DOI : 10.5958/0974-0112.2020.00004.3

Indian J. Hort. 77(1), March 2020: 56-64

Role of membrane lipid hydrolysis genes in the aroma formalion of Chinese white pear 'Xiang Mian Li'

Xingkai Yi, Zhenghui Gao, Jinyun Zhang, Xiaoling Zhang[**], **Haifa Pan, Yongjie Qi, Gaihua Qin, Chunyan Liu, Zhengfeng Chen**[***], **Bo Li**[***] **and Yiliu Xu**[*]

Key Laboratory of Genetic Improvement and Ecophysiology of Horticultural Crops, Institute of Horticulture, AAAS, Hefei 230031, China

ABSTRACT

Pear is an economically important fruit worldwide. We recently reported a crispy texture pear cultivar 'Xiang Mian Li' (XML) with intense aroma as a good candidate for Chinese white pear breeding. Chinese white pears usually have faint odor, thus it's interesting to uncover the molecular mechanisms for the development of aroma in 'XML'. Freshly plucked faint odor 'XML' fruit (XML-FA) and postharvest ripened fruit with intense aroma (XML-IA) were studied through RNA-Seq. A total of 3758 differentially expressed genes (DEGs) were found between XML-FA and XML-IA, which significantly enriched in 288 Gene Ontology (GO) terms and 101 KEGG pathways. Besides the expected "Plant hormone signal transduction" and "ethylene biosynthetic process", changes in volatile biosynthesis related pathways were also found. Highly expressed *LOX* and *lipase* were found to be the most important genes involved in the characteristic aroma formation of 'XML', suggesting the great role of membrane lipid hydrolysis in the mechanism of aroma formation in 'XML' pear. Additionally, *PDC2*, *ADH*, *FAD*, *PLD* and *4CL* were also closely related to the formation of 'XML' aroma. Our data can be applied to improve practically the breeding program for elite Chinese white pear cultivars.

Key words: *Pyrus bretschneideri*, transcriptome analysis, aroma formation, volatile compound biosynthesis, differentially expressed genes.

INTRODUCTION

Flavour is one of the most important factors that determines fruit quality and consumer acceptability. There are four main cultivated species of pear in China, each with distinct flavour and taste. *Pyrus ussuriensis*, which needs postharvest ripening, is the widest consumed pear with rich aroma, however, it has a shorter shelf life than other pears. *P. bretschneideri*, a widely consumed pear species, is famous for its big fruit size and crispy texture, but has faint odor (Teng and Tanabe, 16). Recently, a new pear cultivar 'Xiang Mian Li' (XML) has been reported having characteristics of both *P. ussuriensis* and *P. bretschneideri*. It has attractive aroma, crispy texture, big fruit size and long shelf-life. Previous study showed that 'XML' volatiles had four esters, three aldehydes, two alcohols, two hydrocarbons and D-limonenel. Volatiles based phylogenetic cluster showed its close affinity towardss *P. bretschneideri*, thus this seems a good candidate cultivar for white pear breeding programme (Yi *et al*., 18).

More than 300 volatile compounds have been detected and identified, including esters, alcohols, terpenoids (Arrieta-Garay *et al*., 1; Rapparini and Predieris, 13) from pears. Esters, especially ethyl hexanotate are the dominant and odour contributing volatiles in *P. ussuriensis*, *P. serotina* and *P. communis* (Li *et al*., 8; Qin *et al*., 12; Yi *et al*., 18). Aldehydes, alcohols and esters are the main compounds in *P. bretschneideri* and *P. pyrifolia* (Chen *et al*., 3; Katayama *et al*., 6). Ethyl hexanotate has been reported to be the most abundant and odour contributor in XML (Yi *et al*., 18).

Biosynthesis of volatile esters is mainly from two unsaturated fatty acids, linoleic and linolenic acids, through lipoxygenase (LOX) with help of β-oxidation pathways (Shi *et al*., 14; Yi *et al*., 18). LOX enzymes catalyze unsaturated fatty acids to hydroperoxides, and then hydroperoxide lyases (HPLs) cleave hydroperoxides to C6 aliphatic aldehydes; alcohol dehydrogenases (ADHs) catalyze the reversible aldehydes to alcohols, and alcohols finally formed corresponding esters with the help of alcohol acyltransferase (AAT). Membrane lipids is an important precursor of unsaturated fatty acid during fruit postharvest ripening (Shi *et al*., 14), during which, phospholipase D (PLD) and lipase have been reported to play an important role (Sun *et al*., 15). Fatty acid desaturase (FAD), ADH, pyruvate decarboxylase isozyme (PDC), LOX, and lipase

*Corresponding author's E-mail: yxk79yl@163.com.
**Institute of Soil and Fertilizer, Hefei 230031, China
***Dangshan Horticulture Field of Anhui Province, Dangshan 235300, China

genes are reported to be vital for *P. ussuriensis* fruit aroma formation (Wei *et al*., 17), while the low expression of 18 LOX genes in 'DangShan Suli' (*P. bretschneideri*) has been thought to be the reason of the fruits with poor odor (Li *et al*., 9).

Transcriptome sequencing is an efficient technology for evaluating the differences between samples, especially caused by environmental factors. 'XML' needs a about 10 days postharvest ripening, which results in rich aroma, before marketing (Shi *et al*., 14; Wei *et al*., 17). In this study, we used the freshly harvested 'XML' fruit (XML-FA) and postharvest ripened 'XML' fruit (XML-IA) for transcriptomic analysis to uncover the molecular mechanisms of its aroma formation. Through differentially enriched GO terms and KEGG pathways, some volatile compounds biosynthetic pathways, e.g. LOX pathway and MEP pathway, were found. Through the analysis of relationship between DEGs and content of volatile compounds, *LOX* and *lipase* were found vital for its characteristic aroma formation, alongwith close relationship of *PDC2*, *ADH*, *FAD*, *PLD* and *4CL* with the formation of 'XML' aroma, but not *AAT* and the hydrolysis of volatile glycosides. Our data also provided valuable data for the research of small species of Chinese pears.

MATERIALS AND METHODS

Immature fruits of the local pear cultivar 'XML' were collected from the Center of Pear Germplasm Collection, Anhui, China. Fifty freshly harvested fruits were directly deep frozen in liquid nitrogen and stored at -80 °C for future control use. Another 50 fruits were put at room temperature 10d for postharvest ripening. And then postharvest ripened fruits were also deep frozen and stored at -80°C.

Total RNA was extracted from pear fruits using the RNAprep pure Plant Kit (TianGen, China). The quality and quantity of RNA were determined using both agarose gel and a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). RNA samples with $A_{260/280}$ ratio between 1.8 to 2.0, $A_{260/230}$ ratio between 2.0 to 2.2 and RNA integrity number more than 8.0, were used for transcriptomic sequencing. Purified RNA samples, done with polyT oligo-attached magnetic beads, were sent to Biomarker Technologies Corporation (Beijing, China) for cDNA library construction and sequencing. Enriched mRNA was fragmented and amplified using an Ovation RNA-Seq System V2 kit (Nugen Technologies, USA) following the manufacturer's protocols. Amplified 1 µg cDNA was used to generate cDNA libraries. Illumina HiSeq TM 2000 platform (Illumina, China) was used for sequencing. Each sample was done with three biological replicates.

The unigenes generated from the mixed pear samples were aligned against the six public libraries COG, GO, KEGG, Swissprot, TrEMBL and NR databases (E-value < = 1E-5) to retrieve functional annotations based on sequence similarity using BLASTX. All usable reads were normalized into FPKM values (Fragments Per Kilobase of transcript per Million fragments mapped) to show their transcript levels. Differentially expressed unigenes (DEGs) between the two samples were calculated based on "base mean" value obtained from the DESeq package, only unigenes with a Log_2 (fold change) > 1 and false discovery rate (FDR) < 0.05, were considered to be DEGs. Gene ontology (GO) analysis and visualization were conducted using the TopGO package. KOBAS software was used to test the DEGs enrichment in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (http://www.genome.jp/kegg/) (Wei *et al*., 17). The GO terms and KEGG pathways with p-values < 0.05 were considered significantly enriched.

qRT-PCR assays were performed on a CFX96 platform (Bio-Rad), using gene specific primers (Supplementary Table S1). Top Green qPCR SuperMix (TransGen, China) was used as mixed polymerase. PCR reaction efficiencies for all genes were more than 90%. Transcript levels were normalized to *Actin* (pyrus_GLEAN_10016054) using the $2^{-\Delta\Delta Ct}$ method. All experiments were done using three replicates and shown as means ± SD. Duncan's new multiple range test was performed for statistical significance analysis using Data Processing System (DPS) version 15.10 (http://www.chinadps.net/dps_eng/).

RESULTS AND DISCUSSION

The newly reported fragrance pear 'XML' formed intense aroma, which were mainly esters, after postharvest ripening (Yi *et al*., 18). Terpenoid compound, which rarely detected in pears, D-limonene was also found (Yi *et al*., 18). In order to understand the molecular mechanisms of the formation of these aroma volatiles, transcriptomic analysis was done for two pear samples, fruits directly plucked with faint aroma (XML-FA) and fruits with intense aroma after 10d postharvest ripening (XML-IA).

Accordingly, two cDNA libraries were constructed and sequenced. After removing the adaptors and low-quality sequences, 23,770,715 and 27,070,335 clean reads were obtained form XML-FA and XML-IA, respectively (Table 1). Q30 value was higher than 90% for each library, all the unigenes covered genome sequences approximately 65% for both samples (Table 1). The length distribution of unigenes had similar patterns among the two libraries (Fig. 1) as well as other transcriptomes.

Table 1. Summary of the RNA-seq data

Samples	XLM-FA	XML-IA
Total Reads	47,541,430	54,140,670
Clean reads	23,770,715	27,070,335
GC Content	46.82%	46.23%
Q30	90.07%	90.29%
Mapped Reads	65.33%	64.91%
Uniq Mapped Reads	57.82%	57.81%
Multiple Map Reads	7.51%	7.09%

Totally 42,868 assembled unigenes were annotated using six public libraries, including 915 newly identified unigenes.

The FPKM fold change of > 2 or < 0.5 (Log_2fold changed >1) and Q-value <0.01 (Benjamini and Hochberg, 2) between two libraries was chose as differentially expressed genes (DEGs), a total of 3758 DEGs were found between the two groups. GO analysis comprised of three parts, namely Biological Process (BP), Cellular Component (CC) and Molecular Function (MF). GO BP analysis revealed 120 altered GO terms (P<0.001) (Supplementary

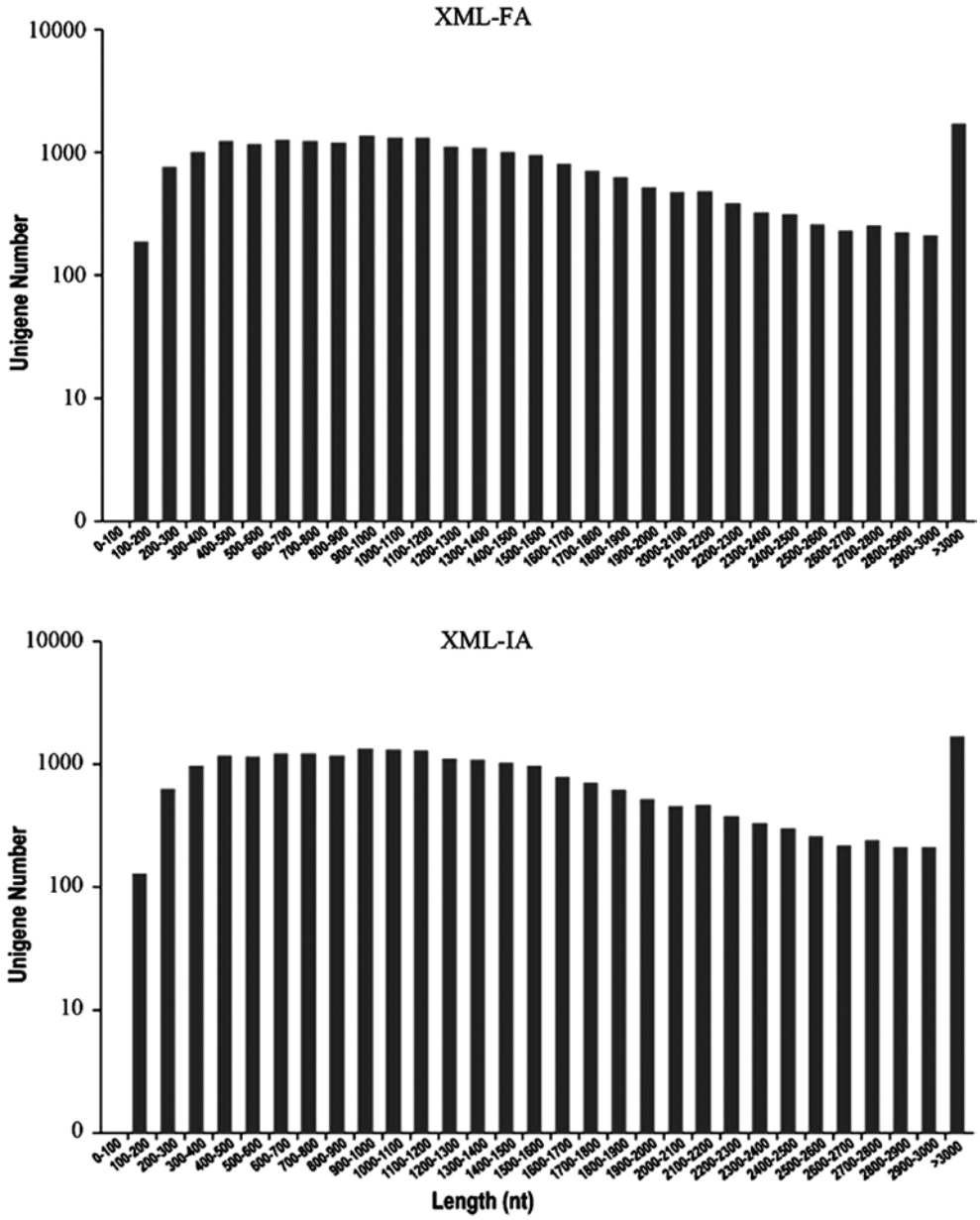

Fig. 1. Length distribution of unigenes in the assembled transcriptome data

Table 2. Top 10 of GO terms Biological process, Cellular component and Molecular function.

GO.ID	Term	P-value
	Biological process	
GO:0015074	DNA integration	7.50E-20
GO:0010200	Response to chitin	1.50E-08
GO:0010193	Response to ozone	1.50E-07
GO:0009863	Salicylic acid mediated signaling pathway	4.30E-07
GO:0002679	Respiratory burst involved in defense response	6.70E-07
GO:0010266	Response to vitamin B1	7.70E-07
GO:0006865	Amino acid transport	1.40E-06
GO:0055114	Oxidation-reduction process	3.00E-06
GO:0019745	Pentacyclic triterpenoid biosynthetic process	4.10E-06
GO:0010117	Photoprotection	6.60E-06
	Cellular component	
GO:0009506	Plasmodesma	0.00014
GO:0005886	Plasma membrane	0.00031
GO:0046658	Anchored component of plasma membrane	0.0004
GO:0009535	Chloroplast thylakoid membrane	0.00055
GO:0009521	Photosystem	0.00108
GO:0030931	Heterotetrameric ADPG pyrophosphorylase complex	0.00173
GO:0005576	Extracellular region	0.0024
GO:0044431	Golgi apparatus part	0.00248
GO:0009505	Plant-type cell wall	0.00261
GO:0048046	Apoplast	0.0027
	Molecular function	
GO:0015020	Glucuronosyltransferase activity	0.000000004
GO:0042299	Lupeol synthase activity	0.000000055
GO:0050403	*trans*-Zeatin O-beta-D-glucosyltransferase activity	0.00000017
GO:0050502	*cis*-Zeatin O-beta-D-glucosyltransferase activity	0.00000017
GO:0090438	Camelliol C synthase activity	0.00000071
GO:0042300	β-Amyrin synthase activity	0.00000092
GO:0043531	ADP binding	0.0000048
GO:0003960	NADPH:quinone reductase activity	0.0000068
GO:0042561	α-Amyrin synthase activity	0.000011
GO:0043864	Indoleacetamide hydrolase activity	0.000014

Table S2) between faint odor and intense aroma pear, including “respiratory burst involved in defense response” (Table 2) and “ethylene biosynthetic process” (GO ID: 0009693, P=0.00039), suggesting the differences between the two samples were truly caused by postharvest ripening. GO CC revealed 93 terms (P<0.001), including “plasmodesma”, “plasma membrane” and “chloroplast thylakoid membrane”. Previous reports have shown that the degradation of the membrane systems contributed a lot on pear aroma formation during postharvest ripening (Liu *et al*., 11; Shi *et al*., 14). GO MF had 75 altered GO terms (P<0.001), including “glucuronosyltransferase activity” and several sesquiterpene biosynthase activities. Moreover, GO terms “geranyl diphosphate (GPP) metabolic process” (GO:0033383, P=4.80E-05), “farnesyl diphosphate metabolic process” (GO:0045338, P=0.00049), “sesquiterpene

biosynthetic process" (GO:0051762, P=0.00986) and "phospholipid biosynthetic process" (GO:0008654, P=0.0059) were found altered between XML-IA and XML-FA in GO BP, GPP is the substrate of monoterpene biosynthesis, e.g. D-limonene; membrane phospholipids hydrolyzed to free fatty acids gave plenty of substrates for pear esters formation (Liu *et al*., 11), thus these altered GO terms were consistent with their different aroma.

As shown in the 50 chosen pathways, "Ribosome" and "Plant hormone signal transduction" were the most affected (Fig. 2). In addition, DEGs involved in "Phenylpropanoid biosynthesis" and "Fatty acid metabolism", which related to volatile compounds formation (Dudareva *et al*., 4), were also found.

In order to understand the mechanism of the formation of the characteristic aroma of XML, DEGs that may be related to aroma formation were also identified. As previously reported, α-linolenic acid metabolism was thought to be vital for pear aroma formation. Accordingly, *LOX* (Li *et al*., 9; Wei *et al*., 17), *ADH* and *AAT* (Li *et al*., 9) , fatty acid desaturase (FAD), pyruvate decarboxylase isozyme (PDC) and *lipase* (Wei *et al*., 17), phospholipase D (PLD) (Shi *et al*., 14) in pear aroma formation. Thus, in this study, whether these genes were differentially expressed were checked one by one. Results showed that no differentially expressed *AAT* was found. Three ω-3 *FAD* and one ω-6 *FAD* were found differentially expressed (Table 3). Lee *et al*. (9) reported that ω-6 and ω-3 FAD played a key role in the synthesis of the unsaturated fatty acids linoleic (18:2) and linolenic (18:3) acids. The 3 up-regulated and 1 down-regulated *FAD* consistent with the higher volatile aroma contents in XML-IA compared with XML-FA. One pyruvate decarboxylase isozyme 2 (PDC2) gene was found. Enhanced PDC activity was correlated with ethanol production in 'Packham's Triumph' pears during fruit ripening, hence the increased transcript level of *PDC2* in this study may have a significant contribution to the aroma formation. Two *PLD* genes and three *ADH* genes were found (Table 3), PLD activity was found positively correlated to the concentration of volatile compounds while ADH was negatively (Shi *et al*., 14). In this study, *PLD* genes in XML-IA were all higher expressed than in XML-FA, while *ADH* genes were lower expressed (Table 3). These data suggested that *PLD* and *ADH* may contributed to 'XML' aroma formation.

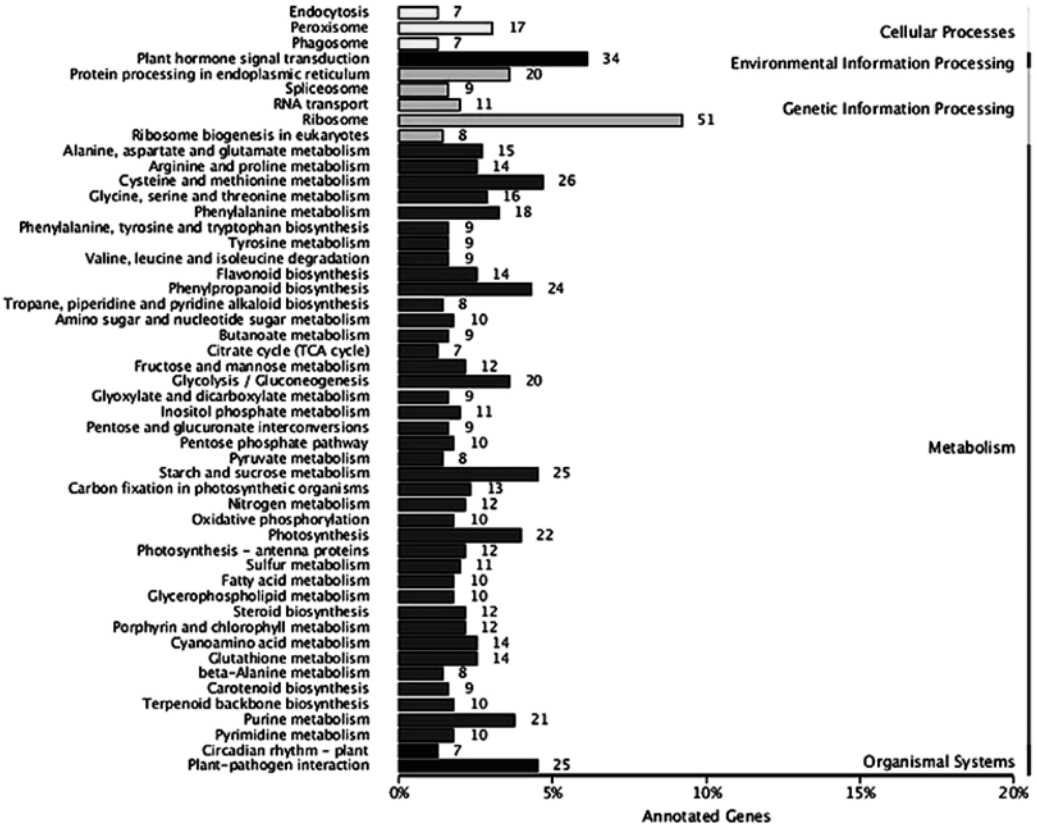

Fig. 2. Most enriched 50 KEGG pathways between XML-FA and XML-IA.

Table 3. DEGs that may involved in volatile aroma compounds biosynthesis.

Gene ID	Annotation	FPKM		XML-IA/ XML-FA
		XML-FA	XML-IA	
	ADH			
pyrus_GLEAN_10041154	Alcohol dehydrogenase	53.65	1.96	0.04
pyrus_GLEAN_10031279	Alcohol dehydrogenase	659.66	24.78	0.04
pyrus_GLEAN_10041153	Alcohol dehydrogenase 1	1.96	0.36	0.19
	AOS			
pyrus_GLEAN_10042093	Allene oxide synthase	2.51	30.92	12.32
	FAD			
pyrus_GLEAN_10024105	Omega-3 fatty acid desaturase	0.23	5.84	25.49
pyrus_GLEAN_10003625	Omega-3 fatty acid desaturase	1.07	0.00	0.00
pyrus_GLEAN_10011425	Omega-3 fatty acid desaturase	3.15	17.85	5.67
pyrus_GLEAN_10041336	Omega-6 fatty acid desaturase	48.58	198.28	4.08
	Lipase			
pyrus_GLEAN_10019923	GDSL esterase/lipase At1g54790	23.78	0.52	0.02
pyrus_GLEAN_10040106	GDSL esterase/lipase EXL3	12.31	197.36	16.03
pyrus_GLEAN_10008276	GDSL esterase/lipase At4g10955	13.87	0.15	0.01
pyrus_GLEAN_10033779	GDSL esterase/lipase 1	0.00	2.23	UP
pyrus_GLEAN_10032411	GDSL esterase/lipase At3g26430	6.09	0.53	0.09
pyrus_GLEAN_10011288	GDSL esterase/lipase 2	0.00	10.78	UP
pyrus_GLEAN_10040107	GDSL esterase/lipase EXL3	3.09	17.49	5.66
CUFF44.37.1	GDSL esterase/lipase 1	20.34	3.89	0.19
pyrus_GLEAN_10011292	GDSL esterase/lipase 1	3.85	0.00	0.00
pyrus_GLEAN_10029420	GDSL esterase/lipase At5g62930	13.63	0.56	0.04
pyrus_GLEAN_10015049	GDSL esterase/lipase At4g28780	1.65	0.00	0.00
pyrus_GLEAN_10034649	GDSL esterase/lipase At2g42990	1.74	10.12	5.82
pyrus_GLEAN_10032408	GDSL esterase/lipase At3g26430	14.26	0.78	0.05
CUFF5.33.1	GDSL esterase/lipase LTL1	4.59	0.00	0.00
pyrus_GLEAN_10013363	GDSL esterase/lipase At1g09390	3.20	0.14	0.04
pyrus_GLEAN_10039600	GDSL esterase/lipase CPRD49	34.55	1.98	0.06
pyrus_GLEAN_10018874	GDSL esterase/lipase At1g29670	3.26	0.00	0.00
	LOX			
pyrus_GLEAN_10032527	Lipoxygenase 6	7.43	0.07	0.01
pyrus_GLEAN_10027674	Linoleate 13S-lipoxygenase 2-1	102.46	14.72	0.14
pyrus_GLEAN_10026046	Linoleate 9S-lipoxygenase 5	0.43	0.00	0.00
pyrus_GLEAN_10017638	Linoleate 9S-lipoxygenase 5	0.05	8.20	161.77
pyrus_GLEAN_10026903	Linoleate 9S-lipoxygenase 5	0.03	0.64	21.61
pyrus_GLEAN_10027670	Linoleate 13S-lipoxygenase 2-1	2.78	31.05	11.17
pyrus_GLEAN_10001337	Linoleate 13S-lipoxygenase 3-1	1.27	20.29	15.93
pyrus_GLEAN_10037931	Linoleate 13S-lipoxygenase 3-1	0.71	3.37	4.76
	PDC			
pyrus_GLEAN_10017851	Pyruvate decarboxylase isozyme 2	13.44	1703.04	126.75
	PLD			
pyrus_GLEAN_10025597	Phospholipase D epsilon	0.00	29.82	UP
pyrus_GLEAN_10011385	Phospholipase D p1	2.09	9.08	4.34
	4CL			
pyrus_GLEAN_10040062	4-coumarate--CoA ligase-like 5	6.03	38.51	6.39

[a]UP means invalid calculation.

It was noteworthy that 8 *LOX* genes and 17 *lipase* DEGs were found (Table 3), LOX is the key enzyme towards esters biosynthesis using unsaturated fatty acids, and lipase is an important enzyme that catalyze the hydrolysis of membrane phospholipid to produce unsaturated fatty acids (Li *et al*., 8; Liu *et al*., 11; Shi *et al*., 14; Wei *et al*., 17; Yi *et al*., 18). Ester ethyl hexanoate was the most contributor of XML aroma through the Odor Active Value (OAV) analysis (Yi *et al*., 18). These data suggested a great role of the two genes on the formation of the characteristic aroma of XML through postharvest ripening. Some of these two genes were higher expressed in XML-IA compared with XML-FA, but others were lower expressed (Table 3), this may because of their different expression pattern and/or functions as found in other pears (Li *et al*., 8). Low *LOX* transcript levels were thought to be the reason of faint odor of Chinese white pear (*Pyrus bretschneideri*) (Li *et al*., 9), then highly expressed pyrus_GLEAN_10027670 and pyrus_GLEAN_10001337, which has high FPKM value (Table 3), can be very important for the formation of XML aroma. Twelve out of the 17 differentially expressed *lipase* genes were lower expressed in XML-IA than in XML-FA (Table 3). Lipase has similar role with PLD in membrane phospholipid hydrolysis (Sun *et al*., 15) and lipase activity was found positively correlated with aroma compound concentration. In this case, either *lipase* did not contribute to XML aroma formation or pyrus_GLEAN_10040106, which has a extremely high FPKM compared to others (Table 3), has a main function on XML aroma formation. Furthermore, two genes involved in α-linolenic acid metabolism were found higher expressed in XML-IA compared to XML-FA through KEGG analysis, named allene oxide synthase (pyrus_GLEAN_10042093) and 4-coumarate: CoA ligase-like 5 (pyrus_GLEAN_10040062), strongly suggested their role in aroma esters formation in XML-IA.

D-Limonene was found in XML aroma compounds (Yi *et al*., 18), monoterpene can be derived from MEP pathway in chloroplast, and/or release from their glycosidically bound aroma through beta-glucosidase (Liu *et al*., 10). In this study, no differentially expressed beta-glucosidase gene was found but some genes involved in MEP pathway were found (Table 4). All MEP pathway genes as well as genes involved in "geranyl diphosphate metabolic process" (GO:0033383) were lower expressed in XML-IA. The homologues of (3*S*,6*E*)-nerolidol synthase 1 in tea can produce D-limonene *in planta* (Liu *et al*., 10). Furthermore, D-limonene did not contribute to sensory quality according to its OAV analysis. However, the relationship between the lower expressed genes and higher D-limonene content need further studies.

In order to validate the reliability of the transcriptome sequencing, the relative expression levels of 7 randomly selected genes were analyzed using qRT-PCR (Fig. 3). The results were consistent with the transcriptome data with a correlation of 0.974 (data not shown). For example, the gene encoding hypothetical protein PRUPE_ppa005432mg was up-regulated in both RNA-seq and qRT-PCR

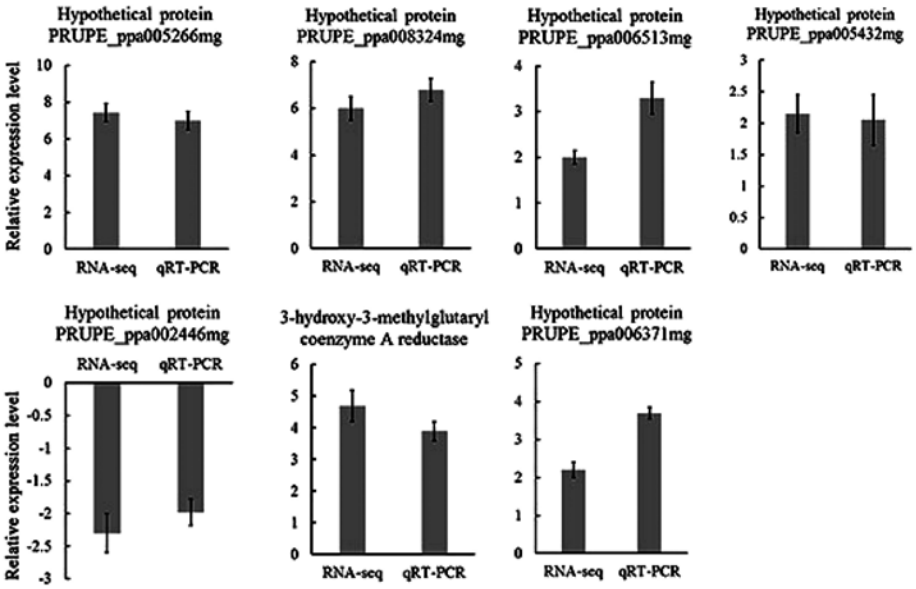

Fig. 3. Correlation between FPKM fold change and genes expression ratios.

Table 4. Relative FPKMs of DEGs involved in MEP pathway and GPP synthesis.

Gene ID	Name	FPKM		IA/ FA
		XML-FA	XML-IA	
pyrus_GLEAN_10014015	1-Deoxy-D-xylulose-5-phosphate synthase	11.81	2.70	0.23
pyrus_GLEAN_10028338	1-Deoxy-D-xylulose-5-phosphate synthase 2	6.11	0.10	0.02
pyrus_GLEAN_10013765	4-Hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	35.67	15.30	0.43
pyrus_GLEAN_10010839	(-)-Germacrene D synthase	3.67	0.63	0.17
pyrus_GLEAN_10001380	(3*S*,6*E*)-Nerolidol synthase 1	1.17	0.06	0.05

analyses, with a similar fold change. Therefore, the transcriptome data were reliable enough.

CONCLUSION

Transcriptomic analysis revealed a total of 3758 DEGs between XML-FA and XML-IA. These DEGs significantly enriched in 120 GO BP terms, 93 GO CC terms and 75 GO MF terms as well as 101 KEGG pathways. The most enriched GO CC terms showed a dramatic change happened to the membrane system. Detailed analysis on the DEGs revealed several pathways may involved in 'XML' pear aroma formation, 8 *LOX* genes together with *ADH*, *AOS* and *4CL* involved in unsaturated fatty acids metabolism and 17 *lipase* genes together with *PLD*, *FAD* and *PDC* involved in the hydrolysis of membrane lipid to produce unsaturated fatty acids. These data strongly suggested the great role of *LOX* and *lipase* together with other genes and the involvement of membrane lipid hydrolysis in 'XML' aroma formation. Q30 value and the good correlation between FPKM value and qRT-PCR results indicated the reliability of our transcriptome data. Our data provided valuable data for the research of other pears and can be applied to improve practically the breeding program for elite Chinese white pear cultivars.

ACKNOWLEDGEMENTS

This project is supported by funding from China pear industrial technology systemthe and Agriculture Research System of Anhui Province, and the Key Laboratory of Genetic Improvement and Eco-physiology of Horticultural Crop, Anhui Province,and Featured small berry innovation team of Anhui Academy of Agricultural Sciences.

The author declared no competing interest.

REFERENCES

1. Arrieta Garay Y., García Liobodanin L., Pérez Correa J.R., López Vázquez, C., Orriols, I. and López, F. 2013. Aromatically enhanced pear distillates from blanquilla and conference varieties using a packed column. *J. Agric. Food Chem.* **61**: 4936-42.
2. Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Sata. Soc. Series B (Methodological)*: 289-300.
3. Chen, J.L., Yan, S., Feng, Z., Xiao, L. and Hu, X.S. 2006. Changes in the volatile compounds and chemical and physical properties of Yali pear (*Pyrus bertschneideri* Reld) during storage. *Food Chem.* **97**: 248-55.
4. Dudareva, N., Klempien, A., Muhlemann, J. K. and Kaplan, I. 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol.* **198**: 16-32.
5. Jiang, S., Zong, Y., Yue, X., Postman, J., Teng, Y., and Cai, D. 2015. Prediction of retrotransposons and assessment of genetic variability based on developed retrotransposon-based insertion polymorphism (RBIP) markers in *Pyrus* L. *Mol. Genet. Genomics*, **290**: 225-37.
6. Katayama, H., Ohe, M. and Sugawara, E. 2013. Diversity of odor-active compounds from local cultivars and wild accessions of Iwateyamanashi (*Pyrus ussuriensis* var. aromatica) revealed by Aroma Extract Dilution Analysis (AEDA). *Breeding Sci.* **63**: 86-95.
7. Lee, K.R., Kim, S.H., Go, Y.S., Jung, S.M., Roh, K.H., Kim, J.B., Suh, M.C., Lee, S. and Kim, H.U. 2012. Molecular cloning and functional analysis of two *FAD2* genes from American grape (*Vitis labrusca* L.). *Gene*, **509**: 189-94.
8. Li, G., Jia, H., Li, J., Wang, Q., Zhang, M. and Teng, Y. 2014a. Emission of volatile esters and transcription of ethylene-and aroma-related genes during ripening of 'Pingxiangli' pear fruit (*Pyrus ussuriensis* Maxim). *Sci. Hort.* **170**: 17-23.

9. Li, M., Li, L., Dunwell, J. M., Qiao, X., Liu, X. and Zhang, S. 2014b. Characterization of the lipoxygenase (LOX) gene family in the Chinese white pear (*Pyrus bretschneideri*) and comparison with other members of the Rosaceae. *BMC Genomics*, **15**: 444.

10. Liu, G.F., Liu, J.J., He, Z.R., Wang, F.M., Yang, H., Yan, Y.F., Gao, M.J., Gruber, M.Y., Wan, X.C. and Wei, S. 2018. Implementation of CsLIS/NES in linalool biosynthesis involves transcript splicing regulation in *Camellia sinensis*. *Plant Cell Environ.* **41**: 176-86.

11. Liu, H., Song, L.L., You, Y.L., Li, Y.B., Duan, X.W., Jiang, Y.M., Joyce, D.C., Ashraf, M. and Lu, W. 2011. Cold storage duration affects litchi fruit quality, membrane permeability, enzyme activities and energy charge during shelf time at ambient temperature. *Postharvest Biol. Tech.* **60**: 24-30.

12. Qin, G., Tao, S., Cao, Y., Wu, J., Zhang, H., Huang, W. and Zhang, S. 2012. Evaluation of the volatile profile of 33 *Pyrus ussuriensis* cultivars by HS-SPME with GC–MS. *Food Chem.* **134**: 2367-82.

13. Rapparini, F. and Predieri, S. 2003. Pear fruit volatiles. *Hort. Rev.,* **28**: 237-24.

14. Shi, F., Zhou, X., Zhou, Q., Tan, Z., Yao, M.M., Wei, B.D. and Ji, S.J. 2018. Membrane lipid metabolism changes and aroma ester loss in low-temperature stored Nanguo pears. *Food Chem.* **245**: 446-53.

15. Sun, J., You, X., Li, L., Peng, H., Su, W., Li, C., He, Q. and Liao, F. 2011. Effects of a phospholipase D inhibitor on postharvest enzymatic browning and oxidative stress of litchi fruit. *Postharvest Biol. Tech.* **62**: 288-94.

16. Teng, Y. and Tanabe, K. 2002. Reconsideration on the origin of cultivated pears native to East Asia. In *XXVI International Horticultural Congress: IV International Symposium on Taxonomy of Cultivated Plants*, ...: 175-182.

17. Wei, S., Tao, S., Qin, G., Wang, S., Tao, J., Wu, J., Wu, J. and Zhang, S. 2016. Transcriptome profiling reveals the candidate genes associated with aroma metabolites and emission of pear (*Pyrus ussuriensis* cv.). *Sci. Hort.* **206**: 33-42.

18. Yi, X.K., Liu, G.F., Rana, M.M., Zhu, L.W., Jiang, S.L., Huang, Y.F., Lu, W.M., and Wei, S. 2016. Volatile profiling of two pear genotypes with different potential for white pear aroma improvement. *Sci. Hortic-AMSTERDAM.* **209**: 221-228.

Received : October, 2019; Revised : February, 2020; Accepted : March, 2020