Pak. J. Bot., 48(4): 1431-1438, 2016.

THE EFFECTS OF A PLANT GROWTH REGULATOR, LEAF REMOVAL, BAGGING, AND HARVEST TIME ON THE LIPOXYGENASE ACTIVITY AND FATTY ACID COMPOSITION OF PINOT NOIR GRAPEVINES

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Abstract

Green leaf volatiles (GLVs) are an important source of grape aromas, and lipoxygenase is a key enzyme involved in the formation of green leaf volatile substances. In addition, fatty acids are the main substrates that compose GLVs and are the main precursor compound utilized in the formation of grape aromas, which are an important index of grape quality. We examined the effects of a plant growth regulator, leaf removal, bagging, and harvest time on the lipoxygenase (LOX) activity, and the fatty acid composition of grapevines were studied. The following four experimental treatments were conducted using Pinot Noir (*Vitis vinifera* L.) grapevines to study the following variables: treatment with a plant growth regulator, leaf removal, fruit bagging, and harvest time. We obtained the following results. (1) 16 types of fatty acids were detected in the grape skins. The unsaturated fatty acid content consisted mainly of linoleic acid, oleic acid and palmitoleic acid; however, no linolenic acid was detected. In addition, the saturated fatty acid content consisted primarily of palmitic acid, stearic acid, behenic acid and arachidic acid. (2) Abscisic acid (ABA), methyl jasmonate (MeJA), light intensity, and harvest time appeared to effect LOX activity. (3) According to a principal component analysis (PCA) of the four treatments and the fatty acid content of the skins, ABA (concentration of 1000 mg/L), MeJA (concentrations of 100 μmol/L, 400 μmol/L and 800 μmol/L) and early harvest treatment were responsible for the changes in fatty acid content. These results could be helpful in vineyard management and in improving the quality of grapes.

Key words: Plant growth regulator, Pinot noir; Lipoxygenase; Fatty acid; PCA.

Introduction

The quality of wine grapes depends on their characteristic aromas, which are primarily derived from green leaf volatiles (GLVs). Lipoxygenase (LOX, linoleate: oxygen oxidoreductase, *E.C.* 1.13.11.12) is an important enzyme involved in the formation of green leaf volatile substances. Furthermore, LOX is ubiquitous in plants and is present in leaves, stems, roots, flowers, fruit pulp and seeds (Agrawal *et al.,* 2004; Andreou *et al.*, 2009; Baysal & Demirdöven, 2007; Feussner & Wasternack, 2002; Gardner, 1991). In addition, LOX is known to catalyze the oxidation of polyunsaturated lipids and esters that contain a 1, 4-cis, cis-pentadiene structure, such as linoleic and linolenic acids, into conjugated hydroperoxides (HPOs) (Hartmut *et al.,* 1999). HPOs are produced by different reactions with different enzymes, and the products are directly involved in the formation of the fruit aromas that determine the fruit's quality (Baldwin, 2002; Hatanaka, 1993; Dai *et al.*, 2000). Furthermore, LOX can regulate the production of jasmonic acid and enhance plant resistance (Hildebrand *et al.,* 1986; Hildebrand *et al.,* 1988). LOX is also related to plant tissue maturation and aging, and plant organs and LOX content are known to affect flavor quality during food storage (Riley *et al.,* 1996; Theerakulkait *et al.,* 1995).

Fatty acids are substrates formed from GLVs and are the main precursor in the formation of grape flavor. LOX catalyzes the transformation of fatty acid to hydroperoxides (HPOD), which can be catalyzed by hydroperoxide lyase (HPL) to form small volatile molecules such as alcohols, aldehydes and esters (Clarke. & Bakker, 2004; Fukushige & Hildebrand, 2005; Pinto *et al.,* 2007; Laurent *et al.,* 2009). These substances are important sources of the aroma of the grape berry, grape juice and wine (Olias *et al.,* 1992).

Abscisic acid (ABA) has a positive regulatory effect on plant growth, and it protects plants from environmental stress (Jan *et al.,* 2003). Treatment with ABA can result in a variety of metabolic problems in plants and can affect various aspects of their physiology (Kim *et al.,* 2014). According to a previous study, ABA plays an important role in the promotion of fruit ripening and quality (Agarwal *et al.,* 2005). In addition, JA and ABA have similar physiological effects in plants, and LOX plays a role in the synthesis of both. Research has shown that after plants are damaged, their ABA and JA contents increase significantly over a short period, indicating that both ABA and JA operate upstream of the adaptive response to stress. In addition, the levels of ABA and JA are significantly impacted by LOX enzyme activity (Matsui, 2006). It is therefore important to study how both of these hormones influence plant growth, plant development and fruit quality.

At present, few studies have examined the effects of vineyard cultivation techniques on the LOX activity and fatty acid composition of wine grapes. In this study, the effects of a plant growth regulator, light intensity, and harvest time on grape berry LOX activity and fatty acid composition were investigated to understand the effects of vineyard cultivation measures on the activity of LOX, a key enzyme in the GLV metabolic pathway, and on fatty acid composition. These results will be useful for vineyard management and for improving the quality of grapes.

Materials and Methods

Materials: *Vitis vinifera* L. 'Pinot Noir' berries were obtained from a vineyard (Yangling, Shaanxi Province). The following vineyard cultivation conditions were used: single-trunk two-arm shaping, a north-south line, a spacing of 0.80 m×1.5 m, general management, and a normal harvest time (August 12, 2013). All samples were frozen in liquid nitrogen, transported to the laboratory within one hour of sampling, and stored at −80°C in a freezer until analysis.

Reagents and instruments: The reagents used in this study, including methyl jasmonate and ABA, were obtained from Sigma-Aldrich (Shanghai, China). Methyl heptadecanoate was purchased from YIFANG S&T Co. (Tianjin, China), and NaOH, Tritonx-100, Tween-20, Polyvingypyrrolidone (PVPP), HPLC grade petroleum ether, ethyl ether, methanol, and formic acid were purchased from the Tianjin Kermel Chemical Reagent Co. (Tianjin, China). The following equipment was used: an Eppendorf 5424R centrifuge, a Milli-Q ultrapure water system, a 101-2AB-type oven, an FA2004 electronic balance, a TGL-16M high-speed desktop refrigerated centrifuge, a Thermo TRACE DSQ gas chromatography-mass spectrometry system, and an RE-52C rotary evaporator.

Methods

Experimental treatments

ABA and MeJA treatments: On a serene evening with calm wind, ABA and MeJA treatments were applied to the surfaces of the grape berries on consistently and moderately growing grape vines during the early stage of grape veraison. Five ABA concentrations (200 mg/L, 400 mg/L, 600 mg/L, 800 mg/L, and 1000 mg/L) and five MeJA concentrations (50 μmol/L, 100 μmol/L, 200 μmol/L, 400 μmol/L, and 800 μmol/L) were used. Each treatment was repeated on three individual vines.

Light intensity treatment: To increase light intensity, old leaves were removed. During the early stage of grape veraison, the old grape leaves, defined as the first 1-5 leaves from the base of a new shoot, as well as the leaves around the grape clusters were removed. Consequently, 75% of the grapes were exposed to sunlight. This treatment was repeated on three individual vines.

To decrease the light intensity, fruit bagging was used. Special grape bagging was used during the early stage of grape veraison, and the treatment was repeated on three individual vines.

Harvest time: The early harvest time was one week sooner than the normal harvest time, and the delayed harvest time was one week later than normal. These treatments were repeated on three individual vines.

Sample collection and treatment: After implementing the treatments, the grape vines were managed normally. When the berries matured (except for the early and delayed harvest treatments), 10 standard grape clusters were randomly selected from each treatment vine. The samples were immediately placed in an icebox after selection and were transported to the laboratory within one hour. The grape skins were carefully peeled using stainless steel tweezers, and the pulp that was attached to the inside of the skin was cleaned. Then, the grape skins were rinsed with distilled water, drained, placed in 10 mL centrifuge tubes, frozen in liquid nitrogen and stored at -80°C in a refrigerator until extraction.

Determination of LOX activity

Substrate preparation: The substrate consisted of 50 μL linoleic acid, 1 mL distilled water and 25 μL Tween-20. After this mixture was shaken, NaOH (1 mol/L) was added until the mixture became clear. Then, the mixture was diluted with distilled water to 10 mL to obtain a 0.5% (v/v) sodium linoleate solution. Next, the substrate was packed and sealed to protect it from light before storing it at -20°C in a refrigerator until use.

Extracting buffer preparation: To prepare the extract buffer, we blended 100 mL of 0.1 mol/L phosphate buffer (pH 6.8) with 1 mL of TritonX-100 and 4 g of PVPP. The mixture was shaken and stored at 4°C in a refrigerator until use.

Extraction of the crude enzyme: The extraction was according to Ju *et al*. (2014). Briefly, an appropriate amount of preserved grape skin was immediately ground in liquid nitrogen. Next, 2 g of powdered grape skin was placed into a centrifuge tube. After adding 4 mL of extraction buffer (pH 6.8), the mixture was centrifuged in a high-speed refrigerated centrifuge for 30 min at 4°C and 12000 r/min. The resulting supernatant was collected to determine its lipoxygenase enzyme activity.

Determination of LOX enzyme activity: The LOX activity was assayed using the method published by Ju *et al*. (2015) and Hwang *et al*. (2010) The reaction mixture contained 33 μL substrate in 900 μL 0.1 mol/L phosphate buffer (pH 6.8) and was heat preserved at 30°C in a water bath for 10 min prior to the addition of 67 μL crude enzyme extract. The absorbance values of the mixture were determined at 234 nm at 30 s, 45 s, 60 s, 75 s, 90 s, 105 s, 120 s, 135 s, and 150 s following extract addition. The enzyme activity was expressed in □ $OD_{234}\cdot g^{-1}FW\cdot min^{-1}$, and each sample measurement was replicated three times. The UV spectrophotometer was warmed and set to zero using distilled water prior to the analysis.

Calculation of LOX enzyme activity: The Calculation of LOX enzyme activity was followed by Ju *et al.* (2015).The absorbance values at 234 nm at different times during the reaction were recorded to generate an OD_{234} time series. According to the initial linear portion of the curve from I (starting point) to F (terminating point), the change in absorbance per minute (□ OD_{234}) was calculated as follows:

$$\square OD_{234} = (OD_{234F} - OD_{234I})/(T_F - T_I)$$

Next, one unit of lipoxygenase activity was defined to equal each of the 0.01 increments of absorbance change in the fruit and vegetable samples (fresh weight) per gram per minute [with units of $0.01 \Delta OD_{234}/(min \cdot g \cdot m_f)$].

$$U = (\Delta OD_{234} * V) / (0.01 * V_S * m)$$

V: total sample extraction volume, mL
Vs: sample extraction volume in the determination, mL
M: sample mass, g

Determination of fatty acid composition

Fatty acid extraction: The extraction was modified according to the method of Jonathan *et al*. (2008). First, 5 g grape skin was immediately and thoroughly ground in liquid nitrogen. Then, a 10 mL mixture of petroleum ether and diethyl ether (4/3, v/v) was added at 4°C for 24 h prior to the extraction. Next, 10 mL of a 0.4 mol/L potassium hydroxide-methanol solution was added, methyl etherification was performed for 2 h at the ambient temperature, and the resulting sample was centrifuged at an ambient temperature (25°C) at 4000 r/min for 10 min. After centrifugation, the organic phase of the upper layer was placed in a 10 mL distillation flask, distilled under reduced pressure, and diluted with distilled water to 5 mL. The experimental system contained 1 mL sample solution and 1 μL 100 mg/mL methyl heptadecanoate as an internal standard for testing.

Determination of fatty acid composition: The fatty acid compositions were determined by thermoelectric TRACE DSQ gas chromatography-mass spectrometry using the method reported by Ozan G *et al*. (2007). As shown in Table 1, 16 types of fatty acids were detected in the skins of the Pinot Noir berries. The unsaturated fatty acids consisted primarily of linoleic acid, oleic acid and palmitoleic acid. Moreover, the linoleic acid content was maximized, but linolenic acid was not detected. The saturated fatty acid consisted primarily of palmitic acid, stearic acid, behenic acid and arachidic acid.

Results and Discussion

Effects of different treatments on the LOX activities of Pinot Noir berry skins

Effects of ABA application on the LOX activity of Pinot Noir berry skins: After treatment with different concentrations of ABA, the ABA concentration increased (except for 200 mg/L) as the LOX activity decreased (Fig. 1A). The LOX activity was highest when the ABA concentration was 400 mg/L and was lowest when the ABA concentration was 1000 mg/L. For this correlation, the LOX activity of the treated groups was significantly higher than that of the control group when the concentration was 400 mg/L. Meanwhile, no significant differences were observed between the control group and the treated groups when the concentration was 600 mg/L. In addition, the LOX activity of the treated groups was significantly lower than that of the control group when the concentration was greater than 800 mg/L.

The effects of MeJA application on the LOX activity of Pinot Noir berry skins: After treatment with MeJA, the LOX activity of the berries decreased as the MeJA concentration increased (except for 200 μmol/L). As shown in Fig. 1B, the LOX activity was highest when the MeJA concentration was 50 μmol/L and was lowest when the MeJA concentration was 800 μmol/L. In addition, the LOX activity was not significantly different between the control group and the treated groups when the MeJA concentration was 50 μmol/L or 100 μmol/L. The LOX activity of the treated groups was significantly lower than that of the control group when the MeJA concentration was greater than 400 μmol/L.

Table 1. Composition and retention time of berry skin fatty acids (methyl ester).

Fatty acid	Molecular formula	Retention time (min)
Tetradecanoic acid	$C_{14}H_{28}O_2$	12.67
Pentadecanoic acid	$C_{15}H_{30}O_2$	13.99
9-Hexadecenoic acid	$C_{16}H_{30}O_2$	15.08
Hexadecanoic acid	$C_{16}H_{32}O_2$	15.42
Cyclopropane octanoic acid	$C_{17}H_{32}O_2$	16.54
Heptadecanoic acid	$C_{17}H_{34}O_2$	16.98
Linoleic acid	$C_{18}H_{32}O_2$	18.17
Elaidic acid	$C_{18}H_{30}O_2$	18.3
Stearic acid	$C_{18}H_{36}O_2$	18.71
14-Methyloctadecanoic acid	$C_{19}H_{38}O_2$	20.63
11-Eicosenoic acid	$C_{20}H_{38}O_2$	22.15
Eicosanoic acid	$C_{20}H_{40}O_2$	22.71
Heneicosanoic acid	$C_{21}H_{42}O_2$	24.88
Docosanoic acid	$C_{22}H_{44}O_2$	27.1
Tricosanoic acid	$C_{23}H_{46}O_2$	29.37
Lignoceric acid	$C_{24}H_{48}O_2$	31.95

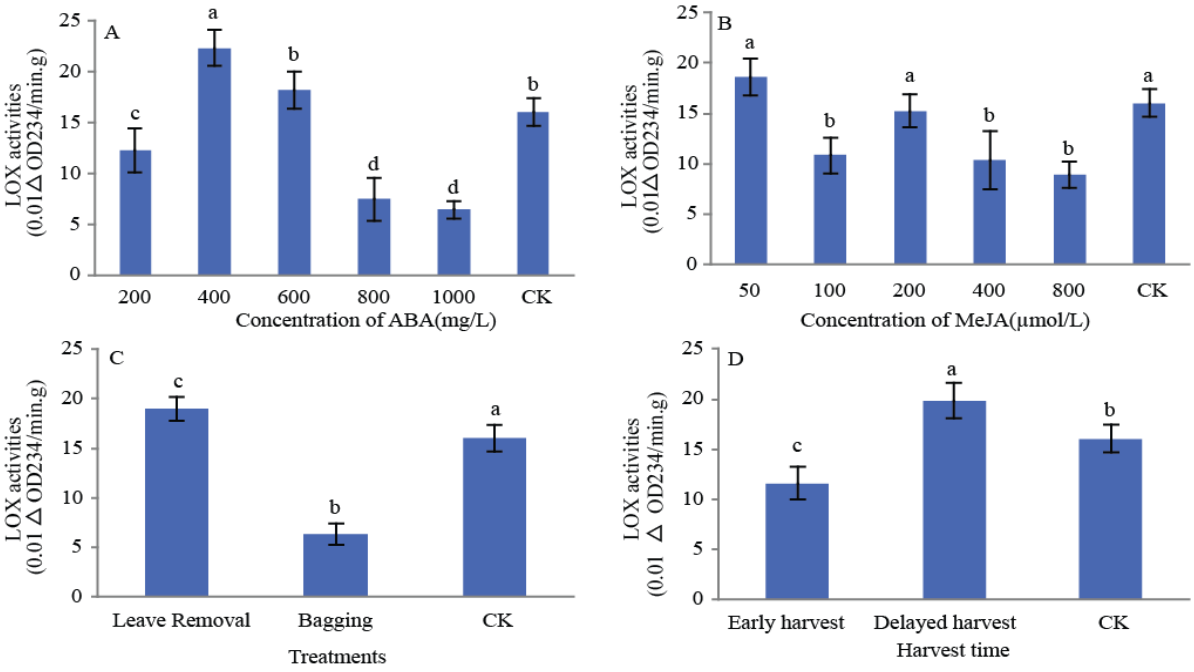

Fig. 1. The effects of ABA (A), MeJA (B), light treatment (C) and harvest time (D) on LOX activity in the skins of Pinot Noir berries.

Effects of light intensity and harvest time on the LOX activity of Pinot Noir berry skins: To study the effect of light intensity on the LOX activity, the light intensity experienced by the berries was altered by removing old leaves and using fruit bagging. All treatments were conducted during the early stages of grape veraison. As shown in Fig. 1C, after removing old leaves, the LOX activity of the treated groups was significantly higher than that of the control group. After bagging, the LOX activity in the skins of the Pinot Noir berries was significantly lower for the treated groups than for the control group.

As shown in Fig. 1D, the LOX activity in the skin of the Pinot Noir berries in the treated groups was significantly lower than that of the control group when harvesting a week early. In addition, the LOX activity in the skins of Pinot Noir berries in the treated groups was significantly higher than that of the control group when harvesting a week later.

Correlation analysis of the fatty acid compositions of Pinot Noir berry skins: Seven major fatty acids were observed in the Pinot Noir grape skins: linoleic acid, oleic acid, palmitoleic acid, palmitic acid, stearic acid, behenic acid and arachidic acid. The correlations among these fatty acid contents were analyzed.

According to Table 2, the linoleic acid content was not only significantly and positively correlated with palmitic acid content but was also significantly and positively correlated with the behenic acid of the Pinot Noir berry skins. The palmitic acid content was significantly and positively correlated with the behenic acid and arachidic acid contents. However, the contents of the other acids were not correlated.

Effects of plant growth regulators, leaf removal, bagging, and harvest time on the fatty acid content of the Pinot Noir berry skins

Effects of ABA application on the fatty acid contents of Pinot Noir berry skins: Table 3 shows that the ABA concentration increased as the linoleic acid concentration gradually increased. The linoleic acid content was lowest when the ABA concentration was 600 mg/L and was highest when the ABA concentration reached 1000 mg/L. The elaidic acid content in the treated groups was lower than that of the control group, with the exception of ABA concentrations of 400 mg/L and 1000 mg/L - the same as the concentration of stearic acid. The docosanoic acid concentration decreased after ABA treatment compared to the control group. In addition, the other fatty acid concentrations increased after ABA treatment.

Effect of MeJA application on the fatty acid content of Pinot Noir berry skins: As the MeJA concentration increased, the fatty acid content decreased and then increased. The fatty acid content reached a minimum value when the MeJA concentration was 200 μmol/L. When the MeJA concentration was 100 μmol/L, 400 μmol/L and 800 μmol/L, the fatty acid contents were higher than those of the control group (Table 3).

Effects of light intensity and harvest time on the fatty acid content of Pinot Noir berry skins: In the early stage of grape veraison, the old grape leaves, defined as the first 1-5 leaves from the base of a new shoot, and the leaves around the grape clusters were removed such that 75% of the grapes were exposed to sunlight. According to Table 3, after the removal of old leaves, the fatty acid content of the Pinot Noir berry skins of the treated groups was lower than that of the control group. The same result was observed for the bagging treatment.

Table 2. Correlation analysis of the fatty acids present in the skins of Pinot Noir berries.

Correlation coefficient	Linoleic acid	Oleic acid	Palmitoleic acid	Palmitic acid	Ptearic acid	Behenic acid	Arachidic acid
Linoleic acid	1.0000						
Oleic acid	0.1797	1.0000					
Palmitoleic acid	0.2451	0.0935	1.0000				
Palmitic acid	0.5469*	0.3326	0.1928	1.0000			
Stearic acid	0.5320*	0.4397	-0.1114	0.3783	1.0000		
Behenic acid	0.2659	0.3991	0.3607	0.5323*	-0.1231	1.0000	
Arachidic acid	0.4021	0.3392	0.3280	0.6316**	0.4234	0.3560	1.0000

Note: **Significant; *Notable

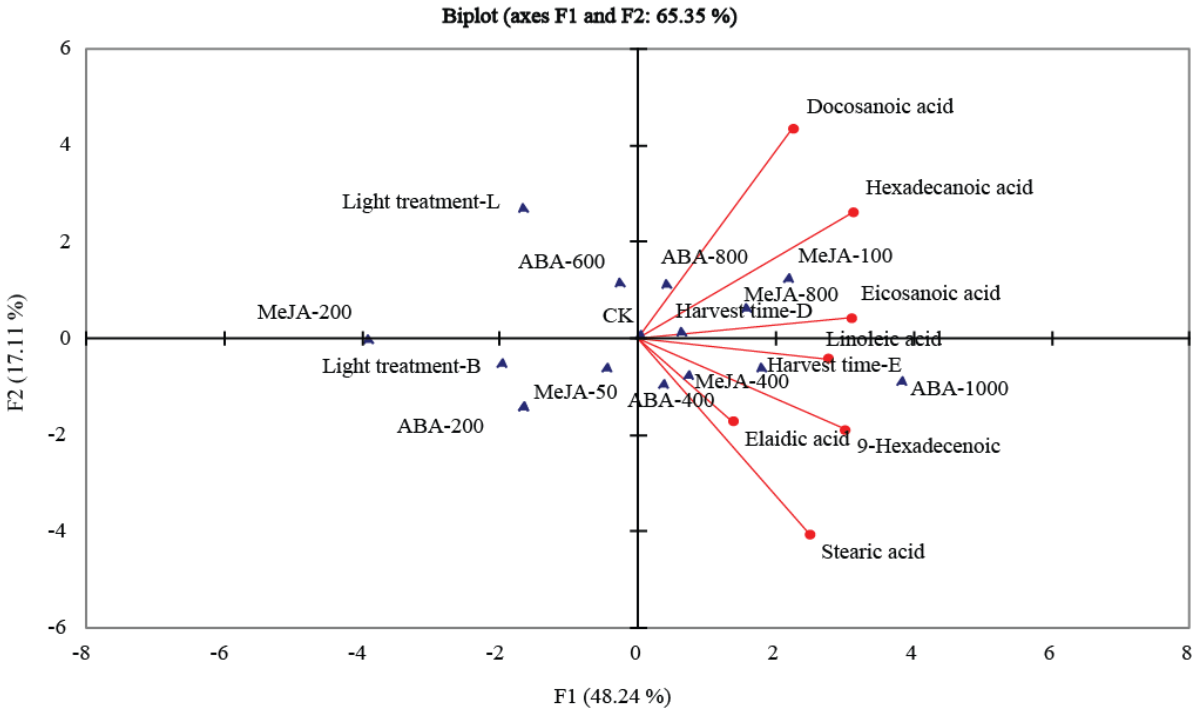

Fig. 2. Scatter plots of the first two principal components analysis of treatment and fatty acid content.

As shown in Table 3, when harvested a week earlier, the fatty acid contents in the treated groups were higher than those in the control group. In addition, no significant differences in fatty acid contents were observed between the treated groups and the control groups when they were harvested a week later. These results indicate that the consumption of linoleic acid increased through the GLV pathway when the harvest was delayed. Accordingly, the delay in harvest has an important effect on the GLV pathway.

Interpreting the results of principal component analysis (PCA): To determine the potential relationships among the measured parameters and to reveal which of these parameters could be responsible for grape fatty acid content after different treatments, principal component analysis (PCA) was performed. PCA was conducted to evaluate the effects of each treatment (ABA, MeJA, leaf removal, and harvest time) on the fatty acid contents in the skins of Pinot Noir berries. The first two factors (F1 and F2) correspond to a high percentage of the variance, which ensures that the maps based on the first two factors are high quality projections of the initial multidimensional table. The first two factors allow us to represent 65.35% (48.24% for F1 and 17.11% for F2) of the initial variability in the data (Fig. 2). The factor scores of the treatments for the first two factors exhibited clear separation. The ABA concentration was 1000 mg/L, the MeJA concentrations were 100 µmol/L and 800 µmol/L, and the early harvest factor scores were high and are located in the positive region of F1. Meanwhile, the ABA concentration was 200 mg/L, the MeJA concentration was 200 µmol/L, and the bagging treatment factor scores were low and are located in the negative region of F1 (Table 5). In this respect, F1 is primarily related to the different treatments. The principal components responsible for differences in the fatty acid composition of the skins are presented in a scatter plot in Fig. 2. Thus, the components responsible for the separation of the fatty acids were linoleic acid, 9-hexadecenoic acid, hexadecanoic acid and eicosanoic acid. These components are positively correlated to F1, ABA (concentration of 1000 mg/L), MeJA (concentrations of 100 µmol/L and 800 µmol/L) and early harvesting (Table 4 and Fig. 2).

Table 3. Effects of ABA, MeJA, light, and harvest time on the fatty acid content of Pinot Noir berry skins.

Treatment	Linoleic acid mg/Kg	Elaidic acid mg/Kg	9-Hexadecenoic mg/Kg	Hexadecanoic acid mg/Kg	Stearic acid mg/Kg	Docosanoic acid mg/Kg	Eicosanoic acid mg/Kg
ABA (200 mg/L)	146.53 ± 3.29	147.01 ± 7.39	0.78 ± 0.034	147.87 ± 5.55	36.64 ± 2.09	12.53 ± 1.02	6.85 ± 0.42
ABA (400 mg/L)	164.00 ± 9.21	180.38 ± 3.35	0.82 ± 0.029	215.11 ± 5.59	39.27 ± 1.99	14.21 ± 0.74	7.52 ± 0.37
ABA (600 mg/L)	154.81 ± 8.34	120.14 ± 6.16	0.59 ± 0.033	226.23 ± 7.62	34.06 ± 2.51	15.67 ± 1.19	9.14 ± 0.65
ABA (800 mg/L)	136.72 ± 5.29	116.56 ± 5.29	0.66 ± 0.040	206.82 ± 3.84	32.90 ± 1.79	16.30 ± 0.55	9.44 ± 0.53
ABA (1000 mg/L)	229.88 ± 6.29	165.18 ± 7.11	1.08 ± 0.044	263.37 ± 3.75	44.46 ± 2.19	15.71 ± 0.96	11.46 ± 0.64
MeJA (50 μmol/L)	164.61 ± 8.38	140.87 ± 5.17	0.78 ± 0.052	198.39 ± 5.58	37.02 ± 2.70	13.50 ± 0.67	7.70 ± 0.35
MeJA (100 μmol/L)	223.49 ± 7.29	183.45 ± 5.02	1.22 ± 0.049	237.26 ± 8.65	33.19 ± 2.01	19.78 ± 1.40	10.46 ± 0.59
MeJA (200 μmol/L)	133.74 ± 8.38	152.73 ± 6.28	0.36 ± 0.078	162.43 ± 9.55	27.52 ± 1.99	11.11 ± 0.45	4.48 ± 0.40
MeJA (400 μmol/L)	189.40 ± 7.27	185.17 ± 4.99	0.74 ± 0.013	210.20 ± 5.55	40.92 ± 2.54	16.16 ± 0.83	6.80 ± 0.27
MeJA (800 μmol/L)	198.28 ± 4.12	168.14 ± 4.36	0.79 ± 0.034	232.28 ± 7.52	36.95 ± 2.80	17.75 ± 1.06	9.09 ± 0.58
Leaf removal	150.29 ± 8.32	112.12 ± 5.69	0.39 ± 0.031	228.31 ± 5.61	29.42 ± 1.11	18.30 ± 0.55	5.62 ± 0.38
Bagging	135.59 ± 9.42	104.97 ± 6.34	0.54 ± 0.029	167.83 ± 9.25	36.97 ± 1.80	12.54 ± 0.50	7.92 ± 0.34
Early harvest	233.07 ± 8.26	108.93 ± 7.83	1.09 ± 0.032	246.09 ± 7.67	40.91 ± 2.72	14.16 ± 0.91	7.83 ± 0.54
Delayed harvest	188.15 ± 7.01	166.66 ± 4.96	0.79 ± 0.025	224.05 ± 6.19	35.85 ± 2.01	15.66 ± 1.14	7.86 ± 0.40
CK	206.59 ± 9.42	160.82 ± 7.87	0.49 ± 0.031	188.97 ± 8.13	39.57 ± 2.21	17.67 ± 0.88	6.51 ± 0.37

Table 4. Factor loading for Pinot Noir skin fatty acid variables.

Variable	Factor loading	
	F1	F2
Linoleic acid	0.70	-0.29
Elaidic acid	0.37	0.06
9-Hexadecenoic	0.82	-0.14
Hexadecanoic acid	0.83	0.31
Stearic acid	0.67	-0.64
Docosanoic acid	0.56	0.74
Eicosanoic acid	0.81	0.07

Table 5. Factor scores for each treatment.

Treatment	Factor scores	
	F1	F2
ABA-200	-1.80	-1.27
ABA-400	0.29	-0.59
ABA-600	-0.36	0.80
ABA-800	0.28	0.40
ABA-1000	3.72	-0.97
MeJA-50	-0.54	-0.70
MeJA-100	2.07	2.15
MeJA-200	-4.01	-0.05
MeJA-400	0.61	-0.47
MeJA-800	1.44	0.70
Light treatment-L	-1.73	2.22
Light treatment-B	-2.08	-0.95
Harvest time-E	1.71	-1.38
Harvest time-D	0.53	0.19
CK	-0.12	-0.10

Regarding F2, the ABA concentration was 600 mg/L, the MeJA concentration was 100 μmol/L and the leaf removal factor scores were high and are located in the positive part of F2. In addition, the ABA concentrations were 200 mg/L and 1000 mg/L, and the early harvest treatment factor scores were low and are located in the negative region of F2 (Table 5 and Fig. 2). The stearic acid content was negatively correlated with F2 and was positively related to the ABA (concentration of 1000 mg/L) and MeJA (concentration of 400 μmol/L) treatments. Docosanoic acid was positively correlated with F2 and was positively related to the MeJA (concentration of 100 μmol/L) treatment (Table 4 and Fig. 2).

Overall, the ABA (concentration of 1000 mg/L), MeJA (concentrations of 100 μmol/L, 400 μmol/L and 800 μmol/L) and early harvest treatments were primarily responsible for the fatty acid content.

Conclusions

The fatty acid compositions of the grapes used in this study were consistent with those reported by Li Bo *et al*. (2012), although the arachidic acid and behenic acid contents were different, potentially due to the difference in test materials. In our study, fresh grape skins were peeled from mature grapes. In contrast, Li Bo *et al*.

(2012) used grape skin residues that consisted of a mixture of grape skin and grape seeds after fermentation rather than fresh grape skins.

When the exogenous ABA concentration was less than 600 mg/L, the fatty acid concentration of the grape berries was less than that of the control group. In addition, ABA appears to play a role in promoting the expression of the GLV pathway. This could be related to the ability of ABA to promote fruit development and maturation as well as the synthesis of anthocyanin in grape skins. However, this hypothesis requires further study. After MeJA treatment, the fatty acid content of the grape berries was reduced compared to grapes in the control group, while JA enhanced the grape GLV pathway. In addition, as the concentration increased, the effects of the exogenous MeJA on the GLV pathway increased and then decreased. This change could be the result from the relationship of mutual promotion and competition between the MeJA production pathway and GLV pathway in plants (Fukushige & Hildebrand, 2005).

After removing the old leaves, the fatty acid content - especially the linoleic acid content - was significantly reduced compared to the control group. This result may be due to the thin skin of Pinot Noir grapes, which are easily affected by adverse external factors. After the bagging treatment, the fatty acid contents were significantly lower than those of the control group because bagging protects the berries from adverse external factors. When harvesting a week earlier, the fatty acid contents were significantly higher than those of the control group. However, when harvesting a week later, the fatty acid contents in the grape skins in the treated groups were not significantly different from those in the control group. The results showed that the consumption of fatty acids (especially linoleic acid) increased in the GLV pathway when the harvest was delayed. Accordingly, this delay in harvest played an important role in promoting the GLV pathway. If the harvest is delayed appropriately, the GLV content in the berries could increase, which could improve the quality of the wine aroma (Clarke & Bakker, 2004; Podolyan *et al.*, 2010). The optimal harvest time also depends on production and climate factors.

The results of this experiment clearly indicate that cultivation measures, including plant growth regulator treatment, light, and harvest time, directly affect the LOX activity and fatty acid composition of the grapes. In addition, this experiment characterized how grapevine cultivation measures affect LOX, the key enzyme involved in the GLV metabolic pathway, as well as the fatty acid composition of the grape skins. These findings could help to improve vineyard management and grape quality.

Acknowledgments

We thank the Cao Xinzhuang vineyard for providing Pinot Noir grapes. This work was supported by the Ministry of Agriculture project “948” (2014k309021401), the National Eleventh Five Year Research Program of China (2012BAD31B07), the Overall Innovation Project of the Shaanxi Province Science and Technology Plan (2013KTCL02-01), and the National Technology System for the Grape Industry (nycytx-30-2p-04). We thank these contributors for their generous financial support.

References

Agarwal, S., R.K. Sairam and G.C. Srivastava. 2005. Changes in antioxidant enzymes activity and oxidative stress by abscisic and salicylic acid in wheat genotypes. *Biol Plant.*, 49: 541-550.

Agrawal, G K., S. Tamogami, O. Han, H. Iwahashi and R. Rakwal. 2004. Rice octadecanoid pathway. *Biochemical and Biophy. Res. Comm.*, 317(1): 1-15.

Andreou, A., F. Brodhun and I. Feussner. 2009. Biosynthesis of oxylipins in non-mammals. *Progress in Lipid Research*, 48(4): 148-170.

Baldwin, E.A. 2002. Fruit volatile metabolism and consumer perceptions. In: Knee. Fruit quality and its biological basis. Boca Raton: Sheffield Academic. 8: 89-106.

Baysal, T. and A. Demirdöven. 2007. Lipoxygenase in fruits and vegetables: A review. *Enzyme & Micro.l Tech.,* 40(4): 491-496.

Clarke, R.J. and J. Bakker. 2004. Wine flavour chemistry. Oxford: Blackwell Pub.

Dai, L.Y., X. Li and K. Luo. 2000. Jasmonic acid and its functions. *J. Hunan Agri. Uni.*, 26(2): 150-154.

Feussner, I. and C. Wasternack. 2002. The lipoxygenase pathway. *Annual Review of Plant Biology*, 53: 275-297.

Fukushige, H. and D.F. Hildebrand. 2005. Watermelon (*Citrullus lanatus*) hydroperoxide lyase greatly increases C6 aldehyde formation in transgenic leaves. *J. Agri. & Food Chem.*, 53(6): 2046-2051.

Gardner, H.W. 1991. Recent investigations into the lipoxygenase pathway of plants. *Biochimica et Biophysica Acta-Lipids and Lipid Metabolism*, 1084(3): 221-239.

Hartmut Kuhn, J. Bernd and Thiele. 1999. The diversity of the lipoxygenase family many sequence data but little information on biological significance. *Febs. Letters*, 44(9): 7-11.

Hatanaka, A. 1993. The bio-generation of odour by green leaf. *Phytochem.*, 34: 1201-1218.

Hildebrand, D.F., T.R. Hamilton and C.S. Legg. 1988. Plant lipoxygenases, occurrence, properties and possible functions. *Current Topics Plant Biochem Phsiol*., 7: 201-219.

Hildebrand, D.F., J.G. Rodriguez and G.C. Brown. 1986. Peroxidative responses of leaf in two soybean gene types injured by two-spotted spider mites. *J. Econ. Entomol*., 79: 1459-1465.

Hwang, I.S. and B.K. Hwang. 2010. The pepper 9-lipoxygenase gene CaLOX1 functions in defense and cell death responses to microbial pathogens. *Plant Physiol.*, 152(2): 948-967.

Jan Smalle, Jasmina Kurepa and Peizhen Yang. 2003. The pleiotropic role of the 26S proteasome subunit RPN10 in arabidopsis growth and development supports a substrate-specific function in abscisic acid signaling. *The Plant Cell*, 15: 965-980.

Ju, Y.L., Y.L. Fang, L. Zhang, J. Zeng and J.B. Liu. 2015. Determination of lipoxygenase activity and the change of the fatty acid composition of cabernet sauvignon grape berries. *Food Sci.*, 36(5): 116-119.

Ju, Y.L., T. Chen, J.S.H. Gao, Y.L. Fang, K. Wang and Z.H.W. Zhang. 2014. Lipoxygenase activity and fatty acid content of cabernet sauvignon grape during berry development and external treatment. *Acta Bot. Boreal. Occident. Sin*., 34(11): 2283-2287.

Jonathan M. Curtis, Natalie Berrigan and Prudence Dauphinee. 2008. The determination of fatty acid in food products containing microencapsulated fish oil using the one step extraction method. Part 1: Measurement in the raw ingredient and dry powdered foods. *J. Am. Oil Chem. Soc*., 85: 297-305.

Kim, Y.J., M.G. Jang and J. Sukweenadhi. 2014. Functional characterization of the pathogenesis-related protein family 10 gene, PgPR10-4, from Panax ginseng in response to environmental stresses. *Plant Cell Tissue and Organ Culture*, 118(3): 531-543.

Laurent, G.D., R.Q. David, D. Alain, G. Jerome, D.W. Matthew, A.S. Karen, J.M. Merillon, J.C. Cushman and G.R. Cramer. 2009. Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics*, 10: 212-244.

Li, B., S.S. Li, Z.M. Wu, Y.Y. Zhang and N. Su. 2012. Analysis of fatty acids of grape skin with different extraction methods. *Liquor-Making Science & Technology*, 9: 39-45.

Matsui, K. 2006. Green leaf volatiles: Hydroperoxide lyase pathway of oxylipin metabolism. *Curr. Opin. in Plant Biol.*, 9(3): 274-280.

Olias, J.M., A.G. Perez and J.J. Rios. 1992. Aroma components and free aroma acids in strawberry variety Chandler during ripening. *Journal of Agricultural Food Chemistry*, 40: 2232-2235.

Ozan, G., G. Duygu and D. Fatih. 2007. Determination of flavan- 3- ols and trans- resveratrol in grapes and wine using HPLC with fluorescence detection. *Food Chemistry*, 100(2): 18- 525.

Pinto, D.M., A.M. Nerg and J.K. Holopainen. 2007. The role of ozone-reactive compounds, terpenes, and green leaf volatiles (GLVs), in the orientation of *Cotesia plutellae*. *Journal of Chemical Ecology*, 33(12): 2218-2228.

Podolyan, A., J. White, B. Jordan and C. Winefield. 2010. Identification of the lipoxygenase gene family from *Vitis vinifera* and biochemical characterisation of two 13-lipoxygenases expressed in grape berries of Sauvignon Blanc.Functional *Plant Biology*. 37(8): 767-784.

Riley, J.M., C. Willemot and J.E. Thompson. 1996. Lipoxygenase and hydroperoxide lyase activities in ripening tomato fruit. *Postharvest Biology and Technology*, 7: 97-107.

Theerakulkait, C., D.M. Barrett and M. Mcdaniel. 1995. Sweet corn germ enzymes affect odor formation. *Journal of Food Science*. 60(5): 1034-1040.

(Received for publication 3 July 2015)