by its odor threshold in the air, were used to assess the relative importance of individual aromatic compounds in rice aroma (Table 3.). To determine the concentrations of the odor-active components, excellent standard curves were established using authentic commercially available standards, with linear correlation coefficients (R^2) ranging from 0.9992 to 0.9999 and relative standard deviations (RSDs) varying from 0.29 to 3.83% (Table 1.). As shown in Table 3., nonanal was the most potent odor-active compound in the Kam sweet rice Gou Cengao within 15 days after pollination (OAV=12.6, 20.4, 15.2, 16.0; relative proportion=49.8%, 32.4%, 33.4%, 50.1%), followed by 2-acetyl-1-pyrroline (OAV=0, 7.7, 19.8, 7.2; relative proportion=0%, 12.2%, 43.5%, 22.5%), octanal (OAV=7.1, 10.4, 3.8, 3.9; relative proportion=27.4%, 16.5%, 8.4%, 12.2%), hexanal (OAV=3.8, 13.5, 3.2, 2.1; relative proportion=14.6%, 21.5%, 7.0%, 6.6%), heptanal (OAV=1.8, 3.5, 1.6, 0.8; relative proportion=6.9%, 5.6%, 3.5%, 2.5%), decanal (OAV=0.6, 1.7, 0.5, 1.7; relative proportion=2.3%, 2.7%, 1.1%, 5.3%), (*E*)-2-octenal (OAV=0, 1.8, 0, 0; relative proportion=0%, 2.8%, 0%, 0%), (*E*)-2-hexenal (OAV=0, 1.4, 0.9, 0; relative proportion=0%, 2.2%, 2.0%, 0%), and 1-octen-3-ol (OAV=0, 1.4,0, 0; relative proportion=0%, 2.2%, 0%, 0%).

Aldehydes, especially nonanal, constituted the greast odor active contributors of the aromatic Kam sweet rice Gou Cengao. Interestingly, C9-aldehydes are significant odorants in many rice types and known products of 9-lipoxygenase and 9-hydroperoxide lyase, which are encoded separately by *OsLOX3* [18] and *OsHPL1* [19] gene, respectively.

Table 3. Odor Activity Values (OAVs) of Major Odor-Active Compounds in Kam Sweet Rice after pollination:6 days,8 days,10 days,15 days

RI [b]	odorant	OAV[a] (n=3)				odor threshold in air(ng/L)
		6 days	8 days	10 days	15 days	
627	pentanol	nd	0.02±0.0003a	nd	nd	153[c]
643	hexanal	3.8±0.08b	13.5±0.17a	3.2±0.01c	2.1±0.004d	1.1[c]
711	(*E*)-2-hexenal	nd	1.4±0.01a	0.9±0.004b	nd	3.1[c]
744	heptanal	1.8±0.03b	3.5±0.02a	1.6±0.01c	0.8±0.002d	0.9[c]
793	2-acetyl-1-pyrroline	nd	7.7±0.15b	19.8±0.21a	7.2±0.06c	0.02[d]
826	benzaldehyde	nd	0.6±0.001a	nd	nd	85[c]
849	1-octen-3-ol	nd	1.4±0.01a	nd	nd	2.7[c]
869	octanal	7.1±0.13b	10.4±0.08a	3.8±0.06c	3.9±0.04c	0.4[c]
906	(*E*)-2-octenal	nd	1.8±0.006a	nd	nd	2.7
920	octanol	nd	0.4±0.002b	0.5±0.0009a	0.2±0.0003c	22[c]
968	nonanal	12.6±0.02d	20.4±0.13a	15.2±0.005c	16.0±0.01b	2.6[c]
1041	decanal	0.6±0.007b	1.7±0.007a	0.5±0.008c	1.7±0.02a	2.6[c]

[a] OAV is obtained by dividing the concentration of an odor-active compound by its odor threshold in air and means of three replicates;means separation within rows by Duncan's multiple-range test $P<0.05$.[b] Retention indexs were counted using a series of n-alkanes C_6-C_{19}.[c] Data from Yang [16].[d] Data from Schieberle [17].

Therefore, to understand the highest OAV of nonanal, we assessed the changes in lipoxygenase activity (Table 4.), relative gene expression of *OsLOX3* and *OsHPL1* (Table 5.) and JA concentrations (Table 5.) in grains 6, 8, 10, and 15 days after pollination of Kam sweet rice Gou Cengao. As shown in Table 2, nonanal was found at all stages of the grain development in Kam sweet rice Gou Cengao. Interestingly, *OsLOX3* expression was significantly higher in Gou Cengao than in Lailong after pollination. However, no constant trend was found for *OsHPL1* expression, which was lower in Gou Cengao compared with Lailong at 6 (1.935 vs. 17.143), 8 (14.713 vs. 18.209) and 15 days (0.1.5 vs. 0.982) but slightly higher at 10 days (14.675 vs. 12.889). Therefore, *OsLOX3* was considered the most critical gene in Gou Cengao. To confirm these findings, enzyme activity of lipoxygenase was assessed in the same samples (Table 4.). In agreement with gene expression data, lipoxygenase activity was markedly increased at all time points during rice filling. Finally, using Pearson correlation analysis, we found that lipoxygenase activity correlated with JA amounts with a positive correlation of r = 0.751 (two-tailed analysis, p <

0.01) and OAV variation in nonanal amounts (6 days vs. 8 days; 8 days vs. 10 days; 10 days vs. 15 days) correlated with JA amounts, with a significant positive correlation of $r = 0.981$ (two-tailed analysis, $p < 0.01$). Interestingly the variation in JA amounts correlated with the *OsLOX3* expression, with a significant positive correlation of $r = 0.936$ (two-tailed analysis, $p < 0.01$) (Table 5.). Using Pearson correlation analysis, we found no significant correlation between JA concentration and *OsHPL1* expression (correlation of $r = 0.045$, two-tailed analysis, $p > 0.05$) (Table 5.). These results suggested that *OsLOX3* was also the most critical gene in JA regulation pathway of nonanal. Importantly, we found that JA had enhancing effects on nonanal biosynthesis. To confirm these findings, nonanal concentrations were assessed in the rice seedlings after application of JA. In agreement with the positive correlation described above between JA and *OsLOX3* expression, nonanal amounts were significantly higher in Gou Cengao seedlings treated with JA than controls (86.238 vs. 54.519) (Table 6.).

Table 4. The enzyme activity of lipoxygenase in Kam Sweet Rice after pollination:6 days,8 days,10 days,15 days

Time	enzyme activity of lipoxygenase($\Delta A_{234}min^{-1}g^{-1}FW$)	
	aromatic Gou Cengao	Lailong as control
6day	0.246±0.047	0.049±0.010
8day	0.229±0.038	0.039±0.0009
10day	0.266±0.031	0.018±0.004
15day	0.080±0.014	0.006±0.001

[a] Time, sampling after rice pollination.

Table 5. The quatification of JA and the relative expression value of *OsLOX3* and *OsHPL1* in Kam Sweet Rice after pollination: 6 days, 8 days, 10 days, 15 days

Time[a]	quatification of JA (ng/g)	relative expression value of *OsLOX3*		relative expression value of *OsHPL1*	
		aromatic Gou Cengao	Lailong as control	aromatic Gou Cengao	Lailong as control
6day	19.480±1.456	4.625±0.059	0.931±0.019	1.935±0.078	17.143±1.624
8day	9.398±0.529	2.043±0.006	0.358±0.047	14.713±0.112	18.209±1.387
10day	14.681±0.554	4.617±0.277	0.614±0.020	14.675±0.356	12.889±2.115
15day	5.522±0.311	0.633±0.034	0.045±0.001	0.105±0.017	0.982±0.025

[a] Time, sampling after rice pollination.

Table 6. The quatification of nonanal in air from the leaves of Kam Sweet Rice seedlings during JA (120mg/L, 48h) treatment

RI[a]	Odorant	JA-treated seedlings (ng/L)	Control (ng/L)	Odor description[b]	Identification[c]
968	nonanal	86.238±0.901	54.519±2.523	green fatty	MS, STD, RI

[a] Retention indexs were counted using a series of n-alkanes C_6-C_{19}. [b] Odorants were described by assessors during GC-O. [c] MS, by comparison of the mass spectrum with the NIST/Wiley mass spectral library;RI, by comparison of RI with those from the literature;STD, by comparison of retention time, spectrum, and odor description of an identified compound with those of an authentic compound.

4. Conclusions

In this study, the aroma profile of Kam sweet rice Gou Cengao was determined by a combination of GC-O and GC-MS. Nonanal and 2-acetyl-1-pyrroline were considered the most potent flavor compounds, with nonanal and 2-acetyl-1-pyrroline displaying the highest OAV at different stages after pollination. In addition, *OsLOX3* gene was expressed in relatively higher levels in aromatic rice and this translated into high lipogenase activity. The variation of *OsLOX3* expression also leaded to the decrease observed for the OAV of nonanal after 8 day. A positive correlation was also found between nonanal concentrations and lipoxygenase activity, suggesting that the high nonanal OAV observed might results from elevated lipoxygenase activity in Gou Cengao rice. The enhancing effect of JA on nonanal biosynthesis was confirmed after application of JA. Indeed, a positive correlation between the endogenous JA and *OsLOX3* gene expression. The findings described herein are different with the past finding that ABA decreased the aroma content of aromatic rice in ABA-treated leaves of rice in our past study [20]. The discrepancy might be due to the different the effects of JA and ABA on the metabolic pathways of nonanal and 2-acetyl-1-pyrroline. Our results suggested that rice of different aromatic compounds might have various flavors according to the hormonal treatment administered.

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