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Genome-Scale Screening, Characterization, and Expression Analysis of Grain Amaranth Small Auxin-Up RNA Gene Family in Response to Drought Stress

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ABSTRACT

The Small Auxin-Up RNA (*SAUR*) gene family represents a class of early auxin-responsive genes that are widely involved in regulating plant cell elongation, tissue differentiation, and environmental adaptation. In this study, we performed a genome-wide analysis of the *SAUR* gene family in grain amaranth (*Amaranthus hypochondriacus*), a nutrient-rich pseudocereal. A total of 80 *SAUR* genes were identified based on the conserved PF02519 domain and were systematically characterized in terms of protein properties, gene structure, and phylogenetic relationships. Most *SAUR* genes in grain amaranth encode small, basic, and hydrophilic proteins, and gene structure analysis revealed that the majority are intronless. Phylogenetic analysis grouped *AhSAUR*s into ten clades alongside *Arabidopsis SAUR*s. Transcriptomic profiling across seven tissues and drought-treated samples showed that although many *SAUR* genes had low or no expression, several genes, including *AhSAUR76, AhSAUR71, AhSAUR65, AhSAUR54*, and *AhSAUR73*, were highly expressed in a tissue-preferential manner and showed responsiveness to drought. These findings highlight the potential regulatory roles of selected *SAUR* genes in growth and stress adaptation, offering a valuable resource for future genetic and functional studies aimed at enhancing agronomic traits in grain amaranth.

1. Introduction

Grain amaranth (*Amaranthus hypochondriacus*), an ancient pseudo-cereal crop, has its origins in Central and South America (Goncalves-Dias *et al*. 2023), where it has been cultivated for millennia by indigenous populations for its high nutritional value (Aderibigbe *et al*. 2022), adaptability, and resilience to adverse environmental conditions (Mukuwapasi *et al*. 2024). Amaranth grains are particularly valued for their exceptional nutritional profile, containing balanced proteins rich in essential amino acids, especially lysine, which is often limited in traditional

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cereals, and high concentrations of minerals such as calcium, magnesium, and iron (Aderibigbe *et al*. 2022; Baraniak & Kania-Dobrowolska 2022). This crop also contains bioactive compounds with antioxidant properties, contributing further to its rising global significance as a functional food that promotes health and dietary diversity (Baraniak & Kania-Dobrowolska 2022). However, the impacts of climate change, characterized by prolonged drought periods, increasingly unpredictable rainfall, and elevated temperatures, severely threaten amaranth production worldwide (Pulvento *et al*. 2022), necessitating a deeper understanding of its drought resistance mechanisms. Consequently, elucidating the molecular, biochemical, and physiological mechanisms underpinning amaranth's stress resilience, particularly its response to water scarcity, is crucial (Sarker & Oba 2018).

The small auxin-up RNA (SAUR) gene family constitutes a critical group of early auxin-responsive genes known to play essential roles in plant growth, development, and adaptive responses to environmental stimuli (Stortenbeker & Bemer 2019). SAUR proteins are generally small (approximately 9-15 kDa), and function primarily by modulating hormonal signaling pathways, notably auxin-mediated processes (Spartz *et al*. 2012). Although first identified in soybean (*Glycine max*) (Gil *et al*. 1994), subsequent research has characterized *SAUR* genes across a broad spectrum of plant species, including model species like *Arabidopsis thaliana* (Gil *et al*. 1994), as well as economically important crops such as potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) (Wu *et al*. 2012), watermelon (*Citrullus lanatus*) (Zhang *et al*. 2017), cotton (*Gossypium* spp.) (Li *et al*. 2017), moso bamboo (*Phyllostachys edulis*) (Bai *et al*. 2017), poplar (*Populus trichocarpa*) (Hu *et al*. 2018), grape (*Vitis vinifera*) (Li *et al*. 2021), apple (*Malus domestica*) (Zhou *et al*. 2022), coffea (*Coffea canephora*) (Zanin *et al*. 2022), Chinese white pear (*Pyrus bretschneideri*) (Wang *et al*. 2022), melon (*Cucumis melo*) (Tian *et al*. 2022), loquat (*Eriobotrya japonica*) (Peng *et al*. 2022), wax gourd (*Benincasa hispida*) (Luo *et al*. 2022), peanut (*Arachis hypogaea*) (Liu *et al*. 2022), pineapple (*Ananas comosus*) (Zhang *et al*. 2023), foxtail millet (*Setaria italica*) (Ma *et al*. 2023), cucumber (*Cucumis sativus*) (Luan *et al*. 2023), longan (*Dimocarpus longan*) (Chen *et al*. 2023) and cocoa (*Theobroma cacao*) (Chu *et al*. 2024). Given their central regulatory roles (Spartz *et al*. 2012), elucidating *SAUR* gene expression profiles under drought conditions at transcriptomic and proteomic levels can offer significant insights into plant resilience mechanisms.

The aim of this study was to perform a comprehensive genome-wide analysis of the SAUR gene family in grain amaranth. Specifically, the study sought to identify and annotate all *SAUR* gene members, characterize their physicochemical properties, and analyze their genomic structure and phylogenetic relationships in comparison with *A. thaliana*. Furthermore, transcriptomic data from public RNA-Seq datasets were re-analyzed to assess the expression patterns of *SAUR* genes across various tissues and under drought stress conditions.

2. Materials and Methods

2.1. Screening of SAURs

To screen for candidate SAURs in grain amaranth, we employed a bioinformatics approach based on sequence homology analysis using the conserved SAUR protein domain PF02519 (Stortenbeker & Bemer 2019). Initially, the genome of grain amaranth (GenBank assembly: GCA_000753965.2) (Clouse *et al*. 2016) is collected from publicly available Phytozome and NCBI portals. Subsequently, the conserved SAUR domain PF02519 sequence (Stortenbeker & Bemer 2019), obtained from the Pfam database (Mistry *et al*. 2021), is utilized as a query to perform Hidden Markov Model (HMM)-based searches using HMMER v3.3 (http://hmmer.org). Candidate SAUR protein sequences in grain amaranth were identified by aligning the proteome against the PF02519 HMM profile using hmmsearch with an E-value threshold of 1e-5. To further validate the identified sequences, all candidate proteins were examined for the presence of the conserved SAUR motif using SMART (http://smart.embl-heidelberg.de/) (Letunic *et al*. 2021) and CDD (NCBI's Conserved Domain Database) (Yang *et al*. 2020). Redundant and incomplete sequences were manually removed.

2.2. Characterization of SAURs

Characterization of the SAURs in grain amaranth was performed using the Expasy ProtParam computational tool (https://web.expasy.org/protparam/, accessed in January 2025) (Gasteiger *et al*. 2005) as previously described (Cao 2022; La *et al*. 2022; Le *et al*. 2022; Tien *et al*. 2024). ProtParam allows for the comprehensive analysis of protein sequences to predict physicochemical

properties, including protein length (number of amino acids), molecular weight, theoretical isoelectric point, hydrophobicity, and aliphatic index. Initially, protein sequences identified as SAUR family members from grain amaranth were retrieved and individually submitted to the Expasy ProtParam server (Gasteiger *et al*. 2005). Subsequently, each protein's physicochemical properties were computed automatically by ProtParam based on amino acid composition and sequence data (Gasteiger *et al*. 2005).

2.3. Phylogeny Analysis of SAURs

Phylogenetic analysis of grain amaranth SAURs was conducted using the Maximum Likelihood approach, utilizing *Arabidopsis* SAUR sequences as references (Spartz *et al*. 2012; Spartz *et al*. 2014). Initially, SAURs sequences from grain amaranth and *Arabidopsis* (Spartz *et al*. 2012; Spartz *et al*. 2014) were aligned using ClustalW v2.1 (Thompson *et al*. 2002; Larkin *et al*. 2007). The Maximum Likelihood-based phylogenetic tree was then generated using MEGA v11 software (Tamura *et al*. 2021) under the JTT+G model, which was selected as the best-fit model based on the lowest Bayesian Information Criterion score. Branch support was evaluated with 1000 bootstrap replicates as previous suggested (Cao 2022; La *et al*. 2022; Le *et al*. 2022; Tien *et al*. 2024). Branch strength was evaluated with 1000 bootstrap replicates.

2.4. Gene Structure of *SAUR*s

To analyze the gene structure of *SAUR*s in grain amaranth, we first retrieved genomic and coding sequences from reference databases in Phytozome. The phylogenetic tree was used to determine the gene order for structural analysis. Using GSDS v2.0 (accessed in January 2025) (Hu *et al*. 2015), we uploaded genomic sequences and corresponding annotation files (GFF3) to visualize exon-intron organization, untranslated regions (UTRs), and conserved domain structures. Structural variations, including exon-intron patterns and duplication events, were examined across phylogenetic clades to identify evolutionary trends and potential functional divergence of *SAUR* genes in grain amaranth.

2.5. Transcriptomic Analysis of *SAUR*s

RNA-Seq dataset (NCBI BioProject accession: PRJNA214804) from a previous study was obtained from the AGRDB portal (Singh *et al*. 2023) to analyze gene expression in cotyledon, leaf, stem, root, flower, developing embryo, mature seed, and drought-treated tissues. The gene expression analysis pipeline involved using the GeneID of each *SAUR* gene as a query to extract corresponding expression data from the AGRDB database. Among these collected samples in the original study, four-week-old grain amaranth plants at the vegetative stage were subjected to drought stress by withholding water for seven days under controlled environmental conditions (25°C, 16 h light/8 h dark photoperiod). Visible signs of wilting confirmed that the plants had reached a moderate to severe level of stress (Sunil *et al*. 2014). Gene expression levels, quantified as RPKM values, were obtained by querying each gene's ID through the AGRDB portal. By using R (R Core Team 2024) v4.4.2, genes with an adjusted p-value (Benjamini-Hochberg correction) < 0.05 and an absolute log2 fold change>1 were considered significantly differentially expressed. The results were visualized using the ggplot2 package.

3. Results

3.1. Identification and Characterization of SAURs in Grain Amaranth

The SAUR family in grain amaranth was newly identified based on the conserved PF02519 domain and subsequently characterized through bioinformatics analyses, resulting in 80 candidate *SAUR* genes, designated as *AhSAUR01* to *AhSAUR80*. The naming principle "*AhSAUR*" incorporates "Ah" for *Amaranthus hypochondriacus*, a representative grain amaranth species, followed by sequential numbering based on chromosomal location. The proteins encoded by the identified *AhSAUR* genes vary widely in length, ranging from 70 amino acids (AhSAUR25) to 184 amino acids (AhSAUR46), and molecular weights span from approximately 7.78 kDa to 21.14 kDa. Additionally, the theoretical isoelectric points of these proteins vary considerably, extending from acidic (4.29 for AhSAUR25) to strongly basic (10.78 for AhSAUR69). Hydrophobicity analysis through the grand average of hydropathicity scores shows a range from highly hydrophilic (-0.92 for AhSAUR54) to relatively hydrophobic (0.48 for AhSAUR08). The aliphatic index values range extensively from 54.68 (AhSAUR70) to 129.32 (AhSAUR59) (Table 1).

3.2. Evolutionary Analysis of SAURs in Grain Amaranth

The phylogenetic analysis clearly illustrated evolutionary relationships among *S*AURs in grain amaranth (AhSAURs) and *Arabidopsis* (AtSAURs),

Table 1. Summary of *SAUR*s in grain amaranth

Gene name	Locus name	Gene length (bp)	Protein length (aa)	mW (kDa)	pI	GRA VY	AI
AhSAUR01	*AH000765*	387	128	14.51	8.88	-0.13	93.83
AhSAUR02	*AH000766*	381	126	14.52	6.82	-0.05	84.37
AhSAUR03	*AH000768*	378	125	14.05	9.26	0.00	100.00
AhSAUR04	*AH001698*	276	91	10.29	7.79	-0.32	83.52
AhSAUR05	*AH001699*	276	91	10.28	6.81	-0.15	87.80
AhSAUR06	*AH001700*	279	91	10.28	6.81	-0.15	87.80
AhSAUR07	*AH001701*	279	92	10.55	8.76	0.12	100.54
AhSAUR08	*AH001702*	240	79	8.83	7.67	0.48	112.15
AhSAUR09	*AH001702*	276	91	10.27	6.82	-0.08	84.62
AhSAUR10	*AH001703*	279	92	10.28	8.62	-0.28	77.28
AhSAUR11	*AH001704*	276	91	10.37	9.34	-0.18	78.13
AhSAUR12	*AH001705*	279	92	10.49	8.98	-0.06	90.98
AhSAUR13	*AH001709*	294	97	11.05	8.85	-0.16	97.32
AhSAUR14	*AH001710*	240	79	8.84	6.56	-0.01	91.27
AhSAUR15	*AH001714*	297	98	11.08	8.88	-0.20	91.33
AhSAUR16	*AH001714*	324	107	12.38	9.17	-0.20	92.80
AhSAUR17	*AH001714*	285	94	10.73	9.06	-0.33	81.81
AhSAUR18	*AH001714*	291	96	10.29	9.46	-0.23	88.12
AhSAUR19	*AH001715*	297	98	11.16	8.84	-0.30	75.61
AhSAUR20	*AH001715*	306	101	11.42	7.85	-0.08	80.89
AhSAUR21	*AH001716*	339	112	12.91	9.30	-0.56	68.75
AhSAUR22	*AH001717*	291	96	10.90	7.82	-0.17	89.27
AhSAUR23	*AH001718*	315	104	11.88	8.51	-0.38	84.33
AhSAUR24	*AH001719*	339	112	12.68	8.82	-0.17	95.71
AhSAUR25	*AH002237*	213	70	7.78	4.29	-0.07	71.14
AhSAUR26	*AH002238*	447	148	17.50	9.44	0.07	75.88
AhSAUR27	*AH002329*	411	136	15.63	9.57	-0.14	92.50
AhSAUR28	*AH002329*	405	134	15.26	9.16	0.00	93.96
AhSAUR29	*AH002329*	240	79	8.89	4.72	-0.01	85.19
AhSAUR30	*AH002330*	381	126	14.54	8.81	-0.24	76.75
AhSAUR31	*AH002331*	405	134	15.24	9.21	-0.07	89.63
AhSAUR32	*AH002331*	362	126	14.66	7.73	-0.16	87.62
AhSAUR33	*AH002332*	357	118	13.52	6.82	-0.05	83.47
AhSAUR34	*AH002331*	408	135	15.60	9.10	-0.13	85.41
AhSAUR35	*AH002331*	381	126	14.43	7.71	-0.08	86.11
AhSAUR36	*AH002336*	378	125	14.29	8.49	-0.18	77.36
AhSAUR37	*AH002336*	381	126	14.56	9.23	-0.15	82.94
AhSAUR38	*AH002336*	378	125	14.25	8.54	-0.08	86.56
AhSAUR39	*AH002336*	408	135	15.64	9.10	-0.11	81.04
AhSAUR40	*AH002336*	372	123	14.50	9.17	-0.39	65.04
AhSAUR41	*AH002336*	381	126	14.45	9.07	-0.17	79.84
AhSAUR42	*AH002336*	408	135	15.64	9.37	-0.13	83.85
AhSAUR43	*AH002336*	408	135	15.45	9.35	-0.02	95.33
AhSAUR44	*AH003217*	516	171	19.61	10.31	-0.52	88.89
AhSAUR45	*AH003753*	312	103	11.75	7.77	-0.30	84.27
AhSAUR46	*AH004565*	555	184	21.14	9.34	-0.67	59.29
AhSAUR47	*AH007102*	411	136	15.55	9.30	0.01	95.44
AhSAUR48	*AH007103*	357	118	13.21	5.79	0.15	92.63
AhSAUR49	*AH008323*	444	147	17.07	10.40	-0.46	82.11
AhSAUR50	*AH008329*	321	106	12.03	6.89	-0.28	82.74
AhSAUR51	*AH009060*	324	107	12.28	7.70	-0.44	87.38
AhSAUR52	*AH009061*	243	80	9.28	9.03	-0.22	98.62
AhSAUR53	*AH009415*	240	79	9.12	8.66	-0.16	87.59

Table 1. Continued

Gene name	Locus name	Gene length (bp)	Protein length (aa)	mW (kDa)	pI	GRA VY	AI
AhSAUR54	*AH009509*	393	130	15.23	7.93	-0.92	69.69
AhSAUR55	*AH010367*	411	126	14.62	7.91	-0.77	68.02
AhSAUR56	*AH010368*	306	101	12.12	8.54	-0.55	85.84
AhSAUR57	*AH012592*	399	132	15.00	8.89	-0.25	79.70
AhSAUR58	*AH012757*	366	121	13.76	8.87	-0.24	99.92
AhSAUR59	*AH012770*	294	73	8.38	6.89	0.42	129.32
AhSAUR60	*AH013127*	312	103	12.04	7.67	-0.30	78.45
AhSAUR61	*AH013496*	546	181	20.56	8.54	-0.46	75.86
AhSAUR62	*AH013498*	438	145	16.86	7.82	-0.62	80.76
AhSAUR63	*AH013918*	420	139	15.73	7.83	-0.88	63.02
AhSAUR64	*AH014308*	435	144	16.91	6.96	-0.67	79.10
AhSAUR65	*AH014310*	351	116	12.96	5.89	-0.18	86.38
AhSAUR66	*AH014311*	396	131	14.11	5.27	-0.24	80.46
AhSAUR67	*AH015201*	498	165	19.06	7.78	-0.66	73.27
AhSAUR68	*AH016694*	309	102	11.41	8.86	-0.08	90.78
AhSAUR69	*AH016735*	441	146	16.80	10.78	-0.50	76.03
AhSAUR70	*AH017350*	411	139	15.88	9.29	-0.68	54.68
AhSAUR71	*AH018382*	345	114	13.24	6.27	-0.24	83.68
AhSAUR72	*AH018555*	528	175	20.06	10.44	-0.46	88.51
AhSAUR73	*AH021073*	393	130	14.71	4.97	-0.59	62.08
AhSAUR74	*AH021794*	378	125	14.37	9.27	-0.31	76.32
AhSAUR75	*AH022002*	369	122	14.09	9.54	-0.22	84.59
AhSAUR76	*AH022329*	375	124	13.94	7.80	-0.22	93.39
AhSAUR77	*AH023136*	294	97	11.13	9.73	-0.10	102.37
AhSAUR78	*AH023404*	426	141	15.88	9.66	-0.36	84.89
AhSAUR79	*AH023429*	378	125	14.33	7.63	-0.55	84.16
AhSAUR80	*AH023586*	327	108	12.56	8.64	-0.49	80.28

categorizing them into ten distinct groups (I - X) (Figure 1). Each group contained varied numbers of AhSAURs. Group V constituted the largest cluster, containing 17 AhSAURs. In contrast, group IX contained 16 AhSAURs closely associated with numerous *Arabidopsis* homologs, suggesting high functional conservation between the two species in this group. Smaller clusters included groups I and III, each comprising six AhSAURs. These groups showed close evolutionary relationships with *Arabidopsis* SAURs. Similarly, group VIII consisted of five AhSAURs, while groups VI and X each contained four AhSAURs. Groups II, IV, and VII each included three AhSAURs (Figure 1).

3.3. Gene Organization of *SAUR*s in Grain Amaranth

The exon-intron organization analysis of AhSAURs in grain amaranth revealed a highly conserved structural pattern characterized by the predominance of intronless gene structures. Based on GSDS visualization, all *AhSAUR*s exhibited a single-exon configuration (Figure 2). The phylogenetic distribution of *AhSAUR* genes indicates that closely related gene clusters share similar exon-intron patterns.

Next, the analysis of the genomic DNA sequences of the SAUR gene family in grain amaranth revealed significant variations in gene length, ranging from 213 bp to 555 bp. The majority of *AhSAUR*s exhibited a compact structure, with genomic lengths predominantly between 240 bp and 450 bp. Notably, genes such as *AhSAUR44* (516 bp), *AhSAUR46* (555 bp), and *AhSAUR61* (546 bp) represent the longest sequences. In contrast, *AhSAUR25* (213 bp) and *AhSAUR14, AhSAUR29, AhSAUR53* (240 bp each) are among the shortest. Several *AhSAUR*s with identical or nearly identical lengths, such as *AhSAUR02, AhSAUR30, AhSAUR35, AhSAUR37*, and *AhSAUR41* (381 bp each) or *AhSAUR04, AhSAUR05, AhSAUR09*, and *AhSAUR11* (276 bp each).

3.4. Expression Patterns of *SAUR*s during The Growth and Development of Grain Amaranth

Based on recent RNA-Seq datasets, our heatmaps reveal that most *SAUR* genes exhibit low or non-differential expression across tissues. However, five genes, including *AhSAUR73, AhSAUR65, AhSAUR54, AhSAUR71*, and *AhSAUR76*, showed tissue-specific expression patterns with high RPKM values (Figure 3). Particularly, *AhSAUR76* exhibited the highest expression

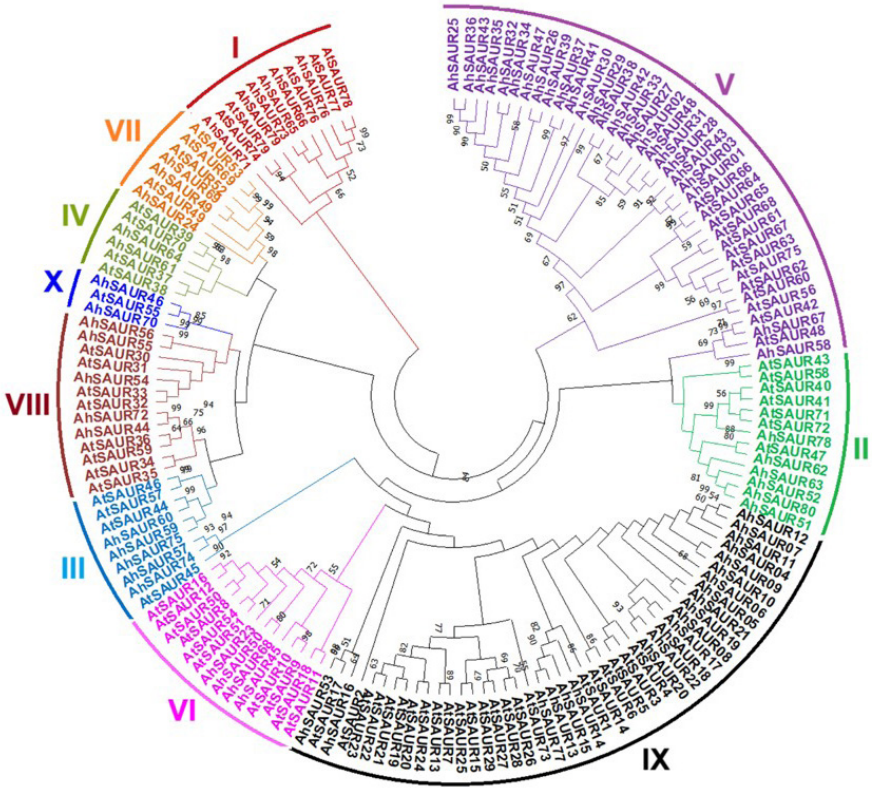

Figure 1. Classification of SAURs in grain amaranth based on well-characterized SAURs in *Arabidopsis thaliana*

level in the stem. This gene is also highly expressed in leaf and flower, while *AhSAUR71* is predominantly expressed in cotyledons and stems, with notable expression in developing embryos. We also found that *AhSAUR65* showed high specificity in leaves and cotyledons, as well as drought-treated tissues, while *AhSAUR73* exhibited high expression in leaves and cotyledons, with moderate levels in stems, roots, and flowers. *AhSAUR54* showed a broad expression pattern with notable activity in cotyledons, developing embryo, and drought-treated tissues.

4. Discussion

4.1. Variation in The Number of Members of The SAURs from Grain Amaranth and Other Species

The *SAUR* gene family represents one of the most rapidly induced primary auxin-responsive gene families in plants (Spartz *et al*. 2012), and its expansion and diversification across species provide critical insights into auxin signaling evolution and plant development (Spartz *et al*. 2012; Stortenbeker & Bemer 2019). Comparative analysis of SAUR gene family members across various plant species, including *Solanaceae* (tomato, potato) (Wu *et al*. 2012), *Rosaceae* (apple, pineapple, Chinese white pear) (Wang *et al*. 2020; Wang *et al*. 2022; Zhou *et al*. 2022; Zhang *et al*. 2023), *Cucurbitaceae* (watermelon) (Zhang *et al*. 2017), and *Amaranthaceae* (grain amaranth). Particularly, in grain amaranth, a total of 55 *SAUR* members were identified and analyzed. Solanaceae species exhibit a broader expansion of the SAUR gene family (Wu *et al*. 2012). For example, tomato and potato possess 99 and 134 *SAUR* genes, respectively (Wu *et al*. 2012), a significantly larger number than in amaranth. Similarly, in watermelon, 65 *SAUR* genes were identified (Zhang *et al*. 2017). In apple, Chinese white pear and cocoa, SAUR gene family members also exhibit expansion (96 in apple, 95 in Chinese white pear and 90 in cocoa) (Wang *et al*. 2020; Wang *et al*. 2022; Zhou *et al*. 2022; Zhang *et al*. 2023; Chu *et al*. 2024).

Additionally, the complete absence of introns in all *AhSAUR* genes is consistent with observations in other plant species, where the SAUR gene family is also largely intronless. Intronless genes are often associated with rapid transcriptional responses, particularly in signaling pathways and stress responses, due to the reduced time and energy required for mRNA splicing. The uniformity of this gene architecture in grain amaranth suggests that

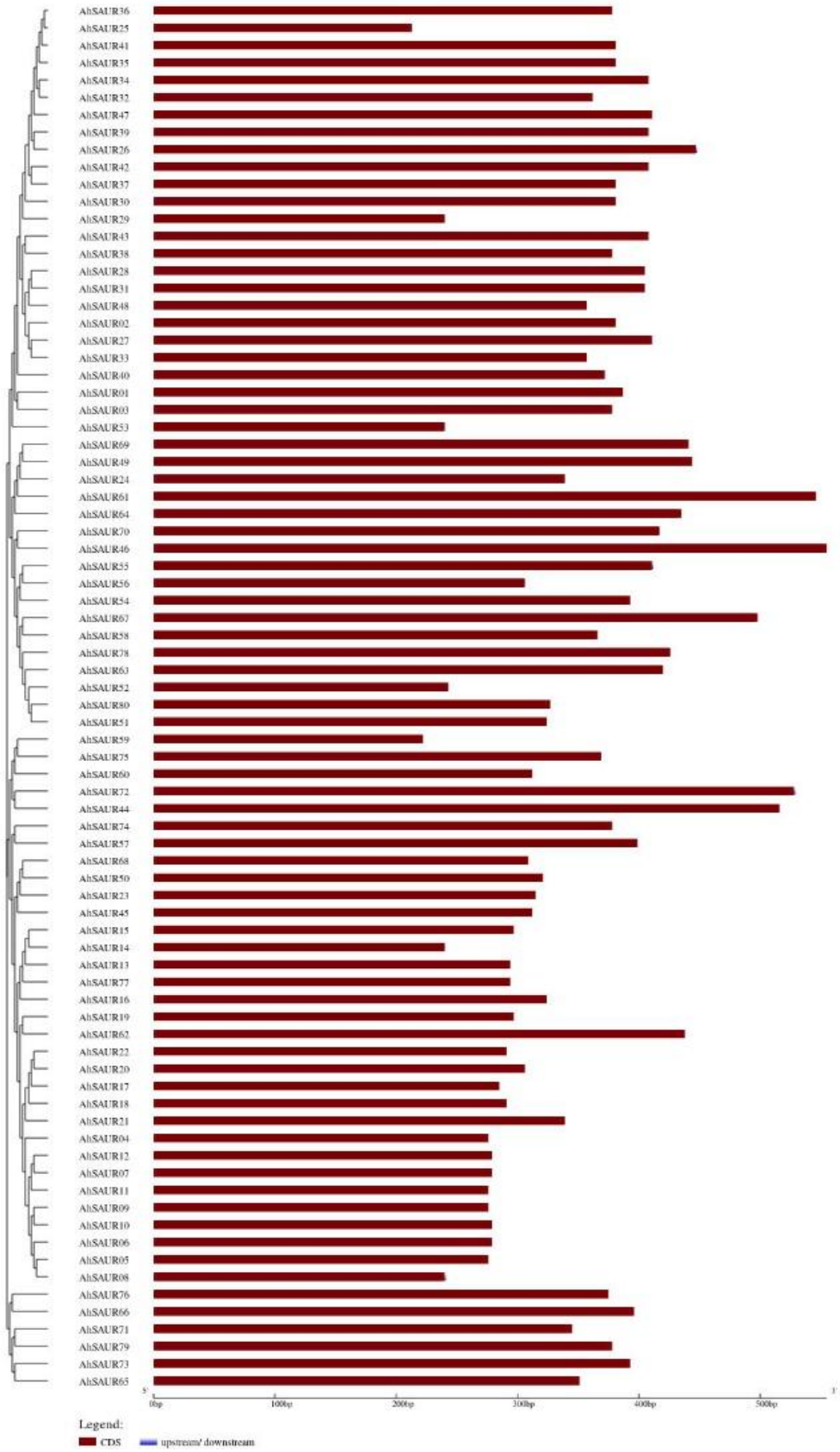

Figure 2. Exon/intron organization of the SAUR gene family in grain amaranth

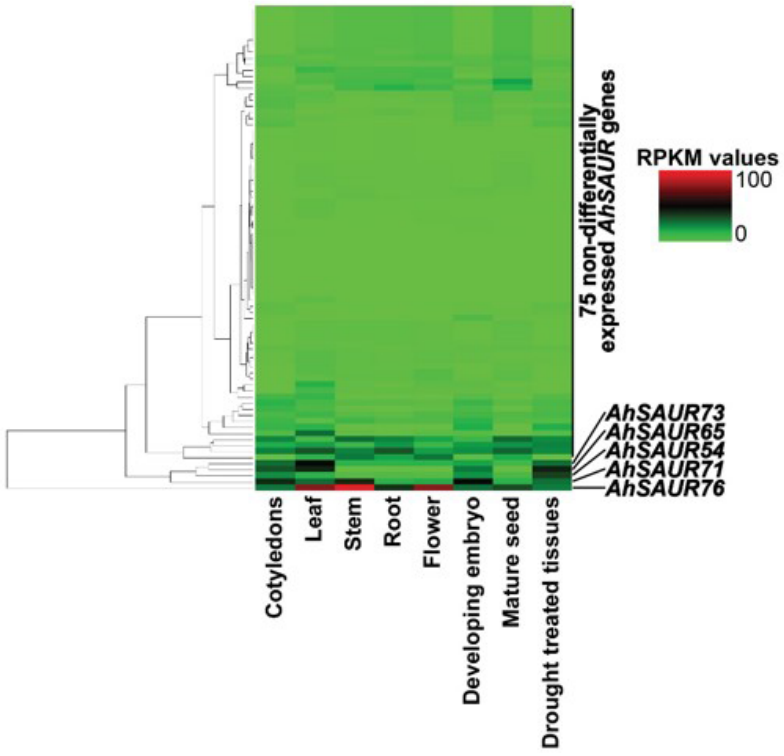

Figure 3. Expression patterns of the SAUR gene family in seven organs and drought-treated tissues of grain amaranth

the intronless structure has been evolutionarily conserved and may be functionally important for ensuring efficient and timely expression of *SAUR* genes under both developmental and stress-related conditions.

4.2. Physicochemical Diversity of SAURs Across Plant Species

In many species, such as *A. thaliana*, tomato, watermelon, and apple, SAUR proteins are generally small-sized, hydrophilic, and basic in nature, typically ranging from 9 to 22 kDa with isoelectric point scores above 8.0 (Wu *et al*. 2012; Zhang *et al*. 2017; Wang *et al*. 2020; Zhang *et al*. 2023). In grain amaranth, a similar trend is observed, with most AhSAUR proteins also being relatively small in molecular weight and basic in nature. However, the range of molecular weight and isoelectric points in grain amaranth appears slightly broader than in some other species. Additionally, the grand average of hydropathicity scores in AhSAUR proteins suggested they are largely hydrophilic. Despite these similarities, notable differences exist in the protein instability index and aliphatic index across species. In grain amaranth, a greater proportion of SAUR proteins are predicted to be stable, compared to a higher frequency of unstable SAUR proteins reported in tomato and watermelon (Wu *et al*. 2012; Zhang *et al*. 2017).

4.3. Tissue-Specific Expression Patterns of SAURs in Plant Species

The expression profiles of *SAUR* genes exhibit substantial variability across plant species. In general, *SAUR* genes are rapidly induced by auxin and are predominantly expressed in tissues undergoing active cell elongation (Chen *et al*. 2023), such as stems, young leaves, and floral organs (Peng *et al*. 2022; Zanin *et al*. 2022; Chen *et al*. 2023; Zhang *et al*. 2023). In *A. thaliana*, numerous *SAUR* genes are strongly expressed in expanding tissues and play roles in auxin-mediated cell elongation, shade avoidance, and tropic responses (Spartz *et al*. 2017; Kathare *et al*. 2018; Chen *et al*. 2023). In crop species, such as tomato and apple, *SAUR* genes also display differential expression across vegetative and reproductive organs (Wu *et al*. 2012; Wang *et al*. 2020; Zhang *et al*. 2023). In tomato, *SAUR* genes show high expression in leaves, flowers, and fruits, with several members being upregulated under abiotic stress conditions such as salinity and drought (Wu *et al*. 2012). Similarly, in apple, a subset of *SAUR* genes is associated with fruit

development and ripening, as well as stress-induced signaling pathways (Wang *et al*. 2020; Zhou *et al*. 2022; Zhang *et al*. 2023;). These tissue- and condition-specific expression patterns point to the functional diversification of *SAUR* family members in different crops. In grain amaranth, transcriptome reanalysis revealed that a subset of *AhSAUR* genes, including *AhSAUR76, AhSAUR71, AhSAUR65, AhSAUR54*, and *AhSAUR73*, exhibited strong tissue-specific expression in organs such as stems, leaves, cotyledons, and developing embryos. This indicated that while the *SAUR* family in amaranth is moderately sized compared to other species, certain members have undergone functional specialization in specific tissues. Moreover, the high expression of *AhSAUR54* and *AhSAUR65* in drought-treated tissues suggests potential roles in stress adaptation.

In conclusion, this study provided the first comprehensive genome-wide identification and characterization of the SAUR gene family in grain amaranth. By integrating genome-wide identification, structural and evolutionary analyses, and expression profiling under drought conditions, we deepen our understanding of how this gene family may contribute to growth regulation and stress adaptation. The predominance of intronless genes and the presence of tissue- and stress-responsive expression patterns suggest that SAURs in amaranth are evolutionarily optimized for rapid transcriptional responses. These findings not only underscore the regulatory importance of SAURs in auxin signaling and environmental adaptation but also establish a foundational resource for functional genomics studies. Future work focusing on gene editing, promoter analysis, and protein-level validation will be essential to unravel the precise roles of key AhSAURs in improving stress resilience and agronomic performance in amaranth and related crops.

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