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GENOME-WIDE IDENTIFICATION AND CHARACTERIZATION OF SAUR1 GENES IN *BRASSICA* SPECIES AND SEEDLING GROWTH

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Abstract

Auxin controls plant growth and development, and the small auxin-up RNA (SAUR) family is the largest group of auxin-responsive genes. SAUR genes are rapidly induced by auxin and regulate cell growth, but their repertoire and activity in Brassica species have not been examined. Here we carried out a genome-wide identification and characterization of SAUR1 genes in six Brassica species (B. rapa, B. nigra, B. oleracea, B. juncea, B. napus and B. carinata). Sixteen SAUR1 genes were identified and analyzed for sequence features, gene structure, conserved motifs and chromosomal location. All of them lacked introns and carried highly conserved motifs, which suggests evolutionary stability. Subcellular localization analysis predicted that SAUR1 proteins are targeted mainly to chloroplasts and mitochondria. Phylogenetic analysis showed strong homology with Arabidopsis SAURs and lineage-specific diversification. Promoter analysis detected multiple auxin- and stress-related cis-elements, which points to roles in development and adaptation. The species differed significantly in root length, shoot length, and fresh and dry biomass (ANOVA), indicating species-specific growth responses. This is the first comprehensive characterization of SAUR1 genes in Brassica, and it gives a basis for studying auxin signaling and growth regulation in the genus.

1. Introduction

The genus *Brassica* belongs to the family *Brassicaceae*, which comprises about 340 genera and 3,709 species. Brassicas are grown for vegetable oil, vegetables, fodder, condiments and biofuel (Downey, 2016), and the oilseed types help meet the vegetable oil demand of a growing population (Ilyas *et al.*, 2022). Cruciferous vegetables and seeds also supply nutrients and phytochemicals such as phenolics, flavonoids and glucosinolates, which show anti-inflammatory, anticancer and antidiabetic activity (Ayadi *et al.*, 2022; Afzal *et al.*, 2025; Siddique *et al.*, 2026).

Rapeseed ranks third among the world's edible oils, after soybean and palm (Hu *et al.*, 2021; Afzal *et al.*, 2025), and oil content varies a great deal among the cultivated species, from 17–24% in *B. nigra* to 38–56% in *B. napus* (Sharafi *et al.*, 2015). Brassica seeds are also rich in carbohydrates, fats, proteins, vitamins and

minerals, and they hold secondary metabolites with therapeutic value (Ayadi *et al.*, 2022).

Six cultivated species form the classical triangle of U. The diploids *B. rapa* (AA, 2n = 20), *B. nigra* (BB, 2n = 16) and *B. oleracea* (CC, 2n = 18) hybridized in pairs to give the allotetraploids *B. juncea* (AABB, 2n = 36), *B. napus* (AACC, 2n = 38) and *B. carinata* (BBCC, 2n = 34) (Choi *et al.*, 2007). Both *B. rapa* and *B. oleracea* went through a whole-genome triplication after they separated from Arabidopsis (Mun *et al.*, 2012), and this event drove much of the diversification of Brassica plants (Cheng *et al.*, 2014). Four of the six species, *B. juncea*, *B. rapa*, *B. carinata* and *B. napus*, are grown mainly for their oilseeds (Katche *et al.*, 2019).

The allopolyploids carry considerable genetic diversity. It may come from repeated hybridization events with different parents, or from backcrossing of the new allotetraploids to the parent species after polyploidization (Katche *et al.*, 2019). Brassica crops are also easy to grow across a wide range of conditions, and interspecific hybridization lets breeders move traits such as stress resistance from one species to another (Thakur *et al.*, 2019).

The species differ widely in form and use. *B. napus* is the most important oilseed of the group (Hu *et al.*, 2021). *B. rapa* includes turnip, Chinese cabbage and oilseed forms (Cheng *et al.*, 2014), whereas *B. oleracea* covers cabbage, cauliflower, broccoli and kale (Singh *et al.*, 2018). *B. juncea* is grown as an oilseed mustard (Shekhawat *et al.*, 2012), *B. nigra* is valued as a condiment (Fahey, 2015), and *B. carinata* carries resistance to several biotic and abiotic stresses (Thakur *et al.*, 2019). Because these species share ancestors but differ in growth habit, they offer a good system for asking which genes shape growth. Growth matters more as the climate becomes less stable. Heatwaves and cold spells are becoming more frequent and more intense, and they can reduce crop production (Yan *et al.*, 2019; Eom *et al.*, 2022; Afzal *et al.*, 2025). Genes that link hormone signals to growth are therefore of direct interest for Brassica breeding.

Auxin regulates cell elongation, division and differentiation, and so affects most aspects of plant growth (Ren and Gray, 2015). The early auxin-responsive genes fall into three families, GH3, Aux/IAA and SAUR (Hagen and Guilfoyle, 2002). The SAUR family is the largest of the three, with roughly 60 to 140 members in most higher plants (Stortenbeker and Bemer, 2019). The proteins are small, usually 60 to 100 amino acids long (Zhang *et al.*, 2021). Most SAUR genes lack introns and sit in clusters produced by tandem and segmental duplication (Li *et al.*, 2017).

Besides auxin, SAUR expression is modulated by cytokinin, gibberellin, brassinosteroid, ethylene, abscisic acid and jasmonate (Stortenbeker and Bemer, 2019). SAUR proteins connect auxin signals to the plasma membrane H^+-ATPase and to environmental cues (Ren and Gray, 2015), and some SAUR genes are down-regulated under abiotic stress, which may be an adaptive response (Zhu *et al.*, 2024). The family has expanded through duplication in many lineages. Rice has about 58 members (Jain *et al.*, 2006), cotton species have 145 to 214 (Li *et al.*, 2017), foxtail millet has 72 (Ma *et al.*, 2023) and soybean, a polyploid, has 236, whereas the moss *Physcomitrella patens* has only 18 (Zhang *et al.*, 2021).

Functional studies show that SAUR proteins control growth. In Arabidopsis, SAUR19 promotes cell expansion, and its constitutive expression in tomato produces auxin-independent hypocotyl elongation (Spartz *et al.*, 2017). Arabidopsis SAUR36 promotes leaf senescence (Hou *et al.*, 2013). In rice, SAUR39 acts as a negative regulator of auxin synthesis and transport, and plants overexpressing it show reduced shoot and root growth (Kant *et al.*, 2009). Genome-wide surveys have been reported for rice (Jain *et al.*, 2006), cotton (Li *et al.*, 2017), foxtail millet (Ma *et al.*, 2023) and *Santalum album* (Zhu *et al.*, 2024). Family size differs among these species, but the sequence features are similar.

Polyploidy has expanded auxin-related gene families in Brassica, as shown for *B. rapa* after the genome triplication (Mun *et al.*, 2012). Because the Brassica genomes have gone through triplication and polyploidization, their SAUR families are expected to be larger than that of Arabidopsis, although gene loss often

follows duplication. Structural variants have also been linked to gene diversification and morphotype traits in a *B. oleracea* pan-genome (Guo *et al.*, 2024). In *B. napus*, a comparison of root transcriptomes found two SAUR-like genes expressed more strongly in plants with short primary roots, which fits a growth-restricting role like that of rice SAUR39 (Dun *et al.*, 2016).

Even so, no genome-wide analysis of SAUR genes has been reported for Brassica, and the six species of the U triangle have not been compared. We therefore used Arabidopsis *SAUR1* as a query to find its orthologs in these species, and we describe their sequence features, gene structure, motifs, predicted localization, phylogeny and promoters. We also compared seedling growth among the species. The objectives of this study were (i) to characterize the *SAUR1* gene in *B. rapa*, *B. nigra*, *B. oleracea*, *B. juncea*, *B. napus* and *B. carinata*, and (ii) to compare growth and development traits among these cultivated species.

2. Materials and methods

2.1 Identification and sequence characterization of SAUR1 genes

The Arabidopsis SAUR1 protein sequence was retrieved from TAIR (Lamesch *et al.*, 2010) and searched for conserved domains with HMMER against the Pfam database (Mistry *et al.*, 2021). The sequence was then used as a BLASTP query against the proteomes of six Brassica species in the BRAD database (Chen *et al.*, 2022): *B. rapa* (Chiifu v3.1), *B. nigra* (San v1.1), *B. oleracea* (JZS v2.0), *B. juncea* (tum v2.0), *B. napus* (ZS v2.0) and *B. carinata* (zd1 v1.0). The E-value cutoff was 10^{-5}, and hits with more than 80% similarity were kept. Gene and transcript IDs, chromosomal positions, strand, CDS, genomic sequences and protein sequences were downloaded from BRAD, and the genes were named with a species prefix and a serial number (for example, BcaSAUR1-01).

Protein length, molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index and grand average of hydropathicity (GRAVY) were calculated with ProtParam on the ExPASy server. Exon-intron structure was drawn in the Gene Structure Display Server (GSDS 2.0) by comparing CDS with genomic sequences. Subcellular localization was predicted with WoLF PSORT, using the plant setting (Horton *et al.*, 2007).

2.2 Motif, phylogenetic and structural analyses

Conserved motifs were identified with the MEME Suite using default parameters (Bailey *et al.*, 2009). Protein sequences were aligned with ClustalW in MEGA 11, and a neighbor-joining tree was built with 1,000 bootstrap replicates and displayed in iTOL. Three-dimensional structures of the proteins were predicted with Phyre2.

2.3 Promoter analysis

The 2,000 bp region upstream of the start codon of each gene was extracted from BRAD and searched for cis-acting regulatory elements in the PlantCARE database (Lescot *et al.*, 2002).

2.4 Plant growth assays and statistical analysis

Ten seeds of each species were placed on moist filter paper in plastic trays and kept at room temperature for 10 days, with daily watering. There were four replications per species. Germination was recorded daily and expressed as the percentage of seeds sown that germinated. At day 10, root length and shoot length were measured with a ruler, and fresh and dry weights of roots and shoots were recorded on a balance. Data were analyzed by analysis of variance (ANOVA) in R for a completely randomized design, with species, replication and their interaction as sources of variation. Differences were considered significant at $P \leq 0.05$.

3. Results

3.1 Identification and chromosomal location of SAUR1 genes

The Arabidopsis SAUR1 protein has 104 amino acids, a molecular weight of 11,317.9 Da and a theoretical pI of 8.49. HMMER detected one auxin-responsive domain (Pfam PF02519) between residues 4 and 101, with an independent E-value of 6.8×10^{-26}. BLASTP searches against the six Brassica proteomes returned 22 unique hits. Six of them came from unplaced scaffolds, which left 16 *SAUR1* genes for analysis. The closest match was

BjuVA08G16710 (93.9% identity, score 492, E = 5.5×10^{-65}), followed by BraA01g003500.3.1C and the two BcaNung genes (94.9% identity, score 486, E = 3.5×10^{-64}).

The 16 genes belonged to four species: four in *B. carinata*, six in *B. juncea*, three in *B. nigra* and three in *B. rapa*. No gene from *B. oleracea* or *B. napus* passed the similarity filter (Table 1). Six genes lie on A-subgenome chromosomes, seven on B-subgenome chromosomes, one on chromosome C1, and two on contig324. Eleven genes are on the minus strand and five on the plus strand. Genomic spans ranged from 231 to 463 bp. All genes had a single exon and no introns.

Table 1: *SAUR1* genes identified in Brassica species, with chromosomal position and exon-intron number

Gene ID	Transcript ID	Chromosome	Start	Stop	Strand	Exons	Introns
BcaSAUR1-01	BcaB03g11810	B3	1658504	1658734	−	1	0
BcaSAUR1-02	BcaC01g00872	C1	10001816	10002133	−	1	0
BcaSAUR1-03	BcaNung00658	Contig324	541198	541512	−	1	0
BcaSAUR1-04	BcaNung00659	Contig324	542726	543040	−	1	0
BjuSAUR1-05	BjuVA01G03740	A1	1790191	1790645	−	1	0
BjuSAUR1-06	BjuVA03G62480	A3	31241997	31242314	+	1	0
BjuSAUR1-07	BjuVA08G16710	A8	13257049	13257363	−	1	0
BjuSAUR1-08	BjuVB02G73420	B2	66313708	66314025	+	1	0
BjuSAUR1-09	BjuVB03G19890	B3	9163308	9163770	+	1	0
BjuSAUR1-10	BjuVB05G03490	B5	2238978	2239292	−	1	0
BniSAUR1-11	BniB036572-PA	B8	43607356	43607673	+	1	0
BniSAUR1-12	BniB040818-PA	B2	44014322	44014636	−	1	0
BniSAUR1-13	BniB048405-PA	B7	33617411	33617725	−	1	0
BraSAUR1-14	BraA01g003500.3.1C	A1	1744623	1744937	−	1	0
BraSAUR1-15	BraA03g060180.3.1C	A3	31307454	31307771	+	1	0
BraSAUR1-16	BraA08g016080.3.1C	A8	12891929	12892243	−	1	0

3.2 Physicochemical properties and predicted localization

The proteins ranged from 76 amino acids (BcaSAUR1-01) to 105 amino acids, with molecular weights of 8,320.46 to 11,529.32 Da and theoretical pI values of 6.24 to 8.66 (Table 2). Ten proteins had a pI above 7 and six had a pI below 7. The instability index was above 40 for six proteins (BcaSAUR1-03, BcaSAUR1-04, BjuSAUR1-05, BjuSAUR1-06, BraSAUR1-14 and BraSAUR1-15), and the aliphatic index ranged from 80.79 to 103.05. GRAVY was positive for 15 proteins (0.039 to 0.198) and negative only for BcaSAUR1-01 (−0.062). Several proteins from different species had identical values in every column: BcaSAUR1-03, BcaSAUR1-04 and BraSAUR1-14; BjuSAUR1-06 and BraSAUR1-15; BjuSAUR1-08 and BniSAUR1-11; BjuSAUR1-09 and BniSAUR1-13; and BjuSAUR1-10 and BniSAUR1-12.

Table 2: Physicochemical properties of the SAUR1 proteins

Gene ID	Amino acids	Molecular weight (Da)	pI	Instability index	Aliphatic index	GRAVY
BcaSAUR1-01	76	8320.46	6.24	24.27	80.79	−0.062
BcaSAUR1-02	105	11436.23	6.90	34.46	103.05	0.182
BcaSAUR1-03	104	11426.27	6.89	40.50	100.38	0.175
BcaSAUR1-04	104	11426.27	6.89	40.50	100.38	0.175
BjuSAUR1-05	104	11454.32	6.89	40.50	102.21	0.198
BjuSAUR1-06	105	11489.26	7.74	41.40	93.81	0.060
BjuSAUR1-07	104	11322.08	7.93	33.88	98.46	0.093
BjuSAUR1-08	105	11529.32	8.66	23.35	95.71	0.057
BjuSAUR1-09	104	11280.00	7.93	30.75	96.63	0.069
BjuSAUR1-10	104	11432.20	7.94	36.29	99.42	0.039
BniSAUR1-11	105	11529.32	8.66	23.35	95.71	0.057
BniSAUR1-12	104	11432.20	7.94	36.29	99.42	0.039
BniSAUR1-13	104	11280.00	7.93	30.75	96.63	0.069
BraSAUR1-14	104	11426.27	6.89	40.50	100.38	0.175
BraSAUR1-15	105	11489.26	7.74	41.40	93.81	0.060
BraSAUR1-16	104	11296.05	7.95	33.88	98.46	0.075

WoLF PSORT gave the highest score to mitochondria for 11 proteins and to chloroplasts for three (BjuSAUR1-05, BjuSAUR1-06 and BraSAUR1-15). The nucleus scored highest for BcaSAUR1-01 and BraSAUR1-16 (Table 3, Fig. 1).

Table 3: Predicted subcellular localization scores of the SAUR1 proteins (WoLF PSORT)

Gene ID	Nucl	Mito	Chlo	Plas	Cysk	Cyto_nucl	Cyto	Chlo_mito	Extr
BcaSAUR1-01	4	2.5	3.5	-	1	-	2	3.5	1
BcaSAUR1-02	2.5	5	4	1	1	2	-	-	-
BcaSAUR1-03	-	7	4	1	1	1	-	-	-
BcaSAUR1-04	-	7	4	1	1	1	-	-	-

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Gene ID	Nucl	Mito	Chlo	Plas	Cysk	Cyto_nucl	Cyto	Chlo_mito	Extr
BjuSAUR1-05	1.5	5	6	-	1	1.5	-	-	-
BjuSAUR1-06	-	5	7	-	1	-	1	-	-
BjuSAUR1-07	3.5	5	3	1	1	2.5	-	-	-
BjuSAUR1-08	1	7	5	-	-	-	1	-	-
BjuSAUR1-09	3.5	5	3	1	1	2.5	-	-	-
BjuSAUR1-10	-	6	4	1	-	2	2.5	-	-
BniSAUR1-11	1	7	5	-	-	-	1	-	-
BniSAUR1-12	-	6	4	1	-	2	2.5	-	-
BniSAUR1-13	3.5	5	3	1	1	2.5	-	-	-
BraSAUR1-14	-	7	4	1	1	1	-	-	-
BraSAUR1-15	-	5	7	-	1	-	1	-	-
BraSAUR1-16	4.5	4	3	3	1	1	-	-	-

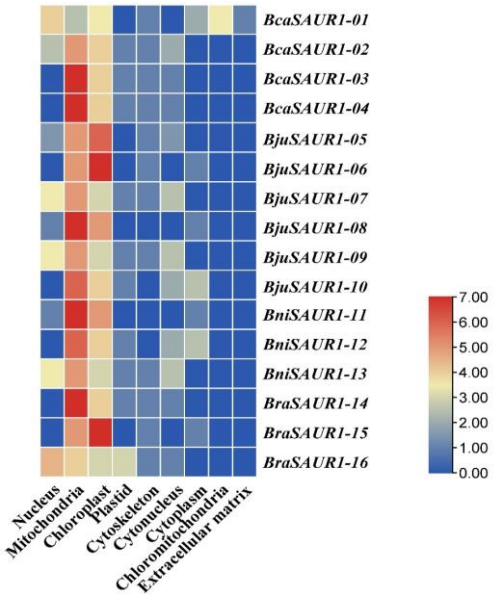


Figure 1: Heat map of predicted subcellular localization scores of the SAUR1 proteins.

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3.3 Gene structure and conserved motifs

All 16 genes consisted of a single exon (Table 1, Fig. 2). BjuSAUR1-05 and BjuSAUR1-09 showed additional upstream or downstream sequence in the display, and BcaSAUR1-01 had the shortest coding sequence, about 230 bp, which matches its 76-residue protein. MEME identified seven motifs (Fig. 3). Most proteins carried the same arrangement from the N terminus: motif 2 (MGIQLIGLSHAKQKLQRSLSAKIASLLAMS GTNNVPKGHVAVYVGETYQR), motif 1 (IPISYLNHPLFQSLLNLAEEEFGFDHPMGG LTIPCTEDYFT) and motif 3 (LASILNGS). BcaSAUR1-01 lacked motif 2 and instead had four short motifs (motifs 4 to 7) at its N terminus, and BcaSAUR1-02 had two small extra motifs near the N terminus.

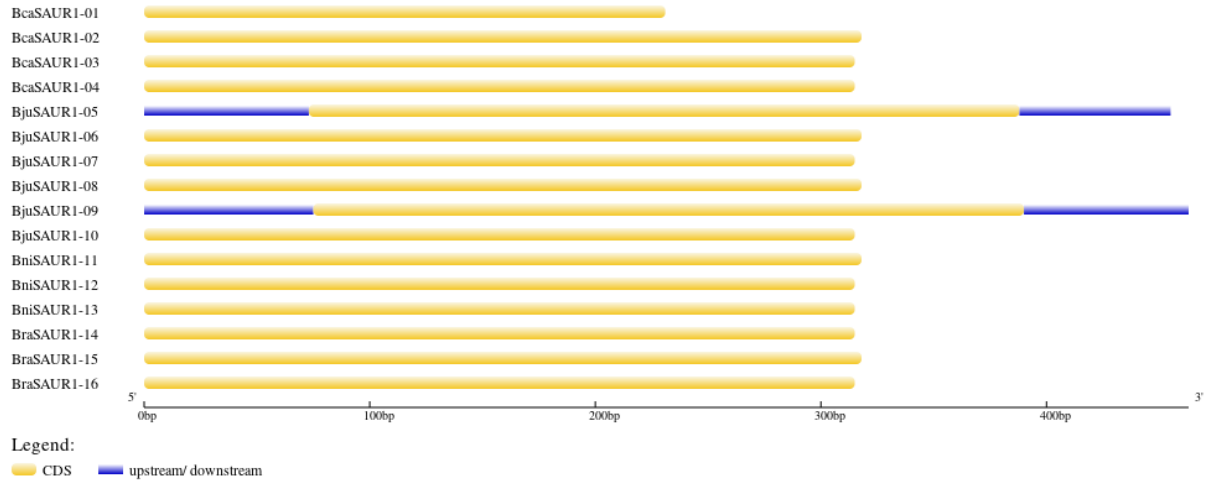


Figure 2: Exon-intron structure of the *SAUR1* genes. Yellow bars show coding sequence and blue bars show upstream or downstream sequence.

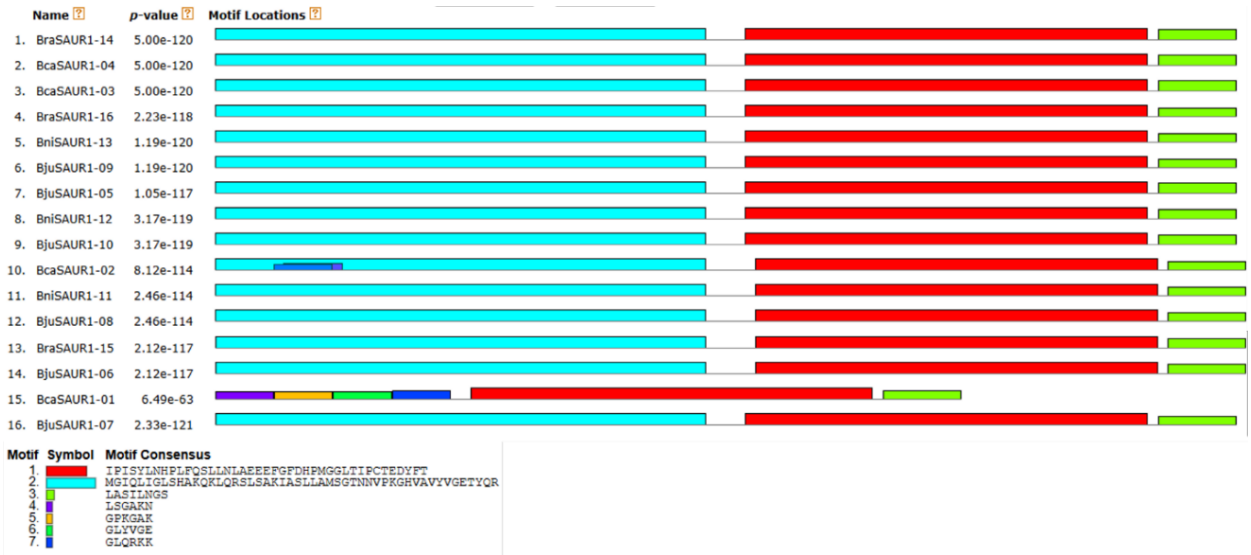


Figure 3: Conserved motifs of the SAUR1 proteins identified by MEME.

3.4 Phylogeny and protein structure

The neighbor-joining tree placed the 16 proteins in three clades (Fig. 4). Proteins from different species grouped together. BniSAUR1-12 clustered with BjuSAUR1-10, BjuSAUR1-09 with BniSAUR1-13, BjuSAUR1-08 with BniSAUR1-11, and BjuSAUR1-06 with BraSAUR1-15. BraSAUR1-14 grouped with BcaSAUR1-03 and BcaSAUR1-04. The three-dimensional models predicted by Phyre2 are shown in Fig. 5.

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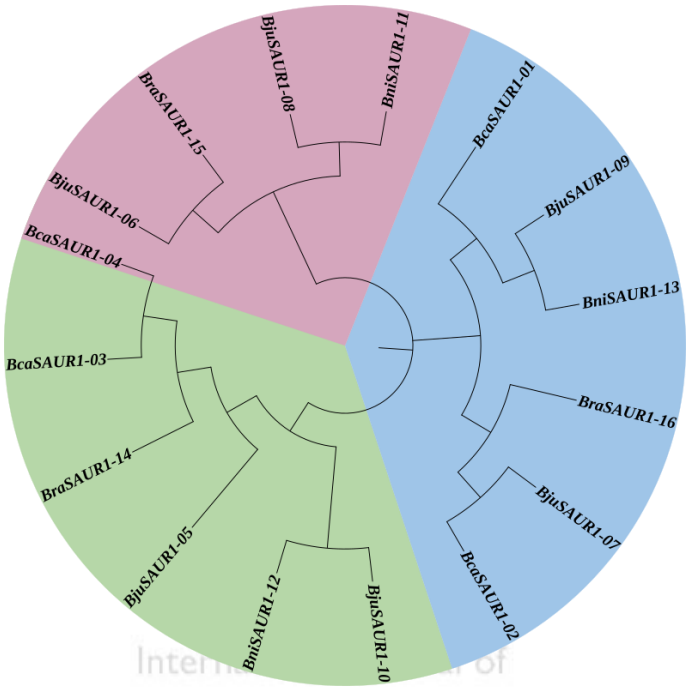


Figure 4: Neighbor-joining tree of the SAUR1 proteins from Brassica species. The three clades are shaded in different colors.

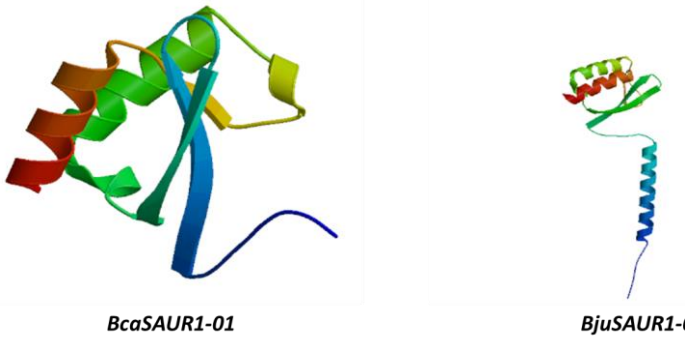


Figure 5: Predicted three-dimensional structures of SAUR1 proteins (Phyre2).

3.5 Cis-acting elements in the promoters

PlantCARE detected many cis-acting elements in the 2,000 bp upstream regions of all 16 genes (Fig. 6). The core elements TATA-box and CAAT-box occurred in every promoter. Hormone-related elements included ABRE, ABRE3a and ABRE4 (abscisic acid), ERE (ethylene), TCA-element (salicylic acid), P-box (gibberellin) and AuxRR-core (auxin). Stress-related elements included MBS (drought inducibility), DRE and DRE core (dehydration), ARE (anaerobic induction), STRE and W box. Light-responsive elements (G-box, Box 4, AE-box, ACE, I-box and MRE) and MYB and MYC binding sites were also present. The number and position of elements varied between genes.

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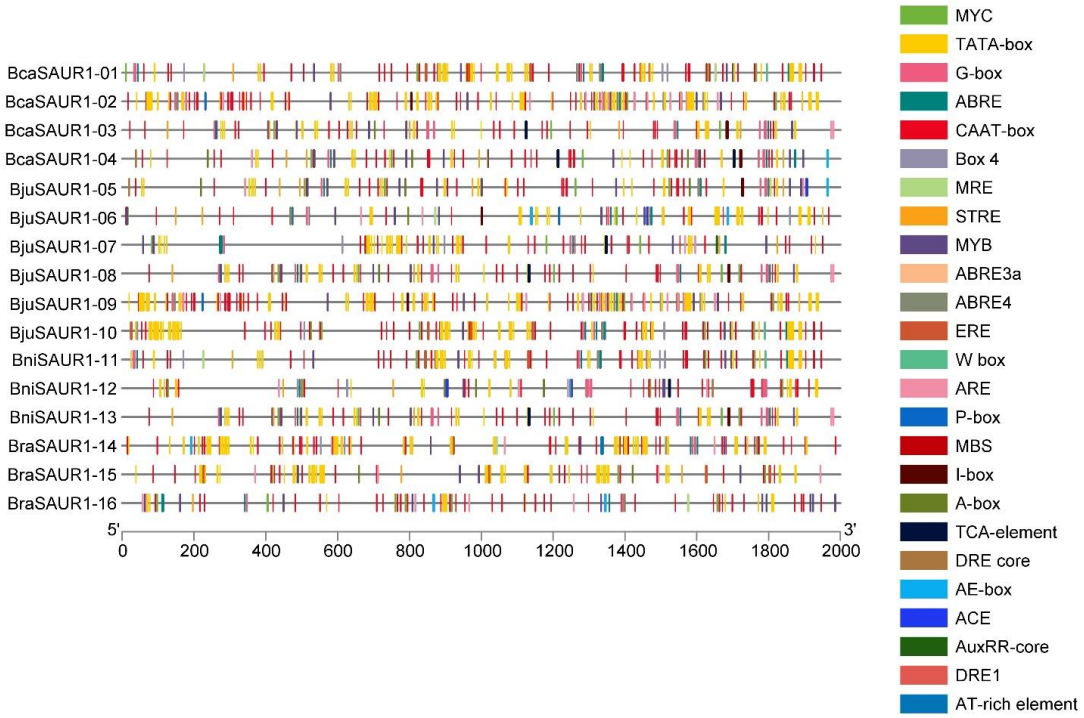


Figure 6: Cis-acting elements in the 2,000 bp promoter regions of the *SAUR1* genes. Each colored box is one element, as shown in the legend.

3.6 Seedling growth

Germination and seedling establishment were recorded daily for all six species (Fig. 7). ANOVA showed significant differences among species for all six growth traits (Tables 4 to 9).

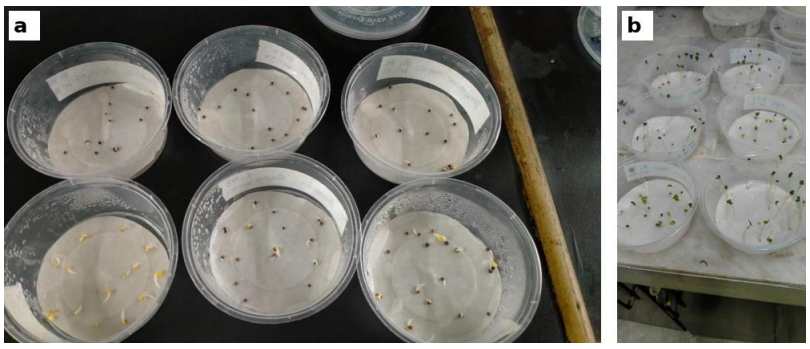


Figure 7: Germination of Brassica seeds on filter paper (a) and seedlings after growth (b).

Mean root length was highest in *B. juncea* (27.2) and *B. rapa* (24.2), intermediate in *B. napus* (18.5) and *B. nigra* (15.0), and lowest in *B. oleracea* (9.5) and *B. carinata* (7.2) (Fig. 8). Species (F = 59.70), replication (P = 0.0099) and their interaction were significant (Table 4).

Table 4: Analysis of variance for root length

Source	DF	SS	MS	F	P
Species	5	384.087	76.8174	59.70	<0.001
Replication	3	16.309	5.4364	4.23	0.0099
Species × replication	15	163.349	10.8899	8.46	<0.001
Error	48	61.761	1.2867		

Source	DF	SS	MS	F	P
Total	71	625.506			

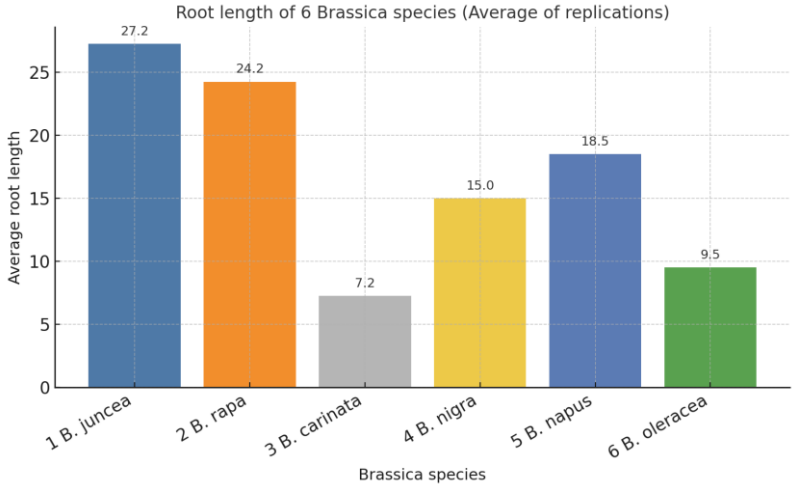


Figure 8: Mean root length of the six Brassica species (1, *B. juncea*; 2, *B. rapa*; 3, *B. carinata*; 4, *B. nigra*; 5, *B. napus*; 6, *B. oleracea*).

Shoot length was greatest in *B. napus* (20.2), followed by *B. juncea* and *B. rapa* (17.0 each) and *B. nigra* (14.5). *B. oleracea* (8.0) and *B. carinata* (7.2) had the shortest shoots (Fig. 9). Species and the species × replication interaction were significant, while replication was not (Table 5).

Table 5: Analysis of variance for shoot length

Source	DF	SS	MS	F	P
Species	5	89.040	17.8080	37.00	<0.001
Replication	3	2.646	0.8820	1.83	0.1539
Species × replication	15	56.537	3.7691	7.83	<0.001
Error	48	23.105	0.4814		
Total	71	171.328			

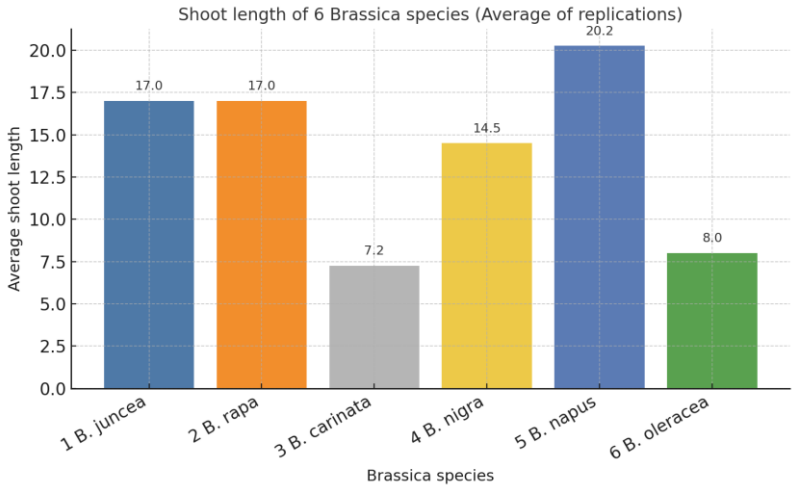


Figure 9: Mean shoot length of the six Brassica species (numbering as in Figure 8).

Fresh root weight was highest in *B. juncea*, followed by *B. rapa* (0.088) and *B. napus* (0.085), and lowest in *B. carinata* (0.028) (Fig. 10). Species, replication and their interaction were all significant (Table 6).

Table 6: Analysis of variance for fresh root weight

Source	DF	SS	MS	F	P
Species	5	0.00121	0.00024	8.45	<0.001
Replication	3	0.01326	0.00442	154.86	<0.001
Species × replication	15	0.00132	0.00009	3.09	0.0015
Error	48	0.00137	0.00003		
Total	71	0.01716			

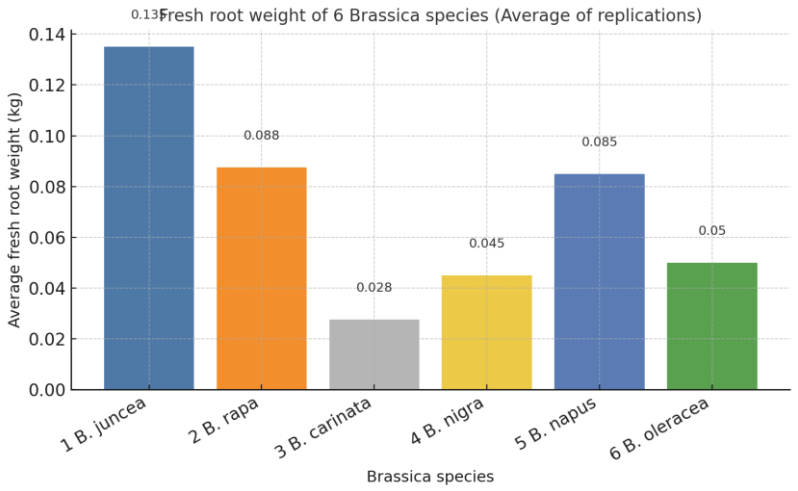


Figure 10: Mean fresh root weight of the six Brassica species (numbering as in Figure 8).

Fresh shoot weight was highest in *B. juncea*, followed by *B. napus* (0.135) and *B. rapa* (0.12), and lowest in *B. carinata* (0.035) (Fig. 11). Species, replication and their interaction were significant (Table 7).

Table 7: Analysis of variance for fresh shoot weight

Source	DF	SS	MS	F	P
Species	5	0.00349	0.00070	15.62	<0.001
Replication	3	0.01038	0.00346	77.55	<0.001
Species × replication	15	0.00267	0.00018	3.98	0.0001
Error	48	0.00214	0.00004		
Total	71	0.01868			

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Figure 11: Mean fresh shoot weight of the six Brassica species (numbering as in Figure 8).

Dry root weight was highest in *B. juncea* (0.00305) and *B. rapa* (0.00295) and lowest in *B. carinata* (0.00092) (Fig. 12). The species effect was significant (P = 0.0168), whereas replication and the interaction were not (Table 8).

Table 8: Analysis of variance for dry root weight

Source	DF	SS	MS	F	P
Species	5	1.338×10^{-6}	2.676×10^{-7}	3.10	0.0168
Replication	3	2.840×10^{-8}	9.4681×10^{-9}	0.11	0.9541
Species × replication	15	2.306×10^{-6}	1.537×10^{-7}	1.78	0.0667
Error	48	4.150×10^{-6}	8.646×10^{-8}		
Total	71	7.823×10^{-6}			

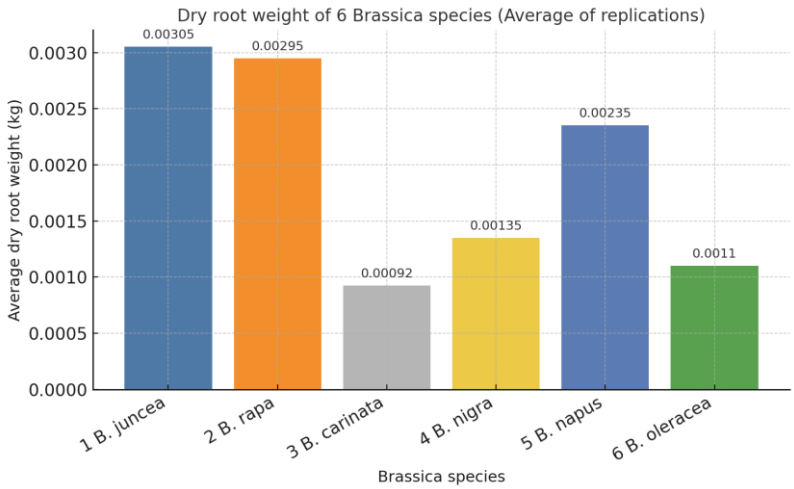


Figure 12: Mean dry root weight of the six Brassica species (numbering as in Figure 8).

Dry shoot weight was nearly the same in *B. napus* (0.00977) and *B. juncea* (0.00975), slightly lower in *B. rapa* (0.00885), and lowest in *B. carinata* (0.00382) and *B. oleracea* (0.00398) (Fig. 13). The species effect (P = 0.0044) and the species × replication interaction (P = 0.0031) were significant, and replication was not (Table 9)

Table 9: Analysis of variance for dry shoot weight

Source	DF	SS	MS	F	P
Species	5	6.701×10^{-6}	1.340×10^{-6}	3.95	0.0044
Replication	3	4.924×10^{-7}	1.641×10^{-7}	0.48	0.6952
Species × replication	15	1.446×10^{-5}	9.643×10^{-7}	2.84	0.0031
Error	48	1.629×10^{-5}	3.394×10^{-7}		
Total	71	3.795×10^{-5}			

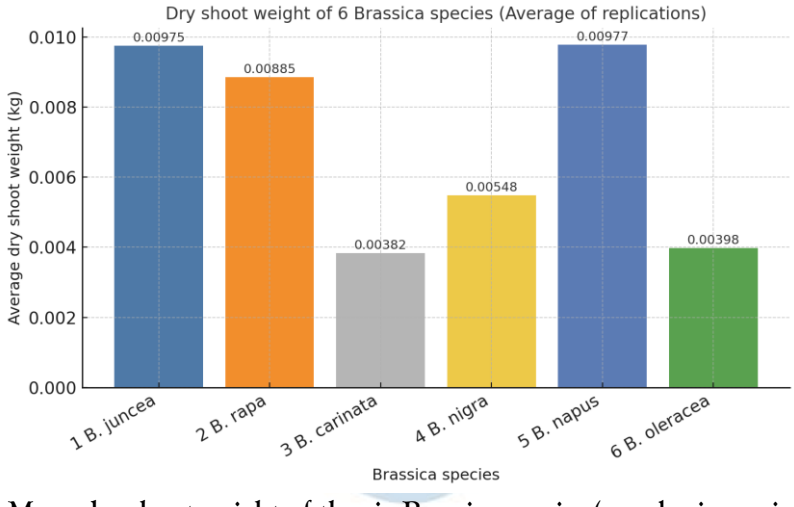


Figure 13: Mean dry shoot weight of the six Brassica species (numbering as in Figure 8).

4. Discussion

Genome-wide analysis revealed a highly conserved yet expanded *SAUR1* gene family across the studied *Brassica* species, suggesting that polyploidy events significantly contributed to the functional diversification of these growth-regulated genes. We found 16 *SAUR1* genes in four of the six species and none in *B. oleracea* or *B. napus* at our similarity cutoff. The distribution fits the polyploid history of the genus. *B. rapa* and *B. nigra* each kept three copies, and the allotetraploid *B. juncea* (AABB) has six. Its three A-subgenome copies lie on chromosomes A1, A3 and A8, the same chromosomes that carry the three *B. rapa* genes, and its three B-subgenome copies lie on B2, B3 and B5. *B. carinata* (BBCC) has one copy on B3, one on C1 and two on an unplaced contig. This pattern agrees with the origin of the allotetraploids from the diploid parents (Choi *et al.*, 2007) and with the retention of several gene copies after whole-genome triplication (Mun *et al.*, 2012; Cheng *et al.*, 2014). The missing genes in *B. oleracea* and *B. napus* are harder to explain. Gene loss after triplication is one possibility, but *B. carinata* carries a C-subgenome copy (BcaSAUR1-02), so an ortholog is expected in *B. oleracea*. Assembly or annotation gaps are therefore another explanation, and the six scaffold hits we set aside should be examined before the genes are called absent.

The proteins are small, 76 to 105 amino acids, which is within the size range reported for SAUR proteins (Zhang *et al.*, 2021). All 16 genes have one exon and no introns, a feature that most SAUR genes share (Li *et al.*, 2017; Stortenbeker and Bemer, 2019). Intronless genes can duplicate in tandem (Li *et al.*, 2017), and genes that are regulated rapidly tend to have few introns (Jeffares *et al.*, 2008; Lan *et al.*, 2013), which suits a gene that responds early to auxin. The Pfam auxin-responsive domain and the arrangement of motifs 2, 1 and 3 were conserved across nearly all proteins. Similar conservation of motifs within groups was reported for *S. album* (Zhu *et al.*, 2024). The exception was BcaSAUR1-01. It is the shortest protein, it lacks motif 2, and it is the only one with a negative GRAVY value. It may be a

truncated or diverged copy, but expression data would be needed to say whether it is functional. Nine proteins from different species had identical physicochemical values, and the tree paired several of them. These values do not prove identical sequences, but the pairs of BjuSAUR1 proteins with BraSAUR1 or BniSAUR1 proteins are what one expects if *B. juncea* inherited its A and B genomes from ancestors close to *B. rapa* and *B. nigra*. The grouping of *B. carinata* and *B. rapa* proteins in one clade is consistent with the same close ancestry. WoLF PSORT predicted mitochondria or chloroplasts as the main location for 14 of the 16 proteins. These predictions need caution. Experimental work places Arabidopsis SAUR19 at the plasma membrane, where it regulates H^+-ATPase activity (Spartz *et al.*, 2017), so the organelle predictions may reflect a bias of the algorithm and not true targeting. Localization of the Brassica proteins should be tested directly, for example with fluorescent protein fusions.

The promoters carried elements for auxin (AuxRR-core), abscisic acid (ABRE), ethylene (ERE), salicylic acid (TCA-element) and gibberellin (P-box), together with elements linked to drought and dehydration (MBS, DRE). This mix suggests that *SAUR1* integrates auxin signals with other hormone and stress signals. It matches the known modulation of SAUR expression by cytokinin, gibberellin, brassinosteroid, ethylene, abscisic acid and jasmonate (Stortenbeker and Bemer, 2019), and the presence of auxin-responsive elements in most cotton SAUR promoters (Li *et al.*, 2017). SAUR genes in *S. album* have also been linked to stress responses (Zhu *et al.*, 2024). The element sets here are predictions, however, and they show only that regulation is possible. The six species differed significantly in all growth traits. *B. juncea* and *B. rapa* had the longest roots and the heaviest roots, *B. napus* had the longest shoots and the heaviest dry shoots, and *B. carinata* had the lowest mean for every trait. Differences in dry weight were smaller ($P = 0.0168$ and 0.0044) than those in length and fresh weight ($P < 0.001$). The significant replication effect for root length and fresh weights, and the significant species × replication interaction for most traits, show that the ranking of species was not the same in every replication. The species means should therefore be read with that variation in mind.

These growth data cannot be tied to *SAUR1* directly. We did not measure gene expression, and copy number does not follow growth: *B. juncea* has six copies and the longest roots, *B. carinata* has four copies and the shortest roots, and *B. napus* retained no copy at our cutoff but had the longest shoots. Growth differences among the species probably reflect genome composition and many other genes. Still, SAUR genes are known to affect growth. SAUR19 enlarges cells (Spartz *et al.*, 2017), rice SAUR39 restricts shoot and root growth (Kant *et al.*, 2009), and SAUR-like genes were more strongly expressed in short-rooted *B. napus* plants (Dun *et al.*, 2016). Expression profiling of each *SAUR1* copy in roots and shoots, with and without auxin, is the next step to test whether the copies contribute to the differences seen here.

Declarations

Conflict of Interest

There is no conflict of interest.

Funding

N/A

Author Contribution

All authors contribute equally.

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