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RESEARCH ARTICLE

Genome-wide identification of SAUR genes in watermelon (*Citrullus lanatus*)

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Abstract The early auxin responsive SAUR family is an important gene family in auxin signal transduction. We here present the first report of a genome-wide identification of SAUR genes in watermelon genome. We successfully identified 65 *ClaSAURs* and provide a genomic framework for future study on these genes. Phylogenetic result revealed a Cucurbitaceae-specific SAUR subfamily and contribute to understanding of the evolutionary pattern of SAUR genes in plants. Quantitative RT-PCR analysis demonstrates the existed expression of 11 randomly selected SAUR genes in watermelon tissues. *ClaSAUR36* was highly expressed in fruit, for which further study might bring a new prospective for watermelon fruit development. Moreover, correlation analysis revealed the similar expression profiles of SAUR genes between watermelon and Arabidopsis during shoot organogenesis. This work gives us a new support for the conserved auxin machinery in plants.

Keywords SAUR · Watermelon · Cucumber · Phylogeny · Expression analysis

Introduction

SAUR genes form an important and large gene family in auxin signal transduction and they are commonly employed as marker genes for early auxin response in model plants (Gil et al. 1994; McClure et al. 1989; Roux et al. 1998). Till now, the function of SAUR genes in auxin signaling has been reported in many other plants, such as mung, tomato, radish, apple, maize, pepper, rice, litchi, potato, cotton, citrus, peach, sorghum and ramie (Huang et al. 2016). The results indicated that SAUR genes would generally regulate auxin-mediated development in plants. Molecular genetic studies have revealed the functions of several SAUR genes in Arabidopsis, such as such as *AtSAUR14*, *15*, *36*, *61–69*, *75* in cell elongation (Chae et al. 2012; Matsui et al. 2005; Roig-Villanova et al. 2007; Stamm and Kumar 2013), *AtSAUR9*, *19–24*, *38*, *40*, *41*, *71*, *72* in cell expansion (Spartz et al. 2012, 2014) and *AtSAUR15*, *50*, *68* in light signaling (Roig-Villanova et al. 2007; Sato et al. 2014). With more and more plant genome information published, SAUR genes have been analyzed at genome-wide level in rice, maize, sorghum, Arabidopsis, tomato and potato (Chen et al. 2014; Jain et al. 2006; Wu et al. 2012). The specie-specific expansion of SAUR genes will be an important research focus to reveal their evolutionary pattern in plant genomes (Chen et al. 2014). In spite of published watermelon and cucumber (*Cucumis sativus*) genome information, there is still no related report on

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SAUR genes in Cucurbitaceae plants (Guo et al. 2013; Huang et al. 2009).

Watermelon is an important cash crop with important nutrient compositions, such as lycopene, sugars and healthy amino acids (Collins et al. 2007). Nowadays, studies on watermelon are mainly focused on the mechanism of fruit development and disease resistance (Guo et al. 2011; Ouibrahim et al. 2014), with general trends to improve fruit yield and quality. However, there are few reports studying the mechanisms of shoot regeneration to improve the efficiency of regeneration and transformation systems published decades ago (Choi et al. 1994). It has been reported that SAUR genes differentially expressed during shoot organogenesis in Arabidopsis and ramie (Che et al. 2006; Huang et al. 2014). Besides, it has been proved the existence of auxin signal regulation during fruit development (Pattison et al. 2014). Related researches would be of great importance and promote the progress of auxin mechanism during shoot regeneration and fruit development in watermelon. In the present study, we conducted the identification of SAUR genes according to watermelon genome of inbred line 97,103. Phylogenetic analysis was employed to investigate the evolutionary history of SAUR proteins among Arabidopsis, cucumber and watermelon. We also conducted the expression analysis of 11 randomly selected SAUR genes in watermelon tissues and different development stages of shoot regeneration. The results would serve as an overview for watermelon SAUR genes and a guidance for future study.

Materials and methods

Sequence retrieval, chromosomal location and subcellular localization prediction

Arabidopsis SAUR sequences were downloaded from The Arabidopsis Information Resource (http://www.Arabidopsis.org) and employed as query sequences by using TBLASTN (Altschul et al. 1997) to search SAUR genes respectively in cucumber and watermelon genome databases (ICuGI) (http://www.icugi.org/cgi-bin/ICuGI/index.cgi) with a cut off of E-value $<10^{-5}$. Obtained sequences were employed as queries to search the two databases again, respectively. Redundant sequences with the same chromosome loci or different identification numbers were removed. The information on chromosome localization and intron for these genes were also obtained from ICuGI. We further used obtained watermelon SAUR genes to search the watermelon unigene database by using BLASTN. Subcellular localization prediction for each genes was conducted using the CELLO software version 2.5 (http://cello.life.nctu.edu.tw/) (Yu et al. 2006).

Phylogenetic, motif and promoter region analysis

Based on neighbor-joining (NJ) method, a phylogenetic tree was constructed for SAUR proteins by using the software MEGA 5.0 (Tamura et al. 2011). Bootstrap values from 1000 trials was used to construct the most parsimonious tree. Multiple Expectation Maximization for Motif Elicitation (MEME) utility was employed to investigate the motifs of watermelon SAUR proteins (http://meme.nbcr.net/meme/) (Bailey et al. 2009).

To investigate cis-elements in promoter sequences of watermelon SAUR genes, we downloaded the upstream genomic DNA sequences (1000 bp) before the initiation codon (ATG) for each gene from the ICuGI. The database of plant cis-acting regulatory DNA elements, PLACE (http://www.dna.affrc.go.jp/PLACE/signalscan.html), was used for searching auxin-responsive elements in the promoter regions of the watermelon SAUR genes (Higo et al. 1999).

Plant materials and sampling

The diploid watermelon inbred line A7 was used for material preparation in this study. The samples of different watermelon tissues (shoot, leaf, stem and flower) were separately collected from 2-month-old flowering plants. The leaves from 1-week-old seedlings were sprayed with IAA (50 mM, Sigma-Aldrich, Saint Louis, MO, USA) and sampled at 0, 15, 60 min according to previous studies (Wu et al. 2012; Huang et al. 2016). Samples form different development stages of shoot regeneration were prepared according to our previous study (Zhang et al. 2015). Decoated seeds were sterilized for 5 min with NaClO solution (10%), washed with sterile distilled water and placed in culture tubes (25 × 150 mm) with Murashige and Skoog medium (20 mL) for germination. Cotyledons from 1-week-old seedlings were cut into segments (0.5 × 0.5 mm) and cultured on MS medium (1.0 mg/L 6-BA and 1.0 mg/L NAA, Sigma-Aldrich, Saint Louis, MO, USA) under 25 ± 2 °C with a photoperiod of 16/8 h (light/dark). Each sample was collected for three times as biological replicates, and froze in liquid nitrogen immediately and stored at −75 °C before RNA isolation.

Quantitative RT-PCR analysis

RNA isolation, reserves cDNA synthesis and quantitative RT-PCR analysis were conducted as previous study (Huang et al. 2014). Total RNA was purified by Tiangen®RNA prep Pure Plant Kit (Tiangen Biomart, Beijing) and used for cDNA synthesis by GoScript™ Reverse Transcription System (Promega, USA), based on the instructions from manufacturer. Quantitative RT-PCR

analysis was conducted by an optical 96-well plate iQ5 multicolor real time PCR system (Bio-RAD, USA). Each reaction (20 μL) consisted of cDNA (1 μL), gene-specific primers (10 nM), iTaq^{TM} Universal SYBR$^{®}$ Green Supermix (10 μL, Bio-RAD, USA) and ddH_2O (7 μL). The watermelon glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) gene was used as a endogenous control (Kong et al. 2014). Specific primers for each genes (Table 1) were designed by using Primer 3 (http://primer3.ut.ee/) and synthesized commercially from *Sunny Biotech*, Shanghai, China. Each sample was performed in triplicates for quantitative RT-PCR analysis. We calculated the relative expression levels as previously reported (Livak and Schmittgen 2001).

Results

Obtained sequences, chromosomal location and subcellular localization of watermelon SAUR genes

After removing the redundant sequences, we obtained 65 SAUR sequences in genome database of watermelon inbred line 97,103 (Table 2). The ORF length of these genes ranged from 252 to 558 bp, encoding polypeptides of 83–185 aa. The locations of the genes were also obtained when sequence retrieval and mapped to chromosomes (Fig. 1). The 65 genes were named from *ClaSAUR1* to *ClaSAUR65* according to their chromosome positions (Table 2). Watermelon SAUR genes spread on the eleven chromosomes. Two gene clusters were found in chromosome 2 and 11, which contained 15 and 23 SAUR genes, respectively. According to the results of predicted protein localization, we found that most watermelon SAUR genes possess signal sequences targeting the mitochondria or the nucleus. Only a few genes were located in extracellular, chloroplast, cytoplasmic or plasma membrane.

Phylogenetic, motif and promoter region analysis

SAUR proteins from Arabidopsis, watermelon and cucumber were selected for the construction of NJ phylogenetic tree to investigate their evolution patterns. The Arabidopsis SAUR proteins were selected as model system. We obtained 73 SAUR genes in cucumber genome, which was used as close-related species (Supplementary Table 1). All SAUR proteins were clustered into eight groups (Fig. 2). Group I contained much more Cucurbitaceae SAUR proteins than Arabidopsis, while group II was on the contrary. There were slightly more Arabidopsis sequences than Cucurbitaceae plants in group IV and V. Nearly the same scale of SAUR sequences in Arabidopsis and Cucurbitaceae plants were clustered into group VI, VII and VIII. Moreover, most watermelon sequences shared highly similarities with cucumber compared to Arabidopsis. Furthermore, we used MEME tool to investigate the conserved domains of watermelon SAUR genes. As a result, a conservative motifs were simultaneously found in most sequences (Fig. 3).

The 1000 bp upstream sequences of *ClaSAURs* were investigated by PLACE, the results revealed seven types of auxin signal transduction related cis-elements (Chen et al. 2014). We found that at least one of the putative cis-elements existed in promoter regions of most *ClaSAURs*, except for *ClaSAUR 10*, *11*, *22*, *32*, *62*, *63* and *64* (Supplementary Figure 1).

Expression analysis of watermelon SAUR genes

The expression patterns of 11 randomly selected *ClaSAURs* were examined by qRT-PCR analysis. These genes were differentially expressed among six watermelon tissues

Table 1 Primers of watermelon GAPDH gene (endogenous control) and 11 SAUR genes used for qRT-PCR

Gene	Forward primer	Reverse primer
GAPDH	TGGAAGAATCGGTAGGTTGG	CTGTCACTGTTTTTGGCGTC
ClaSAUR11	CGTTTTGTCATCCCACTGTCTTA	ACGAGCCATTCCAACTCTGA
ClaSAUR16	GACGACATGTGATTCCGATTTCT	CAATTAGCCAAGCGAGAAGTGA
ClaSAUR19	GACTCCTCCCAATCCAACTACT	TTGAGCCGATTTCTCCAGTAAAG
ClaSAUR27	CATTTCGTTGTATATGTCGGCCA	GAGAAAGACGTGTTCATCGCAAG
ClaSAUR32	GCTATCAAAGTTGGACATGAAAGTG	GCTTGAACATACCTAAATTGCTCC
ClaSAUR36	CCAAAACAACTCCAAGGACCTTC	ATGACAACCTCACAAGAAACCAC
ClaSAUR41	GATTCTCCACCATCGTCGGA	AGACCACTTTTCTGATCAAAACCA
ClaSAUR44	ATTGTGCAGTTTATGTTGGGGAA	CATCGCTGCAAGGAATAGTGAG
ClaSAUR46	TTCAGAGGAAGCGATTTGTGA	TGCAATCTAGAAGTGAGATCGATAA
ClaSAUR54	TGAAGATGCAGTCAGGTTTTACA	AAGCCAAATTCTTCCTCTGCATA
ClaSAUR56	GGATTCGTTTGCTATCCTTGGT	TGTTGGAATGATGGATGGTTTAAGT

Table 2 SAUR gene family in watermelon

Gene	Accession	Location	ORF(bp)	Predicted protein (aa)	Group	CELLO localization	Unigene
ClaSAUR1	Cla004867	Chr01:21697–22068(−)	372	123	I	Nuclear (2.372)	WMU38606
ClaSAUR2	Cla014274	Chr01:29617657–29617980(−)	324	107	IV	Mitochondrial (1.351)/Nuclear (1.232)	WMU13974
ClaSAUR3	Cla015782	Chr02:3475294–3475740(+)	447	148	IV	Chloroplast (1.832)	
ClaSAUR4	Cla015856	Chr02:4172956–4173294(−)	339	112	I	Mitochondrial (1.869)	WMU13304
ClaSAUR5	Cla015857	Chr02:4189805–4190107(−)	303	100	I	Mitochondrial (1.556)	
ClaSAUR6	Cla015858	Chr02:4196874–4197155(+)	282	93	II	Mitochondrial (2.233)	
ClaSAUR7	Cla015859	Chr02:4199877–4200149(−)	273	90	I	Nuclear (1.430)/Extracellular (1.307)	
ClaSAUR8	Cla015860	Chr02:4209324–4209578(−)	255	84	I	Extracellular (1.093)/Nuclear (1.084)	
ClaSAUR9	Cla015862	Chr02:4222092–4222361(+)	270	89	I	Nuclear (1.484)/Extracellular (1.059)	WMU77806
ClaSAUR10	Cla015863	Chr02:4231480–4231773(+)	294	97	I	Nuclear (2.038)	
ClaSAUR11	Cla015864	Chr02:4233449–4233751(+)	303	100	I	Nuclear (1.661)/Mitochondrial (1.184)	
ClaSAUR12	Cla015865	Chr02:4237014–4237310(+)	297	98	I	Nuclear(1.200)/ Mitochondrial(1.035)	
ClaSAUR13	Cla015866	Chr02:4240319–4240618(+)	300	99	I	Nuclear (1.598)/Mitochondrial (1.414)	
ClaSAUR14	Cla015867	Chr02:4244885–4245187(+)	303	100	I	Nuclear (1.921)	
ClaSAUR15	Cla015868	Chr02:4248467–4248757(+)	291	96	I	Nuclear (1.889)	
ClaSAUR16	Cla015869	Chr02:4253824–4254075(+)	252	83	II	Chloroplast (1.424)/ Mitochondrial (1.164)	
ClaSAUR17	Cla015870	Chr02:4273922–4274221(+)	300	99	IV	Mitochondrial (2.558)	
ClaSAUR18	Cla015871	Chr02:4294325–4294783(−)	459	152	V	Nuclear (1.981)	
ClaSAUR19	Cla013463	Chr02:29119351–29119773(+)	423	140	V	Nuclear (1.764)/Mitochondrial (1.331)	WMU43911
ClaSAUR20	Cla008714	Chr02:31662116–31662526(+)	411	136	V	Cytoplasmic (1.862)	
ClaSAUR21	Cla005087	Chr03:2977473–2977904(+)	432	143	V	Cytoplasmic (1.481)/Chloroplast (1.174)/Nuclear (1.057)	WMU63127
ClaSAUR22	Cla008029	Chr04:1428526–1428864(+)	339	112	VII	Nuclear (2.154)	WMU40749
ClaSAUR23	Cla018306	Chr04:20658563–20658937(−)	375	124	VII	Extracellular (2.806)	
ClaSAUR24	Cla018405	Chr04:21691123–21691527(+)	405	134	VI	Nuclear (1.680)/Chloroplast (1.118)	
ClaSAUR25	Cla021120	Chr05:584905–585219(−)	315	104	VI	Mitochondrial (1.414)/Nuclear (1.356)/Cytoplasmic (1.238)	WMU17151
ClaSAUR26	Cla021848	Chr05:6844117–6844623(+)	507	168	VI	Nuclear (2.333)/Mitochondrial (1.910)	
ClaSAUR27	Cla020821	Chr05:26464978–26465277(+)	300	99	IV	Mitochondrial (1.279)/ Extracellular (1.263)	
ClaSAUR28	Cla020819	Chr05:26500696–26501148(−)	453	150	V	Mitochondrial (1.671)/Nuclear (1.139)	
ClaSAUR29	Cla009987	Chr05:33006683–33007192(+)	510	169	VI	Nuclear (2.475)	
ClaSAUR30	Cla001500	Chr06:1949767–1950294(+)	528	175	VI	Nuclear (3.672)	WMU11144
ClaSAUR31	Cla006646	Chr06:2643211–2643735(+)	525	174	VI	Mitochondrial (2.911)	WMU15601
ClaSAUR32	Cla006703	Chr06:3446468–3446770(−)	303	100	VI	Cytoplasmic (1.512)/Nuclear (1.145)	
ClaSAUR33	Cla012544	Chr07:23968425–23968793(−)	369	122	VIII	Mitochondrial (1.883)/ Extracellular (1.615)	WMU32892

Table 2 continued

Gene	Accession	Location	ORF(bp)	Predicted protein (aa)	Group	CELLO localization	Unigene
ClaSAUR34	Cla013759	Chr08:16935542–16935865(−)	324	107	VII	Cytoplasmic (2.273)	
ClaSAUR35	Cla022435	Chr08:23597574–23597900(−)	327	108	IV	Mitochondrial (1.785)/Nuclear (1.322)	
ClaSAUR36	Cla015521	Chr09:808924–809295(−)	372	123	VIII	Mitochondrial (1.701)/ Chloroplast (1.181)	WMU31548
ClaSAUR37	Cla015473	Chr09:1282152–1282628(+)	477	158	VI	Nuclear (2.472)	
ClaSAUR38	Cla016189	Chr09:11958879–11959304(+)	426	141	VIII	Nuclear (2.953)	
ClaSAUR39	Cla005501	Chr09:34581012–34581398(+)	387	128	V	Extracellular (1.562)/ Nuclear(1.232)/ Mitochondrial(1.090)	WMU32933
ClaSAUR40	Cla005678	Chr10:3551181–3551636(+)	456	151	VI	Mitochondrial(2.459)	
ClaSAUR41	Cla017679	Chr10:25175996–25176355(−)	360	119	VI	Nuclear (1.690)/Cytoplasmic (1.228)	
ClaSAUR42	Cla016616	Chr11:23256389–23256850(+)	462	153	V	Nuclear (3.575)	WMU50780
ClaSAUR43	Cla016617	Chr11:23280608–23280940(−)	333	110	IV	Mitochondrial (2.200)	WMU53688
ClaSAUR44	Cla016619	Chr11:23304625–23304918(−)	294	97	I	PlasmaMembrane (1.350)/ Nuclear (1.029)	
ClaSAUR45	Cla016620	Chr11:23308654–23308941(−)	288	95	I	PlasmaMembrane (1.450)	
ClaSAUR46	Cla016621	Chr11:23321892–23322179(+)	288	95	I	Mitochondrial (1.474)/Nuclear (1.134)	
ClaSAUR47	Cla016622	Chr11:23327375–23327662(+)	288	95	I	Mitochondrial (2.112)	
ClaSAUR48	Cla016623	Chr11:23332560–23332853(+)	294	97	I	Mitochondrial (1.518)/Nuclear (1.478)/PlasmaMembrane (1.156)	
ClaSAUR49	Cla016625	Chr11:23341471–23341767(+)	297	98	I	Nuclear (1.238)/Mitochondrial (1.225)	
ClaSAUR50	Cla016626	Chr11:23343447–23343740(+)	294	97	I	Mitochondrial (1.494)	WMU79661
ClaSAUR51	Cla016627	Chr11:23346057–23346350(+)	294	97	I	Mitochondrial (1.925)	
ClaSAUR52	Cla016628	Chr11:23348490–23348783(+)	294	97	I	Mitochondrial (1.597)/ PlasmaMembrane (1.172)	
ClaSAUR53	Cla016629	Chr11:23351128–23351412(+)	285	94	I	Mitochondrial (1.373)/ Extracellular (1.023)	
ClaSAUR54	Cla016630	Chr11:23353693–23353986(+)	294	97	I	Extracellular (1.479)/ Mitochondrial (1.351)	
ClaSAUR55	Cla016631	Chr11:23355704–23355997(+)	294	97	I	Mitochondrial (2.344)	
ClaSAUR56	Cla016632	Chr11:23360937–23361230(+)	294	97	I	Mitochondrial (2.041)	
ClaSAUR57	Cla016633	Chr11:23362408–23362689(+)	282	93	I	Mitochondrial (1.393)/ Extracellular (1.100)/Nuclear (1.046)	
ClaSAUR58	Cla016634	Chr11:23363443–23363736(−)	294	97	I	Mitochondrial (1.874)	
ClaSAUR59	Cla016635	Chr11:23371966–23372316(+)	351	116	I	Mitochondrial (2.419)	
ClaSAUR60	Cla016636	Chr11:23374694–23374987(−)	294	97	I	Mitochondrial (1.897)	
ClaSAUR61	Cla016637	Chr11:23378192–23378485(+)	294	97	I	Mitochondrial (2.264)	
ClaSAUR62	Cla016638	Chr11:23385046–23385339(+)	294	97	I	Mitochondrial (2.358)	
ClaSAUR63	Cla016640	Chr11:23388432–23388725(+)	294	97	I	Mitochondrial (2.332)	
ClaSAUR64	Cla016641	Chr11:23391929–23392222(+)	294	97	I	Mitochondrial (2.570)	
ClaSAUR65	Cla001873	Chr11:27065514–27066071(+)	558	185	VI	Nuclear (2.144)/Cytoplasmic (1.774)	

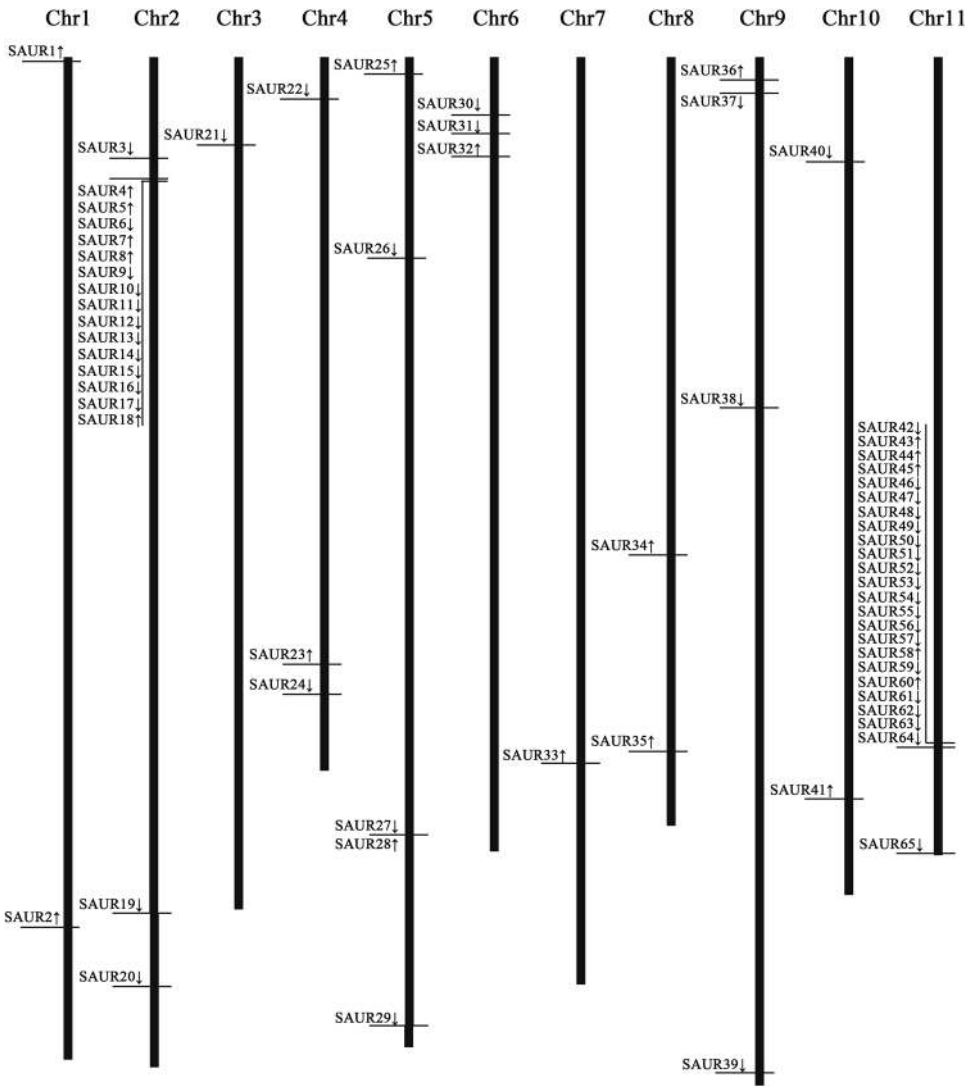

Fig. 1 Chromosomal position of SAUR genes. The chromosome number is labeled on the *top* of each chromosome. The *arrows* next to the genes indicated the transcription direction

(Fig. 4a). Five genes were mainly expressed in leaf and flower tissues and other five in leaf, bud and flower tissues. Interestingly, *ClaSAUR36* was highly expressed in fruit. All these genes were expressed at relatively low levels in stem and root tissues. We further analyzed the expression patterns of these genes response to IAA treatment (Fig. 4b). Four genes were slightly up-regulated after IAA treatment, while the other seven genes were decreased more than 2-fold at 30 min.

Auxin is an important regulator during shoot organogenesis (Duclercq et al. 2011). For this reason, we examined the expression of SAUR genes during shoot organogenesis of watermelon. The results indicated that nine *ClaSAURs* were down-regulated at 3, 14 and 28 days, while up-regulated at 7 and 21 days (Fig. 4c). The other two *ClaSAURs* were down-regulated after 7 days. Moreover, we compared the expression pattern of *ClaSAURs* with the expression data of Arabidopsis homologs (Supplementary Table 2) during shoot organogenesis in a previous study (Che et al. 2006). By calculating correlation coefficient in SPSS (Liu et al. 2003), we found that *ClaSAUR* genes showed moderate relationship ($R = 0.529$, significant at 0.01 level) with Arabidopsis homologs (Fig. 5).

Discussion

Identification of SAUR genes in watermelon

Genome-wide identification has revealed the scale of SAUR family in model plants, such as Arabidopsis (79), rice (56), tomato (74), potato (134), maize (75) and sorghum (71) (Chen et al. 2014; Jain et al. 2006; Wu et al. 2012). In the present study, we successfully identified 65 watermelon SAUR genes, which was at a moderate scale compared with model plants. The cucumber contained 73 SAUR genes even though its genome was smaller than

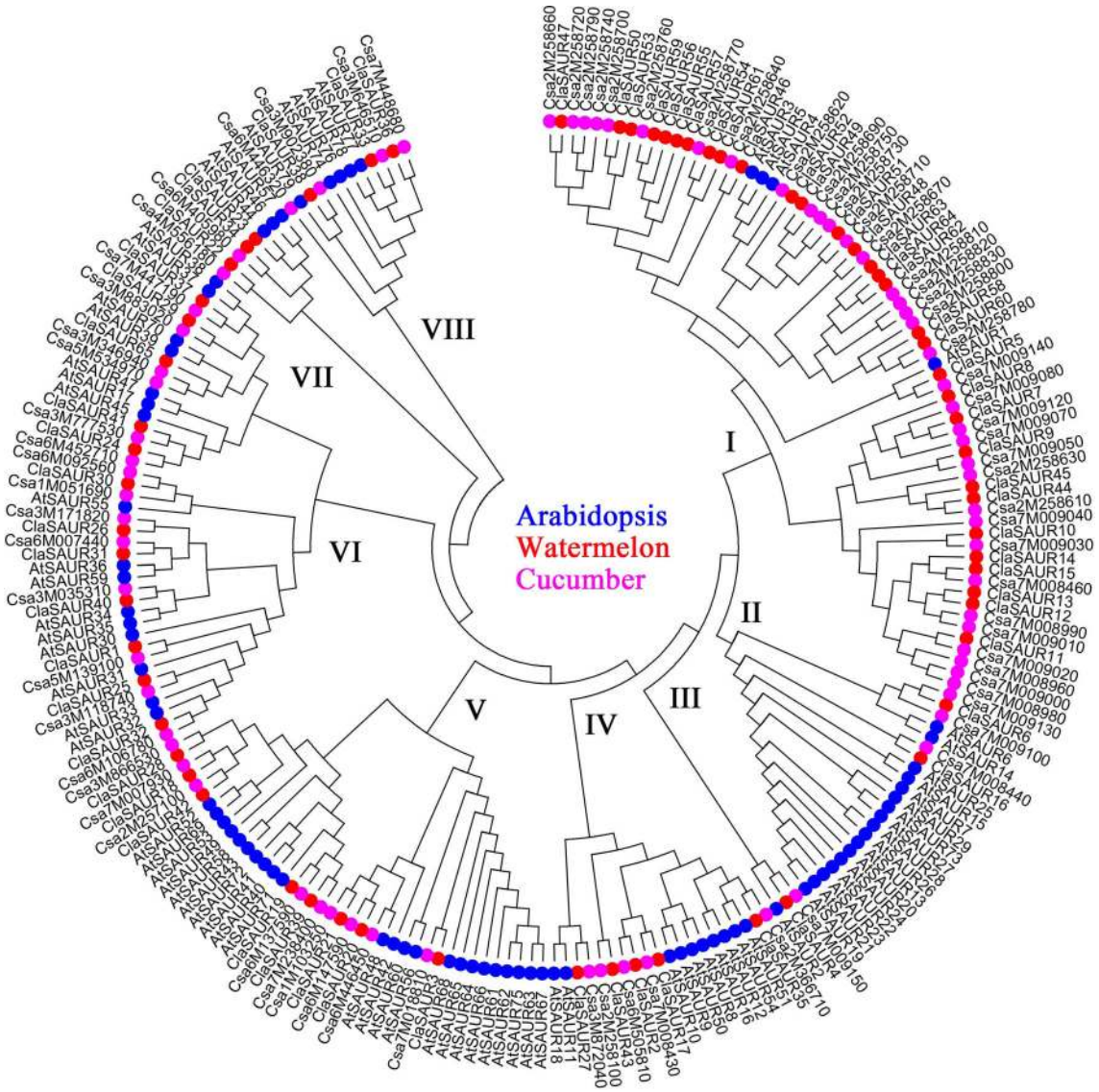

Fig. 2 Phylogenetic tree of SAUR proteins from Arabidopsis (*blue*), watermelon (*red*) and cucumber (*pink*). Phylogenetic inference was conducted using MEGA 5.0. Branch width corresponds to support values (color figure online)

Fig. 3 The conserved consensus motif among watermelon SAUR proteins according to MEME. The symbol heights indicated the relative frequency for each residue

watermelon, which might be caused by whole genome duplication (Guo et al. 2013; Huang et al. 2009). In the 65 genes, no intron was found, while six cucumber SAUR genes contained introns (Supplementary Table 1). Introns were also found in several tomato, potato and maize SAUR genes, which might affect the expression of these genes (Chen et al. 2014; Wu et al. 2012). Among the 65 genes, 16 could match the unigenes in a previous study (Table 2); (Guo et al. 2011).

Cucurbitaceae-specific expansion of SAUR genes

The watermelon genome contained two SAUR clusters consisted of 15 and 23 genes. We also found two SAUR

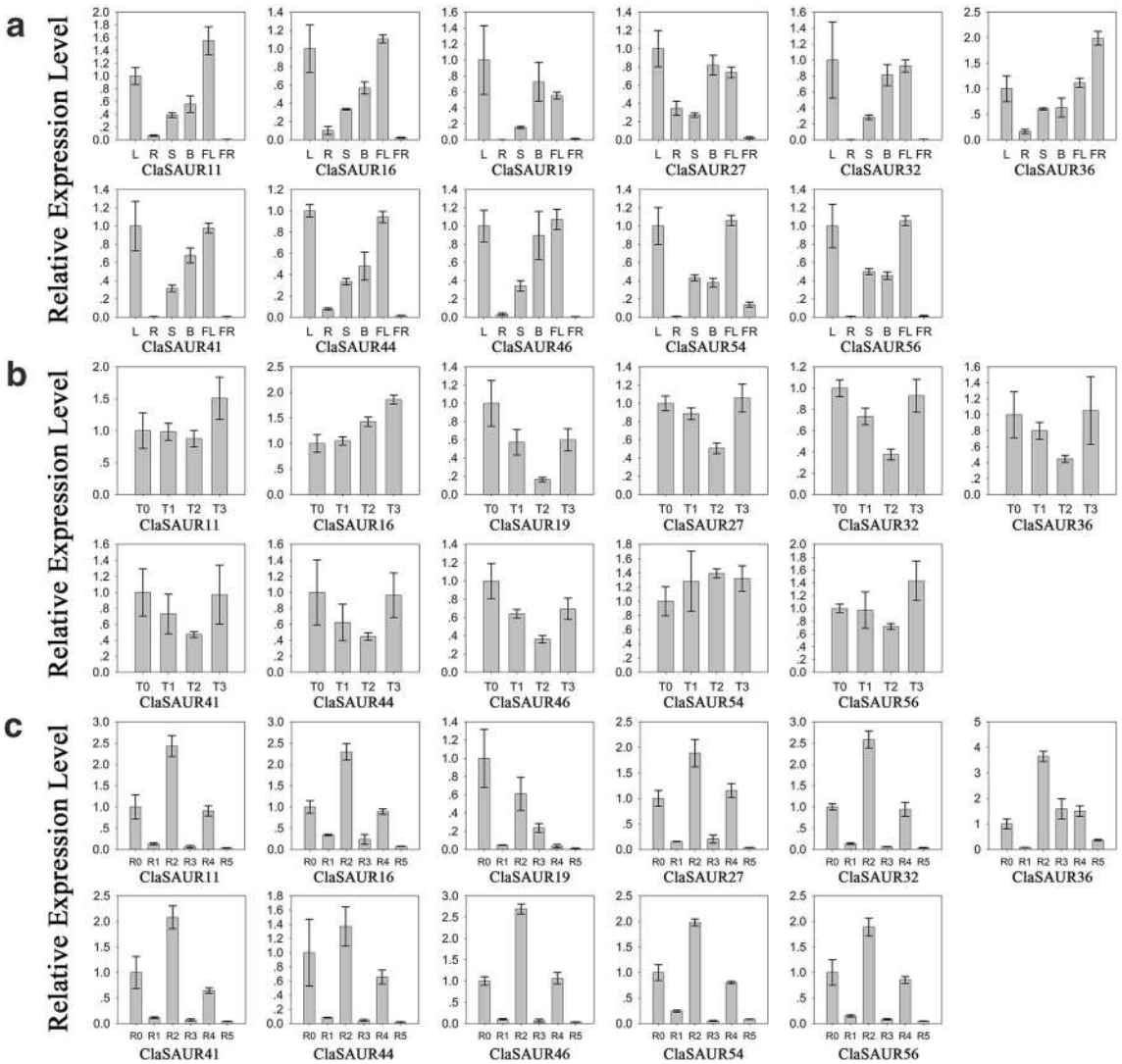

Fig. 4 **a** Expression of SAUR genes in different watermelon tissues. The capital letters, R, L, S, B, FL and FR on X-axis represent respectively for root, leaf, stem, bud, flower and fruit. **b** Expression patterns of SAUR genes after IAA treatment. T0, T1, T2 and T3 of X-axis represent 0, 10, 30 and 60 min after IAA treatment, respectively. **c** Expression patterns of SAUR genes during watermelon shoot organogenesis. R0, R1, R2, R3, R4 and R5 of X-axis represent cotyledon segments incubated for 0, 3, 7, 14, 21 and 28 days, respectively

clusters in cucumber, which possessed 20 and 23 members, respectively (Supplementary Table 1). Most of these watermelon and cucumber genes were grouped together by phylogenetic analysis (Fig. 2). The results indicated that the clusters in watermelon chromosome 2 and 11 were highly homologous with those in cucumber chromosome 7 and 2, respectively. SAUR clusters were also reported in rice, tomato and maize, in which most genes tended to be grouped together by phylogenetic analysis (Chen et al. 2014; Jain et al. 2006; Wu et al. 2012), indicating the specie-specific expansion was commonly existed in SAUR family. In plants, gene expansion generally accompanied with plant evolution under environment factors (Lespinet et al. 2002). It has been reported that rapid gene expansion occurred in morphological development and stress response related gene families (Hanada et al. 2008). We here present the first report the specific SAUR expansion in Cucurbitaceae plants, which might contribute to Cucurbitaceae-specific morphological development. Much more molecular genetic or biochemical analyses would be further needed to reveal the functional evolution of these Cucurbitaceae-specific SAUR genes.

Differentially expressed ClaSAUR genes

We randomly selected 11 *ClaSAURs* to examine their expression patterns in watermelon tissues. The results indicated all these genes were highly expressed in leaf,

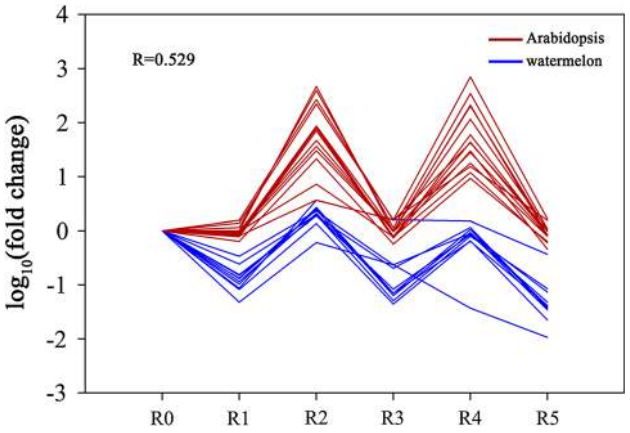

Fig. 5 Comparison of expression pattern between *ClaSAURs* (*bule*) and Arabidopsis homologs (*red*) during shoot organogenesis. The correlation coefficient was calculated by SPSS (color figure online)

shoot and flower tissues of growing watermelon plants. These genes might share the similar functions with Arabidopsis SAUR genes. The functions of SAUR genes in actively dividing tissues, such as cell elongation and cell expansion in Arabidopsis, have been revealed. Moreover, transcriptome profiling has identified 3023 differentially expressed genes during watermelon fruit development and ripening (Guo et al. 2011). However, in this study, only *ClaSAUR36* was highly expressed in developing fruit of watermelon, which indicated that *ClaSAUR36* was a specific one from SAUR family. More works are necessary to reveal the function of *ClaSAUR36*, which will significantly enrich the functional diversity of SAUR family.

Under IAA treatment, the expression of seven *ClaSAURs* decreased more than twofold. Particularly, the expression of *ClaSAUR19* reduced more than threefold, which indicated it was an IAA-responsive gene. The other four *ClaSAURs* were slightly responsive to IAA treatment even if the existence of auxin-responsive cis-elements in their promoter regions. They might function in other phytohormone signaling. In Arabidopsis, only 27 SAUR genes were responsive to auxin treatment (Paponov et al. 2008). It still remained unclear that why nearly two third of *AtSAURs* were insensitive to auxin.

Similar SAUR expression between Arabidopsis and watermelon during shoot organogenesis

Transcription profilings have revealed complex auxin signaling pathway during shoot organogenesis of Arabidopsis, poplar and ramie (Bao et al. 2009; Che et al. 2006; Huang et al. 2014). Many SAUR genes differentially expressed during Arabidopsis and ramie shoot organogenesis. In this study, all the 11 *ClaSAURs* were differentially expressed during the process and the complex expression pattern of *ClaSAURs* indicated complex auxin signaling pathway during watermelon shoot organogenesis. We further investigated the correlation between the expression pattern of *ClaSAURs* and Arabidopsis homologs (Fig. 5). The correlation coefficient indicated the expression of *ClaSAURs* was similar with those in Arabidopsis during the process, which supported the conservation of auxin machinery in plants (De Smet et al. 2011; Finet and Jaillais 2012). However, more functional characterizations would be still needed to reveal how SAUR genes regulate shoot organogenesis.

Conclusion

In this study, the results provided a genomic framework for future characterization of watermelon SAUR genes. The phylogenetic analysis revealed a Cucurbitaceae-specific SAUR subfamily and contributed to revealing the evolutionary pattern of plant SAUR genes. Quantitative RT-PCR analysis demonstrated the existed expression of 11 randomly selected SAUR genes in different watermelon tissues. *ClaSAUR36* was highly expressed in fruit, for which further study might bring a new prospective for watermelon fruit development. Moreover, correlation analysis revealed the similar expression profiles of SAUR genes between watermelon and Arabidopsis during shoot organogenesis. This work gives us a new support for the conserved auxin machinery in plants and lay the foundation of further studies for auxin signaling during shoot regeneration and fruit development in watermelon.

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