RESEARCH ARTICLE

Identification of small auxin-up RNA (*SAUR*) genes in Urticales plants: mulberry (*Morus notabilis*), hemp (*Cannabis sativa*) and ramie (*Boehmeria nivea*)

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Abstract

Small auxin-up RNA (*SAUR*) genes are important gene families in auxin signalling transduction and are commonly used as early auxin responsive markers. Till date, no SAUR gene is identified in Urticales plants despite of the published bioinformation of mulberry, hemp and ramie. In this study, we used Arabidopsis sequences as query to search against mulberry, hemp genomes and ramie transcriptome database. In total, we obtained 62, 56 and 71 SAUR genes in mulberry, hemp and ramie, respectively. Phylogenetic analysis revealed the Urticales specific expansion of *SAUR* genes. Expression analysis showed 15 randomly selected ramie *SAUR* genes that were diversely functioned in ramie tissues and revealed a series of IAA-responsive, drought-responsive and high temperature-responsive genes. Moreover, comparison of qRT-PCR data and previous RNA-Seq data suggested the reliability of our work. In this study, we first report the identification of *SAUR* genes in Urticales plants. These results will provide a foundation for their function validation in Urticales plant growth and development.

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Introduction

SAUR genes, an important gene family in auxin signalling transduction are commonly used as early auxin-responsive markers in soybean, *Arabidopsis* and tobacco (McClure *et al*. 1989; Gil *et al*. 1994; Roux *et al*. 1998). *SAUR* genes have been reported in many plants, such as mung (Yamamoto 1994), tomato (Zurek *et al.* 1994), radish (Anai *et al.* 1998), apple (Watillon *et al.* 1998), maize (Yang and Poovaiah 2000), pepper (Marois *et al.* 2002), rice (Jain *et al.* 2006), litchi (Kuang *et al.* 2012), potato (Wu *et al.* 2012), cotton (Yang *et al.* 2012), citrus (Licciardello *et al.* 2013), peach (Tatsuki *et al.* 2013), sorghum (Chen *et al.* 2014b) and ramie (Huang *et al.* 2014). Molecular-genetics methods have revealed the function of many *Arabidopsis SAUR* genes in regulating auxin-mediated development, such as *AtSAUR9*, *19-24*, *38*, *40*, *41*, *71*, *72* in cell expansion (Spartz *et al.* 2012; Qiu *et al.* 2013; Spartz *et al.* 2014) and *AtSAUR14*, *15*, *36*, *61-69*, *75* in cell elongation (Matsui *et al.* 2005; Roig-Villanova *et al.* 2007; Chae *et al.* 2012; Stamm and Kumar 2013). With the increasing number of available plant genomes, genomewide analysis of *SAUR* genes has been conducted in *Arabidopsis*, rice, tomato, potato, maize and sorghum (Jain *et al.* 2006; Wu *et al.* 2012; Chen *et al.* 2014b). The species-specific expansion of *SAUR* genes has been reported, which is a new prospective to investigate their evolutionary pattern in plant genomes (Chen *et al.* 2014b). Despite published information on mulberry, hemp genomes and ramie transcriptome there is still no related information on Urticales plants (van Bakel *et al.* 2011; He *et al.* 2013; Chen *et al.* 2014a; Huang *et al.* 2014; An *et al.* 2015).

Urticales is an important cash crop and provides three kinds of natural fibres directly or indirectly. Mulberry is an economic food crop for the domesticated silkworm more than 5000 years (Barber 1991). Bast fibre of hemp has been used in textile production in China more than 6000 years (Li 1974). Ramie is also famous for its bast fibre with smooth long appearance and excellent tensile strength (Bruhlmann *et al*. 2000). The molecular basis of Urticales plant still remains low even with available bioinformation. In the

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present study, we first identified *SAUR* genes in Urticales plants: mulberry, hemp and ramie. Phylogenetic analysis of SAUR proteins was conducted to investigate their evolutionary pattern in *Arabidopsis* and Urticales plants. We further examined the expression patterns of 15 randomly selected ramie *SAUR* genes in different tissues under indole-3-acetic acid (IAA), drought and high temperature treatments. Besides, the expression data of qRT-PCR was compared with previous RNA-Seq data to investigate the reliability. The results provide an overview for Urticales *SAUR* genes and will serve as a guideline for future study.

Materials and methods

Sequence retrieval and subcellular localization prediction

To identify the *SAUR* family genes in mulberry and hemp, the BLAST searches were performed in the Morus Genome Database (http://morus.swu.edu.cn/morusdb/) and the Cannabis Genome Database (http://genome.ccbr.utoronto.ca/) by using 79 SAUR protein sequences from the Arabidopsis Information Resource (TAIR) (https://www.arabidopsis.org/) as query sequences. All *Arabidopsis*, mulberry and hemp protein sequences were further used as query sequences to search against three ramie transcriptome databases, respectively (Chen *et al.* 2014a; Huang *et al.* 2014; An *et al.* 2015). The sequences obtained from three databases were aligned based on the nucleotide sequence using ClustalX (Thompson *et al*. 1997). The genes from these three different databases that had an overlap of more than 50 bp were further assembled. If two or three genes from the three databases overlapped completely then that gene with the longer nucleotide sequence was reserved. Additionally, the genes which only appeared in a single database were set aside. Finally, all genes that were assembled and set aside were analysed to obtain coding sequence (CDS) by using open reading frame finder (ORF finder, http://www.ncbi.nlm.nih.gov/projects/gorf/). Obtained sequences were sub mitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) with accession numbers, see table 1.

Phylogenetic and motif analysis

A neighbour-joining (NJ) phylogenetic tree was constructed for SAUR proteins using MEGA 5.0 software (Tamura *et al.* 2011). The most parsimonious tree with boot strap values from 1000 trials was used. Multiple expectation maximization for motif elicitation (MEME) utility was used to investigate motifs of SAUR proteins (http://meme-suite.org/) (Bailey *et al.* 2009).

Expression profiling of Urticales SAUR genes

To further investigate the SAUR expression in different organs, previous transcriptome data were employed. Mulberry and hemp transcriptome data were downloaded from Morus Genome Database (http://morus.swu.edu.cn/morusdb/) and Cannabis Genome Database (http://genome.ccbr.utoronto.ca/). Ramie transcriptome data was according to the previous studies (Chen *et al.* 2014a; An *et al.* 2015). These data were presented as heat maps in red/green code using R software (http://www.r-project.org/).

Plant materials and sampling

Plants of ramie cv. 1504 were grown in Ramie Germplasm Repository of Huazhong Agricultural University (Wuhan, China). Leaves, shoots, roots and stem barks were separately sampled from 2-month-old plants. For IAA and abiotic stress treatment, young plants (about 15 cm from top) were cut and the notches were soaked in 0.02% $KMnO_4$ for two days and tap water for rooting. After rooting, all plants were grown in Hoagland's nutrient solution. The leaves of plants cultured without soil were sprayed with 50 mM IAA (Sigma-Aldrich, Saint Louis, USA) and then sampled at 15 and 60 min intervals (Wu *et al.* 2012); treated with 20% PEG 6000 as drought treatment and sampled at 8 h (An *et al.* 2015); grown in incubator at 40°C as high temperature treatment and sampled at 24 h; untreated leaves were sampled as control. All the samples were immediately frozen in liquid nitrogen and stored at −80°C. Three samples for each tissue and treatment were separately stored as three biological replicates.

Quantitative RT-PCR analysis

Total RNA was isolated and reverse transcribed separately using the Tiangen RNA Prep Pure Plant kit (Tiangen Biomart, Beijing, China) and the GoScript Reverse Transcription System (Promega, Madison, USA), under the manufacturer's instructions. The qRT-PCR analysis was conducted by an optical 96-well plate iQ5 multicolour real-time PCR system (Biorad, Hercules, USA). Each 20 μL reaction volume was mixed with 1 μL of cDNA template, 10 nM gene-specific primers, 10 μL of iTaq Universal SYBR Green Supermix (Biorad) and 7 μL of double distilled water. The ramie glyceraldehyde-3-phosphate-dehydrogenase (*GAPDH*) gene was selected as the endogenous control (Kong *et al*. 2014). Gene-specific primers (table 2) were designed online (http://primer3.ut.ee/) and commercially synthesized (Sunny Biotech, Shanghai, China). The thermal cycle used was as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. After amplification, a dissociation stage was carried out to detect any complex products. The qRT-PCR was performed in triplicate for each sample. Relative expression levels were calculated as the previous study (Livak and Schmittgen 2001).

Results

Obtained sequences and subcellular localization of Urticales SAUR genes

Sixty-two and 56 SAUR sequences were separately retrieved from published mulberry and hemp genomes, respectively

Table 1. *SAUR* gene family in ramie.

Gene	GenBank accession	CDS (bp)	Predicted protein (aa)	CELLO localization
BnSAUR01	KR076440	282	93	Mitochondrial (1.011)
BnSAUR02	KR076441	270	89	Nuclear (1.16) / chloroplast (1.084) / mitochondrial (1.047)
BnSAUR03	KR076442	282	93	Nuclear (1.27) / mitochondrial (1.174)
BnSAUR04	KR076443	288	95	Chloroplast (1.297) / mitochondrial (1.293)
BnSAUR05	KR076444	306	101	Nuclear (1.316) / chloroplast (1.296) / mitochondrial (1.225)
BnSAUR06	KR076445	282	93	Nuclear (1.323) / mitochondrial (1.133)
BnSAUR07	KR076446	276	91	Mitochondrial (1.334)
BnSAUR08	KR076447	408	135	Nuclear (1.345) / mitochondrial (1.198) / chloroplast (1.168)
BnSAUR09	KR076448	303	100	Nuclear (1.351) / chloroplast (1.289) / mitochondrial (1.006)
BnSAUR10	KR076449	417	138	Mitochondrial (1.395) / nuclear (1.341)
BnSAUR11	KR076450	258	85	Cytoplasmic (1.433) / mitochondrial (1.004)
BnSAUR12	KR076451	282	93	Nuclear (1.45) / mitochondrial (1.132)
BnSAUR13	KR076452	282	93	Nuclear (1.45) / mitochondrial (1.132)
BnSAUR14	KR076453	285	94	Mitochondrial (1.47) / nuclear (1.031)
BnSAUR15	KR076454	297	98	Mitochondrial (1.512) / nuclear (1.231) / cytoplasmic (1.019)
BnSAUR16	KR076455	423	140	Plasma Membrane (1.563)
BnSAUR17	KR076456	309	102	Mitochondrial (1.576) / nuclear (1.552)
BnSAUR18	KR076457	438	145	Nuclear (1.577) / mitochondrial (1.347)
BnSAUR19	KR076458	282	93	Mitochondrial (1.578) / chloroplast (1.142)
BnSAUR20	KR076459	444	147	Mitochondrial (1.628) / plasma membrane (1.162)
BnSAUR21	KR076460	306	101	Chloroplast (1.631) / mitochondrial (1.153)
BnSAUR22	KR076461	282	93	Mitochondrial (1.636) / nuclear (1.36)
BnSAUR23	KR076462	300	99	Chloroplast (1.674) / mitochondrial (1.431)
BnSAUR24	KR076463	309	102	Mitochondrial (1.72) / nuclear (1.578)
BnSAUR25	KR076464	270	89	Chloroplast (1.723)
BnSAUR26	KR076465	303	100	Nuclear (1.732) / mitochondrial (1.632)
BnSAUR27	KR076466	339	112	Mitochondrial (1.733) / nuclear (1.642)
BnSAUR28	KR076467	327	108	Mitochondrial (1.741) / chloroplast (1.438)
BnSAUR29	KR076468	333	110	Cytoplasmic (1.743) / chloroplast (1.409)
BnSAUR30	KR076469	303	100	Chloroplast (1.764) / mitochondrial (1.074)
BnSAUR31	KR076470	459	152	Nuclear (1.768) / mitochondrial (1.287)
BnSAUR32	KR076471	309	102	Mitochondrial (1.775) / chloroplast (1.136)
BnSAUR33	KR076472	456	151	Nuclear (1.781) / mitochondrial (1.306) / extracellular (1.304)
BnSAUR34	KR076473	315	104	Mitochondrial (1.798) / nuclear (1.113)
BnSAUR35	KR076474	399	132	Cytoplasmic (1.799) / nuclear (1.387)
BnSAUR36	KR076475	294	97	Mitochondrial (1.828) / cytoplasmic (1.594)
BnSAUR37	KR076476	294	97	Mitochondrial (1.834) / cytoplasmic (1.374)
BnSAUR38	KR076477	330	109	Mitochondrial (1.858) / nuclear (1.436)
BnSAUR39	KR076478	294	97	Mitochondrial (1.892)
BnSAUR40	KR076479	318	105	Mitochondrial (1.908)
BnSAUR41	KR076480	297	98	Mitochondrial (1.91)
BnSAUR42	KR076481	291	96	Chloroplast (1.936)
BnSAUR43	KR076482	477	158	Mitochondrial (1.969) / nuclear (1.518)
BnSAUR44	KR076483	459	152	Nuclear (1.972) / mitochondrial (1.46)
BnSAUR45	KR076484	309	102	Mitochondrial (2.003)
BnSAUR46	KR076485	306	101	Chloroplast (2.004)
BnSAUR47	KR076486	423	140	Cytoplasmic (2.004)
BnSAUR48	KR076487	402	133	Nuclear (2.032)
BnSAUR49	KR076488	297	98	Mitochondrial (2.038)
BnSAUR50	KR076489	537	178	Mitochondrial (2.046) / nuclear (1.81)
BnSAUR51	KR076490	294	97	Mitochondrial (2.05)
BnSAUR52	KR076491	450	149	Chloroplast (2.054)
BnSAUR53	KR076492	471	156	Mitochondrial (2.184)
BnSAUR54	KR076493	357	118	Nuclear (2.248)
BnSAUR55	KR076494	294	97	Mitochondrial (2.261)
BnSAUR56	KR076495	294	97	Mitochondrial (2.268)
BnSAUR57	KR076496	294	97	Mitochondrial (2.279)
BnSAUR58	KR076497	297	98	Mitochondrial (2.281)
BnSAUR59	KR076498	321	106	Chloroplast (2.289)
BnSAUR60	KR076499	318	105	Mitochondrial (2.311)
BnSAUR61	KR076500	369	122	Cytoplasmic (2.325)
BnSAUR62	KR076501	294	97	Mitochondrial (2.33)
BnSAUR63	KR076502	447	148	Nuclear (2.377)

Table 1 (*contd*)

Gene	GenBank accession	CDS (bp)	Predicted protein (aa)	CELLO localization
BnSAUR64	KR076503	522	173	Nuclear (2.47)
BnSAUR65	KR076504	303	100	Chloroplast (2.5)
BnSAUR66	KR076505	255	84	Nuclear (2.599)
BnSAUR67	KR076506	324	107	Mitochondrial (2.636)
BnSAUR68	KR076507	324	107	Mitochondrial (2.791)
BnSAUR69	KR076508	387	128	Extracellular (2.791)
BnSAUR70	KR076509	522	173	Mitochondrial (2.919)
BnSAUR71	KR076510	522	173	Nuclear (3.199)

(table 3). After removing the redundant sequences, we obtained 71 SAUR sequences from published transcriptome databases which were named from *BnSAUR1* to *BnSAUR71* (table 1). These genes ranged from 255–537 bp in CDS with predicted proteins of 84–178 aa. The predicted protein localization was conducted online by a subcellular localization predictor (CELLO, http://cello.life.nctu.edu.tw/). We found that 35, 19 and 10 BnSAURs were more likely to possess signal sequences targeting the mitochondrial, nucleus and chloroplast, respectively. Only five BnSAURs were located in cytoplasm. Moreover, BnSAUR19 was located in plasma membrane, while BnSAUR69 was extracellular.

Phylogenetic and motif analysis

The phylogenetic tree was conducted by NJ method. We selected Arabidopsis SAUR proteins as a model system to investigate the evolutionary relationships. Mulberry, hemp and ramie were used as closely-related Urticales species. All SAUR proteins in four species were clustered into six groups (figure 1). Ramie and hemp possess larger scales of *SAUR* genes than Arabidopsis and mulberry in group I. More Arabidopsis and ramie sequences were found in group II. There were more Arabidopsis sequences than Urticales plants in group III, IV and VI. In group V, more Arabidopsis and mulberry sequences were clustered together than ramie and hemp. Moreover, most Urticales sequences shared high similarities when compared with Arabidopsis. We further used MEME to investigate the conserved motif of SAUR proteins. As a result, the conservative motif with 69 aa were simultaneously found in most sequences (figure 2).

Expression profile of Urticales SAUR genes and qRT-PCR validation of BnSAURs

The expression profiles of Urticales *SAUR* genes are shown in figure 3. Most genes were actively expressed at least in one organ. Fifteen randomly selected *BnSAURs* were examined by qRT-PCR analysis. Major expressed tissues were designated when relative expression level was 3-fold over other organs. As a result, we found that four genes were mainly expressed in leaf, three in root and one in shoot, excluding three genes highly expressed in both leaf and root (figure 4a). We further analysed the expression of these 15 genes under IAA treatment (figure 4b). Nine genes were significantly upregulated at 15 min and downregulated at 60 min after IAA treatment, while *BnSAUR44* showed conflicting expression. *BnSAUR52* and *BnSAUR71* were downregulated only at 60 min and so *BnSAUR69* at both 15 and 60 min.

We also conducted drought and high temperature stresses in this study. The results showed that all 15 *BnSAURs* were upregulated or downregulated by more than 3-fold when

Table 2. Primers of ramie GAPDH gene (endogenous control) and 11 *SAUR* genes used for qRT-PCR.

Gene	Forward primer	Reverse primer
GAPDH	TGGAAGAATCGGTAGGTTGG	CTGTCACTGTTTTTGGCGTC
BnSAUR02	CAAGTGTGATCAGAAAGTCCTCA	AATCCAAATTCTCGTTCGGCTT
BnSAUR15	ATGGTTCGCACTTTTATCAGAAA	TCACAACAAACCTCTTCTTCTCC
BnSAUR16	ATGAAGGCCGTTTTGTTATCTAC	GAACGAGCAAGACAATGTAATTCA
BnSAUR20	AACGAAAAGAAGCGGTTTGTAGT	TGTAGAGCAGAGATTGAGAGTAGA
BnSAUR33	AGAAGATCACAAAAGCTCAACCA	ACCTCACACTTCATCACCAAAAC
BnSAUR36	ATGTTGGAGAGGAGGAGAAGAAG	AGAAGTGAGGTCAATGAAAACGT
BnSAUR43	CGAGTGAGTATGGGTTTTGGGTT	GCTTCAAATTTCGCCAGAGAGTT
BnSAUR44	AGGAAACAAACAAACCAACCGAA	CACGACGAATCTCCTGCACC
BnSAUR50	TGAAGCCTCGTATCTTTCTCATGT	AACTCTCTCCCGTCTTACCATGT
BnSAUR52	CTGAGAGCTTCTTCTTTGAGTGG	GCCTTCCTTGACGTCGTTG
BnSAUR58	AGGCACAACGTCCCATATTACAT	ATCGAGAAATAAAAGCCAGCGTG
BnSAUR61	CGATGCCACTGATTTTGACGA	CTCAAGTAGCCTCAGAAACGC
BnSAUR69	ACGTGAATAAAGTGAGTCCTTGT	CCTCTTGTAGTATTCGTCTTTCCC
BnSAUR70	GAGTGACGATCGAGGTGTAGATG	CCATGGGGCTTATTCGGTTTT
BnSAUR71	CTCGGTCTCATCGTGTTACTCAT	CACTTTCCCTTGCCTTTGATCTG

Table 3. *SAUR* gene family in mulberry and hemp.

MGD accession	CDS (bp)	Predicted protein (aa)
Morus000422	303	100
Morus000538	300	99
Morus000888	315	104
Morus000889	312	103
Morus000894	366	121
Morus002133	456	151
Morus002170	309	102
Morus002171	384	127
Morus002172	393	130
Morus002175	282	93
Morus002176	366	121
Morus002177	384	127
Morus002178	387	128
Morus002582	438	145
Morus003321	528	175
Morus003324	342	113
Morus003581	324	107
Morus005029	399	132
Morus005030	324	107
Morus005120	462	153
Morus008396	366	121
Morus008756	282	93
Morus008816	510	169
Morus010409	309	102
Morus010413	228	75
Morus010414	309	102
Morus010415	279	92
Morus012229	384	127
Morus012231	282	93
Morus012234	594	197
Morus012237	447	148
Morus013050	399	132
Morus013820	531	176
Morus014349	480	159
Morus014350	498	165
Morus014351	294	97
Morus014352	456	151
Morus014353	456	151
Morus014484	531	176
Morus014966	309	102
Morus014967	297	98
Morus015476	294	97
Morus015477	213	70
Morus015478	303	100
Morus015480	318	105
Morus015481	309	102
Morus015482	294	97
Morus015483	294	97
Morus016032	318	105
Morus016238	435	144
Morus017482	540	179
Morus019007	537	178
Morus019148	396	131
Morus020601	432	143
Morus020868	504	167
Morus023265	405	134
Morus023452	387	128
Morus024347	417	138
Morus024348	387	128
Morus024769	363	120
Morus024893	411	136
Morus026699	318	105

Table 3 (*contd*)

CGD accession	CDS (bp)	Predicted protein (aa)
Cas100372	267	88
Cas102200	279	92
Cas109218	297	98
Cas112007	297	98
Cas113119	444	147
Cas114107	414	137
Cas116704	402	133
Cas118029	297	98
Cas122305	270	89
Cas127869	408	135
Cas14397	372	123
Cas161351	306	101
Cas163223	285	94
Cas16493	333	110
Cas165009	282	93
Cas166988	435	144
Cas1704	327	108
Cas172168	297	98
Cas17879	420	139
Cas19244	297	98
Cas21903	567	188
Cas22718	282	93
Cas22777	303	100
Cas23328a	282	93
Cas23328b	303	100
Cas24402	390	129
Cas25226	495	164
Cas27761	579	192
Cas27935a	270	89
Cas27935b	282	93
Cas28054	303	100
Cas29883	405	134
Cas34614	300	99
Cas35397	270	89
Cas3638	519	172
Cas37497	483	160
Cas41889	330	109
Cas43744	450	149
Cas44040a	345	114
Cas44040b	486	161
Cas44137	315	104
Cas4417	378	125
Cas46554	270	89
Cas47265	303	100
Cas47563	471	156
Cas48141	456	151
Cas48552	426	141
Cas56591	249	82
Cas57286	297	98
Cas59356	279	92
Cas63924	303	100
Cas6669	336	111
Cas69160	303	100
Cas69370	336	111
Cas73278	312	103
Cas8004	357	118

stressed and were considered as stress-responsive genes (table 4; figure 4c). Among these genes, 10 and 12 genes were regulated by drought and high temperature, respectively. There were eight genes regulated by both stresses. Among these, four were both upregulated and the others were downregulated under the two stresses. Besides, four *BnSAURs* were downregulated only under high temperature.

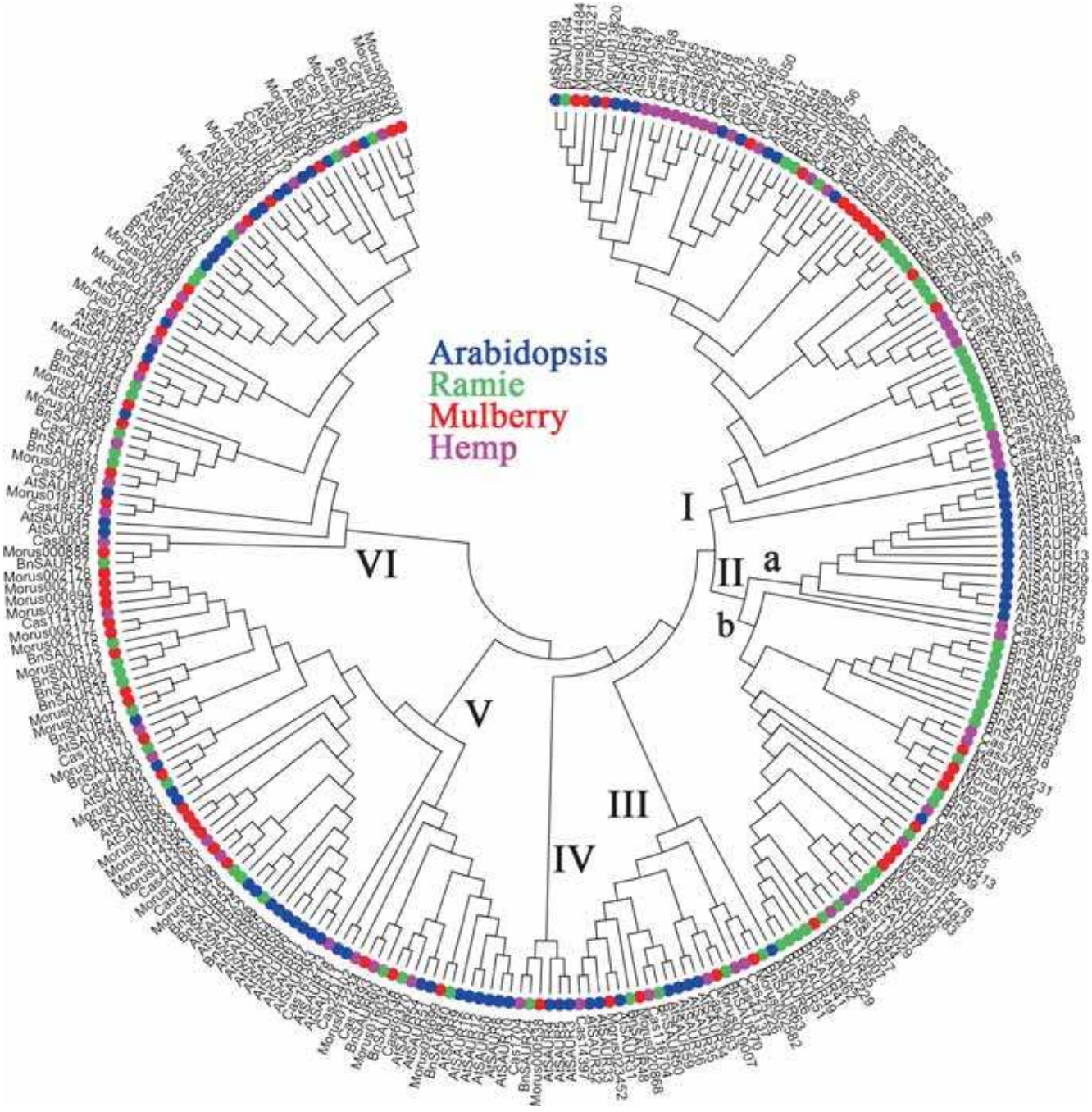

Figure 1. Phylogenetic analysis of SAUR proteins in *Arabidopsis*, mulberry, hemp and ramie. Phylogenetic inference was conducted using MEGA 5.0. Branch width corresponds to support values. The *Arabidopsis* proteins are shown in blue, ramie in green, mulberry in red and hemp in pink.

BnSAUR43 was upregulated and *BnSAUR44* downregulated only under drought.

Discussion

Identification of SAUR genes in ramie

Genomewide analysis has revealed the scales of SAUR family in model plants, such as 79 in Arabidopsis, 56 in rice, 134 in potato, 74 in tomato, 71 in sorghum and 75 in maize (Jain *et al.* 2006; Wu *et al.* 2012; Chen *et al.* 2014b). In this study, we successfully identified 71 ramie *SAUR* genes, which were a moderate scale compared to model plants. The other two Urticales plants, mulberry and hemp, each contained 62 and 56 *SAUR* genes in their genome, respectively. Their smaller scales of SAUR family might be due to whole genome duplication (Jaillon *et al.* 2009). Overall, we might obtain more *SAUR* genes even if the ramie genome information has not been accessible.

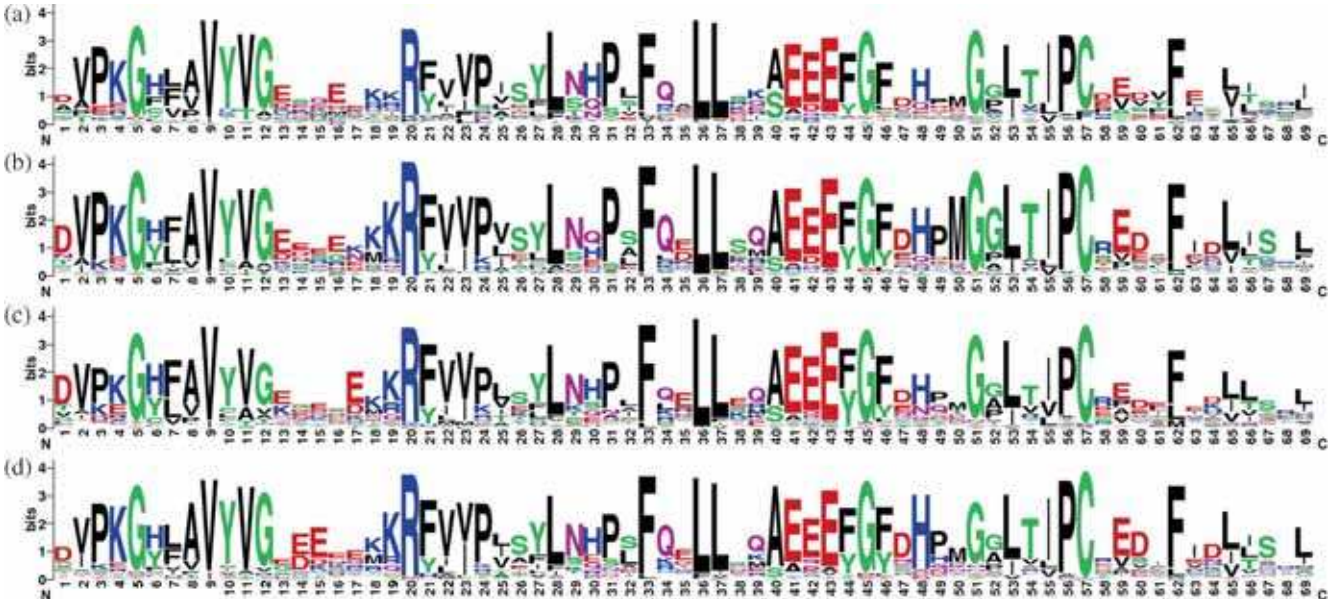

Figure 2. The conserved consensus motif in SAUR proteins. (a) *Arabidopsis*; (b) ramie; (c) mulberry; (d) hemp were found by MEME. The symbol heights represent the relative frequency of each residue.

Urticales specific expansion of SAUR genes

All *Arabidopsis*, ramie, mulberry and cannabis SAUR proteins were employed in phylogenetic analysis to investigate the evolutionary pattern. Many sequences of the four species were clustered in group I, III–VI. In these groups SAUR proteins might be more conserved in their functionary evolution. The results have also revealed specific gene clusters in Urticales plants, such as subgroups IIa and IIb (table 5). This phenomenon indicated the distinct evolutionary pattern between Urticales and *Arabidopsis*. The species-specific expansion of *SAUR* genes has been reported in rice, tomato and maize (Jain *et al.* 2006; Wu *et al.* 2012; Chen *et al.* 2014b). In plants, gene expansion generally accompanied by environment stresses drives plant evolution (Lespinet *et al.* 2002). Rapid gene expansion has been reported in multigene families related to morphological development and stress response (Hanada *et al.* 2008; Lehti-Shiu *et al.* 2009; Albert *et al.* 2013). Here, we first report the specific SAUR expansion in Urticales plants which might be caused by Urticales-specific morphological development. Besides, the subgroups IIa and IIb also showed distinct scales in different Urticales plants. Further molecular genetics or biochemical analyses is needed to reveal the evolutionary distinctions of *SAUR* genes in different Urticales plants.

Auxin-responsive and abiotic stress-responsive BnSAUR genes

SAUR genes have been reported to be involved in auxin signalling pathway of ramie (Huang *et al.* 2014). Within the 15 randomly selected *BnSAURs*, seven (*BnSAUR2*, *15*, *43*, *44*, *50*, *52*, *71*) were differentially expressed during auxin-mediated *in vitro* organogenesis in the previous study (Huang *et al.* 2014). We further analysed their expression under IAA-treatment. Seven (*BnSAUR2*, *16*, *36*, *43*, *44*, *58* and *69*) and five (*BnSAUR15*, *20*, *52*, *61* and *71*) of them were upregulated or downregulated more than 3-fold at 15 min and 60 min after IAA-treatment, respectively. These genes should be considered as auxin-responsive *BnSAUR* genes. Besides, *BnSAUR2* and *BnSAUR36* were upregulated more than 10-fold and could be used as early auxin marker genes in future studies.

A previous transcriptome analysis has revealed the expression pattern of ramie genes under PEG-induced drought stress (An *et al.* 2015). According to the RNA-Seq data, we found that two (*BnSAUR50* and *BnSAUR58*) of the 15 selected *BnSAURs* were differentially expressed during the process. The qRT-PCR analysis revealed another eight drought-responsive *BnSAURs* (table 4). *SAUR* genes have also been reported to be drought responsive in rice (Jain and Khurana 2009). Similar to drought, high temperature was another main abiotic stress during ramie development. It has been reported that a group of *Arabidopsis SAUR* genes expressed at high temperature (Franklin *et al.* 2011). Our results also revealed 12 high temperature responsive *BnSAURs*. These genes were more likely to share the same function with those *Arabidopsis* genes. The character of abiotic responsive BnSAURs would be valuable for application in molecular breeding.

Comparison of qRT-PCR and previous RNA-Seq analysis

To validate the qRT-PCR data in this study, we further collected RNA-Seq data from previous studies (Chen *et al.* 2014a; An *et al.* 2015). The expression pattern of 15 genes by RNA-Seq is shown in heatmap (figure 4). The Pearson correlation coefficient was calculated to assess the

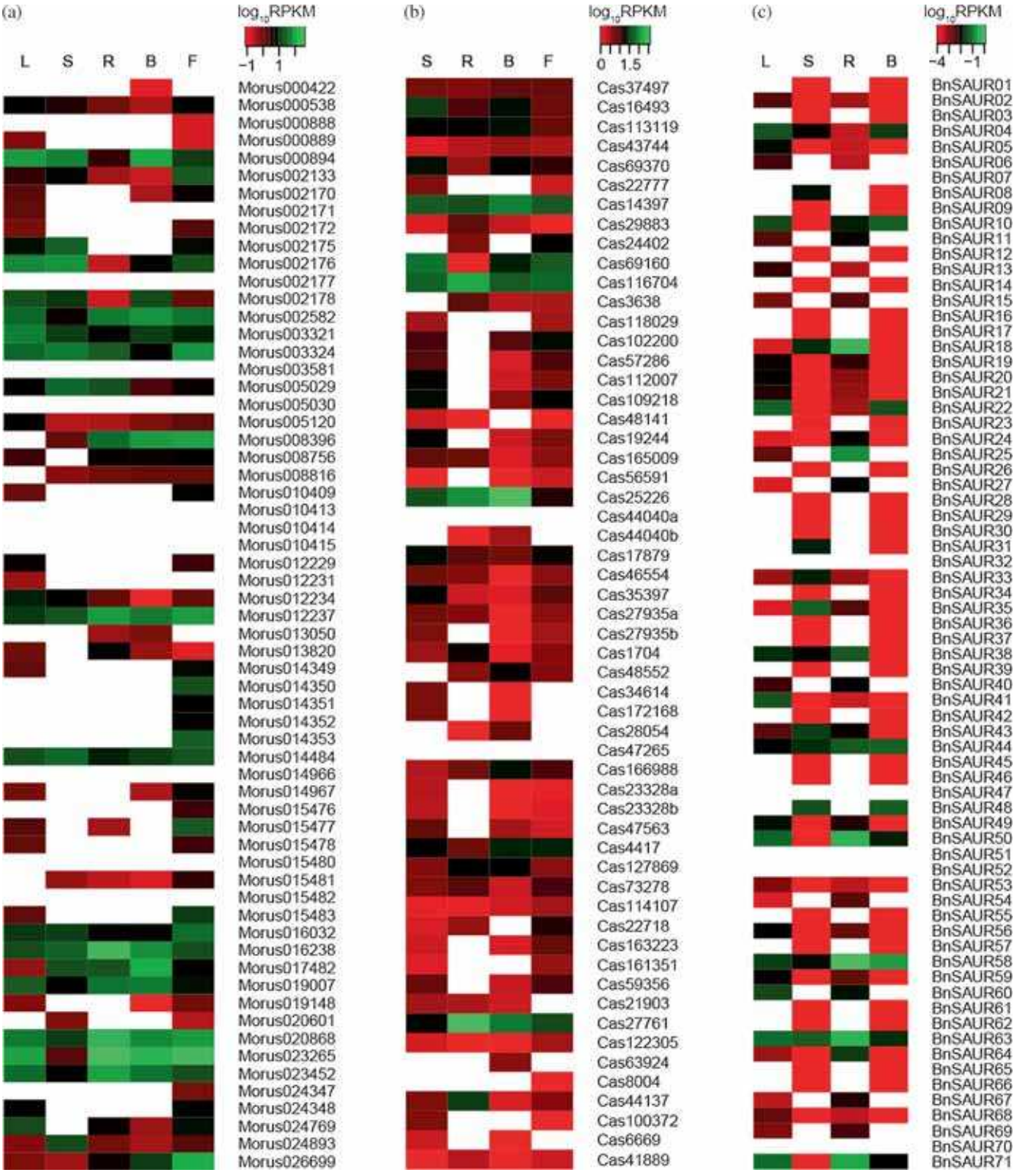

Figure 3. Expression patterns of Urticales *SAUR* genes different organs. L, S, R, B and F represent leaf, shoot, root, bark and flower, respectively. Mulberry and hemp data were downloaded from Morus Genome Database (http://morus.swu.edu.cn/morusdb/) and Cannabis Genome Database (http://genome.ccbr.utoronto.ca/). Ramie data was according to previous RNA-Seq data (Chen *et al.* 2014a; An *et al.* 2015). The blank area represent no published expression data.

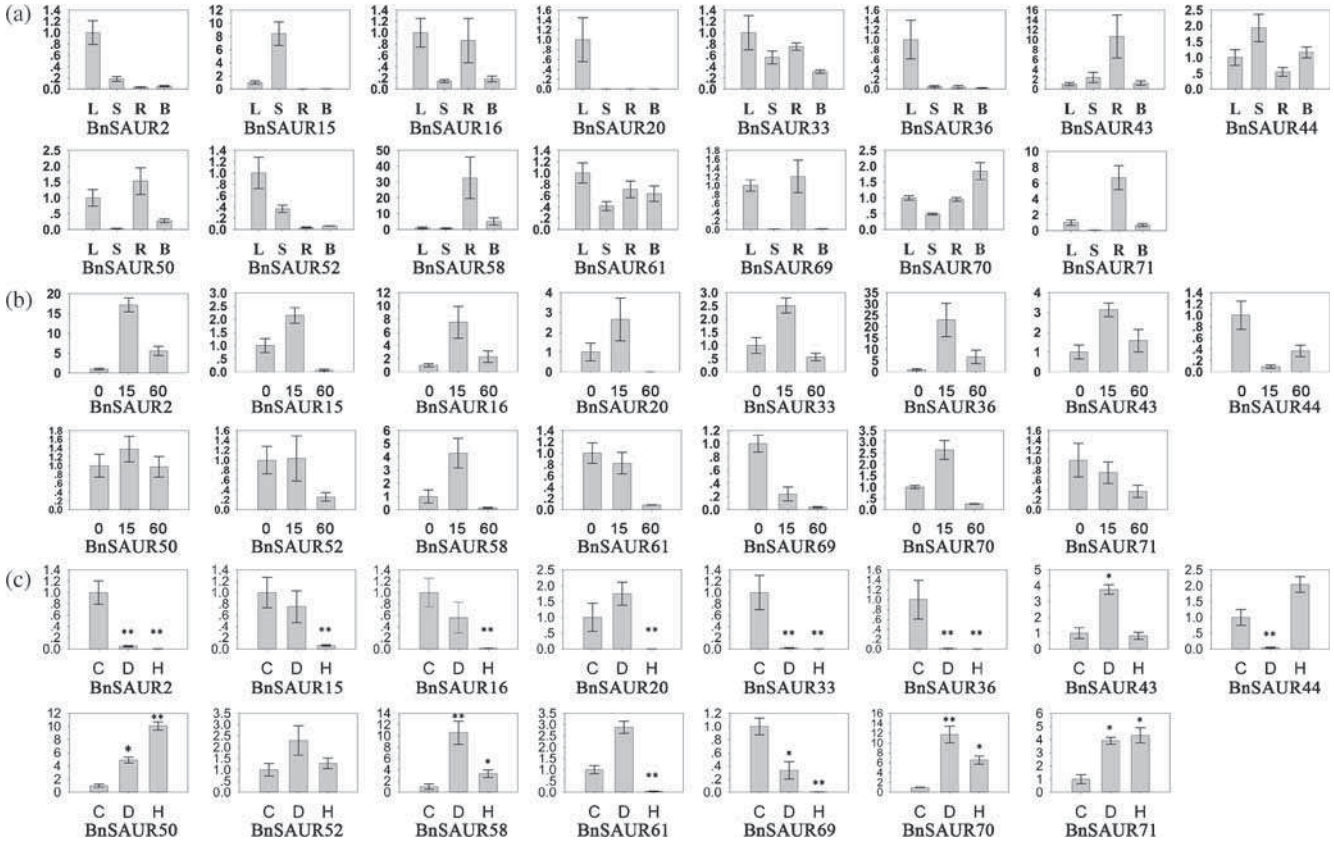

Figure 4. (a) Expression patterns of 15 *SAUR* genes in ramie tissues. L, S, R and B of *x*-axis represent leaf, shoot, root and stem bark, respectively. (b) Expression patterns of *SAUR* genes after IAA treatment. *X*-axis represent 0, 15 and 60 min after IAA treatment, respectively. (c) Expression patterns of *SAUR* genes under drought and high temperature treatment. C, D and H of *x*-axis represent control, drought and high temperature treatment, respectively. *, ** Expression level was upregulated or downregulated by more than 3-fold and 10-fold, respectively. The error bar represents the standard error.

correlation between different platforms by SPSS. The validation of expression patterns in ramie tissues and under drought stress, both showed a moderate correlation (figure 5a, $R = 0.652$; figure 5b, $R = 0.405$ correlation was significant at the 0.01 level). The results suggested the reliability of our qRT-PCR validation.

Table 4. Expression analysis of 15 ramie *SAUR* genes.

Gene	Major tissue expressed	IAA response (sequential comparison)		Stress response	
		15 min	60 min	Drought	High temperature
BnSAUR02	Leaf	Up	Down	Down	Down
BnSAUR15	Shoot	Up	Down	–	Down
BnSAUR16	Leaf/root	Up	Down	–	Down
BnSAUR20	Leaf	Up	Down	–	Down
BnSAUR33	Constitutive	Up	Down	Down	Down
BnSAUR36	Leaf	Up	Down	Down	Down
BnSAUR43	Root	Up	Down	Up	–
BnSAUR44	Constitutive	Down	Up	Down	–
BnSAUR50	Leaf/root	–	–	Up	Up
BnSAUR52	Leaf	–	Down	–	–
BnSAUR58	Root	Up	Down	Up	Up
BnSAUR61	Constitutive	–	Down	–	Down
BnSAUR69	Leaf/root	Down	Down	Down	Down
BnSAUR70	Constitutive	Up	Down	Up	Up
BnSAUR71	Root	–	Down	Up	Up

Table 5. Number of *SAUR* genes of Arabidopsis, ramie, mulberry and hemp in groups I–VI.

Species	I	II	III	IV	V	VI	Total
Arabidopsis	11	15	8	3	23	19	79
Ramie	20	22	4	1	13	11	71
Mulberry	12	9	4	1	20	15	61
Hemp	20	9	5	1	9	12	56

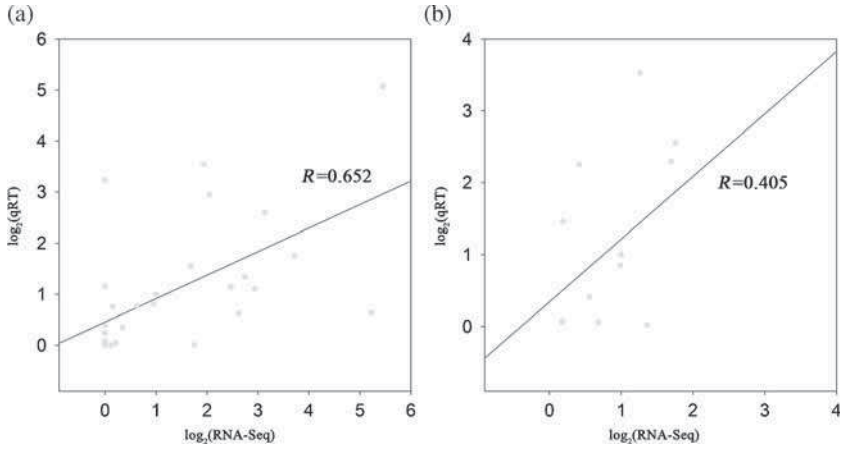

Figure 5. Comparison of qRT-PCR and previous RNA-Seq data in ramie tissues (a) and PEG-induced drought stress (b).

Conclusion

The results of this study provide a frame work for further research on Urticales *SAUR* genes. Phylogenetic analysis revealed an Urticales-specific SAUR expansion and contribute to understanding of the evolutionary mode of plant *SAUR* genes. Most Urticales *SAUR* genes were actively expressed according to previous transcriptome data. Expression validation by qRT-PCR showed 15 randomly selected ramie *SAUR* genes that were diversely functioned in ramie tissues and revealed a series of IAA-responsive, drought-responsive and high temperature-responsive genes. Moreover, comparison of qRT-PCR data and previous RNA-Seq data suggest reliability of our work. Here, we first report the identification of *SAUR* genes in Urticales plants. The results will provide foundation for their function validation in Urticales plant growth and development.

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