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ORIGINAL ARTICLE

Transcriptome profiling of postharvest strawberry fruit in response to exogenous auxin and abscisic acid

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Abstract

Main conclusion **Auxin and abscisic acid regulate strawberry fruit ripening and senescence through cross-talk of their signal transduction pathways that further modulate the structural genes related to physico-chemical properties of fruit.**

The physiological and transcriptomic changes in harvested strawberry fruits in responses to IAA, ABA and their combination were analyzed. Exogenous IAA delayed the ripening process of strawberries after harvest while ABA promoted the postharvest ripening. However, treatment with a combination of IAA and ABA did not slow down nor accelerate the postharvest ripening in the strawberry fruits. At the molecular level, exogenous IAA up regulated the expressions of genes related to IAA signaling, including *AUX/IAA*, *ARF*, *TOPLESS* and genes encoding E3 ubiquitin protein ligase and annexin, and down regulated genes related to pectin depolymerization, cell wall degradation, sucrose and anthocyanin biosyntheses. In contrast, exogenous ABA induced genes related to fruit softening, and genes involved in signaling pathways including *SKP1*, *HSPs*, *CK2*, and *SRG1*. Comparison of transcriptomes in responses to individual treatments with IAA or ABA or the combination revealed that there were cooperative and antagonistic actions between IAA and ABA in fruit. However, 17 % of the differentially expressed unigenes in response to the combination of IAA and ABA were unique and were not found in those unigenes responding to either IAA or ABA alone. The analyses also found that receptor-like kinases and ubiquitin ligases responded to both IAA and ABA, which seemed to play a pivotal role in both hormones' signaling pathways and thus might be the cross-talk points of both hormones.

Keywords Abscisic acid · Auxin · *Fragaria* × *ananassa* · Postharvest senescence · Signal transduction · Transcriptome

Abbreviations

ABA	Abscisic acid
ABI3	Abscisic acid insensitive 3
AIP2	ABI-interacting protein 2
ARF	Auxin response factor
ARP	Auxin-repressed protein
BAG	Bcl-2 associated athanogene family
CAD	Cinnamyl alcohol dehydrogenase
CK	Casein kinase
DGE	Digital gene expression
GH3	Indole-3-acetic acid-amido synthetase
HSPs	Heat-shock proteins
IAA	Auxin
KO	KEGG Ortholog database
KOG/COG	Clusters of orthologous groups of proteins
NCED	9-cis-epoxycarotenoid dioxygenase
RLKs	Receptor-like kinases
SKP1	S-phase kinase-associated proteins 1

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VLCFA	Very-long-chain fatty acid synthesis
XT1	Xylosyltransferase 1
XTH	Xyloglucan endotransglucosylase/hydrolase

Introduction

The octoploid strawberry (*Fragaria × ananassa*) is one of the important economic fruit species, cultivated and consumed around the world for its pleasant flavor and nutritional values (Li et al. 2013). However, strawberry as a perishable fruit is prone to water loss, decay, physiological deterioration and fungal pathogen infection, due to its rapid ripening and senescence after harvest (Shin et al. 2008). The ripening and senescence in strawberry fruits are genetically programmed complex processes that involve many changes in gene expression and metabolism, including anthocyanin accumulation, chlorophyll degradation, cell wall breakdown, biosynthesis of sugars, flavors and aroma volatiles (Knee et al. 1977; Miszczak et al. 1995; Ornelas-Paz et al. 2013; Chen et al. 2014).

The initiation and progression of fruit ripening and senescence can be regulated by such plant hormones as abscisic acid (ABA) and ethylene (Mcatee et al. 2013). Ethylene is a vital hormone for climacteric fruit ripening and ABA is believed to indirectly regulate ripening via ethylene. In contrast, for non-climacteric fruit, ABA plays a major role in regulating the ripening and senescence (Mcatee et al. 2013). Previous studies showed that exogenous ABA application could increase anthocyanin contents, enhance sucrose accumulation and accelerate the ripening processes in strawberry (OfosuAnim et al. 1996; Jiang and Joyce 2003; Jia et al. 2011).

The earlier work involving a single plant hormone species greatly advanced our understanding of the effects of hormones on fruit ripening and senescence. However, it is now evident that the ripening and senescence processes are regulated in a complex way involving cross-talks among different classes of plant hormones (Munne-Bosch and Muller 2013). For example, IAA, an auxin compound, has been reported to cross-talk with ethylene during ripening in tomato and peach because ethylene production increases with elevation of IAA contents and the transcript levels of IAA-signaling components, and the increased ethylene production can in turn up-regulate IAA biosynthesis and transcription of IAA-signaling components (Jones et al. 2002; Trainotti et al. 2007). In strawberry fruit, IAA suppresses the expression of the 9-cis-epoxy-carotenoid dioxygenase genes *FaNCED1*, *FaNCED2,* and *FaCYP707A1* that are involved in ABA biosynthesis (Ji et al. 2012). Naphthalene acetic acid (NAA), a synthetic auxin compound, has also been observed to delay anthocyanin accumulation, chlorophyll loss, softening and the subsequent ripening of strawberry (Given et al. 1988a; Symons et al. 2012). It has also been reported that the levels of IAA in strawberry fruit are high during its early development but reduce to low levels prior to coloration, which is in contrast to ABA levels that are low at anthesis and gradually increase with fruit development and ripening (Symons et al. 2012). Similar changes in ABA and IAA levels also occurred during storage of strawberry fruits that were harvested at white stage (Chen et al. 2014). Given et al. (1988b) proposed that the decline in IAA concentration in the achenes of strawberry maturing fruits modulated the speed of fruit ripening. All these researches suggest that the ripening and senescence processes of non-climacteric fruits involve complex cross-talks among multiple plant hormones. The underlying mechanisms of the cross-talks between ABA and IAA or ethylene in fruit ripening are yet to be deciphered.

Our previous study found that there was an accelerated reduction in IAA contents and an increase in ABA levels in detached strawberry fruits compared to those in *in planta* fruits, which might be the reason for accelerated senescence in harvested fruits (Chen et al. 2014). To further understand the molecular basis of the cross-talk between ABA and IAA in regulating the postharvest ripening and senescence of strawberry fruit, we used *de novo* assembly and digital gene expression (DGE) methods to establish three transcriptomes of harvested strawberry fruits in response to individual treatments with IAA, ABA or their combination, and we then performed comparative analyses of these transcriptomes.

Materials and methods

Plant materials and treatments

Strawberry plants (*Fragaria × ananassa*) were grown in a plastic greenhouse (Chen et al. 2014), and fruits at 23 days after anthesis were harvested. The harvested fruits were of similar size, and free of diseases and insect pests. The fruits were quickly transported to the laboratory within 1 h of harvest. The fruits were surface sterilized by immersion in 0.1 % sodium hypochlorite solution for 2 min, followed by three rinsings in ddH_2O (distilled and deionized water) and brief surface drying in a laminar flow hood. Then, these fruits were randomly divided into four groups, with each containing eighty fruits. For hormone treatments, hormone solution or water (as a control) was injected with 100-μL microsyringe into the fruit core from the pedicel. Group IAA fruits were injected with 100 μL of 1 mM IAA. Similarly, Group ABA fruits were injected with 100 μL of 1 mM ABA, and Group IAA + ABA with 100 μL of

mixture solution of IAA (1 mM) and ABA (1 mM). The control fruits were injected with 100 μL water. All these fruits were kept at 20 ± 2 °C, with 90 ± 5 % relative humidity under darkness for 2 days, then quickly frozen in liquid nitrogen and stored in a −80 °C freezer for further analyses.

Determination of tristimulus color and firmness

Tristimulus color was measured on two opposite sides (equatorial area) of each fruit using Chroma Meter CR-400 (Konica Minolta Sensing Inc., Osaka, Japan) and *a** value representing red/green ratio was recorded.

Firmness was also measured, using the TA-XT2i Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK), on the area where tristimulus color was quantified. The depth of puncture was 7 mm at a rate of 0.5 mm/s. The maximum force (in Newton) needed to puncture the fruits was recorded.

Determination of endogenous ABA and IAA levels in strawberry fruits

ABA and IAA contents were simultaneously determined using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) as described by Symons et al. (2012). Briefly, the frozen fruit sample (10 g) was ground in liquid nitrogen using a mortar and pestle. One gram powder was mixed with 10 mL 80 % methanol containing 1 mg of 2, 6-di-tert-butyl-4- methylphenol (BHT > 99.0 %, Aladdin Industrial Inc., Shanghai, China). The mixture was kept at 4 °C overnight, and then filtered through a Whatman No. 1 filter paper. The liquid was concentrated to less than 1 mL using speed vacuum at 35 °C. The concentrate was then taken up in 3 × 3 mL of 10 % (v/v) methanol-0.4 % (v/v) acetic acid and injected into a Sep-Pak C_{18} cartridge (6 cc/500 mg, Waters Corporation, Milford, MA, USA). Plant hormones were eluted from the Sep-Pak with 0.4 % (v/v) acetic acid–methanol solution after a rinse with 10 % (v/v) methanol-0.4 % (v/v) acetic acid. The eluates were vacuum dried, the residue was re-suspended in 20 % (v/v) methanol-0.4 % (v/v) acetic acid and centrifuged at 12,000*g* for 3 min. The ABA and IAA content in the sample were then analyzed using an Agilent 6460 Triple quadruple LC/MS system (Agilent Technologies Inc., Santa Clara, CA, USA).

Isolation of total RNA

Strawberry fruits were grounded in liquid nitrogen and one gram powder was collected for total RNA extraction using cetyltrimethylammonium bromide (CTAB) protocol as described by Chang et al. (1993).

Construction of cDNA libraries and RNA sequencing

To obtain a transcriptome of *Fragaria* × *ananassa* fruit as a reference for DGE, a cDNA library was constructed using the total RNAs pooled from aforementioned four groups (i.e., IAA, ABA, IAA + ABA, and the control) described above. Individual cDNA libraries representing each group of fruit samples were constructed with total RNA from five fruits in that group. Two biological replicates per group were used. Three μg total RNA per group was used as input material for the RNA sample preparations. mRNA was purified from total RNA using poly-T oligo-attached magnetic beads.

Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's instruction. PCR products were purified using the AMPure XP system and library quality was assessed using an Agilent Bioanalyzer 2100 system.

The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq SR Cluster Kit v3-cBot-HS (Illumina) according to the manufacturer's instruction. After cluster generation, the library preparations were sequenced on an Illumina Hiseq 2000/2500 platform and 125 bp paired-end reads (50 bp single-end reads for DGE) were generated.

De novo assembly and gene functional annotation

For quality control, raw data (raw reads) of fastq format were processed through the in-house perl scripts to remove low-quality reads and reads containing adapters and/or poly (N), resulting in clean data (clean reads). Q20, Q30, GC content and sequence duplication levels of the clean data were also calculated. The high-quality clean data were used for all further analyses.

For transcriptome assembly, ref-1 files and ref-2 files from all libraries were pooled to form one big left.fq file and one big right.fq file, respectively. The left.fq and right.fq files were then subject to the Trinity assembly process (Grabherr et al. 2011) with min-kmer-cov set to 2 and all other parameters to default settings.

Gene function was annotated based on the following databases: NCBI non-redundant protein sequences (Nr), NCBI non-redundant nucleotide sequences (Nt), Protein family (Pfam), Clusters of Orthologous Groups of proteins (KOG/COG), the manually annotated and reviewed protein sequence database (Swiss-Prot), KEGG Ortholog database (KO), and Gene Ontology (GO).

Quantification of gene expression levels and differential expression analysis

Gene expression levels were estimated by RSEM (Li and Dewey 2011) for each sample. Prior to differential gene expression analysis, for each sequenced library, the read counts were adjusted by edgeR program package through one scaling normalized factor. Differential expression analysis of two samples was performed using the DEGseq R package (Wang et al. 2010). *P* value was adjusted using *Q* value (Storey and Tibshirani 2003). *Q* value <0.005 and |log2 (fold change)| > 1 was set as the threshold for significantly differential expression.

RT-qPCR verification of RNA sequencing transcripts

Real-time quantitative PCR (RT-qPCR) was used to validate the digital gene expression data obtained by RNA sequencing. The cDNA of group IAA, ABA, IAA + ABA and control obtained was 10 × diluted and 5 μL was used as the template in each qPCR reaction using the SYBR® Premix Ex Taq™ (TaKaRa Biotechnology, Dalian, China) and the reactions were run using the Applied Biosystems 7500 Real-Time PCR System (Life Technologies Corporation, Beverly, MA, USA). Genes and primers for the qPCR were listed in Supplemental Table S1. Relative expression was determined with the $2^{-\Delta\Delta T}$ algorithm by normalizing to the transcript levels of related genes in the control group.

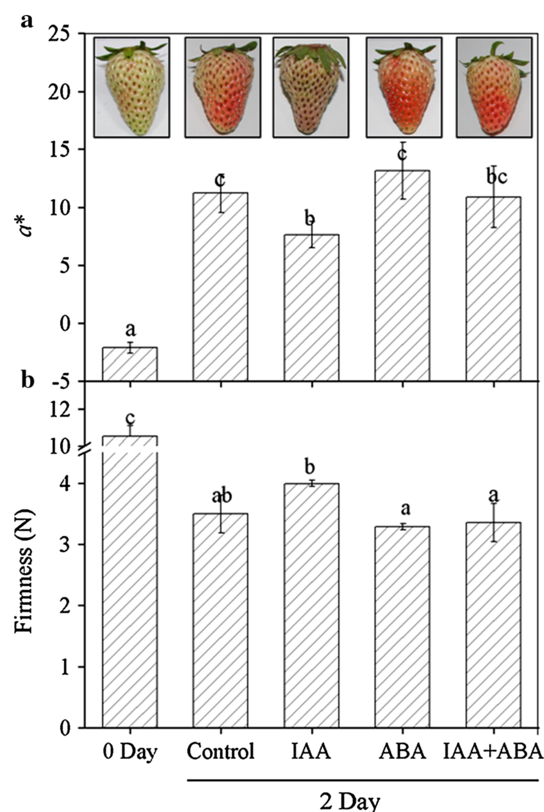

Fig. 1 Color **a** and firmness **b** of strawberry fruits in response to exogenous IAA and ABA. 0 day represents the freshly harvested fruits (23 days after anthesis). Control, IAA, ABA, and IAA + ABA represent fruits treated with water, IAA, ABA, and a mixture of IAA and ABA for 2 days, respectively. *Lower-case letters* (*a–c*) on the *bar chart* indicate the significant differences between different treatments (Duncan's test, $P < 0.05$)

Results

Changes in maturity and hormone levels in strawberry fruits in response to exogenous IAA and ABA

Strawberry fruits at white stage (approximately 23 days after anthesis) were harvested and subject to IAA and ABA treatments. Two days after the respective treatments, a^* value, an indicator of color, increased in all four groups, including IAA, ABA, IAA + ABA, and control (Fig. 1a). The IAA-treated fruits had the lowest a^* value. Although the ABA-treated fruits appeared to have the highest a^* value, there was no significant difference among ABA, IAA + ABA and control (Fig. 1a). In contrast to the color changes, the firmness of all fruits decreased 2 days after harvest, and there was no significant difference in firmness among the four groups of fruits (Fig. 1b).

There were lower levels of IAA in the control and ABA groups and higher levels of IAA in IAA and IAA + ABA groups 2 days after harvest than those of day 0 (freshly harvested) fruits (Fig. 2a). Regarding ABA contents, higher levels of ABA were detected in all groups of fruits on day 2, compared with those of day 0 fruits. In addition, fruits treated with ABA and IAA + ABA had high levels of ABA that were nearly three times of those in control and IAA groups (Fig. 2b).

RNA sequencing, *de novo* assembly and gene annotation of *Fragaria* × *ananassa* fruit transcriptome

To obtain an overview of *Fragaria* × *ananassa* fruit transcriptome, cDNA library constructed from the total RNA of fruits was subject to 125 bp pair-end reads using Illumina Hiseq platform. After eliminating adapters, ambiguous nucleotides and low-quality sequences, 86,234,158 clean reads of 10.78 Gb were obtained, and the average GC content was 45.78 % (Table S2). The clean reads were then assembled into 44,457 unigenes, with an N50 length of 1595 bp and an average size of 852 bp

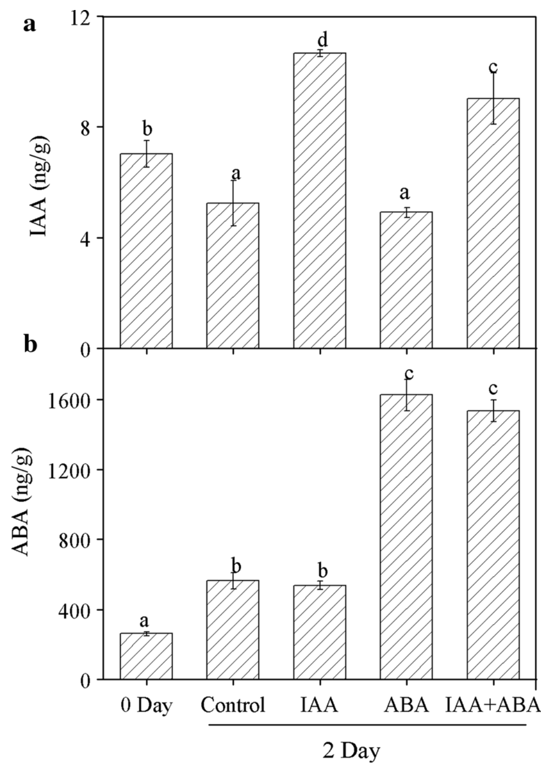

Fig. 2 IAA **a** and ABA **b** contents in strawberry fruits 0 day, Control, IAA, ABA, IAA + ABA are noted as in the legend to Fig. 1. *Lower-case letters* (*a*–*d*) on the *bar chart* indicate the significant differences between different treatments (Duncan's test, $P < 0.05$)

(Table S1). Assembled unigenes ranged from 201 to 15,670 bp and about half of them (24,791, 55.76 %) were 200–500 bp in length (Fig. S1).

To functionally annotate these unigenes, sequences were compared against public databases for Blast analysis, with a threshold of 10^{-5}. 58.51 % of unigenes (26,013) were annotated (Table S3), 46.78 % of them or 20,801 unigenes had significant similarity to known sequences in the NCBI non-redundant protein sequences (Nr) database (Table S3). 23,377 (52.58 %) unigenes were annotated based on sequence search against the NCBI non-redundant nucleotide sequences (Nt) database and 15,675 (35.25 %) the SwissProt database (Table S3). In addition, querying against the Protein family (Pfam) suggested that there were 15,645 (35.19 %) unigenes containing known protein domains. To functionally classify the transcriptomes, the assembled unigenes were also searched against such databases as Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO) and euKaryotic Ortholog Groups (KOG), which allowed us to place 6360 unigenes into 262 KEGG pathways and 8497 unigenes in all 26 KOG functional categories (Table S3; Figs. S2, S3). However, 17,310 unigenes were assigned to 47 level-two GO terms (Table S3; Fig. S4).

Global transcriptional profiling of harvested strawberry fruit in responses to IAA and ABA

To reveal the molecular events in immature strawberry fruits (23 days after anthesis) in response to IAA and ABA, four DGE libraries representing transcripts from the control, IAA, ABA and their combination (IAA + ABA) fruits, respectively, were constructed. Using an Illumina 2000 RNA sequencing platform, we obtained a total of 11.41 G reads with an average length of 50 bp, approximately 93.31–93.97 % clean reads were mapped into the transcriptome reference database described above (Table S4).

RNA sequence data analyses revealed that, when compared to the control, there were only 153 genes that were significantly differentially transcribed in the IAA-treated fruits (Q value < 0.005 and $|\log2$ (fold change)$| > 1$). Among them, 111 unigenes were up-regulated and 42 unigenes were down-regulated (Table S5). In the ABA-treated fruits, 132 unigenes were significantly differential expressed, with 48 up-regulated and 84 down-regulated genes (Table S6). In the IAA + ABA fruits, there were 147 significantly differentially expressed unigenes, including 54 up-regulated and 94 down-regulated genes (Table S7).

H cluster display revealed that strawberry fruits treated with a combination of exogenous IAA and ABA had a different transcript pattern from that treated with IAA or ABA alone (Fig. 3a). As shown in the Venn diagrams in Fig. 3b, there was an overlap of 27 unigenes between IAA and ABA groups, 58 unigenes between IAA and IAA + ABA, and 84 unigenes between ABA and IAA + ABA (Fig. 3b). There were only 20 significantly differential unigenes that were commonly shared in IAA, ABA and IAA + ABA groups (Fig. 3b). Interestingly, there were 25 unigenes that were specific to the IAA + ABA group (Fig. 3b). These data suggested that the expression pattern in response to the combination of ABA and IAA was not just an additive or subtractive algorithm of those responding to IAA or ABA alone.

Expression analysis of postharvest strawberry fruit in response to exogenous IAA

Among the transcripts significantly elevated in IAA group, there were some unigenes involved in IAA conjugation and signaling pathways (Tables 1, S5, S8). For example, comp50466_c0, a significantly up-regulated unigene, encodes an indole-3-acetic acid-amido synthetase (GH3) and is possibly involved in IAA conjugation (Tables 1, S5). Another unigene named comp45854_c0 having a 2.2-fold increase in its transcript levels encodes an IAA-responsive protein and belongs to the *AUX/IAA* family (Tables 1, S5). Comp45854_c0 and comp46511_c0, up-regulated by 2.2-

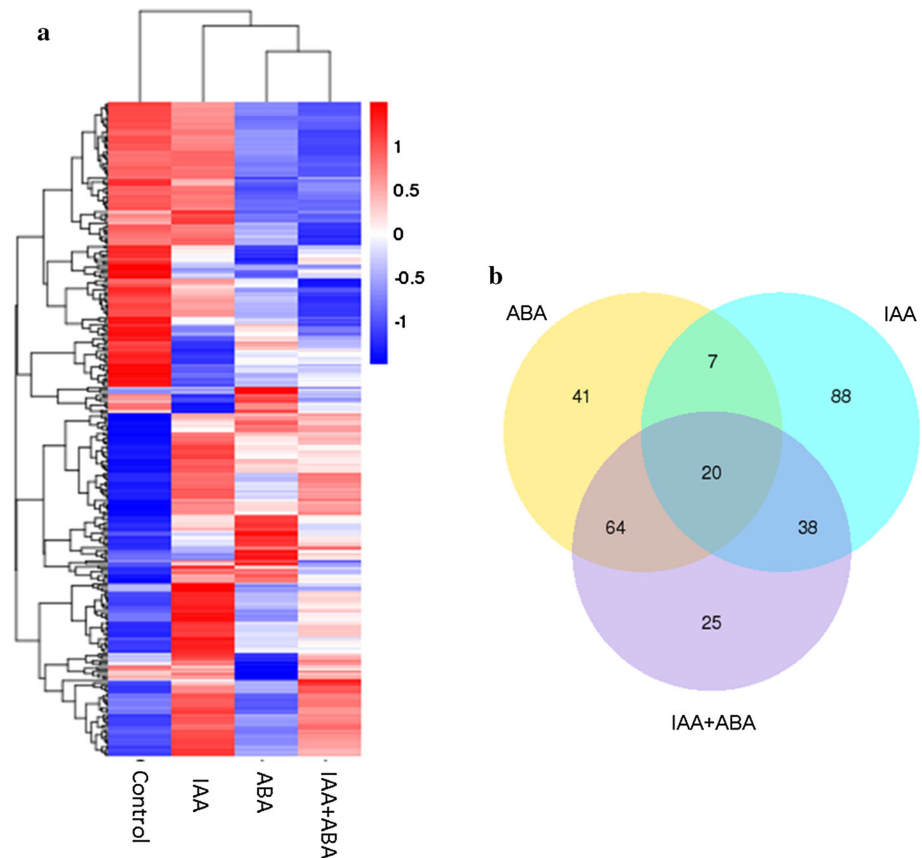

Fig. 3 H cluster **a** and Venn diagram **b** showing the number of unigenes significantly differentially transcribed in response to exogenous IAA and ABA. The heat map shows transcript levels across treatments. *Color scale* $\log_2$ 'read count' of genes

and 24.5-fold, respectively, are two auxin response factor (ARF) unigenes (Tables 1, S5). Two putatively encoding annexin unigenes (comp44021_c0 and comp44021_c1) were substantially increased by 15.6- and 13.2-fold, respectively (Tables 1, S5). Intriguingly, the unigene comp53054_c0, encoding a putative E3 ubiquitin protein ligase, was down-regulated while another E3 ubiquitin protein ligase-like gene (comp46194_c0) was up-regulated (Tables 1, S5). Similarly, transcript levels of three putative receptor-like kinases genes (comp51887_c0, comp52501 and comp47297_c0) were decreased while the transcript levels of two other putative receptor-like kinase genes (comp51143_c0, comp47443_c0) were increased (Tables 1, S5).

Another class of significantly differentially expressed unigenes upon IAA treatment was heat-shock protein-related genes. For example, the unigene comp43575 with 3.9-fold induction by IAA encodes an *arg2*-like heat-shock protein (Tables 1, S5). *Arg2* was shown to be an IAA-induced heat-shock protein gene in mung bean (*Vigna radiata*) hypocotyls (Yamamoto et al. 1992). Transcript levels of some unigenes related to degradation of pectin and other cell wall components were also shown to be significantly elevated (Tables 1, S5). These genes are generally associated with fruit softening. There was an increase in the expression of comp48185_c0 and comp54649_c0; these two unigenes encode putative xylosyltransferase 1 (XT1) and xyloglucan endotransglucosylase/hydrolase (XTH), respectively (Tables 1, S5), which have roles of catalyzing the endo-cleavage of xyloglucan polymers and transferring of the newly generated reducing ends to other xyloglucans.

Two unigenes, comp54082_c0 and comp49931_c0 encoding proteins having acetyl-CoA-carboxylase activity that catalyzes the ATP-dependent formation of malonyl-CoA from acetyl-CoA and bicarbonate (Ohlrogge and Jaworski 1997), were significantly down-regulated by exogenous IAA, with 2.4- and 2.00-fold, respectively (Tables 1, S5). However, the unigene encoding butyrate-CoA synthase was up-regulated. The transcript of a putative β-ketoacyl-CoA synthase (KAS) family gene (comp55300_c0) notably increased by 8.4-fold, which encodes an enzyme that catalyzes the synthesis of long saturated acyl chains of fatty acid (Fofana et al. 2004). The cytochrome P450 86B1-like gene (comp39724_c0) encoding protein owning fatty acid ω-hydroxylase activity associated with suberin synthesis, increased by twofold (Tables 1, S5).

Exogenous IAA also decreased the transcript of the unigene comp42246_c0 encoding sucrose synthase

Table 1 Differently expressed genes potentially related to hormone signaling and fruit ripening and senescence in response to exogenous IAA (Q value < 0.005 and |log2 (fold change)| > 1)

Gene_ID	log2 (Fold_change)	Q value	Putative functional identification
comp50466_c0	3.312	0.002	Indole-3-acetic acid-amido synthetase GH3.1
comp46511_c0	4.613	0.001	ARF domain class transcription factor
comp45854_c0	1.130	0.000	IAA-responsive protein
comp44817_c0	1.171	0.001	IAA-responsive protein
comp43575_c0	1.959	0.000	Indole-3-acetic acid-induced protein ARG2
comp47770_c0	−1.149	0.001	TOPLESS
comp44021_c0	4.152	0.000	Annexin protein
comp44021_c1	4.076	0.000	Annexin protein
comp52628_c1	−1.861	0.000	E2 ubiquitin-conjugating enzyme
comp53054_c0	−1.298	0.000	E3 ubiquitin protein ligase
comp46194_c0	1.665	0.002	RING-H2 finger protein (E3 ubiquitin ligase)
comp52780_c0	2.945	0.004	F-box/LRR-repeat protein
comp51887_c0	−2.158	0.000	Leucine-rich repeat receptor-like kinase
comp52501_c0	−1.475	0.003	Leucine-rich repeat receptor-like kinase
comp47297_c0	−1.047	0.002	Serine/threonine protein receptor-like kinase
comp51143_c0	1.722	0.000	Serine/threonine protein receptor-like kinase
comp47443_c0	2.080	0.000	Receptor-like kinase
comp41618_c0	2.720	0.000	Calcium-binding protein
comp51089_c0	−1.355	0.000	β-D-xylosidase
comp42060_c0	−1.272	0.000	β-galactosidase
comp46483_c0	−1.293	0.000	Glucan endo-1,3-β-glucosidase
comp42142_c0	−1.871	0.000	Glucan endo-1,3-β-glucosidase
comp43192_c0	2.060	0.001	Glucuronosyltransferase
comp54082_c0	−1.279	0.000	Acetyl-CoA carboxylase
comp48499_c0	−1.618	0.000	Endo-1,3(4)- β-glucanase
comp54924_c0	−1.316	0.000	Endoglucanase
comp34077_c0	−1.894	0.000	Pectate lyase
comp34077_c1	−1.921	0.001	Pectate lyase
comp36978_c0	1.349	0.000	Pectinesterase/pectinesterase inhibitor
comp36335_c0	−2.224	0.001	Expansin
comp34773_c0	−1.253	0.000	Chitinase
comp54649_c0	1.135	0.000	Xyloglucan endotransglucosylase
comp48185_c0	1.171	0.000	Xylosyltransferase
comp54082_c0	−1.279	0.000	Acetyl-CoA carboxylase
comp49931_c0	−1.027	0.000	Methylcrotonoyl-CoA carboxylase subunit α
comp43332_c0	2.655	0.004	Butyrate-CoA ligase
comp55300_c0	3.067	0.000	β-ketoacyl-CoA synthase
comp48375_c0	−1.873	0.000	Linoleate 13S-lipoxygenase
comp39724_c0	1.100	0.000	Cytochrome P450 (fatty acid ω-hydroxylase)
comp29094_c0	1.503	0.000	Jasmonate O-methyltransferase
comp54105_c1	2.973	0.000	Gibberellin 20 oxidase
comp41021_c0	1.764	0.000	Gibberellin 20 oxidase
comp43212_c0	1.227	0.000	Glutathione S-transferase
comp38169_c0	3.117	0.005	Glutathione S-transferase
comp44552_c0	1.123	0.000	Glutathione S-transferase
comp41637_c0	3.355	0.000	Metallothionein protein type 2 MET1
comp48366_c0	−1.142	0.000	Phenylalanine ammonia-lyase
comp42246_c0	−1.282	0.000	Sucrose synthase
comp46753_c0	2.326	0.000	UDP-glucose flavonoid 3-O-glucosyltransferase
comp48158_c0	1.511	0.000	UDP-glucose flavonoid 3-O-glucosyltransferase
comp46225_c0	−1.054	0.000	UDP-rhamnose: rhamnosyltransferase (GT4)

(Tables 1, S5), the activity of which increased with the increase in sucrose concentration during the ripening of strawberries (Hubbard et al. 1991). Additionally, the unigene comp48366_c0 encoding phenylalanine ammonia-lyase (PAL) was down-regulated with the low activity of UDP-rhamnose: rhamnosyltransferase gene (*FaRT1*) owning glycosyltransferase 4 (FaGT4) activity (Tables 1, S5), suggesting a suppression of IAA to anthocyanin biosynthesis as reported by Given et al. (1988a). Interestingly, two putative UDP-glucose flavonoid 3-O-glucosyltransferase 3 (*FaGT3*) unigenes comp46753_c0 and comp48158_c0 were up-regulated (Tables 1, S5) although FaGT3 did not form glucose (Glc) esters and was not involved in the biosynthesis of phenylpropanoyl- and benzoyl-D-Glc derivatives (Lunkenbein et al. 2006).

Glutathione S-transferase (GST) was involved in the metabolism during fruit senescence (Shi et al. 2014). There were three putative *GST* genes (comp43212_c0, comp38169_c0, comp44552) increased by exogenous IAA (Tables 1, S4), which was similar to *PpGST1* and *PpGST2* remarkably induced by IAA treatment in pear fruits (Shi et al. 2014).

Expression analysis of harvested strawberry fruit in response to exogenous ABA

ABA generally induces genes involved in ABA metabolism and signaling. A putative E3 ubiquitin ligase encoding unigene comp41565_c0 was significantly up-regulated. Another unigene named comp54510_c0 encoding a SKP1-like protein with homology to a subunit of E3 ubiquitin ligase was also significantly up-regulated (Tables 2, S6). The SKP1-like protein has been shown to be involved in ABA signaling in *Arabidopsis thaliana* (Li et al. 2012). In contrast to the above up-regulated unigenes, the transcript levels of comp4449_c0 decreased by 7.0-fold upon the ABA treatment. Interestingly, comp44449_c0 has 98 % identity with *SAR5* (Genbank: L44142.1, an auxin-repressed protein gene) (Tables 2, S6); *SAR5* has been shown to be absent or at very low levels in breaker and ripe fruits (Reddy and Poovaiah 1990). ABA also increased the transcripts of two putative annexin (comp44021_c0 and comp44021_c1) and a putative receptor-like kinase (comp47443_c0) (Tables 2, S6). However, the exogenous ABA treatment did not strongly elevate the transcript levels of genes involved in ABA biosynthesis and signaling (Tables S6, S9). The transcripts of unigenes comp50323_c0 (a homologue of *NCED1*), comp41907_c0 (*FaPYR1*) and comp46342_c0 and comp46342_c1 (both are ABI1 protein phosphatase 2C family gene) were slightly up-regulated (only 1.3-, 1.5-, 1.5- and 1.2-fold, respectively) (Table S9).

Exogenous ABA treatment also effected the expression of heat-shock proteins (HSPs) genes. The unigene comp47381_c0 encoding a low-molecular-weight heat-shock protein (LMW HSP17.4) was down-regulated (Tables 2, S6). In contrast, the unigene comp50216_c0, a putative HSP 70 protein gene, was up-regulated (Tables 2, S6). The homologue of this putative HSP 70 protein in maize was shown to regulate the ABA-induced antioxidant in response to combined drought and heat stress (Hu et al. 2010). In addition, a Bcl-2-associated athanogene 6-like (*BAG6*-like) gene comp53095_c0 was down-regulated in the strawberry fruits treated with ABA (Tables 2, S6). The *BAG6*-like gene encodes a BAG protein that mediates direct interaction with the ATPase domain of HSP70 molecular chaperones (Kabbage and Dickman 2008).

Several fruit softening-related genes were also affected by exogenous ABA. The unigenes comp42142_c0 (a glucan endo-1,3-β-glucosidase-like gene), comp34077_c0 and comp34077_c1 (pectate lyase gene), comp54649_c0 (xyloglucan endotransglucosylase gene) and comp54649_c0 (a putative XTH gene) were up-regulated (Tables 2, S6). Comp18699_c0 and comp43648_c0 (both are cinnamyl alcohol dehydrogenase (CAD)-like genes) were also up-regulated (Tables 1, 2). In contrast, two expansin genes, comp39227_c0 and comp43102_c0, were down-regulated (Tables 2, S6).

In addition, some fruit ripening and senescence-related genes were up-regulated by the ABA treatment. The transcript levels of comp39127_c0 had 2.2-fold increase (Tables 2, S6). This unigene is a homologue of *AtCK2α1* encoding a subunit of casein kinase CK2 (formerly known as casein kinase II)-like protein; this protein was shown to be involved in responses to various hormones (Mulekar et al. 2012). Similarly, comp40864_c0, a putative senescence-related gene (*SRG1*) was up-regulated; SRG1 protein is a member of the Fe(II)/ascorbate oxidase superfamily (Callard et al. 1996). The expression of comp41637-c0 (a metallothionein 1-like gene *MET2*) also had 1.4-fold increase (Tables 2, S6). In contrast to these up-regulated unigenes, comp41879_c0 encoding a putative metallothionein-like protein 1 was down-regulated (Tables 2, S6). The metallothionein has been reported to be both developmental and ripening genes (Esaka et al. 1992; Whitelaw et al. 1997; García-Hernández et al. 1998).

Interestingly, ABA enhanced Fe recycling-related unigenes. comp50647_c0, a unigene encoding a putative ferric reductase defective 3 (*FRD3*), was up-regulated (Tables 2, S6). The unigene comp54516_c0 encoding an isoflavone reductase (IFR)-like protein was also up-regulated (Tables 2, S6). IFR may promote production of phenolics that facilitate the reuse of Fe (Lei et al. 2014). In *Arabidopsis*, ABA was shown to be involved in the reutilization and transport of Fe from root to shoot (Lei et al. 2014).

Table 2 Differently expressed genes potentially related to hormone signaling and fruit ripening and senescence in response to exogenous ABA (Q value < 0.005 and |log2 (fold change)| > 1)

Gene_id	log2 (Fold_change)	Q value	Putative functional identification
comp54510_c0	1.495	0.000	S-phase kinase-associated proteins 1 (SKP 1)
comp53095_c0	−1.977	0.000	Bcl-2 associated athanogene (BAG) 6
comp44021_c0	1.725	0.000	Annexin protein
comp44021_c1	1.451	0.000	Annexin protein
comp44449_c0	−2.810	0.000	IAA-repressed 12.5 kDa protein
comp52501_c0	−1.802	0.000	Leucine-rich repeat e protein receptor-like kinase
comp47443_c0	2.009	0.000	Receptor-like protein kinase
comp41565_c0	1.963	0.000	RING-H2 finger protein (E3 ubiquitin ligase)
comp37348_c0	−5.113	0.000	Serine/threonine protein phosphatase 7
comp39127_c0	1.161	0.000	Casein kinase II subunit α-1
comp43866_c0	1.348	0.002	Protein kinase-like protein
comp55199_c0	1.308	0.001	BURP domain-containing protein 3
comp40392_c0	−1.438	0.000	Defensin D2
comp40864_c0	1.535	0.000	Senescence-related gene (SRG1)
Comp50216_c0	1.070	0.000	Heat-shock 70 kDa protein
comp47381_c0	−1.495	0.000	LMW heat-shock protein
comp41879_c0	−1.142	0.002	Metallothionein protein 1
comp41637_c0	1.411	0.000	Metallothionein protein type 2 MET1
comp39227_c0	−1.275	0.001	Expansin 4
comp43102_c0	−1.100	0.000	Expansin-A1
comp34077_c0	2.131	0.000	Pectate lyase
comp34077_c1	2.370	0.000	Pectate lyase
comp42142_c0	2.353	0.000	Glucan endo-1,3-β-glucosidase
comp54649_c0	1.324	0.000	Xyloglucan endotransglucosylase
comp18699_c0	1.065	0.000	Cinnamyl alcohol dehydrogenase (CAD)
comp43648_c0	1.120	0.000	Cinnamyl alcohol dehydrogenase (CAD)
comp45921_c0	−1.397	0.000	Glucose and ribitol dehydrogenase
comp54536_c0	1.260	0.000	Dihydroflavonol 4-reductase
comp29240_c0	1.518	0.000	Carboxyl/carbamoyltransferase
comp50687_c0	−1.628	0.000	Galactinol-sucrose galactosyltransferase
comp43192_c0	2.442	0.000	Glucuronosyltransferase PGSIP7
comp41886_c0	1.280	0.000	Inositol-pentakisphosphate 2-kinase
comp29094_c0	3.126	0.000	Jasmonate O-methyltransferase
comp56684_c0	2.209	0.000	Jasmonate O-methyltransferase
comp41021_c0	−2.219	0.000	Gibberellin 20 oxidase 1
comp48373_c0	−2.852	0.000	ω-3 fatty acid desaturase (FAD)
comp49239_c0	−1.728	0.000	ω-6 fatty acid desaturase (FAD)
comp48375_c0	−1.293	0.000	Linoleate 13S-lipoxygenase
comp50647_c0	1.459	0.000	Ferric reductase defective 3a (FRD3)
comp54516_c0	1.151	0.000	Isoflavone reductase protein (IFR)
comp50388_c0	1.037	0.000	NADH dehydrogenase (ubiquinone)
comp45309_c1	−1.285	0.000	Peroxygenase 2
comp42477_c0	1.087	0.000	Phenylalanine zipper/dehydratase large subunit
comp50981_c0	−1.490	0.000	UDP-glucuronate 4-epimerase 6

Expression analysis of harvested strawberry fruit in response to the combination of ABA and IAA

The treatment with combination of IAA and ABA shared an overlap of 58 unigenes with the IAA treatment (Fig. 3b). Except for 15 unigenes whose functions are unknown, most of these unigenes were related to fruit softening. Some unigenes (e.g., unigenes encoding pectate lyase, 1,3-β-glucosidase and pectinesterase) in response to the treatment with both IAA and ABA displayed changes similar to those responding to the treatment with IAA alone but some other unigenes (e.g., unigene encoding β-galactosidase) showed an opposite pattern in the transcript levels (Tables 1, 3).

In contrast, the treatment with combination of IAA and ABA shared an overlap of 84 unigenes with the ABA treatment, including 21 uncharacterized unigenes (Fig. 3b). The majority of these unigenes encode HSP, BAG, fatty acid desaturase, lipoxygenase, metallothionein, defensin, oleosin, and vicilin-like antimicrobial peptides (Tables 2, 3).

However, some unigenes were uniquely dysregulated by the combination treatment, such as, comp48717_c0 (a 17.2 kDa class II heat-shock protein), comp50306_c0 (a MAPKKK-like gene), and comp50021_c0 (a putative cytochrome *c* oxidase gene).

Notably, the expression of some unigenes involved in IAA or ABA signaling was only moderately altered by the treatment with a mixture of IAA and ABA. For example, the transcripts of comp41907_c0 (*FaPYR1*), comp46342_c0 and comp46342_c1 (two ABI1 protein phosphatase 2C family genes) were decreased by 1.4-, 1.6-, and 1.3-fold, respectively (Tables S7, S10). The transcripts of comp44817_c0 and comp45854_c0 (two putative *AUX/IAA* family genes) were increased by 2.1-fold and twofold, respectively (Tables S7, S10).

RT-qPCR validation of digital expression patterns revealed by RNA sequencing

Twelve unigenes that were significantly differentially expressed as revealed by above-mentioned digital analyses of RNA sequencing data were randomly selected for validation using RT-qPCR. Overall, the RT-qPCR results were consistent with the RNA-seq data. Linear regression analysis indicated that there was a high correlation between the RT-qPCR data and the RNA-seq data ($R^2 = 0.95$) (Fig. S5).

Discussion and conclusion

Our previous study showed that postharvest strawberries experienced accelerated ripening and senescence processes that were correlated with enhanced decrease of endogenous auxin (IAA) levels and increase of ABA contents, compared to those *in planta* fruits(Chen et al. 2014). The physico-chemical parameters examined in this study revealed that exogenous IAA delayed the ripening processes of harvested strawberry fruits, whereas ABA promoted the processes; the treatment with a mixture of IAA and ABA, however, showed little effect on the ripening processes. More importantly, the current study investigated into the underlying molecular events by establishing and comparative analyses of four strawberry fruit transcriptomes in response to respective treatments with IAA, ABA, IAA + ABA, or water (control).

Auxin is produced in the achenes and stimulates receptacle expansion during fruit development, but inhibits fruit ripening (Given et al. 1988b). For harvested strawberries, exogenous IAA delayed pectin depolymerization and degradation of cell wall components by suppressing genes encoding pectate lyase, β-D-xylosidase, endoglucanase, β-galactosidase, endo-1,3-β-glucosidase, endo-1,3(4)-β-glucanase. Interestingly, IAA promoted the transcript levels of pectinesterase/pectinesterase inhibitor-like gene (Table 1), which at least in part accounts for IAA's role in hindering pectin degradation during ripening of harvested strawberries. IAA also promoted the reorganization of cellulose–xyloglucan framework by activating the expressions of *XT1* and *XTH* (Table 1), which is consistent with a previous report that tomato *LeEXT1* (XTH) was up-regulated by IAA (Catalá et al. 2000). In addition, exogenous IAA suppressed the expression of genes encoding sucrose synthase, phenylalanine ammonia-lyase and glycosyltransferase (Table 1). IAA also induced the expression of glutathione S-transferases (GSTs) as reported in pear fruit (Shi et al. 2014), which would protect tissues against oxidative stress in fruit development and senescence. These findings were consistent with the quality attributes shown in Fig. S6, suggesting that exogenous IAA delayed the ripening processes in strawberry fruits after harvest.

As expected, the auxin signaling pathway and components for keeping auxin homeostasis in harvested strawberry fruits were activated by exogenously applied IAA. AUX/IAA proteins, the repressors heterodimerizing with auxin responsive factor (ARF), may provide specificity in IAA responses (Pierre-Jerome et al. 2013). Exogenous IAA induced the expression of putative *AUX/IAA* as well as *ARF* in this study (Table 1). Similar result was also reported by Liu et al. (2011) who found that naphthalene acetic acid (NAA, a synthetic species of auxin) activated the accumulations of *AUX/IAA* when applied either at large green or white stage of *in planta* strawberry fruits. Notably, the putative *GH3.1* gene encoding GH3 that conjugates IAA to amino acids was also induced by IAA (Table 1), confirming its involvement in the establishment and

Table 3 Differently expressed genes potentially related to hormone signaling and fruit ripening and senescence in response to a combination of exogenous IAA and ABA (Q value < 0.005 and |log2 (fold change)| > 1)

Gene_ID	log2 (Fold_change)	Q value	Putative functional identification
comp53095_c0	−2.053	0.000	BAG family 6
comp55199_c0	1.349	0.001	BURP domain-containing protein 3
comp44021_c0	3.963	0.000	Annexin protein
comp44021_c1	3.722	0.000	Annexin protein
comp50306_c0	3.979	0.000	Mitogen-activated protein kinase kinase kinase 3
comp50216_c0	−1.228	0.000	Heat-shock 70 kDa protein
comp48717_c0	−1.339	0.000	Heat-shock protein 20 (17.2 kDa class II)
comp47381_c0	−1.980	0.000	LMW heat-shock protein(17.4 kDa)
comp49488_c0	1.931	0.001	Heat-shock 70kDa protein 5
comp52501_c0	−1.416	0.004	Leucine-rich repeat receptor protein kinase
comp52146_c0	−1.367	0.003	Receptor protein kinase
comp51143_c0	1.459	0.000	Receptor-like protein kinase
comp47443_c0	2.198	0.000	Receptor-like protein kinase
comp42740_c0	−1.932	0.004	Reticulon-like protein
comp40392_c0	−1.712	0.000	Defensin D2
comp50900_c1	−2.502	0.002	Defensin
comp45194_c0	−1.721	0.000	Universal stress protein YxiE
comp42542_c0	−2.520	0.000	Metallothionein protein
comp41637_c0	2.220	0.000	Metallothionein protein type 2 MET1
comp41886_c0	1.146	0.000	Inositol-pentakisphosphate 2-kinase
comp43470_c0	2.966	0.000	C2 domain-containing protein
comp42060_c0	1.010	0.000	β-galactosidase
comp48499_c0	1.207	0.000	Endo-1,3(4)- β-glucanase
comp46483_c0	−1.387	0.000	Glucan endo-1,3-β-glucosidase
comp42142_c0	−2.980	0.000	Glucan endo-1,3-β-glucosidase
comp34077_c0	−1.361	0.001	Pectate lyase
comp36978_c0	1.259	0.000	Pectinesterase/pectinesterase inhibitor
comp46986_c0	1.476	0.000	Mannan endo-1,4-β-mannosidase 4
comp49049_c0	1.436	0.000	Rhamnose biosynthetic enzyme
comp50687_c0	−1.705	0.000	Galactinol-sucrose galactosyltransferase
comp45921_c0	−2.044	0.000	Glucose and ribitol dehydrogenase
comp49465_c0	−1.615	0.000	Malate synthase
comp48375_c0	−1.539	0.000	Linoleate 13S-lipoxygenase
comp55300_c0	2.696	0.000	β-ketoacyl-COA synthase family protein
comp48373_c0	−2.873	0.000	ω-3 fatty acid desaturase, putative
comp49239_c0	−1.006	0.002	ω-6 fatty acid desaturase, putative
comp44014_c0	−1.646	0.000	Oxaloacetate decarboxylase
comp50021_c0	−1.172	0.001	Cytochrome c oxidase assembly protein
comp44125_c0	2.967	0.000	Cytosolic sulfotransferase
comp48964_c1	1.112	0.000	Ferritin-3
comp29094_c0	2.113	0.000	Jasmonate O-methyltransferase
comp29262_c0	1.086	0.000	Monomethylxanthine methyltransferase 2
comp48514_c0	1.576	0.000	NAD(P)H dehydrogenase B2
comp45309_c1	−1.279	0.000	Peroxygenase 2

maintenance of low IAA levels during fruit ripening (Böttcher et al. 2010). In grape berry (*Vitis Vinifera* L.), the transcript levels of *VvGH3-1* increased at the onset of ripening (veraison), accompanying the IAA sequestration in the ripening berries (Böttcher et al. 2010). Therefore, the intervention of exogenous IAA influenced the IAA homeostasis between anabolism and catabolism, and made the dynamic balance towards the conjugation of IAA in fruits.

Very-long-chain fatty acids (VLCFAs) have been found to be probably required for polar auxin transport or tissue patterning in plants, which has been demonstrated in *pas1* (acetyl-CoA carboxylase PASTICCINO3) mutants of *Arabidopsis* (Roudier et al. 2010). In this study, exogenous IAA also affected fatty acid metabolism in harvested strawberry fruits by altering the transcript levels of acetyl-CoA-carboxylase, cytochrome P450 86B1-like protein, especially β-ketoacyl-CoA synthase (KAS) (Table 1). KAS is the first-step elongase in the VLCFA biosynthesis; VLCFAs function in cell expansion as essential lipids at plasma membrane (Roudier et al. 2010).

The role of ubiquitin in hormone signaling cascades has been previously reported. For example, E3 ubiquitin ligase CUL1 (cullin) and RBX1 (RING-H2 finger protein) have been shown to be subunits of the SCF^{TIR1} complex, which is required for the response to auxin (Gray et al. 2002). Reduced auxin response is associated with increased RELATED TO UBIQUITIN (RUB) modification of CUL1 when overexpression of RBX1 in Arabidopsis (Gray et al. 2002). In the harvested strawberries, the RING-H2 finger protein-like gene as well as ubiquitin-conjugating enzyme E2-like gene induced by IAA (Table 1) suggests that ubiquitin may affect the IAA signaling through its E3 ubiquitin ligase activity controlling the sensitivity of fruits to IAA, and subsequently regulate fruit development. In addition, *Arg2* and TOPLESS-like gene also respond to IAA in harvested fruits in mung bean and *Arabidopsis* (Yamamoto et al. 1992; Szemenyei et al. 2008). *Arg2* has been speculated to be a heat-shock cognate gene and TOPLESS (TPL) is a transcriptional co-repressor that can direct interaction with ARF5/MONOPTEROS regulated by IAA12/BDL (Yamamoto et al. 1992; Szemenyei et al. 2008). The specific regulatory mechanisms of auxin in fruit development and ripening need to be further studied.

ABA has a more dominant role in ripening of non-climacteric fruits including strawberries than climacteric fruits (Mcatee et al. 2013). In contrast to IAA, exogenous ABA had antagonist effects on the expression of cell wall degradation-related genes such as glucan endo-1, 3-β-glucosidase and pectate lyase gene (Table 2), resulting in promoting fruit softening. The transcript levels of an *XTH* gene (comp54649_c0), a homologous gene of *FcXTH1* (Genbank: GQ367550), were also induced by ABA (Table 2). In addition, two expansin genes (comp39227_c0 and comp43102_c0) were also negatively regulated by ABA (Table 2). Thus, the repression of cell expansions may be one of the aspects that ABA suppressed the development and promoted the ripening of strawberries.

ABA moderately up-regulated genes in ABA biosynthesis and signaling, including *FaNCED1*, *FaPYR1* and *FaABI1* (Table S6) and strongly activated a putative RING-H2 finger E3 ubiquitin ligase gene and a subunit of the SCF complex E3 ligases, SKP1 (Table 2). Previous studies showed that ABA-insensitive 3 (ABI3) derogation was mediated, at least in part, by the ABI-interacting protein 2 (AIP2), a RING-type E3 ubiquitin ligase (Zhang et al. 2005). These findings suggest that ubiquitin-mediated proteolysis also acts upstream of the ABA signaling cascades.

Heat-shock proteins (HSPs)/chaperones play crucial roles in protecting plants against stresses by re-establishing normal protein conformation and thus cellular homeostasis (Wang et al. 2004). In strawberry, low-molecular weight HSP (LMW HSP) was expressed in fruits at elongation stages and gradually decreased in the turning stage and the red stage, which was not controlled by auxin (Medina-Escobar et al. 1998). In this study, LMW HSP was found not to be regulated by IAA too (Tables 1, S5), but was significantly decreased by the ABA treatment (Table 2). The transcript levels of *HSP70* were elevated by ABA, whereas the *BAG6*-like gene was repressed (Table 2). The knock-out mutant of *BAG6* exhibited early flowering and a hastened senescence in *Arabidopsis* (Doukhanina et al. 2006). Therefore, the interaction of HSPs and BAG may participate in the regulation of ABA-mediated ripening and senescence in strawberry fruits.

This study also revealed several critical genes that have not been fully characterized yet were regulated by ABA. Casein kinase CK2-like protein, a ubiquitous Ser/Thr kinase, consists two catalytic α subunits and two regulatory β subunits (Mulekar et al. 2012). In Arabidopsis, CK2α mutants displayed hyposensitive responses to ABA associated with later flowering under both long- and short-day conditions (Mulekar et al. 2012). In harvested strawberries, a putative *CK2α1* gene was found to be induced by exogenous ABA (Table 2), suggesting that *CK2α1* may also involve in the ABA signaling. In addition, ABA may participate in the Fe recycling during the postharvest ripening in strawberries. The Fe(II)/ascorbate oxidase superfamily gene *SRG1* and metallothionein *MET2* were also induced by ABA. Exogenous ABA up-regulated the genes related to Fe reutilization, *FRD3* and isoflavone reductase gene *IFR* (Table 2).

In strawberry fruits treated with a combination of IAA and ABA, there was overlap of genes with fruits treated with IAA or ABA alone. The overlap mainly involved in pectin esterlysis, cell wall degradation, fatty acid metabolism, and transmembrane signal transduction. To some extent, the responses to the combination treatment displayed the cooperation or antagonism between IAA and ABA treatments. For example, both genes encoding pectinesterase/pectinesterase inhibitors induced by IAA and genes encoding HSPs induced by ABA were also regulated by their mixture. However, expansins, which were induced by IAA and/or repressed by ABA, were not significantly altered by the IAA and ABA combination.

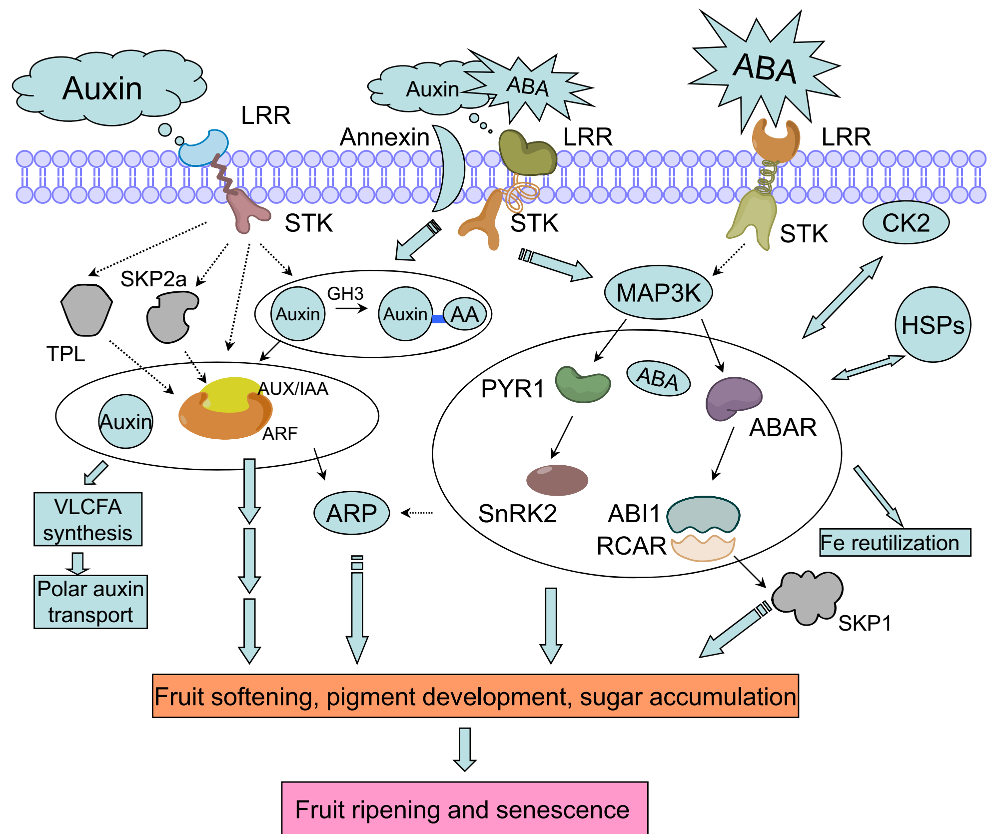

Fig. 4 A putative regulatory network of auxin and ABA on ripening and senescence of postharvest strawberry fruits. *Dashed arrows* hypothetical pathways. *AA* amino acid, *ABA* abscisic acid, *ABAR* ABA responsive proteins, *ABI1* ABA-insensitive 1, *ARF* IAA-responsive factor, *ARP* IAA-repressor protein, *AUX/IAA* IAA/indole-3-acetic acid protein, *CK2* casein kinase 2, *HSPs* heat-shock proteins, *LRR* leucine-rich repeat receptor-like protein kinase, *MAP3K* mitogen-activated protein kinase kinase kinase 3, *PYR1* pyrabactin resistance 1, *RCAR* regulatory components of ABA receptors, *SKP1* S-phase kinase-associated proteins 1, *SnRK2* sucrose non-fermenting protein-related kinase, *STK* serine/threonine receptor-like protein kinase, *TPL* TOPLESS, *Ub* Ubiquitin ligase, *VLCFA* very-long-chain fatty acid

The strawberry fruits responded to the treatment with the mixture of IAA and ABA in a different way than individual treatments with IAA or ABA alone. Approximately 17 % of differentially expressed unigenes responding to the combination were not found in the differentially expressed unigenes responding to IAA or ABA. Among these unigenes, 17.2 kDa class II heat-shock protein (LMW HSP) was up-regulated specifically by the combination but neither by IAA nor ABA (Table 3). MAPKKK-like gene was also uniquely elevated by the combination (Table 3). In *Arabidopsis*, MAPKKKs are known to participate in hormone-mediated signaling and defense responses (Rodriguez et al. 2010). In contrast, cytochrome *c* oxidase gene was specially down-regulated by the combination (Table 3); this gene encodes the key enzyme in cell respiration. These unique genes may represent novel gene networks in response to the combination of IAA and ABA.

Annexins belong to an evolutionarily conserved multi-gene family of Ca^{2+}-dependent and phospholipid binding proteins and play a significant role in protecting plants from both abiotic and biotic stresses (Jami et al. 2012). A previous study also certified that annexins mediated osmotic stress and ABA signal transduction in Arabidopsis (Lee et al. 2004). In this study, two annexins were involved in the response of harvested fruits to both IAA and ABA (Tables 1, 2, 3), suggesting annexins in harvested fruits may not only participate in ABA signal transduction but also in IAA signal transduction.

It is known that receptor-like kinases (RLKs) play critical roles in signaling pathways that regulate growth, development, hormone perception, and pathogenic defense responses (Gou et al. 2010; ten Hove et al. 2011). Both IAA and ABA repressed the expression of leucine-rich repeat receptor-like protein kinase (LRR-RLK) (Tables 1, 2). LRR-RLKs represent one of the largest transmembrane receptor kinase families. In *Arabidopsis thaliana* roots, no LRR-RLKs were observed to respond to ABA but T-DNA insertion lines corresponding to 16 RLK genes showed a consistently enhanced root length, indicating an increased resistance to IAA (ten Hove et al. 2011). So far, the functions of the majority of LRR-RLKs have not been elucidated. In addition, a putative serine/threonine kinase (STK), one of the RLK subclass, was induced by IAA and ABA (Tables 1, 2), suggesting that RLKs may play important roles in plant hormone signaling pathways during postharvest fruit ripening.

In conclusion, the physiological processes of fruit ripening and senescence are regulated in a complex way by cross-talks among multiple hormones. This study advanced our understanding of global molecular events in harvested strawberry fruits in responding to exogenous hormones. As

proposed in Fig. 4, either exogenous IAA or ABA transmits its signaling to intracellular substances through receptor-like kinases (RLKs) and further affects the inherent signaling pathways of hormones. The signaling of exogenous IAA disrupts intracellular IAA homeostasis and its original signaling by regulating such genes as *GH3*, *Ubiquitin ligase* (*Ub*), *TOPLESS* (*TPL*). On the other hand, the exogenous ABA signaling disturbs the normal expression of such genes as *CK2* and *HSP*s that may interact with intracellular ABA signaling. *SKP1* functions as a signal in the downstream of the ABA signaling that may regulate some structural genes. The negative regulation of ARP by ABA implies complex roles of *ARP* in the ripening and senescence processes of the strawberry fruits. The response of fruits to the mixture of IAA and ABA can be either cooperative or antagonistic between the two hormones. Our findings suggest that a marginal fluctuation in plant hormone signaling may cause substantial changes in metabolism probably due to their cascading effects. In addition, genes encoding receptor-like kinases and ubiquitin ligases may play pivotal roles in IAA and ABA signaling pathways and may be the cross-talk points of the hormones.

Author contribution statement MLC and CJX conceived and designed the experiments. CJX and LWJ performed the experiments. CJX analyzed the data and wrote the manuscript. YTJ and LZS also have contributed to the data interpretation and writing.

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