



# Genome-wide analysis, evolutionary expansion, and expression of early auxin-responsive *SAUR* gene family in rice (*Oryza sativa*)

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## Abstract

Small auxin-up RNAs (*SAURs*) are the early auxin-responsive genes represented by a large multigene family in plants. Here, we report the identification of 58 *OsSAUR* gene family members from rice (*Oryza sativa japonica* cv Nipponbare), the model monocot plant, by a reiterative database search and manual reannotation; 2 of these are pseudogenes. The coding sequences of *OsSAURs* do not possess any intron. Most of the predicted *OsSAUR* protein sequences harbor a putative nuclear localization signal at their N-terminus. Localized gene duplications appear to be the primary genetic event responsible for *SAUR* gene family expansion in rice. Interestingly, the duplication of *OsSAURs* was found to be associated with the chromosomal block duplication as well. The phylogenetic analysis revealed that the *SAUR* gene family expanded in rice and *Arabidopsis* due to species-specific expansion of the family in monocots and dicots. The auxin-responsive elements and downstream element are conserved in the upstream and downstream sequences, respectively, of *OsSAURs*. In addition to the 21 *OsSAURs* with full-length cDNA sequences and 20 with expressed sequence tags, gene expression analyses of at least 7 *OsSAURs* by RT-qPCR indicated that the majority of identified *OsSAURs* most likely are expressed in rice. The transcript abundance of the *OsSAURs* examined increased within a few minutes of exogenous auxin application with varying kinetics. The present study provides basic genomic information for the rice *SAUR* gene family and will pave the way for deciphering the precise role of *SAURs* in plant growth and development.

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The phytohormone auxin exerts a pleiotropic effect on various aspects of plant growth and development, including cell elongation, cell division, differentiation, root initiation, apical dominance, and tropic responses. Auxin mediates these effects at the molecular level by altering the expression of numerous genes [1,2]. The early auxin-responsive genes, which are specifically induced within minutes of auxin application, have been broadly grouped into three major classes: auxin/indoleacetic acid (*Aux/IAA*), *GH3*, and small auxin-up RNA (*SAUR*) gene families [3]. Apart from auxin, *Aux/IAAs* and *SAURs* can be induced by cycloheximide, a translational inhibitor, indicating that their transcription is regulated by a short-lived repressor. There is increasing evidence that the *Aux/IAA* proteins act as repressors of gene transcription, regulating their

own transcription [4]. However, the function of *SAURs* largely remains obscure.

Following the initial identification of *SAUR* genes from soybean [5], members of this class have been isolated from mung bean [6], pea [7], *Arabidopsis* [8], tobacco [9], and, more recently, maize [10,11]. *SAURs* are represented as a large multigene family in the *Arabidopsis* genome comprising more than 70 members [2]. The *SAURs* encode highly unstable mRNAs with a very high turnover rate [12,13] that are induced within minutes by auxin application. The instability of *SAUR* mRNAs has been attributed due to the presence of a conserved downstream (DST) element in their 3'-untranslated regions [8,14]. There is evidence that the *SAURs* are regulated at the posttranscriptional and posttranslational levels, too [10,15]. Recently, the calcium-dependent in vitro binding of *SAUR* proteins with calmodulin has been demonstrated [10,11], which provides a link between the  $Ca^{2+}$ /calmodulin second messenger system and auxin signaling.

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The SAUR proteins remain largely uncharacterized. Recently, the analysis of a nuclear-localized *Zea mays* SAUR protein, ZmSAUR2, has revealed that the SAUR proteins are short-lived, with a half-life of about 7 min [11]. The SAUR transcripts are expressed mainly in the elongating tissues of soybean and maize [11,12,16], indicating their role in auxin-mediated cell elongation. However, the exact role of SAURs in the auxin signaling hierarchy is still unknown.

To understand the function and evolution of the SAURs in plants, we analyzed the SAUR gene family in rice (*Oryza sativa*). Rice is one of the most important food crops and is considered the model monocot plant for molecular and genetic studies. The complete rice genomic sequences are now available for two of the subspecies, *indica* [17] and *japonica* [18,19]. Earlier, we identified and comprehensively analyzed the early auxin-responsive GH3 and Aux/IAA gene families from rice [20,21]. Here, we report the genome-wide analysis of the rice SAUR (*OsSAUR*) gene family. The work involved the identification of putative *OsSAURs* by reiterative database searches and manual reannotation. The phylogenetic analysis of SAURs from rice and *Arabidopsis* was performed to understand the possible mechanisms of gene family expansion. Also, the expression of *OsSAURs* has been analyzed using the full-length cDNAs and ESTs available in databases. The real-time PCR analysis of certain members demonstrated that *OsSAURs* are expressed in various rice organs/tissues and are induced very rapidly by exogenous auxin.

## Results and discussion

### Identification of the SAUR gene family in rice

The SAURs are present as a multigene family in *Arabidopsis* comprising over 70 members [2]. The availability of the complete rice genome sequence [17–19] provided us the opportunity to find the *OsSAURs* in rice. The *OsSAURs* were identified in four steps from *japonica* subsp. cv Nipponbare. The first step involved a BLASTP search of annotated proteins at The Institute for Genomic Research (TIGR) Rice Genome Annotation database using *Arabidopsis* SAUR protein sequences [2] as query. The second step aimed at a complete search for putative *OsSAURs* in rice and was performed by BLASTP and TBLASTN searches of the *japonica* cv Nipponbare genome sequences in GenBank at the National Center for Biotechnology Information (NCBI) utilizing *Arabidopsis* SAURs and all the rice SAUR proteins identified in the first step. All the *OsSAURs* identified were annotated as auxin-responsive/induced proteins at TIGR. However, many of the *OsSAURs* identified in the present study were annotated as unknown/hypothetical or expressed proteins and others as auxin-responsive/induced proteins at GenBank.

In the third step, identical sequences present on the same or overlapping contigs in both the databases were identified and removed to obtain a set of nonredundant *OsSAURs*. Each putative *OsSAUR* protein sequence was manually assessed for its sequence similarity to other putative *OsSAURs* or *Arabidopsis thaliana* (At) SAURs. Manual reannotation was

performed to correct and reannotate the misannotated putative *OsSAURs*. Finally, as a last step, each predicted *OsSAUR* protein sequence was confirmed by a Pfam search for the presence of an auxin-inducible signature (PF02519) conserved in other SAUR proteins [22]. The overall analysis revealed that the *OsSAUR* gene family comprises 58 members in rice, including 2 pseudogenes. They were designated as *OsSAUR1* to *OsSAUR58* according to their position on the rice chromosomes, 1 to 12, and from top to bottom (Table 1, Fig. 1A). The annotation of the two predicted pseudogenes seems to be correct as both of them are present on the completely sequenced bacterial artificial chromosomes (BACs); one of them (*OsSAUR43*) showed the presence of a premature stop codon and the 5' end was missing for the other (*OsSAUR50*). Both the pseudogenes are present in the cluster of 17 *OsSAURs* on chromosome 9 and may have become nonfunctional after duplication. The predicted cDNA and protein sequences of all 58 *OsSAURs* are provided in Supplemental Data I and II.

### Genomic organization of *OsSAURs*

The family of 58 *OsSAURs* identified is distributed on 10 of the 12 rice chromosomes; no *OsSAUR* could be located on chromosomes 5 and 11. Nineteen *OsSAURs* are present on chromosome 9; 9 on chromosome 2; 6 on chromosomes 4, 6, and 8 each; 5 on chromosome 3; 3 on chromosome 1; 2 on chromosome 12; and 1 each on chromosomes 7 and 10 (Fig. 1A). The position (in bp) and direction of transcription (arrows) of each gene were determined on the International Rice Genome Sequencing Project (IRGSP) rice chromosome pseudomolecules (Fig. 1A, Supplemental Table S1). The BAC or PAC (P1 phage-derived artificial chromosome) clones carrying the *OsSAURs* were also identified (Table 1). The approximate chromosome map positions of BACs/PACs in centimorgans from the top of the chromosome and their nearest markers are listed in Table 1.

The fine-mapping analysis revealed that most of the *OsSAURs* are clustered in the rice genome. There are examples of the localized tandem gene duplication on chromosomes 2 (*OsSAUR4* and *OsSAUR5*; *OsSAUR8* and *OsSAUR9*), 3 (*OsSAUR14* to *OsSAUR17*), 4 (*OsSAUR22* and *OsSAUR23*), 6 (*OsSAUR25* and *OsSAUR26*; *OsSAUR27* and *OsSAUR28*), 8 (*OsSAUR31* and *OsSAUR32*; *OsSAUR34* and *OsSAUR35*), and 9 (*OsSAUR37* and *OsSAUR38*; *OsSAUR39* to *OsSAUR55*), either in the same or the inverse orientation (Fig. 1A). It is noteworthy that among the 19 *OsSAURs* (30% of total) present on chromosome 9, 17 are clustered together at a single locus in tandem (Fig. 1B). Among the 9 nonoverlapping duplicated blocks described by Paterson et al. [23], 4 occurring between chromosomes 2 and 6 (*OsSAUR4* and *OsSAUR28*; *OsSAUR5* and *OsSAUR27*; *OsSAUR6* and *OsSAUR25*), chromosomes 2 and 4 (*OsSAUR11* and *OsSAUR19*), chromosomes 3 and 12 (*OsSAUR14* and *OsSAUR58*), and chromosomes 8 and 9 (*OsSAUR33* and *OsSAUR37*; *OsSAUR36* and *OsSAUR54*) gave rise to *OsSAUR* gene duplications. The duplicated block between chromosomes 2 and 6 contains two adjacent duplicated *OsSAURs*, suggesting the occurrence of localized

gene duplications prior to the chromosomal segment duplication. However, in only a few cases a single *OsSAUR* is present on a chromosome, for example, *OsSAUR30* and

*OsSAUR56* on chromosome 7 and 10, respectively. These results suggest that the expansion of *OsSAURs* has occurred due to both local tandem duplications and large-scale

Table 1  
*SAUR* gene family in rice

Gene name <sup>a</sup>	ORF length <sup>b</sup> (bp)	Deduced polypeptide <sup>c</sup>			NLS <sup>d</sup>	Chr. No. <sup>e</sup>	Genomic locus <sup>f</sup>			Nearest marker <sup>g</sup>	FL-cDNA/EST <sup>h</sup>
		Length (aa)	Mol wt (kDa)	pI			BAC/PAC name	Accession No.	cM position		
<i>OsSAUR1</i>	276	91	9.88	9.44	Yes	1	P0011G08	AP003225	12.5–13.1	C804	No
<i>OsSAUR2</i>	369	122	12.45	5.15	No	1	B1143G03	AP003371	133.9	R3347	Yes
<i>OsSAUR3</i>	522	173	18.06	9.15	Yes	1	B1033B05	AP004223	160.4–161.5	R2833	No
<i>OsSAUR4</i>	363	120	12.77	9.67	Yes	2	OSJNBa0064G16	AP005649	11.6	R10479S	No
<i>OsSAUR5</i>	393	130	14.63	8.76	Yes	2	OSJNBa0064G16	AP005649	11.6	R10479S	Yes
<i>OsSAUR6</i>	378	125	13.11	9.28	Yes	2	OSJNBa0085K21	AP005804	17.1	R2242S	Yes
<i>OsSAUR7</i>	432	143	15.31	6.36	No	2	P0543C11	AP005743	52.2	E488S	Yes
<i>OsSAUR8</i>	291	96	10.70	7.82	Yes	2	OJ1116_C12	AP004134	54.6	E61875S	Yes
<i>OsSAUR9</i>	327	108	11.55	7.24	Yes	2	OJ1116_C12	AP004134	54.6	E61875S	Yes
<i>OsSAUR10</i>	501	166	17.38	8.39	Yes	2	OJ1789_D08	AP005299	59.5–62.2	G132	Yes
<i>OsSAUR11</i>	573	190	21.04	11.64	Yes	2	OP1282_H11	AP005291	103.9–105.8	R857S	Yes
<i>OsSAUR12</i>	387	128	13.74	9.64	Yes	2	OJ1767_D02	AP004125	138–140.9	S13245	Yes
<i>OsSAUR13</i>	624	207	21.92	4.92	No	3	OSJNBb0027B12	AC137075	44.4–46.6	R2849	No
<i>OsSAUR14</i>	357	118	12.29	8.20	Yes	3	OSJNBb0065L20	AC139174	101.9–115.6	E3781S	No
<i>OsSAUR15</i>	321	106	11.10	7.00	No	3	OSJNBb0065L20	AC139174	101.9–115.6	E3781S	No
<i>OsSAUR16</i>	405	134	14.14	8.37	Yes	3	OSJNBb0065L20	AC139174	101.9–115.6	E3781S	No
<i>OsSAUR17</i>	321	106	10.85	7.12	No	3	OSJNBb0065L20	AC139174	101.9–115.6	E3781S	No
<i>OsSAUR18</i>	390	129	14.27	7.33	Yes	4	OSJNBa0073E02	AL731616	78.2–81.7	C60048S	No
<i>OsSAUR19</i>	504	167	18.54	11.20	Yes	4	OSJNBa0091D06	AL606459	82.5	L1091	Yes
<i>OsSAUR20</i>	531	176	18.94	8.92	Yes	4	OSJNBa0060N03	AL606691	102.7	C810	Yes
<i>OsSAUR21</i>	759	252	26.62	10.64	Yes	4	OSJNBa0093O08	AL606648	102.7–107.4	E50452S	Yes
<i>OsSAUR22</i>	432	143	15.50	8.84	Yes	4	OSJNBa0084K01	AL606999	120.3	S12653S	Yes
<i>OsSAUR23</i>	462	153	16.89	10.01	Yes	4	OSJNBa0084K01	AL606999	120.3	S12653S	Yes
<i>OsSAUR24</i>	360	119	13.26	8.71	Yes	6	P0548D03	AP003526	9.0	C52026	Yes
<i>OsSAUR25</i>	423	140	14.65	7.80	Yes	6	OSJNBa0032M14	AP005610	109.5	C52865S	Yes
<i>OsSAUR26</i>	402	133	13.39	9.06	Yes	6	OSJNBa0032M14	AP005610	109.5	C52865S	Yes
<i>OsSAUR27</i>	405	134	14.75	8.50	Yes	6	P0596H10	AP003726	117.0	C69	No
<i>OsSAUR28</i>	423	140	15.11	9.57	Yes	6	P0596H10	AP003726	117.0	C69	Yes
<i>OsSAUR29</i>	426	141	14.18	9.08	No	6	OJ1136_F03	AP004678	120.1–121.7	R1479	Yes
<i>OsSAUR30</i>	363	120	12.54	10.03	No	7	P0571D04	AP004315	57.5–60.8	R646	Yes
<i>OsSAUR31</i>	330	109	11.94	8.35	No	8	OJ1005_B05	AP003925	12.8	R3003	Yes
<i>OsSAUR32</i>	309	102	10.86	9.04	No	8	OJ1005_B05	AP003925	12.8	R3003	No
<i>OsSAUR33</i>	402	133	14.17	6.49	Yes	8	OJ1117_F10	AP003871	85.1	E61231	Yes
<i>OsSAUR34</i>	432	143	15.34	6.02	Yes	8	P0702C09	AP005528	110.1	R3961S	No
<i>OsSAUR35</i>	432	143	15.34	6.02	Yes	8	P0702C09	AP005528	110.1	R3961S	Yes
<i>OsSAUR36</i>	432	143	15.85	8.19	Yes	8	OSJNBb0011H15	AP005251	119.3	S1570	Yes
<i>OsSAUR37</i>	498	165	16.64	8.51	No	9	OJ1328_D07	AP005833	58.3–60.8	C2070	Yes
<i>OsSAUR38</i>	573	190	20.42	4.54	No	9	OJ1328_D07	AP005833	58.3–60.8	C2070	No
<i>OsSAUR39</i>	516	171	19.20	9.13	Yes	9	P0705E11	AP006548	88.2	C12375S	Yes
<i>OsSAUR40</i>	417	138	15.22	7.89	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR41</i>	426	141	15.68	7.03	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR42</i>	423	140	15.06	7.30	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	No
<i>OsSAUR43*</i>	420	Stop codon within ORF				9	OSJNBa0038K02	AP005862	90.1	E60222S	No
<i>OsSAUR44</i>	435	144	15.96	8.90	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR45</i>	426	141	15.62	8.12	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR46</i>	423	140	15.50	7.94	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR47</i>	333	110	12.55	9.10	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR48</i>	474	157	17.53	8.39	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	No
<i>OsSAUR49</i>	435	144	15.98	8.89	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR50*</i>	231	5' end missing				9	OSJNBa0038K02	AP005862	90.1	E60222S	No
<i>OsSAUR51</i>	435	144	15.62	6.33	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR52</i>	426	141	15.52	8.59	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR53</i>	435	144	15.91	8.49	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR54</i>	426	141	15.60	7.87	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR55</i>	453	150	15.98	8.11	Yes	9	OSJNBa0038K02	AP005862	90.1	E60222S	Yes
<i>OsSAUR56</i>	378	125	13.94	7.61	No	10	OSJNBa0005K07	AC087192	55.6–57.5	G4003	Yes
<i>OsSAUR57</i>	522	173	18.12	8.49	Yes	12	OSJNBa0002L05	AL731881	99.7–100.9	C51368	Yes
<i>OsSAUR58</i>	393	130	13.49	6.34	Yes	12	OSJNBb0016P08	AL831803	107.4	R496	Yes

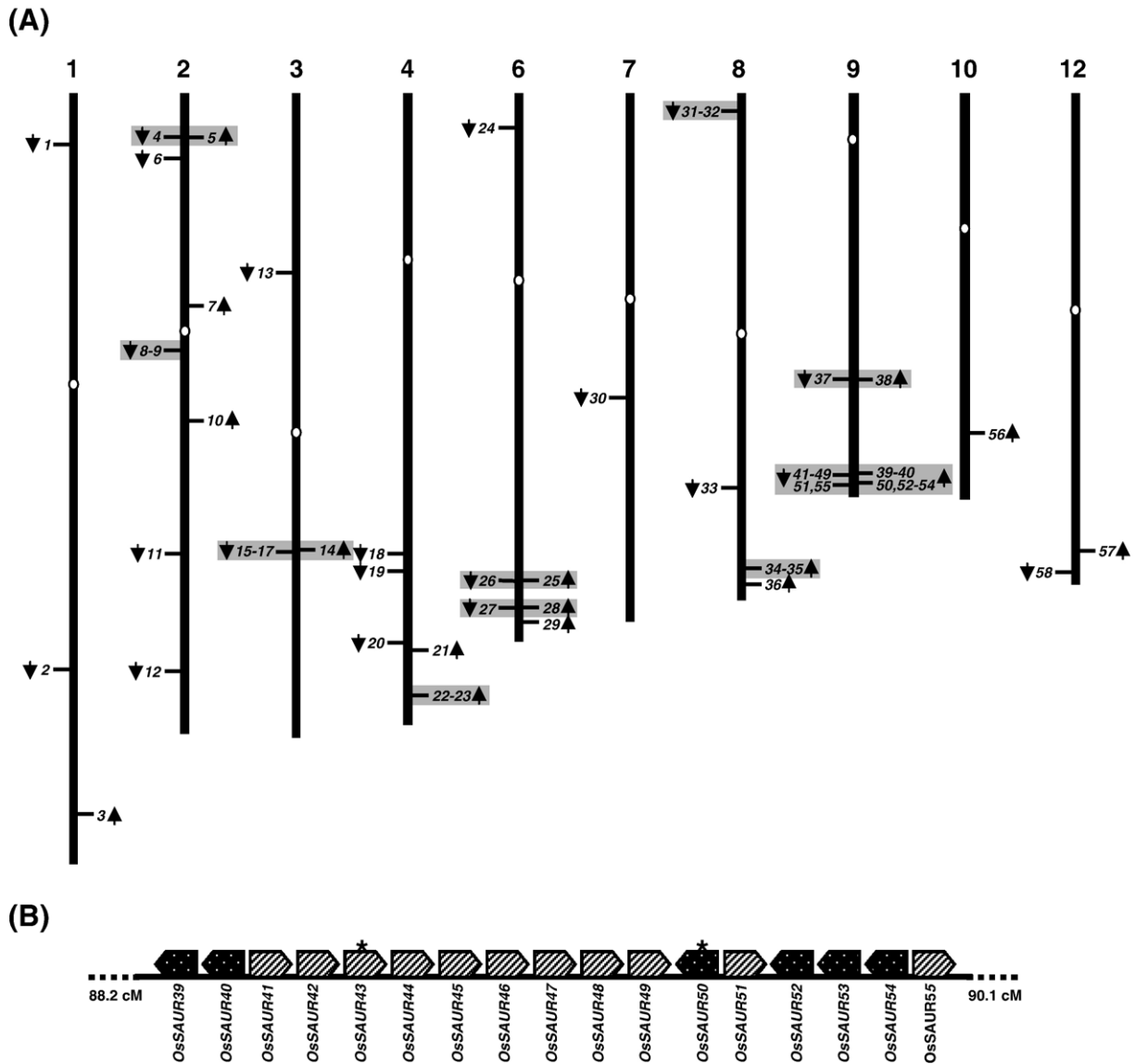


Fig. 1. (A) Genomic distribution of *OsSAURs* on rice chromosomes. White ovals on the chromosomes (vertical bars) indicate the positions of centromeres. Arrows next to gene names show the direction of transcription. Chromosome numbers are indicated at the top of each bar. The adjacent genes representing localized gene duplications are highlighted in gray boxes. The position of each *OsSAUR* on IRGSP rice chromosome pseudomolecules in base pairs is given in Supplemental Table S1. (B) Diagrammatic representation of 17 *OsSAURs* clustered together on chromosome 9. Shading represents direction of transcription. The two pseudogenes are marked with an asterisk.

genomic duplications in rice. In soybean and *Arabidopsis*, too, the *SAURs* were found to be clustered [2,22]. A large number of examples of expansions of large gene families in eukaryotes are available, which may in part be explained due to extensive gene duplications.

#### Sequence analysis of *OsSAUR* proteins

The *SAURs* stand out from a vast majority of other plant genes in their paucity of introns. All the *SAURs* characterized so far, with one exception of *AtSAUR11*, lack introns [2,22]. A

#### Notes to Table 1:

- <sup>a</sup> Systematic designation given to rice *SAURs* (*OsSAURs* marked with asterisk represent pseudogenes).
- <sup>b</sup> Length of open reading frame in base pairs.
- <sup>c</sup> Length (number of amino acids), molecular weight (kilodaltons), and isoelectric point (pI) of the deduced polypeptide.
- <sup>d</sup> Putative nuclear localization signal (NLS) has been predicted or not.
- <sup>e</sup> Chromosomal localization of *OsSAUR* gene.
- <sup>f</sup> Name, accession number, and approximate centimorgan position of the BAC/PAC clone in which *OsSAUR* gene is present.
- <sup>g</sup> Nearest marker to the *OsSAUR* gene.
- <sup>h</sup> Corresponding full-length cDNA or EST is available or not.

comparison of the coding sequence with the corresponding genomic DNA sequences showed that none of the *OsSAURs* harbor any intron. Such intronless gene families can evolve rapidly either by gene duplication or by reverse transcription/integration [24,25]; however, no obvious traces of such events persist. Intronless genes are in general a characteristic feature of prokaryotes; therefore, *SAURs*, like other single-exonic genes, make good candidates for comparative genomics among different domains of life and evolutionary studies. The deduced open reading frame of *OsSAURs* encodes small proteins with molecular mass ranging from 10 kDa for *OsSAUR1* to 27 kDa for *OsSAUR21* (Table 1). The pair-wise analysis of the full-length *OsSAUR* protein sequences indicated that the overall identities range from 13% (between *OsSAUR13* and *OsSAUR21*) to 100% (between *OsSAUR34* and *OsSAUR35*) (Supplemental Table S2). The multiple sequence alignments of the full-length protein sequences showed that the core region is highly conserved among the *OsSAURs* and *SAUR* proteins from other plant species (Figs. 2A and 2B). However, the homology at the N- and C-termini is rather low. *OsSAURs* did not show the presence of any known conserved motif or domain

except for the invariable auxin-inducible signature as revealed by the Pfam search. Most of the *OsSAUR* proteins are basic ( $pI > 7.0$ ) in nature and exhibit the presence of a putative nuclear localization signal (NLS) at their N-terminus (Table 1, Fig. 2B). However, the functional validation of these putative NLSs will be required to ascertain their *in vivo* role in the subcellular localization of *OsSAUR* proteins. Recently, the NLS present at the N-terminus of *ZmSAUR2* was found to be functional in translocating the GUS fusion protein to the nucleus in onion epidermal cells [11].

#### Evolutionary expansion of *OsSAURs*

To explore the evolutionary expansion of *OsSAURs* in the rice genome, an unrooted tree was constructed from alignments of their full-length protein sequences (Fig. 3). This analysis revealed that all the *OsSAURs* grouped broadly into two major groups (A and B) with well-supported bootstrap values. Eighteen and thirty-eight *OsSAURs* were included in group A and B, respectively. Fifty-six of the *OsSAURs* formed 21 sister pairs, 13 of which had very strong bootstrap support (>95%).

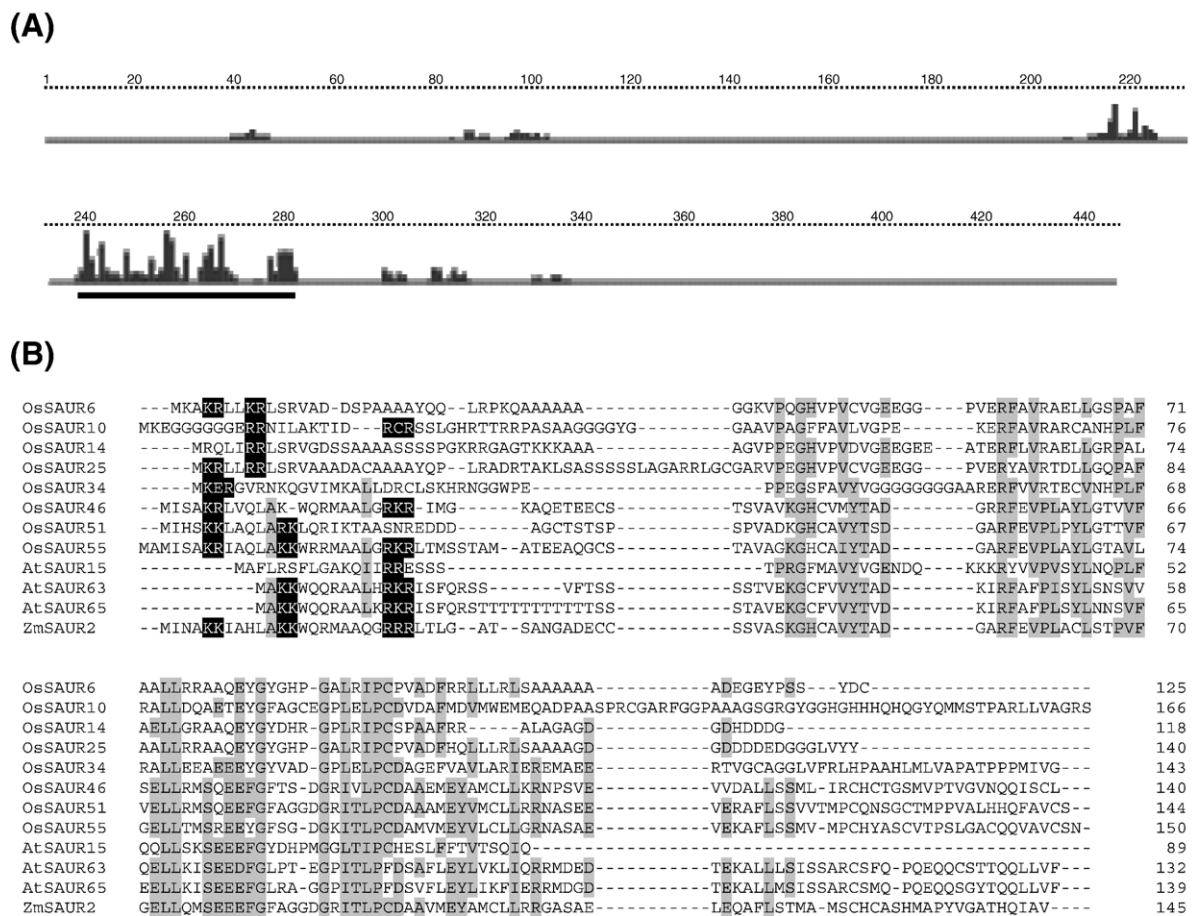


Fig. 2. (A) Alignment profile of all the full-length rice SAUR proteins obtained with the ClustalX program. The height of the bars indicates the number of identical residues per position. The conserved core region is underlined. Complete alignment of all the *OsSAURs* is given in Supplemental Fig. 1. (B) Multiple alignment of representative full-length *OsSAUR*, *AtSAUR*, and *ZmSAUR2* proteins obtained with ClustalX and manual correction. Conserved residues (present in equal to or more than 50% of aligned sequences) are highlighted in gray boxes. Amino acids considered conserved are K and R, D and E, and I, L, and V. Gaps (marked with dashes) have been introduced to maximize the alignments. Nuclear localization signals are highlighted in black boxes. The respective amino acid position is given on the right of each sequence.

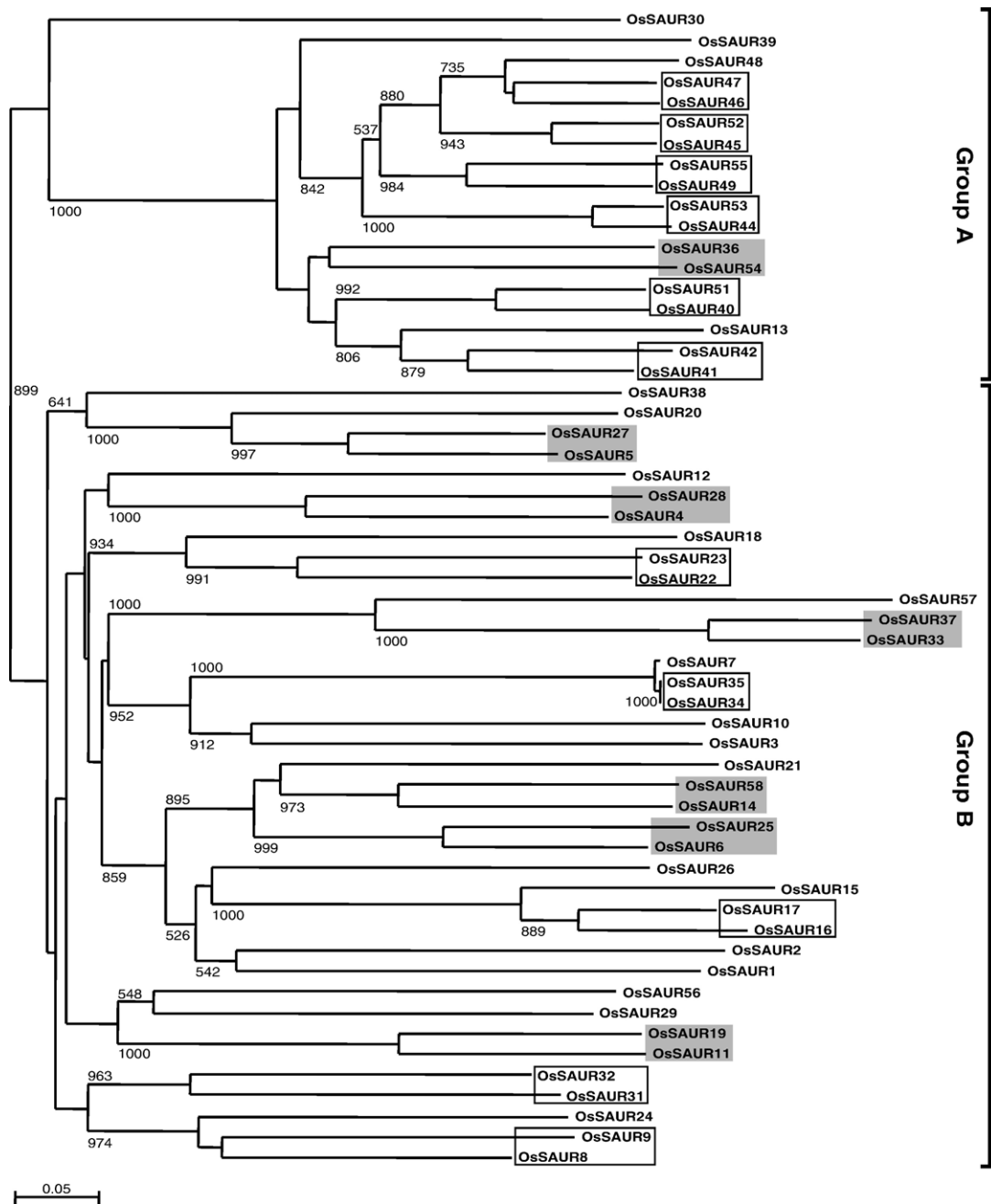


Fig. 3. Phylogenetic relationship among the OsSAUR proteins. The unrooted tree was generated using the ClustalX program and the neighbor-joining method. Bootstrap values (above 50%) from 1000 replicates are indicated at each node. Sister pairs representing localized gene duplication are given in rectangles. Sister pairs present on duplicated chromosomal segments are highlighted in gray boxes.

Interestingly, 11 sister pairs represented the localized gene duplications and 7 others were located on the duplicated chromosomal blocks of rice as described above. These results suggest that the expansion of *OsSAURs* was probably, in large part, due to localized gene duplications. Recently, Yu et al. [26] presented evidence for ongoing individual gene duplications in rice, which provide never-ending raw material for studying gene genesis and their functions. The duplication of the *OsSAURs* is also associated with chromosomal block duplications in rice. It is remarkable that the duplicated *OsSAURs* have been retained evolutionarily. Earlier, the preferential retention of the dupli-

cated *Aux/IAA* genes in rice and *Arabidopsis* was explained as maintaining the proper dosage relationships with interacting proteins such as ARFs [21,27]. It has been demonstrated that the interaction of specific pairs of ARF and Aux/IAA proteins generates the specificity of the auxin response at different developmental stages and physiological levels [28]. Recently, the binding of two *Z. mays* SAUR proteins with calmodulin (CaM) has been shown in vitro [10,11]. Calmodulin is a small Ca<sup>2+</sup>-binding protein that acts to transduce second messenger signals into a wide array of cellular responses and is represented by a multigene family in higher plants, including rice,

*Arabidopsis*, wheat, and potato [29]. The *Arabidopsis* genome encodes a total of 50 CaM or CaM-like (CML) proteins [30,31], and a similar number of CaM/CML genes can be expected in rice also. Therefore, it is tempting to speculate that the retention of duplicated *OsSAURs* may also help maintain the proper dosage relationship with CaM's/CMLs (or other unknown interacting proteins), and the specificity of their interaction may be critical for regulating the specificity of the auxin response during various stages/processes of plant growth and development. The retention of the duplicated *SAURs* also strengthens the idea that the genes involved in transcription and signal transduction have been preferentially retained [32]. The fact that the *SAUR* gene family in both rice and *Arabidopsis* showed a high degree of duplicated gene retention is particularly interesting as both species experienced similar evolutionary mechanisms, i.e., polyploidization followed by diploidization [23,33,34]. Taken together, these observations throw some light on the evolutionary steps encountered during the diversification of *SAURs*.

To examine the phylogenetic relationship of rice and *Arabidopsis* SAUR proteins, an unrooted tree was constructed from alignments of their full-length protein sequences (Fig. 4). The *Arabidopsis* SAURs were clustered distinctly into two groups along with rice *OsSAURs* (groups A and B). In addition, this analysis revealed that most *OsSAURs* and *Arabidopsis* SAURs cluster in species-specific distinct clades. This result indicates that most *OsSAURs* and *Arabidopsis* SAURs expanded in a species-specific manner; probably only a few members originated from the common ancestral genes that existed before the divergence of monocots and dicots. This type of divergence between a monocot (rice) and a dicot (*Arabidopsis*) species has been observed for other large gene families as well [35,36]. Moreover, the *SAUR* gene family is expanded more in *Arabidopsis* than in rice. Genes from groups A and B are present both in *Arabidopsis* and in rice, indicating that these existed before the divergence of monocots and dicots.

#### Identification of putative auxin-responsive elements in *OsSAURs*

A number of auxin-responsive elements (AuxREs) have been defined within upstream promoter regions of auxin-responsive genes, including *SAURs*, such as one or more copies of a conserved motif, TGTCTC, and/or DUE/NDE element GGTCCCAT or some variation of these, which confer auxin responsiveness [3,37–40]. The investigation of 1000 bp upstream sequences of *OsSAURs* representing their promoter region by PLACE and manual search revealed the presence of a few to several putative AuxREs for most of them (Supplemental Data III), which may be responsible for their auxin responsiveness. In addition, the upstream flanking regions of nine pairs of sister loci (*OsSAUR34* and *OsSAUR35*, *OsSAUR15* and *OsSAUR16*, *OsSAUR33* and *OsSAUR37*, *OsSAUR40* and *OsSAUR51*, *OsSAUR41* and *OsSAUR42*, *OsSAUR44* and *OsSAUR53*, *OsSAUR45* and *OsSAUR52*, *OsSAUR46* and *OsSAUR47*, and *OsSAUR49* and *OsSAUR55*)

were highly similar (Fig. 5A). The upstream sequence of *OsSAUR7* also showed 100% similarity with upstream sequences of sister pair *OsSAUR34* and *OsSAUR35*. Likewise, the upstream sequence of *OsSAUR48* was highly similar to sister pair *OsSAUR46* and *OsSAUR47*. In these pairs, the regions of apparent homology contain multiple putative AuxREs (TGTCTC and GGTCCCAT or a variation of these) (Fig. 5A). These conserved regions with AuxREs are located approximately 50 to 250 bp upstream of the start codon. The retention of duplicated *OsSAURs* along with their promoter sequences and individual *cis*-regulatory elements may be critical for their specific or overlapping expression and function. A genome-wide expression analysis by massively parallel signature sequencing in *Arabidopsis* revealed that more than two-thirds of duplicated genes exhibit divergence in their expression characteristics [41], although they showed the preservation of promoter sequences as well as individual *cis*-elements between them. The expression divergence results in neo- or subfunctionalization and might represent an important evolutionary mechanism for the retention of duplicated genes [42]. However, the preservation of promoter sequences as well as individual *cis*-elements between duplicated genes indicates that the process of transcriptional neo- and subfunctionalization is restricted to only a fraction of *cis*-elements [41].

#### Identification of putative mRNA-destabilizing DST elements in *OsSAURs*

The stability of eukaryotic mRNAs varies over a wide range and greatly regulates gene expression posttranscriptionally. The stability of *SAUR* transcripts is determined by a highly conserved DST element, an approximately 40-base sequence present in their 3' untranslated region (UTR) [12,43,44]. The DST element of *SAURs* is defined by two functionally important conserved regions, ATAGAT and GTA, as revealed by mutational analyses [44]. To investigate the presence of DST elements in *OsSAURs*, 500 bp of genomic sequence downstream of the stop codon of each *OsSAUR* was retrieved and analyzed for the presence of conserved nucleotides manually. The exact consensus DST element with ATAGAT (or a variation only at the first nucleotide position) and GTA regions could be identified in 10 *OsSAURs* (Fig. 5B). The predicted DST element was present within the 3' UTR of the two *OsSAURs* (*OsSAUR9* and *OsSAUR30*) for which corresponding full-length cDNA is available (described later). However, putative DST elements with some variation of conserved regions are also present in most of the *OsSAURs* (Supplemental Data IV). The presence of a DST element in the downstream region of *OsSAURs* may be responsible for the instability of their mRNAs and thereby regulating their expression. However, their functional validation remains to be done. Two *dst* mutants that show an increased abundance of DST-containing *SAUR-AC1* mRNA have been isolated from *Arabidopsis* [45]. The microarray analysis of the *dst1* mutant and further studies demonstrated a potential link between the DST-mediated decay pathway and circadian rhythm in plants [46,47]. Further studies on the DST-mediated decay pathway in rice and *Arabidopsis* may provide new

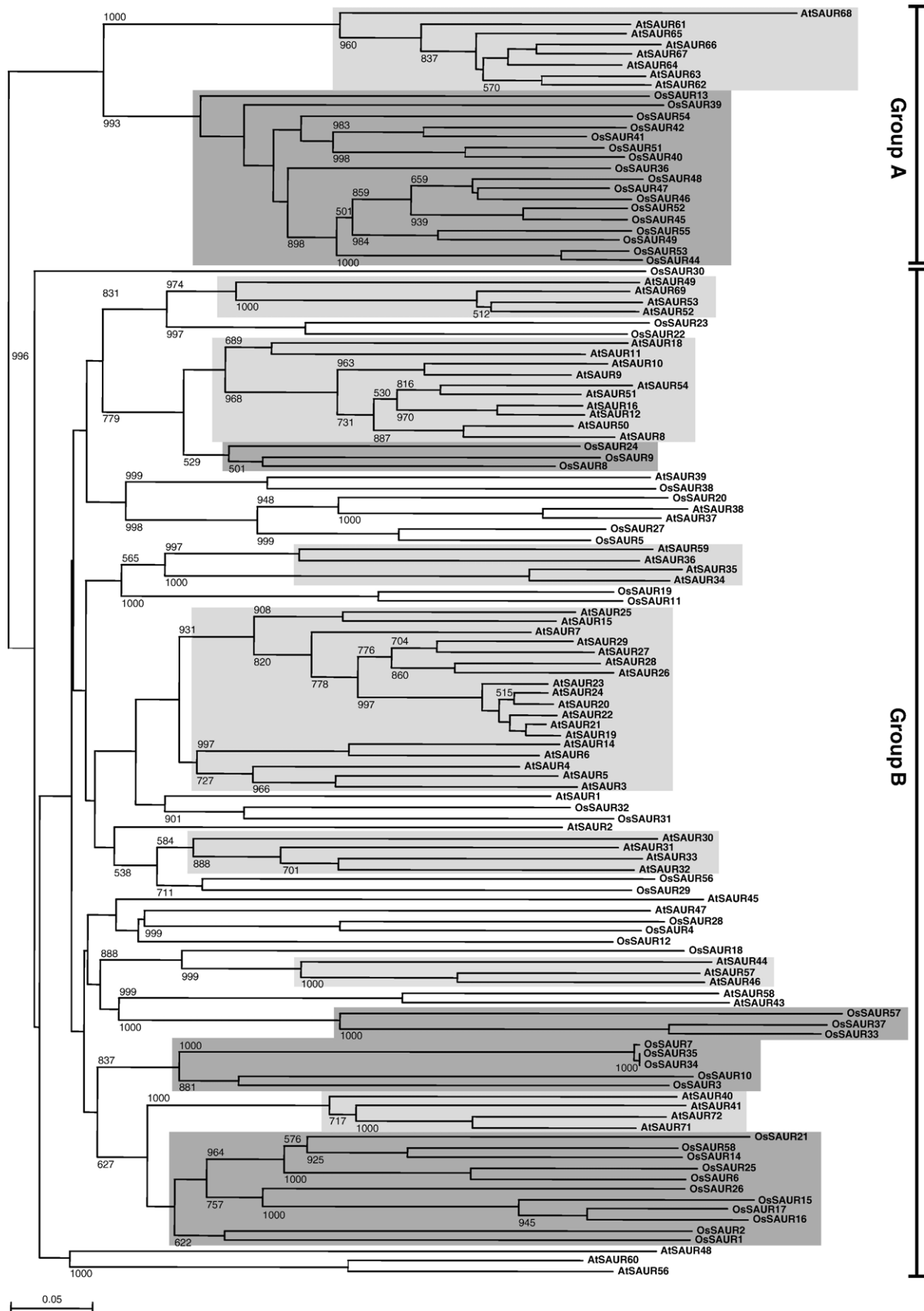


Fig. 4. Phylogenetic relationship of rice and *Arabidopsis* SAUR proteins. The unrooted tree was generated using the ClustalX program and the neighbor-joining method. Bootstrap values (above 50%) from 1000 replicates are indicated at each node. Differently shaded boxes represent the distinct species-specific clades of SAURs in rice and *Arabidopsis*.





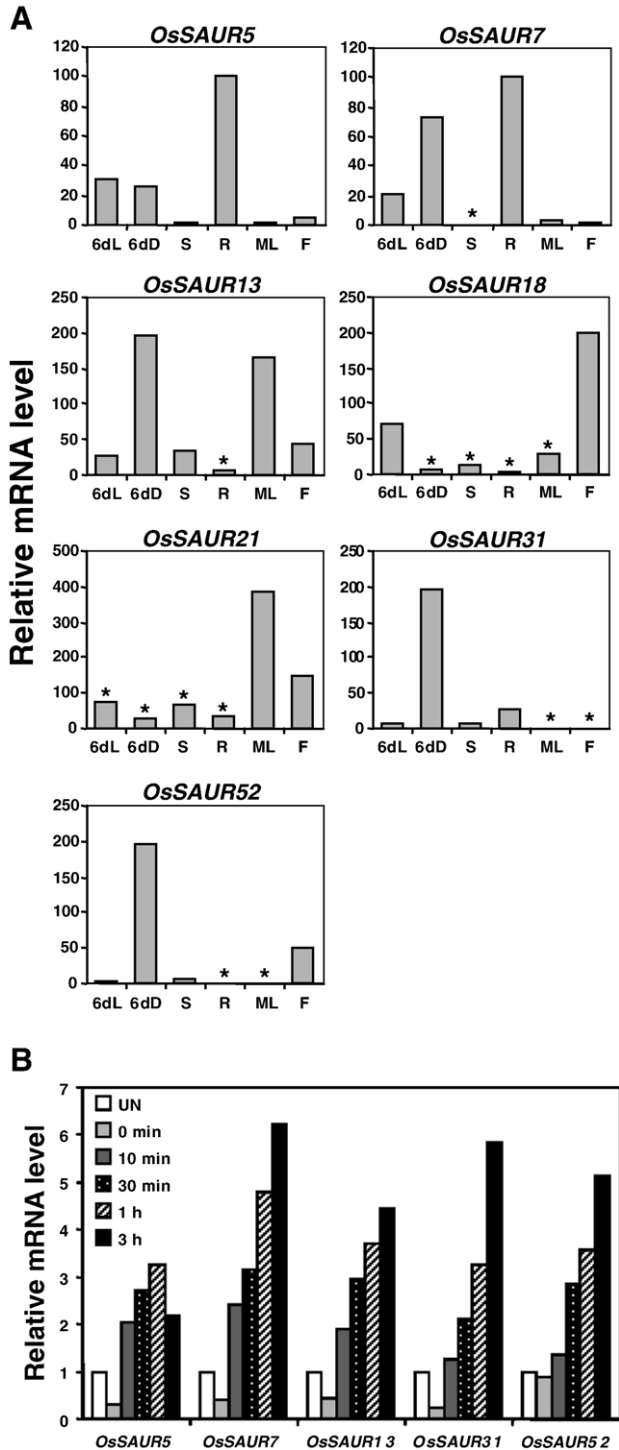


Fig. 6. Expression analysis of *OsSAURs*. (A) Real-time PCR analysis showing expression profiles of seven individual *OsSAURs* in different tissues. Relative mRNA levels of individual *OsSAURs* were normalized with respect to the housekeeping gene *UBQ5* in different tissues (6dL, 6-day-old light-grown seedlings; 6dD, 6-day-old etiolated seedlings; S, green shoot; R, root; ML, mature leaf; F, flower). Asterisk indicates that the expression was close to the detection limit. (B) Kinetics of induction of five *OsSAURs* after exogenous auxin application. Transcript levels of each *OsSAUR* in untreated 3-day-old etiolated rice coleoptiles (UN), after depletion for 3 h in KPSC buffer (0 min), and after treatment with IAA for 10 min, 30 min, 1 h, and 3 h were plotted as the relative expression (fold) compared to expression in UN coleoptiles. The expression of *OsSAUR18* and *OsSAUR21* was close to the detection limit in rice coleoptiles and did not increase further after auxin treatment.

differences were also found in the transcript abundance of *OsSAURs* in green and etiolated seedlings (Fig. 6A). The transcript levels of *OsSAUR7*, *OsSAUR13*, *OsSAUR31*, and *OsSAUR52* were significantly higher in etiolated seedlings, while that of *OsSAUR18* was higher in green seedlings, indicating their roles in light and hormone interaction. The transcript levels of most of the *OsSAURs*, except for *OsSAUR18* and *OsSAUR21*, were upregulated within 10 min of exogenous auxin application in etiolated rice coleoptiles, although to varying degrees (Fig. 6B). In addition to factors such as tissue- or cell-type-dependent auxin perception, the different turnover rates of the DST-element-harboring *OsSAUR* mRNAs may contribute to their variable kinetics of auxin induction.

#### Putative functions of SAURs?

The *SAURs* represent a class of early auxin-responsive genes that encode short-lived nuclear proteins and may play a role in auxin-mediated cell elongation [2,5,11,16]. However, their exact function is still unknown. Recently, the binding of a SAUR protein from *Z. mays* with a small  $\text{Ca}^{2+}$ -binding protein, CaM, has been demonstrated in vitro. The  $\text{Ca}^{2+}$ /CaM cascade has been implicated in various signal transduction pathways, including auxin, brassinosteroid, light, mechanical perturbation, and stress [10,11,30,31,50]. The *OsSAURs* were found to be differentially expressed in various rice tissues and in response to light and auxin stimuli. The specificity of the interaction of SAURs with CaM's/CMLs in different tissues may provide a specific auxin response during various stages/processes of plant growth and development. The recent studies on SAURs strengthen the view that auxin signal transduction does employ the  $\text{Ca}^{2+}$ /calmodulin second messenger system for eventual realization of the response.

To have an inkling about the functions of *OsSAURs*, we investigated the phenotypes of rice *Tos17* retrotransposon insertion mutants [51] of these genes with the aid of a *Tos17* mutant panel database (<http://tos.nias.affrc.go.jp/>) of rice. We could enlist several insertion mutants corresponding to *OsSAUR5* and to the locus on chromosome 9 where 17 *OsSAURs* are clustered (Supplemental Table S4). The phenotypes of the insertion mutants of these genes showed dwarfism, sterility, late heading, low tillering, and altered yield. From these phenotypes, it can be speculated that these genes may play a critical role in different metabolic pathways and cellular processes in rice.

In conclusion, the results of this study provide the genomic framework for further in-depth study of the functions of *OsSAURs* in the rice. It is quite apparent that this gene family has expanded more in *Arabidopsis* than in rice. Whether the degree of expansion is species-specific or if in general there has been less expansion in monocots than in the dicots will be revealed only as more and more genome sequences become available. The very fact that most of the duplicated *OsSAURs* are indeed expressing indicates that they may perform specific or redundant cellular functions. The unraveling of roles of

individual members of this family in auxin signaling will require a concerted effort by adoption of diverse approaches, including molecular genetic analysis.

## Materials and methods

### Identification of SAUR gene family in rice

The NCBI (<http://www.ncbi.nlm.nih.gov>) and TIGR Rice Genome Annotation (<http://www.tigr.org/tdb/e2k1/osa1>) database resources were used for the identification of putative SAURs in rice. The amino acid sequences of *Arabidopsis* SAUR proteins [2] were downloaded from The *Arabidopsis* Information Resource (<http://www.arabidopsis.org>). BLAST search tools BLASTP and TBLASTN [52] were used to identify putative *OsSAURs* in rice using *Arabidopsis* SAUR protein sequences as queries. The hits with an optimized cutoff value of bit score of 50 or more and expect value of less than 0.1, without any filter, were used for further analysis. The Pfam database (<http://www.sanger.ac.uk/Software/Pfam/search.shtml>) was used to confirm each predicted *OsSAUR* protein sequence as an auxin-responsive SAUR protein.

### Mapping of *OsSAURs* on rice chromosomes

All the sequenced contigs of *japonica* cv Nipponbare have been physically constructed as pseudomolecules by the IRGSP (<http://rgp.dna.affrc.go.jp/IRGSP>), representing the 12 rice chromosomes, and are available in GenBank (Accession Nos. AP008207-AP008218). Each of the *OsSAURs* was positioned on these rice chromosome pseudomolecules by the BLASTN search.

### Sequence and phylogenetic analysis

The Gene Runner (version 3.04) and DNASTAR programs were used for the DNA and protein sequence analysis. Multiple sequence alignment analysis was done using the ClustalX (version 1.83) program with default parameters. The phylogenetic analysis was carried out using the neighbor-joining method and the unrooted tree was displayed using the NJPLOT program.

### Upstream and downstream sequence element search

Once the *OsSAURs* were mapped to the rice genome, the 1000 bp of genomic sequences upstream of the ATG of each gene was downloaded for upstream element search. PLACE (<http://www.dna.affrc.go.jp/PLACE/signalscan.html>), a database of plant *cis*-acting regulatory DNA elements, was used for searching auxin-responsive elements. The DST elements were searched manually for the presence of conserved nucleotides in the 500 bp of genomic sequence downstream of the stop codon of each *OsSAUR* retrieved from the GenBank.

### Plant material and growth conditions

Rice seeds (*O. sativa* L. ssp. *indica* var. Pusa Basmati 1) were treated and grown as described previously [20]. For auxin treatment, the coleoptile apical segments (10 mm) from the 3-day-old etiolated rice seedlings were incubated in KPSC buffer (10 mM potassium phosphate, pH 6.0, 2% sucrose, 50  $\mu$ M chloramphenicol) for 3 h to deplete endogenous auxin. The buffer was changed every half-hour and the coleoptile segments were transferred to fresh buffer with 30  $\mu$ M concentration of IAA and incubated for specified duration.

### RNA isolation and real-time PCR analysis

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions, followed by DNase I treatment to remove any genomic DNA contamination. The quantitative real-time PCR analysis was performed as described [20]. The cDNA samples synthesized using the High Capacity cDNA Archive Kit (Applied Biosystems, USA) were used as template and mixed with 200 nM each primer and SYBR Green PCR Master Mix (Applied Biosystems) for real-time PCR analysis, using an ABI Prism 7000 sequence detection

system and software (PE Applied Biosystems) according to the manufacturer's instructions. The primers were designed by Primer Express 2.0 software (PE Applied Biosystems). The primer sequences are listed in Supplemental Table S5. The specificity of the reactions was verified by melting curve analysis. The relative mRNA levels for each *OsSAUR* in various tissue RNA samples were quantified with respect to the internal standard, *UBQ5*. At least two independent RNA isolations were used for cDNA synthesis and each cDNA sample was subjected to real-time PCR analysis in triplicate.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ygeno.2006.04.008](https://doi.org/10.1016/j.ygeno.2006.04.008).

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