Original article

Microarray analysis of gene expression profile induced by pre-storage application of oxalic acid in tomato fruit under chilling stress

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Summary

To further illuminate the mechanism of oxalic acid (OA) alleviating chilling injury in tomato fruit under chilling stress, the differentially expressed genes were screened by using Affymetrix tomato GeneChip arrays after the mature-green tomato fruit were dipped in 10 mM OA solution for 10 min and then stored at $4 \pm 0.5^\circ\text{C}$ for 20 days. The results showed that 1,623 genes (540 up-regulated and 1,083 down-regulated genes) with more than two-fold differences in expression level were identified between OA treatment and the control. Differentially expressed genes mainly participate in signal transduction, defense, stress, primary metabolism, energy metabolism, cell-wall related, transcription related, protein metabolism and carotenoid metabolism. All differentially expressed genes were annotated by Gene Ontology (GO) annotation based on biological process, molecular functions and cellular components. Based on the level of molecular function, differentially expressed genes were found to be involved in binding, catalytic activity, lyase activity and peroxidase activity, while according to biological process categorization, those genes were involved in defense response, stress response, metabolic process, oxidation-reduction process, phosphorylation and cellular process. In addition, OA treatment could obviously up-regulate 1-aminocyclopropane-1-carboxylate synthase (ACS), 1-aminocyclopropane-1-carboxylate oxidase 1 (ACO), heat shock protein (HSP) and phosphoenolpyruvate carboxylase kinase gene and succinodehydrogenase gene expression, down-regulate pathogenesis-related proteins gene, sucrose synthetase and expansin gene expression. It was suggested that OA treatment improved the chilling tolerance in postharvest tomato fruit during storage at chilling temperature, due mainly to regulate genes expression such as ethylene biosynthesis, heat shock protein, pathogenesis-related protein and energy metabolism.

Keywords

differentially expressed gene, energy metabolism, ethylene, heat shock protein, pathogenesis-related protein

Significance of this study

What is already known on this subject?

- Tomato fruit is susceptible to chilling injury when continuously exposed to low temperature. Oxalic acid treatment could alleviate chilling injury in many postharvest fruits such as mango, kiwifruit and Hami melon. Previous works have established that oxalic acid treatment also could reduce chilling injury in tomato fruit and maintain its quality. However, the specific mechanism is still not clear, especially in the differentially expressed genes in tomatoes caused by oxalic acid treatment.

What are the new findings?

- Oxalic acid treatment could reduce chilling injury and cause differential genes expression in tomato fruit during cold storage. Differentially expressed genes mainly participate in signal transduction, defence, stress, primary metabolism, energy metabolism, cell-wall related, transcription related, protein metabolism and carotenoid metabolism. Oxalic acid might attribute to regulating tomato genes expression such as ethylene biosynthesis, heat shock protein, pathogenesis-related protein and energy metabolism, thereby alleviating chilling injury in cold storage.

What is the expected impact on horticulture?

- The results of this study identified the differentially expressed genes associated with chilling injury in oxalic acid treated tomato fruit during cold storage, which could provide the research basis for controlling chilling injury in tomato fruits and other cold sensitive fruits.

Introduction

Tomato fruit (*Solanum lycopersicum* L.) is susceptible to chilling injury when stored at low temperature for a long time, which results in reduced fruit quality and shelf life. Application of exogenous chemicals such as melatonin (Jannatizadeh, 2019), 2,4-epibrassinolide (Gao et al., 2016), methyl jasmonate (Wang et al., 2015) and γ -aminobutyric acid (Wang et al., 2014) have shown efficacy in reducing chilling injury in harvested banana, pomegranate, peach, tomato and banana fruit. OA is an organic acid occurring widely in various organisms, especially in plants, and plays different roles in living organisms such as controlling tissue browning, inducing systemic resistance, retarding ripening and controlling decay.

Many studies have reported that pre-storage application of OA delays ripening and senescence process in tomato (Kant et al., 2013), mango (Zheng et al., 2007a), peach (Zheng et al., 2007b) and kiwi fruit (Zhang et al., 2006). Interestingly, pre-storage application of OA alleviates the chilling injury in harvested mango (Ding et al., 2007; Xue et al., 2012; Li et al., 2014), peach fruit (Jin et al., 2014), sweet persimmon (Li et al., 2018), Hami melons (Wang et al., 2018) and tomato fruit (Li et al., 2016) under chilling stress, which collectively attributes to enhancing membrane integrity, increasing antioxidant capacity and energy status. Thus, the application of OA is a promising method for improving chilling tolerance and alleviating chilling injury in chilling-sensitive fruit during storage at lower temperature.

Microarray analysis has become an indispensable tool for understanding the biological processes elicited during biotic or abiotic stresses in genomes. The microarray technique has been used mainly for identification of differentially expressed genes in fruit development or preservation (Li et al., 2012). For example, the different expression genes in the tomato fruit during storage in response to postharvest UV-C irradiation (4 kJ m^{-2}) have been identified by using the microarray technique (Liu et al., 2012). However, no information is available concerning transcript modification in tomato fruit in response to the application of OA under chilling stress. Therefore, to further clarify the mechanism of OA alleviating chilling injury in fruit, the objective of this study was to analyze the gene expression profile in tomato fruit treated with OA under chilling stress, and then to identify the differentially expression genes associated with chilling tolerance in tomato fruit.

Materials and methods

Fruit material and experiments

Tomato fruits (*Solanum lycopersicum* L. cv. Oumeiyuan) were harvested at mature-green stage from a farm in Hangzhou, China. The harvest fruits were selected for uniformity of shape and color. 120 tomato fruits without mechanical damage were randomly divided into two groups. One group was dipped in 10 mM OA solution at room temperature for 10 min (OA-treated), while the other was dipped in water (as control). After air drying, tomatoes were placed inside clean plastic boxes wrapping in a 0.02-mm-thick polyethylene

bag and were held in low temperature humidity chambers at $4 \pm 0.5^\circ\text{C}$ for 20 days. Each treatment was replicated three times. Analysis in triplicate of ten fruits each was undertaken at 20 days cold storage for subsequent GeneChip analyses.

Extraction of RNA

Total RNAs were extracted from approximately 2 g of frozen tissue using mirVana™ RNA Isolation Kit (Applied Biosystem p/n AM1556), and were purified by QIAGEN RNeasy® Kit. Quantification and integrity of extracted total RNA were assessed by Bioanalyzer (Agilent 2100, USA) and capillary electrophoresis (Agilent G1602A, USA), respectively.

GeneChip experiments and data analysis

Differentially expressed genes of tomato fruit with pre-storage application of OA under chilling stress were identified by using Affymetrix tomato GeneChip arrays. Hybridization temperature was 45°C , hybridization speed was 60 rpm, and hybridization time was 16 h. The chip was scanned by Affymetrix GeneChip Scanner 3000. The differentially expressed genes of tomato fruit were classified by Gene Ontology.

Quantitative real-time PCR (qPCR)

Total RNA from tomato fruit was extracted using a RNA extraction kit. Six differentially expressed genes were selected for mRNA quantification of expression levels by real-time PCR. The primer sequences of the six genes are listed in Table 1. Reaction mix for all RT-PCR were performed in a final volume of 25 μL containing 1.0 μL cDNA, 10.5 μL H_2O_2 , 12.5 μL SYBR Premix Ex Taq™, 0.5 μL PCR-F (10 μM) and 0.5 μL PCR-R (10 μM). The qPCR amplification protocol consisted of a denaturation step at 95°C for 1 min, followed by 40 cycles of 95°C for 10 s and 62°C for 25 s. To check the annealing specificity of each oligonucleotide, melting curve analysis ($55 \sim 95^\circ\text{C}$) was carried out at the end of amplification. All reactions were performed with three replicates. The relative expression levels of selected unigenes normalized to 18srRNA was calculated using $2^{-\Delta\Delta\text{Ct}}$ method.

Statistical analysis

All data were expressed as mean values $\pm$ SE and were analyzed by one-way analysis of variance (ANOVA) using with SPSS Version 14.0. Differences were considered to be significant if p value was smaller or equal to 0.05.

TABLE 1. Sequence of primers used for gene expression analysis by qPCR.

Gene	Accession	Primer sequences(5' to 3')	Size (bp)	Annealing ($^\circ\text{C}$)
Tomato HSP21	NM_001246971.1	AAATGTTAGCGTTGGTGAACCTC GTAGCCCGAGCTACCAGCCTAGT	114	63
Tomato CHI9	NM_001247474.1	CAAGGTAGCCCTGGCGATTACT GCTCTTCCACATGGCCCATAGTT	125	63
Tomato LOC543878	NM_001247422.2	AATTTTGATGGTGTGGTAGAGGTT GCCGAAAGACATAGGGATGTTGA	180	63
Tomato LOC543987	NM_001247876.1	ACATTTACAAAGCAATCGGTGAAGC CATCGTTCTCAAATGAACCCTGTGA	120	63
Tomato PR1b1	NM_001247385.1	AAGTCGGAGTCGGGCTATGT GCTGCCCTCCCCGTGAAGT	151	63
Tomato LOXD	XM_004235501.1	ACGATCCCAGATATGCGGTGT GTCCAAGTAGACGGTTGCTGTCTT	166	63
Tomato 18S (reference gene)	JX444500	GCTCAAAGCAAGCCCAAGCTCT CCGAAACCGAACGCCAACGCAA	76	63

TABLE 2. Differentially expressed genes number and fold change in tomato fruit treated by OA after stored at 4°C for 20 days.

Differentially expressed genes	Gene number			Fold change		
	Total	Known	Unknown	> 10	5–10	< 5
All	1,623	243	1,380	152	157	1,314
Up-regulated	540	85	455	46	53	441
Down-regulated	1,083	158	925	106	104	873

Results

Gene expression microarrays analysis in tomato fruit with OA treatment stored for 20 days at 4°C

The gene expression profile microarray that contained a total of 9,254 expressed sequence tags and printed in duplicate spots were used to identify differentially expressed genes, and 1,623 differentially expressed genes were screened out among the 9,254 genes ($P < 0.05$). Differentially expressed genes number and fold change in tomato fruit stored for 20 days at 4°C were shown in Table 2. Comparative analyses between OA-treated and control, the results showed that 540 genes were up-regulated (85 known, 455 unknown) (Table 3), whereas 1,083 genes were down-regulated (158 known, 925 unknown) (Table 4). Among 85 up-regulation differentially expressed known genes, 3.53%, 5.88%, 15.29%, 14.12%, 4.71%, 11.76%, 8.24%, 4.71% and 1.18% were related to signal transduction, defense, stress, primary metabolism, energy metabolism, cell-wall related, transcription related, protein metabolism and carotenoid metabolism, respectively. Moreover, 6.69%, 7.59%, 3.16%, 12.03%, 3.16%, 13.29%, 5.70%, 10.13% and 0.63% of the down-regulation differentially expressed known genes respectively accounted for signal transduction, defense, stress, primary metabolism, energy metabolism, cell-wall related, transcription related, protein metabolism and carotenoid metabolism.

GO annotation and analysis of genes differentially expressed

BLAST2GO was utilized to separately annotate the differentially expressed gene sequences. Differentially expressed genes were classified into three different categories belonging to molecular function, biological process and cellular components according to level 2 (Table 5). On the categorization of molecular function, differentially expressed genes with known or predicted functions were classified into fifteen groups based on their similarities as shown in Table 5. The top four proportions of up/down-regulated differentially expressed genes were shown to involve in binding (50.67%), catalytic activity (14.67%), lyase activity (10.22%) and peroxidase activity (7.11%). Based on the level of biological process, a large number of genes were found to involve in defense response (3.62%), stress response (6.97%), metabolic process (42.08%), oxidation-reduction process (5.88%), phosphorylation (5.43%), and cellular process (3.62%), while according to cellular component categorization, the most of genes were involved in cytoplasm (22.39%), nucleus (20.90%), membrane (14.93%) and extracellular region (14.93%).

Identification of differentially expressed genes by qPCR

To confirm the findings of the microarray, six differentially expressed genes (4 up-regulated and 2 down-regulated) were randomly selected, and their expression patterns in OA treated and control tomato fruit stored for 20 days at

4°C were analyzed using qRT-PCR. As shown in Figure 1, qRT-PCR expression patterns of the six genes were in agreement with their microarray analysis results, which verified the consistency of the differentially expressed genes.

Discussion

Pre-storage application of OA has shown to be effective in reducing chilling injury in harvested tomatoes (Li et al., 2016). The present date showed that differentially expressed genes in tomato fruit with OA treatment under chilling stress by microarray technology, since 1,623 genes with more than two-fold differences, and 309 genes with more than five-fold differences in expression level were identified between OA treated fruit and the control. According to the annotation results the majority (67.42%) of differentially expressed genes with known functions corresponded to defense response, stress response, metabolic process, oxidation-reduction process, phosphorylation and cellular process at the biological process level; 82.67% and 73.15% of functionally assigned genes fell into the four categories of binding, catalytic activity, lyase activity and peroxidase activity as well as cytoplasm, nucleus, membrane and extracellular region, respectively. Therefore, the effect of OA in alleviating chilling injury in tomato fruit might attribute to its important role in regulating the expression of genes associated with defense response, stress response, metabolism process, oxidation-reduction process, phosphorylation and cellular process.

Ethylene, a kind of important plant growth regulator, could participate in adversity response process, under light, temperature and drought stress (Wang et al., 2019; Yu et al., 2019). Application of ethephon solution to normal 'Pearson' tomato seedlings led to enhancing ethylene biosynthesis rate and improving cold resistance at chilling temperature (Ciardi et al., 1997). Chen et al. found that treatment with ethylene before storage prevented chilling injury of tomato fruit during storage at chilling temperature, and prolonged the marketable life of tomatoes (Chen et al., 2008). Chen et al. further found that treatment by suitable concentration exogenous ethylene could increase the endogenous ethylene synthesis and improve cold tolerance of tomato fruit (Chen et al., 2008). From the above it was suggested that the endogenous ethylene biosynthesis was involved in the development of chilling tolerance. ACC Synthase (ACS) and ACC Oxidase (ACO) were rate-limiting enzymes of ethylene biosynthesis, and both of them were important for regulation of ethylene biosynthesis. In our study, ACS4 and ACO1 were found to be strongly up-regulated 22.74 and 2.11 times, while ACS1A, ACS6 and ACS2 were found to be obviously down-regulated 5.59, 3.80 and 3.40 times in OA treated tomato fruit, respectively. This finding suggested that ethylene biosynthesis in tomato fruit were significantly regulated by OA treatment, which might contribute to decrease chilling injury during cold storage.

Heat shock proteins (HSPs) are closely related to plant cold resistance, which enhance cold-resistant in plants during cold stress by alleviating cell membrane damage (Collins, 1993;

TABLE 3. Up-regulation differential expression genes in tomato fruit treated by OA after stored at 4°C for 20 days.

Entrez gene	UniGene ID	Gene title	Gene symbol	Fold
<i>Signal transduction (3)</i>				
544052	Les.11125	1-aminocyclopropane-1-carboxylate oxidase 1	ACO1	2.11
778356		1-aminocyclopropane-1-carboxylate synthase	ACS4	22.74
606712	Les.10341	Ethylene-responsive factor 1	ERF1	4.52
<i>Defense (5)</i>				
543758	Les.3462	Pi1 protein	PI1	10.44
543979	Les.84	NP24 protein precursor	LOC543979	2.69
543987	Les.3468	Beta-1,3-glucanase	LOC543987	12.66
543878	Les.13553	PR5-like protein	LOC543878	24.51
543591	Les.3751	Heat-induced chilling tolerance protein HCT6	HCT6	2.35
<i>Stress (13)</i>				
543955	Les.10341	Wound-induced proteinase inhibitor II prepeptide	LOC543955	2.00
543953	Les.9077	Proteinase inhibitor I	ER1	2.52
544031	Les.3483	Ethylene-responsive late embryogenesis-like protein	ER5	2.28
544130	Les.392	Glutathione peroxidase	GPXle-2	2.27
543577	Les.591	Ethylene-responsive catalase	ER60	2.85
543848	Les.4798	Type I small heat shock protein 17.6 kda isoform	LOC543848	2.15
544024	Les.13217	Heat shock protein	HSP21	8.31
544282	Les.3043	Class II small heat shock protein Le-HSP17.6	LOC544282	5.54
543846	Les.480	Small heat shock protein	VIS1	9.46
543580	Les.3584	ER6 protein	LOC543580	2.68
543574	Les.1892	Fruit-ripening protein	LOC543574	2.30
543576	Les.13764	Ethylene-responsive heat shock protein cognate 70	ER21	2.34
544180	Les.3738	Anaerobic basic leucine zipper protein	ABZ1	3.00
<i>Primary metabolism (12)</i>				
543818	Les.25631	Lipoxygenase	LOC543818	2.95
543997	Les.19838	Lipoxygenase	LOXB	2.13
544085	Les.19348	Histidine decarboxylase	HDC	4.34
544049	Les.21900	Protein kinase	PK7	2.37
100147727	Les.19516	Carbonic anhydrase	CA3	22.32
544236	Les.331	Alkaline alpha-galactosidase seed imbibition protein	SIP	5.23
544136	Les.3632	Uncharacterized LOC544136	LOC544136	7.98
544275	Les.22	Glutamine synthetase	LOC544275	2.07
100037489	Les.3762	6-deoxocastasterone oxidase	CYP85A1	3.94
100037489	Les.3967	6-deoxocastasterone oxidase	CYP85A1	7.79
544209	Les.3525	Ornithine decarboxylase	ODC	2.49
543736	Les.4317	Ss-galactosidase	TEG3	4.07
<i>Energy metabolism (4)</i>				
543568	Les.3766	Succinate dehydrogenase	LOC543568	2.90
543647	Les.1841	Light dependent NADH:protochlorophyllide oxidoreductase 2	POR2	4.06
544276	Les.3769	Isocitrate lyase	LOC544276	12.81
543633	Les.3523	Phosphoenolpyruvate carboxylase kinase	LOC543633	5.25
<i>Cell-wall related (10)</i>				
100134906	Les.3234	Beta-expansin precursor	LOC100134906	5.85
543876	Les.19392	SNF1 kinase complex anchoring protein	GAL83	2.04
543582	Les.13645	Expansin	EXP2	2.17
544289	Les.2560	Pectin esterase	PME2.1	3.34
544090	Les.3733	Pectin esterase	PME1.9	2.77
543853///778216	Les.141	Xyloglucan-specific fungal endoglucanase inhibitor protein precursor/// Xyloglucan-specific fungal endoglucanase inhibitor protein precursor	ACI25///Xegip	4.11
543515	Les.369	Beta-D-xylosidase 2	XYL2	2.83
544265	Les.2688	Xyloglucan endotransglucosylase-hydrolase XTH6	XTH6	7.43
543619	Les.3747	Xyloglucan endotransglycosylase lexet2	XET2	3.54
544148	Les.3619	Chitinase	CHI9	4.44

TABLE 3. Continued.

Entrez gene	UniGene ID	Gene title	Gene symbol	Fold
<i>Transcription related (7)</i>				
778357	Les.1409	Lysyl-trna synthetase	LYSRS	2.52
778200	Les.335	NAC domain protein	NAC-NOR	9.23
543711	Les.5	Dehydration responsive element binding protein	DREB1	2.52
543708	Les.2189	MADS-box transcription factor	MADS-RIN	24.50
544222	Les.13855	Ripening regulated protein DDTFR8	DDTFR8	2.14
544294		Transcription factor	THM27	2.92
100736457	Les.13	TCP transcription factor 14	TCP14	2.49
<i>Protein metabolism (4)</i>				
543536		Gamma-glutamylcysteine synthetase	GSH1	2.48
778274	Les.19018	Cysteine protease	CP	6.64
543836	Les.20919	Glutathione S-transferase-like protein	LOC543836	4.10
543628	Les.20918	Glutathione S-transferase/peroxidase	BI-GST/GPX	3.71
<i>Carotenoid metabolism (1)</i>				
543988	Les.3593	Phytoene synthetase	PSY1	2.64
Others (26)				
Unknown functions (455)				

TABLE 4. Down-regulation differential expression genes in tomato fruit treated by OA after stored at 4°C for 20 days.

Entrez gene	UniGene ID	Gene title	Gene symbol	Fold
<i>Signal transduction (11)</i>				
543517	Les.9493	Alpha-L-arabinofuranosidase	ARF1	2.11
544028	Les.1032	1-aminocyclopropane-1-carboxylate synthase	ACS1A	5.59
606302	Les.19087	1-aminocyclopropane-1-carboxylate synthase	ACS6	3.80
606304	Les.22659	Ripening-related ACC synthase 2	ACS2	3.40
543544	Les.10802	IAA10 protein	IAA10	9.89
543542	Les.4385	IAA7 protein	IAA7	2.10
543543	Les.11310	IAA9 protein	IAA9	5.49
543540	Les.19902	IAA3 protein	IAA3	2.02
543866	Les.8467	Ethylene response factor 2	ERF2	2.40
543527	Les.207	Jasmonic acid 1	JA1	12.22
543701	Les.13064	Auxin-regulated protein	LOC543701	2.81
<i>Defence (12)</i>				
544286	Les.9593	Metalloproteinase inhibitor	MCPI	49.87
544069	Les.19935	Pathogenesis-related protein P2	PR-P2	10.33
544123	Les.13247	PR protein	PR1B1	11.26
544185	Les.6883	Pathogenesis-related protein P4	P4	15.49
543551	Les.19537	Root-knot nematode resistance protein	MI-1.2	12.72
544210	Les.12860	Plant resistance protein	MI-1.1	8.00
543551///544210	Les.19818	Plant resistance protein///root-knot nematode resistance protein	MI-1.1///MI-1.2	2.70
543788	Les.11331	Hero resistance protein	HERO	5.30
544042	Les.13414	Pti5	LOC544042	3.74
544235	Les.8092	Dehydration responsive element binding protein	DREB2	2.87
544146	Les.7365	Chitinase	LOC544146	27.42
543989	Les.8276	Prosystemin	SYSTEMIN	4.55
<i>Stress (5)</i>				
543806	Les.19027	Alpha-dioxygenase 2	ALPHA-DOX2	3.81
544293	Les.13210	Peroxidase	CEVI16	12.97
544056	Les.13248	TAS14 peptide (AA 1-130)	TAS14	14.13
100736433	Les.3409	Heat shock protein cognate 70	HSC70	2.14
100301927	Les.12513	Salt responsive protein 1	SRG1	10.18

TABLE 4. Continued.

Entrez gene	UniGene ID	Gene title	Gene symbol	Fold
<i>Primary metabolism (19)</i>				
100134875	Les.9582	Glycolate oxidase	GLO2	2.27
543649	Les.11171	Chromoplast-specific lycopene beta-cyclase	LOC543649	3.86
100736464	Les.11650	Prephenate aminotransferase	PAT	2.33
543875	Les.25716	Asparagine synthetase	AS1	4.17
543660	Les.10881	Plasma membrane H ⁺ -atpase	LHA2	2.06
544068	Les.12723	Enolase	PGH1	7.75
543961	Les.19757	Sucrose synthase	LOC543961	2.41
100316873	Les.2073	Chloroplast sedoheptulose-1,7-bisphosphatase	SBPASE	2.14
543804	Les.12837	1,4-alpha-glucan-maltohydrolase	BMV3	2.07
544307	Les.2922	N-hydroxycinnamoyl-coa:tyramine N-hydroxycinnamoyl transferase THT7-8	THT7-8	9.90
543835	Les.9204	N-hydroxycinnamoyl-coa:tyramine N-hydroxycinnamoyl transferase THT7-1	THT7-1	4.53
544207	Les.19562	Amino acid transporter	AAP2	2.91
543529	Les.9593	Amino acid transporter	AAP1	16.09
778364	Les.3308	Alternaria stem canker resistance protein	ASC-1	2.86
544297	Les.8194	Beta-carotene hydroxylase	CRTR-B2	4.23
544009	Les.22384	Lipoxygenase	LOXD	4.58
544239	Les.9557	12-oxophytodienoate reductase	OPR1	2.29
543763	Les.19015	12-oxophytodienoate reductase 3	OPR3	2.08
544203	Les.3337	Omega-3 fatty acid desaturase	FAD7	4.39
<i>Energy metabolism (5)</i>				
543728	Les.3290	Hexose transporter protein	LOC543728	2.24
543737	Les.19240	Hexose transporter 1	HT1	8.83
544283	Les.463	ADP-glucose pyrophosphorylase large subunit	AGP-S1	4.81
544039	Les.19917	ADP-glucose pyrophosphorylase large subunit	AGPL3	3.92
543502	Les.4414	Acid invertase	WIV-1	5.31
<i>Cell-wall related (21)</i>				
778302	Les.3301	Pectinesterase	LOC778302	2.31
100134886	Les.2940	Expansin-like protein precursor	EXLA1	8.20
543557	Les.2940	Expansin A4	EXPA4	11.78
543558	Les.10205	Expansin	EXPA5	8.62
543794	Les.11375	Expansin11	EXP11	10.34
544159	Les.19070	Extensin	LOC544159	2.99
778277	Les.19861	Alpha-galactosidase	AGAL	3.26
543610	Les.9881	Beta-galactosidase	TBG7	38.83
543599	Les.3017	Polygalacturonase	XOPG1	3.25
544272	Les.2938	Endo-xyloglucan transferase	XTH1	5.24
543914	Les.12855	Xyloglucan endotransglucosylase-hydrolase XTH3	XTH3	4.74
543916	Les.2998	Xyloglucan endotransglucosylase-hydrolase XTH9	XTH9	4.25
544100	Les.2247	Xyloglycan endo-transglycosylase	TXET-B2	3.04
778213	Les.5930	Xyloglucan endotransglycosylase/hydrolase 16 protein	XTH16	3.20
543583	Les.1659	Endo-beta-1,4-D-glucanase	CEL8	3.75
543637	Les.1561	Xyloglucan endotransglycosylase	BR1	2.04
543607	Les.19592	Pirin	LOC543607	2.94
544160///544195	Les.3403	Arabinogalactan///uncharacterized LOC544160	AGP-1C/// LOC544160	2.51
544059	Les.4300	Extensin (class I)	LOC544059	3.27
778328	Les.3085	Methionine rich arabinogalactan	LOC778328	4.40
778266	Les.7292	Plant cell wall protein sltfr88	LOC778266	4.93

TABLE 4. Continued.

Entrez gene	UniGene ID	Gene title	Gene symbol	Fold
<i>Transcription related (9)</i>				
543857	Les.1409	WRKY transcription factor iid-3	WRKYIID-3	4.18
100191120	Les.1757	WRKY	LOC100191120	3.87
100736444	Les.2164	WRKY transcription factor	WRKY3	3.03
100736506	Les.19291	Heat stress transcription factor A3	HSFA3	2.18
543840	Les.2294	TAG1 transcription factor	LOC543840	2.19
544255	Les.2294	TDR6 transcription factor	LOC544255	2.27
544124	Les.10981	RNA-directed RNA polymerase	RDRP	2.02
544120	Les.244	Myb-related transcription factor	THM16	6.59
100736516	Les.22920	TCP transcription factor 5	TCP5	21.22
<i>Protein metabolism (16)</i>				
778229	Les.4300	Dehydroascorbate reductase	DHAR1	2.33
544268	Les.11716	Chloroplast-specific ribosomal protein	LOC544268	3.11
543756	Les.4849	Gts1 protein	GTS1	2.05
543537	Les.8398	Glutathione synthetase	GSH2	2.02
543622	Les.8625	Meiotic serine proteinase	TMP	10.61
544017	Les.1544	Leucine aminopeptidase	LAP2	2.06
778274	Les.2366	Cysteine protease	CP	2.56
778273	Les.10949	Phytophthora-inhibited protease 1	PIP1	12.94
543684	Les.6258	Auxin-regulated dual specificity cytosolic kinase	LOC543684	4.13
543561	Les.16905	LSTK-1-like kinase	LOC543561	2.44
544139	Les.16754	Cyclin-dependent protein kinase p34cdc2	CDC2A-1	2.06
100316879		Calcium-dependent protein kinase	LOC100316879	2.81
544184		3-phosphoinositide-dependent protein kinase-1	PDK1	3.19
543641		Uncharacterized LOC543641	LOC543641	4.62
543595	Les.11757	DnaJ-like protein	LOC543595	2.28
100037515	Les.7687	DNAJ-like protein	DNAJ	5.11
<i>Carotenoid metabolism (1)</i>				
543935	Les.12874	Carotenoid cleavage dioxygenase 1-1	CCD1-1	2.95
Others (59)				
Unknown functions (925)

FIGURE 1. Comparison of fold changes in differentially expressed genes between microarray and qPCR. The horizontal axis represents genes; the vertical axis represents fold changes; the red column represents microarray analysis; the blue-coloured column represents qPCR. Results were presented as mean $\pm$ standard deviation. Values with different letters for each differentially expressed gene were significantly different at $P < 0.05$.

TABLE 5. GO functional categories of known differential expression genes.

Ontology and GO term	Up-regulated genes of function annotation		Down-regulated genes of function annotation		All function annotation genes	
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
Molecular function	91	100	134	100	225	100
Binding	47	51.65	67	50.00	114	50.67
Catalytic activity	16	17.58	17	12.69	33	14.67
Lyase activity	7	7.69	16	11.94	23	10.22
Peroxidase activity	4	4.40	12	8.96	16	7.11
Transferase activity	6	6.59	4	2.99	10	4.44
Transport activity	5	5.49	2	1.49	7	3.11
Enzyme inhibitor activity	4	4.40	1	0.75	5	2.22
Protein dimerization activity	0	0.00	5	3.73	5	2.22
Protein kinase activity	2	2.20	2	1.49	4	1.78
Hormone activity	0	0.00	2	1.49	2	0.89
Isomerase activity	0	0.00	2	1.49	2	0.89
Nutrient reservoir activity	0	0.00	1	0.75	1	0.44
Structural constituent of cell wall	0	0.00	1	0.75	1	0.44
Ion channel activity	0	0.00	1	0.75	1	0.44
Ligase activity	0	0.00	1	0.75	1	0.44
Biological process	93	100	128	100	221	100
Defense response	1	1.08	7	5.47	8	3.62
Response to stress	10	10.75	5	3.91	15	6.79
Metabolic process	41	44.09	52	40.63	93	42.08
Oxidation-reduction process	2	2.15	11	8.59	13	5.88
Phosphorylation	2	2.15	10	7.81	12	5.43
Cellular process	4	4.30	4	3.13	8	3.62
Others	33	35.48	39	30.47	72	32.58
Cellular component	58	100	76	100	134	100
Cytoplasm	22	37.93	8	10.53	30	22.39
Nucleus	9	15.52	19	25.00	28	20.90
Membrane	6	10.34	14	18.42	20	14.93
Extracellular region	8	13.79	12	15.79	20	14.93
Cell wall	3	5.17	6	7.89	9	6.72
Chloroplast	5	8.62	7	9.21	12	8.96
Others	5	25.86	10	13.16	15	11.19

Yang et al., 2007). Salicylic acid treatment alleviated chilling injury in peaches fruit during cold storage might attribute to its ability to induce HSP101 and HSP73 (Wang et al., 2006). Heat shock treatment induced the expression of VcHSP17.7 in blueberry fruit, which was involved in enhancing chilling tolerance (Shi et al., 2017). Methyl jasmonate and methyl salicylate induced the accumulation of sHSP transcripts in tomato, especially class II sHSPs, which were involved in the protection of tomato fruit against chilling injury (Ding et al., 2001). The transgenic tomato plants that were transformed into the full-length endoplasmic reticulum small heat shock protein (ER-sHSP) cDNA, exhibited slighter cold-injured symptoms under chilling stress, which indicated HSPs played an important role in improvement of the chilling resistance in tomato plants (Zhao et al., 2006). Overexpression of chloroplast-localized small molecular heat shock protein could enhance chilling tolerance in tomato plant (Wang et al., 2005). Data here showed that heat shock protein, class II small heat shock protein, and small heat shock protein genes expression in OA treated tomato fruit were up-regulated 8.31, 5.54 and 9.46 times, respectively. It suggests that OA

treatment induced the expression of HSP, which may be involved in enhancing chilling resistance in postharvest tomato during storage at chilling temperature.

Pathogenesis-related proteins are also induced or increasingly expressed in postharvest fruit in response to pathogen infection and many environmental factors, which have attracted increasing attention due to being bound up with plant disease resistance. Salicylic acid treatment leads the pathogenesis-related protein to express and improve stress resistance of tomato fruit (Guo et al., 2012). OA treatment induces local and systemic resistance in cucumber against downy mildew (Li et al., 2006). In our work, pathogenesis-related proteins genes, such as PR-P2, PR₁-B1, PR-P4 and chitinase, were found to be strongly down-regulated (10.33, 11.26, 15.49 and 27.42 times, respectively) in OA treated tomato fruit, which suggested that OA treatment might decrease chilling injury in tomato fruit, and thus resulted in reducing expressing of pathogenesis-related protein. Our results were consistent with prior studies that OA treatment could reduce postharvest fruit decay incidence during cold storage (Zheng et al., 2007a).

Energy deficit impedes membrane integrity and results in chilling injury in postharvest fruit (Jin et al., 2014; Liang et al., 2017). Succinodehydrogenase is the key enzyme in sugar metabolic process, which is closely related to energy metabolism and plant stress resistance. Cold stress reduces the activity of succinodehydrogenase in postharvest fruit along with lower levels of energy, which results in the occurrence of chilling injury in fruit (Chen et al., 2012). Qi et al. (2012) have found that the chilling-tolerant tomato enhances higher level of total carbohydrate content to acclimate chilling stress under short-time low night temperature, while chilling-sensitive tomato responses to low temperature by decreasing it. Thus, it is suggested that sucrose-metabolizing enzymes have a closely correlation to low temperature response. Our present results showed that phosphoenolpyruvate carboxylase kinase gene and succinodehydrogenase gene were up-regulated by 5.25 and 2.90 times in OA treated fruit, respectively, while sucrose synthetase gene was down-regulated 2.41 times, indicating that OA could maintain high levels of energy status by regulating the key enzymes of sugar metabolism, which contribute to alleviating chilling injury in postharvest tomato fruit.

In conclusion, OA treatment resulted in significant change of gene expression in postharvest tomato fruit, and OA improved the chilling resistance in tomato fruit under low temperature stress by regulating genes expression of ACS, ACO, HSP, pathogenesis-related proteins and key enzymes of sugar metabolism.

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