Genome-wide identification and analysis of bZIP gene family reveal their roles under salt stress in Suaeda australis

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Abstract

Background

Suaeda australis is one of typical halophyte owing to high levels of salt tolerance. In addition, the *bZIP* gene family assumes pivotal functions in response to salt stress. However, there are little reports available regarding the *bZIP* gene family in *S. australis*.

Results

In this study, we successfully screened 44 *bZIP* genes within *S. australis* genome. Subsequently, we conducted an extensive analysis, encompassing investigations into chromosome location, gene structure, phylogenetic relationship, promoter region, conserved motif, and gene expression profile. The 44 *bZIP* genes categorized into 12 distinct groups, exhibiting an uneven distribution among the 9 chromosomes of *S. australis* chromosomes, but one member (*Sau23745*) was mapped on unanchored scaffolds. Examination of cis-regulatory elements reveled that bZIP promoters were closely related to anaerobic induction, transcription start, and light responsiveness. Expression patterns analyses clearly discovered the role of several *SabZIPs* including *Sau08107*, *Sau08911*, *Sau11415*, *Sau16575*, and *Sau1927*6, which showed higher expression levels in higher salt concentration than low concentration and obviously response to salt stress. These expression patterns were corroborated through RT-qPCR analysis.

Conclusions

Our findings offer valuable insights into the evolutionary trajectory of the *bZIP* gene family in *S. australis* and shed light on their roles in responding to salt stress. In addition to fundamental genomic information, these results would serve as a foundational framework for future investigations delving into the regulation of salt stress responses in *S. australis*.

Background

Coastal wetlands play crucial roles in climate change mitigating, carbon sequestering, and biodiversity support [1, 2]. Unfortunately, over the past few decades, more than one-third of coastal wetlands have experienced a decline in their ecological functionality [3]. The presence of plants is crucial for the preservation of ecological function in coastal wetlands, particularly in terms of enhancing biodiversity [4]. However, substantial variations in salinity levels within coastal wetlands can directly impair plant capabilities, leading to inhibited growth or, in severe cases, plant mortality [5].

Suaeda australis belonging to the genus *Suaeda* (Chenopodiaceae), is mainly distributed in the subtropical and tropical coastal areas of China, Japan and Oceanica [6]. Pharmacological studies showed that antioxidant and antibacterial activities were found in the extracts of *S. australis* leaves [7]. Owing to the high nutritional value, the leaves of *S. australis* have long been used as food ingredient and supplementary feed [8]. Especially, *S. australis* is a typical halophyte, which can improve the coastal marsh saline-alkali land, reduce pollutants, and protect the coastline [9]. However, the growth zone of *S. australis* gradually moved from intertidal zone with higher salt concentration to suprapatid zone with lower salt concentration, which is caused by extreme fluctuations in salinity in coastal wetlands. More severely, the amounts of *S. australis* of suprapatid zone had fallen sharply in the past decades. Hence, it is of great scientific significance and ecological value to investigate the salt tolerance mechanism of *S. australis* in depth for the conservation of *S. australis*, utilization of saline soil resources, improvement of coastal salt-alkali environment, and development of marine agricultural economy.

Many valuable genes associated with stress responses need to be identified to enhance stress tolerance effectively for *S. australis*. As one of the greatest family encoding transcription factors (TFs), the basic leucine zipper (*bZIP*) gene family plays fundamental roles in environmental and developmental signalling as well as stress response in plants [10]. Previous studies reported that bZIP TFs play pivotal roles in salt stress response [11]. The bZIP TFs typically consist of 60–80 amino acids, containing a bZIP domain characterized by a highly conserved basic region and a variable leucine zipper region [12]. bZIP TFs were categorized into different subgroups based on conserved domains [13]. In addition, the *bZIP* genes involved in salinity stress varied among different plants.

For example, the *OsbZIP12*, *OsbZIP72* and *OsbZIP46* genes confer stronger salt tolerant than other members in rice bZIP family [14], while *GmbZIP 62*, *GmbZIP44*, and *GmbZIP 78* genes of soybean are significantly upregulated in reaction to salt stress [15–17].

So far, little reports on the functional characterization of bZIP TFs are available. To identify potential *bZIP* genes involved in regulation of salt stress responses, this study systematically identified and characterized all *bZIP* genes in *S. australis*. We performed a detailed analysis of genome distribution, gene structure, and expression patterns in response to salt stress. Finally, we identified the candidate *bZIP* genes that exhibit potential responsiveness to salt stress through transcriptome analysis and quantitative real-time PCR (qRT-PCR) evaluation.

Results

Genome-wide identification of the SabZIP gene family in S. australis

SabZIP genes were identified in *S. australis* genome based on HMM program and the local BLASTP analyses. As a result, 44 identified *SabZIP* genes were serially named as *SabZIP01* through *SabZIP44* for convenience (Table 1). Additionally, it was observed that substantial variations existed among the *SabZIP* genes in terms of mRNA transcript length and the protein sequences they encoded. Additionally, the results indicated considerable diversity among *SabZIP* genes in terms of mRNA transcript lengths and the corresponding protein sequences. The mRNA products (CDS) of 44 *SabZIP* genes exhibited lengths spanning from 420 to 2352 bp and the corresponding translated protein sequences displayed variations in amino acid counts ranging from 140 to 784. Among these sequences, *SabZIP23* featured the shortest amino acid sequence, comprising only 143 amino acid residues, whereas *SabZIP20* was characterized by the longest sequence, encompassing 784 amino acid residues.The theoretical isoelectric point (PI) values ranged from 4.91 (*SabZIP14*) and 9.69 (*SabZIP15*), and the molecular mass of bZIP members ranged from 16.12 (*SabZIP11*) to 86.06 kDa (*SabZIP20*) (Table 1).

Table 1
Physical and chemical properties of *bZIP* genes in *Suaeda australis* (CDS: coding DNA sequence; PI: isoelectric point; MW: molecular weight).

Gene Name	Gene ID	Exon Count	Chromosome Localization	Genomic Sequence (bp)	CDS (bp)	Amino Acid (aa)	PI	MW (kDa)
SabZIP01	*Sau00495*	1	Chr1:5207106–5207780	675	675	225	6.14	25.52
SabZIP02	*Sau03578*	6	Chr2:9378604–9387860	9257	1320	440	6.05	47.72
SabZIP03	*Sau05007*	4	Chr2:45209273–45214027	4755	1050	350	5.84	38.48
SabZIP04	*Sau07061*	9	Ch3:35544832–35550965	6134	1092	364	6.05	41.48
SabZIP05	*Sau07497*	11	Chr3:40242813–40248800	5988	1239	413	6.36	46.04
SabZIP06	*Sau07765*	3	Chr3:43094703–43105365	10663	801	267	9.64	29.34
SabZIP07	*Sau07938*	12	Chr3:44765727–44772850	7124	1065	355	5.26	37.16
SabZIP08	*Sau07974*	2	Chr3:45121201–45122500	1300	504	168	5.04	18.95
SabZIP09	*Sau08107*	2	Chr3:46830205–46831007	803	672	224	6.31	25.57
SabZIP10	*Sau08108*	2	Chr3:46848167–46848788	622	516	172	5.86	20.04
SabZIP11	*Sau08911*	2	Chr4:6935364–6936552	1189	420	140	6.14	16.12
SabZIP12	*Sau10955*	13	Chr4:46054419–46061506	7088	1197	399	6.09	42.47
SabZIP13	*Sau11293*	9	Chr5:2527343–2536797	9455	1002	334	8.96	37.30
SabZIP14	*Sau11415*	4	Chr5:4389963–4392148	2186	1206	402	4.91	45.12
SabZIP15	*Sau11436*	4	Chr5:4644947–4649485	4539	501	167	9.69	17.99
SabZIP16	*Sau12558*	6	Chr5:35387367–35392916	5550	1020	340	5.80	37.64
SabZIP17	*Sau13274*	2	Chr5:42215204–42216039	836	555	185	5.23	21.39
SabZIP18	*Sau13718*	2	Chr6:890832–892489	1658	561	187	4.94	21.08
SabZIP19	*Sau14074*	13	Chr6:4579899–4584824	4926	1248	416	8.33	43.95
SabZIP20	*Sau14574*	12	Chr6:9104604–9114480	9877	2352	784	8.36	86.06
SabZIP21	*Sau14952*	4	Chr6:14900300–14907016	6717	975	325	5.99	35.71
SabZIP22	*Sau15162*	9	Chr6:21374565–21381493	6929	1095	365	6.12	41.27
SabZIP23	*Sau15737*	2	Chr6:39514216–39515062	847	429	143	9.14	16.89
SabZIP24	*Sau16019*	3	Chr6:42592107–42595286	3180	609	203	8.85	23.24
SabZIP25	*Sau16553*	4	Chr7:3801227–3804100	2874	1050	350	5.71	38.42
SabZIP26	*Sau16575*	12	Chr7:4008426–4019303	10878	1566	522	7.12	57.89

Gene Name	Gene ID	Exon Count	Chromosome Localization	Genomic Sequence (bp)	CDS (bp)	Amino Acid (aa)	PI	MW (kDa)
SabZIP27	*Sau16748*	5	Chr7:5747950–5752831	4882	1467	489	9.51	51.60
SabZIP28	*Sau18144*	7	Chr7:36424263–36429240	4978	1422	474	8.58	52.39
SabZIP29	*Sau18379*	5	Chr7:39698150–39706292	8143	1404	468	7.35	50.68
SabZIP30	*Sau19002*	4	Chr8:3042910–3046518	3609	1173	391	6.35	42.65
SabZIP31	*Sau19276*	13	Chr8:5941229–5948356	7128	1062	354	5.34	37.35
SabZIP32	*Sau19467*	1	Chr8:8030605–8031144	540	540	180	6.74	20.26
SabZIP33	*Sau19742*	2	Chr8:10604210–10605284	1075	573	191	7.0	20.32
SabZIP34	*Sau19839*	4	Chr8:11774599–11777788	3190	474	158	8.99	18.21
SabZIP35	*Sau20932*	11	Chr8:38738491–38750498	12008	1776	592	7.46	65.15
SabZIP36	*Sau21103*	4	Chr8:40408664–40413058	4395	996	332	7.99	36.07
SabZIP37	*Sau21199*	2	Chr8:41312321–41315385	3065	2085	695	5.69	75.71
SabZIP38	*Sau21237*	11	Chr8:41789118–41796975	7858	1293	431	5.70	47.69
SabZIP39	*Sau21412*	5	Chr9:1456870–1460041	3172	1731	577	7.23	62.80
SabZIP40	*Sau22170*	3	Chr9:8664026–8669668	5643	873	291	5.94	31.61
SabZIP41	*Sau22785*	4	Chr9:24283457 – 21291627	8171	1023	341	6.11	38.03
SabZIP42	*Sau23047*	8	Chr9:32719285–32728695	9411	702	234	9.00	26.78
SabZIP43	*Sau23067*	4	Chr9:33310036–33314904	4869	648	216	9.11	24.13
SabZIP44	*Sau23745*	11	Scaffold_136:35494–42293	6800	2130	710	8.50	78.94

Chromosome location and synteny analysis of SabZIP genes

Except for *SabZIP44*, all *SabZIP* genes in *S. australis* were mapped onto nine chromosomes (Chrs), as illustrated in Fig. 1. The highest count of *SabZIP* genes per chromosome was observed on Chr8, where nine *SabZIP* genes were located. Seven genes per chromosome were located on Chr3 and Chr6; five genes each were found on Chr5, Chr7, and Chr9; two genes each were located on Chr2 and Chr4. Chr1 and Scaffold_136 contained the lowest counts of *SabZIP* genes, with only one each. Among *SabZIP* genes in *S. australis*, a total of four pairs of tandem duplicated genes (*Sau08107* and *Sau08108*, *Sau11415* and *Sau11436*, *Sau16553* and *Sau16575*, *Sau23047* and *Sau23067*) were observed, as illustrated in Fig. 1.

To examine the evolutionary relationships among the *bZIP* genes in these three species (*A. thaliana*, *B. vulgaris*, and *O. sativa*), syntenic analysis was performed (Fig. 2). A total of 32, 41, and 22 orthologous gene pairs of orthologous *SabZIP* genes were identified between *S. australis* and *A. thaliana*, *S. australis* and *B. vulgaris*, and *S. australis* and *O. sativa*, respectively (Fig. 2). In contrast, 19 collinear gene pairs initially identified between *S. australis* and *A. thaliana* were also found in the comparison between *S. australis* and *B. vulgaris*. Similarly, 11 collinear gene pairs initially associated with *S. australis* and *A. thaliana* were also observed when comparing *S. australis* with *O. sativa*. Furthermore, all 10 collinear gene pairs initially detected between *S. australis* and *O.*

sativa were likewise identified when comparing *S. australis* with *B. vulgaris* (Fig. 2). Additionally, it's worth noting that each of the following species: *S. australis*, *A. thaliana*, *B. vulgaris*, and *O. sativa* shared 9 common collinear gene pairs (Fig. 2).

Phylogeny, structural, and conserved motifs analysis of *SabZIP* genes

To investigate the evolutionary relationships among *SabZIP* genes, we constructed a neighbor-joining (NJ) phylogenetic tree based on the full-length protein sequences (Fig. 3). *SabZIP* genes were categorized into 12 subfamilies from *S. australis* (Fig. 3). The A subfamily containing eight *SabZIP* family members, was the most extensive clade. In contrast, both subfamilies F and J were the smallest, each comprising only one member. Furthermore, subfamilies H, C, K, B, E, I, G, D, and S consisted of 2, 2, 2, 2, 2, 5, 4, 7, and 7 members, respectively.

The gene structure analysis of the 44 *SabZIP* genes in *S. australis* is illustrated in Fig. 4. Our observations revealed notable variability in the gene structure of *SabZIP* members, with exon numbers ranging from 1 to 13. Notably, members of the G subfamily, namely Sau10955, Sau14074, and Sau19276 (except Sau07938), displayed a maximum of 13 exons, a distinctive feature distinguishing them from members of other subfamilies, where exon numbers ranged from 1 to 12. By contrast, the *Sau23745* of B subfamily contained 11 exons, but the other member *Sau21199* contained only two exons. Additionally, some *SabZIP* members within the same group exhibited similar gene structures. For instance, the exon numbers of H subfamily and S members were both 1 and lacked introns. All members of C, K, and E subfamily had 6, 4, and 4 exons, respectively. These results suggested that the structural features of *SabZIP* genes exhibit noticeable variations, even when considering members in the same gene subfamily.

A total of ten different motifs were identified in *SabZIP* gene family of *S. australis* (Fig. 4). All of *SabZIP* genes contained a bZIP domain (PF00170) represented by motif 1. Motif 7 and motif 8 were specific to the A subfamily of SabZIP family. Moreover, motif 2, motif 3, motif 4, and motif 6 occurred only in D subfamily. Each member belonging to S subfamily and H subfamily exhibited a shared pattern of two motifs (motif 1 and motif 10) within their gene structures. Motif 5 was shared only by two subfamilies (E and I). This collective evidence provid support for the notion that members in the same subfamily exhibit identical conserved motifs, suggesting a potential relationship between these conserved motifs and specific biological processes.

Estimation of nonsynonymous (Ka) and synonymous (Ks) substitution rates and Ka/Ks values

To investigate the selection pressures acting on *SabZIP* genes following duplication during their evolutionary history, we computed the ratios of nonsynonymous (Ka) to synonymous (Ks) substitutions for each paralogous pair, as presented in Table 2. A total of four pairs of tandem duplications in *S. australis* were identified. The Ka/Ks ratio spanned from 0.7221 (*Sau23047* and *Sau23067*) to 1.0019 (*Sau23047* and *Sau23067*) with an average ratio of 0.8925. The Ka/Ks ratios of three paralogous *SabZIP* gene pairs (*Sau08107* and *Sau08108*, *Sau11415* and *Sau11436*, and *Sau23047* and *Sau23067*) were less than 1, indicating that these genes are subject to purifying selection. Conversely, the Ka/Ks value of (*Sau16553* and *Sau16575*) was slightly greater than 1, suggesting positive selection following duplication. Collectively, these results imply that the majority of these gene pairs have been subjected to purifying selection pressure, and the duplication events may contributed significantly to the expansion of the SabZIP family in *S. australis*.

Table 2
Analysis of tandem duplication events of *SabZIP* gene pairs in *Suaeda australis*.

Tandem duplicated genes	Ka	Ks	Ka/Ks	Purifying selection
Sau08107 and *Sau08108*	0.6874	0.7340	0.9365	Yes
Sau11415 and *Sau11436*	0.9783	1.0754	0.9096	Yes
Sau16553 and *Sau16575*	1.0004	0.9986	1.0019	No
Sau23047 and *Sau23067*	0.3744	0.5186	0.7221	Yes
Promoter region analysis of SabZIP genes				

To discovered cis-acting elements associated with mechanisms pertaining to the response to salt stress, we analyzed the promoter sequences of *S. australis SabZIP* genes. In *S. australis*, the cis-acting elements in promoters' regions of majority *SabZIP*

genes (more than 35 members) were mainly associated with anaerobic induction, transcription start, and light responsiveness (Fig. 5 and Table 3). In contrast, we also identified several cis-elements (RY-element, WUN-motif, TATC-box, HD-Zip 3, MBSI, ABRE, A-box, AACA_motif), which were present in a minority of members (less than 6 members) (Table 3). Phytohormone response elements, including abscisic acid, auxin, gibberellin, and MeJA were also widely observed. Moreover, *SabZIP* members were also related to low-temperature responsiveness, circadian control, flavonoid biosynthetic genes regulation, drought-inducibility, seed-specific regulation, and stress responsiveness, suggesting their role in the response to various stresses and plant growth. These results offer valuable insights into the regulatory roles of *SabZIP* gene family during both stress responses and plant development.

Table 3
Cis-element and corresponding functions of *SabZIP* genes in *Suaeda australis*.

Cis-element	Function	members
ARE	Cis-acting regulatory element essential for the anaerobic induction	44
TATA-box	Core promoter element around − 30 of transcription start	39
G-Box	Cis-acting regulatory element involved in light responsiveness	35
TGACG-motif	Cis-acting regulatory element involved in the MeJA-responsiveness	30
LTR	Cis-acting element involved in low-temperature responsiveness	26
O2-site	Cis-acting regulatory element involved in zein metabolism regulation	20
TC-rich repeats	Cis-acting element involved in defense and stress responsiveness	17
GCN4_motif	Cis-regulatory element involved in endosperm expression	15
AE-box	Part of a module for light response	10
circadian	Cis-acting regulatory element involved in circadian control	10
AuxRR-core	Cis-acting regulatory element involved in auxin responsiveness	9
MBS	MYB binding site involved in drought-inducibility	9
CAT-box	Cs-acting regulatory element related to meristem expression	8
RY-element	Cis-acting regulatory element involved in seed-specific regulation	5
WUN-motif	Wound-responsive element	5
TATC-box	Cis-acting element involved in gibberellin-responsiveness	4
HD-Zip 3	Protein binding site	4
MBSI	MYB binding site involved in flavonoid biosynthetic genes regulation	3
ABRE	Cis-acting element involved in the abscisic acid responsiveness	1
A-box	Sequence conserved in alpha-amylase promoters	1
AACA_motif	Involved in endosperm-specific negative expression	1

Expression profiles of SabZIP genes under different salt concentrations

Pairwise comparison between ST1 and ST2 samples identified 2,434 DEGs. Among them, 1,568 and 866 genes were respectively up-regulated and down-regulated in ST2 compared to ST1. GO and KEGG enrichment analysis revealed that the 2,434 DEGs were significantly enriched in some primary biological pathways, such as response to reactive oxygen species, response to hypoxia, regulation of cytokine production, photosynthesis, biosynthesis of nucleotide sugars, starch and sucrose metabolism, which were closely related to salt response-regulating signaling pathways (Fig. 6).

To investigate potential functions of *SabZIP* genes among *S. australis* under salt stress, we conducted RNA-seq analysis to investigate expression patterns of 44 *SabZIP* genes. We used FPKM values to build a heatmap (Fig. 7). Of 44 *SabZIP* genes, 35 were expressed in ST1 samples and 39 were expressed at in ST2 samples (FPKM > 0). Among different samples, *SabZIP* genes

showed differences in expression patterns (Fig. 7). For instance, *Sau08911*, *Sau11415*, *Sau08107*, *Sau19276*, and *Sau16575* showed higher expression levels under higher salt concentration stress (ST2) than ST1 leaves. These results indicated that *SabZIP* genes may play significant role for salt tolerance of *S. australis*. Further, we found that the expression levels of subfamily S members (*Sau08107*, *Sau19742*, *Sau08108*, *Sau00495*, *Sau15737*, *Sau13274*) except for *Sau08911*, were lower than the other 11 subfamily members. However, the S subfamily *Sau08107* gene was highly expressed among two salt treatments of *S. australis* leaves. Furthermore, the majority of gene pairs undergoing tandem duplications exhibited distinct expression patterns. For example, *Sau11436* was strongly expressed in ST2 leaves, but *Sau11415* was weakly expressed in them (Fig. 7).

In addition, a total of six *SabZIP* genes, namely, S subfamily *SabZIP09* (*Sau08107*) and*SabZIP11* (*Sau08911*) genes; I subfamily *SabZIP14* (*Sau11415*) gene; D subfamily *SabZIP26* (*Sau16575*) gene; G subfamily *SabZIP31* (*Sau19276*) gene; and A subfamily *SabZIP36* (*Sau21103*) gene, were chosen for qRT-PCR analysis (three replicates for each gene). In general, the relative expression levels of selected genes were in agreement with FPKM values obtained from RNA-seq (Fig. 8), verifying the reliability of our transcriptomic expression profiles.

Discussion

bZIP transcription factors represent a prominent protein family in flowering plants engaged in stress responses, pathogen defence, light signalling, flower development, and seed maturation [13]. In this study, a genome-wide identification of the bZIP protein family was carried out in *S. australis*. The 44 identified bZIP proteins in *S. australis* were categorized into 12 distinct subfamilies, which correspond to the classification and identification results in *A. thaliana* [10]. Upon comparing the subfamily composition, we found that seven, two, five, and one SabZIP members in subfamilies S, C, I, and F were less than ten, four, seven, and three in *A. thaliana*, respectively (Fig. 3). In contrast, subfamily A and D exhibited a higher count of members, with eight and seven SabZIP members compared to seven and five in *A. thaliana*, respectively (Fig. 3). It is worth noting that previous studies have documented the identification of *bZIP* genes in numerous other plant species, such as 92 members in *Oryza sativa* [18], 125 members in *Zea mays* [19], 114 members in *Malus domestica* [20], and 86 members in poplar [21]. Similar to the *S. australis* family, the number of bZIP family in *Ziziphus jujuba* Mill. (45 bZIPs) and *Prunus mume* (49 bZIPs) show the relatively low level [22, 23], indicating the contraction of bZIP family during evolution. Furthermore, the assembled genome size of *S. australis* (437.17 Mb) was notably larger than that of *A. thaliana* (119.67 Mb) [24], indicating that there was no substantial correlation between genome size and the quantity of members. In a word, comparison of the *bZIP* genes number among various plants showed that the number of various species are also different.

Except for minority of subfamilies (S and H), the gene structures of *SabZIP* members exhibited visible variations, even in the same gene subfamily, particularly in terms of exon length and quantity (Fig. 4). Similar findings have been documented in cucumber [25] and *Cyclocarya paliurus* [26]. An analysis of motifs (Fig. 5) revealed the presence of 10 motifs in *S. australis*, designated as motif 1 through motif 10. Beside, motifs compositions remained consistent within the same subfamily but exhibited variations among different subfamilies, indicating that the functional divergence of *SabZIP* genes may be influenced by subfamily-specific motifs. These findings were consistent with previous study of *Carthamus tinctorius* [27] and *Glycyrrhiza uralensis* [28]. Moreover, the analysis of the promoter region revealed that a majority of the cis-element components in *SabZIP* genes were associated with temperature responsiveness, circadian control, flavonoid biosynthetic genes regulation, drought-inducibility, seed-specific regulation, and stress responsiveness (Fig. 5).

Based on the expression profiles analysis, it was evident that genes in the same subfamily exhibited diverse expression patterns among two salt treatment samples, but small amounts of subfamily genes (*Sau08107*, *Sau19742*, *Sau08108*, *Sau00495*, *Sau15737*, and *Sau13274*) showed the similar expression profiles. The study suggested that genes in the same subfamily displayed distinct and varied expression patterns throughout the evolutionary process. In addition, these results could be attributed to the intrinsic requirement for paralogous genes of the same origin to prevent functional redundancy while generating subfunctions and novel functions [29]. As a typical euhalophyte, *S. australis* possesses a special structure with succulent leaves, which may effectively store water, reduce the concentration of salt ions in leaf cells, maintain the osmotic balance of leaf cells, and heavily contribute to salt-stress adaptability [30]. In our study, five *bZIP* DEGs contained *Sau08107*, *Sau08911*, *Sau11415*, *Sau16575*, and *Sau1927*6 showed higher expression levels in ST2 leaves than ST1. Among them, *Sau08107* and *Sau08911* belonging to group S, could be directly activated via the ABA-dependent pathway, and form heterodimeric complexes with other members of the bZIP family under

the salt stress [31]; group I gene (*Sau11415*) is involved in cellcycle regulation and salt stress response [10]; *Sau16575* belongs to group D, which can regulate the growth and development and decrease the sensitivity to salt stress in plants [32]; group G *bZIP* gene (*Sau1927*6), has been demonstrated as an inhibitor of salt stress tolerance in tomato [33]. In conclusion, our results demonstrated the significant roles of the *SabZIP* gene family in salt stress and plant growth.

Conclusions

S. australis is a typical euhalophyte distributed in subtropical and tropical coastal areas. Research has mainly focused on increasing yield, quality, and stress tolerance in *S. australis*. The *bZIP* gene family is significantly associated with plant growth, development, and the tolerance to salt stress. In this study, we identified 44 members of *bZIP* gene families in *S. australis*. Expression patterns analyses clearly discovered the role of several *SabZIPs* including *Sau08107*, *Sau08911*, *Sau11415*, *Sau16575*, and *Sau1927*6, which showed higher expression levels in higher salt concentration than low concentration and obviously response to salt stress. These results improve our understanding of the role of *bZIPs* in developmental processes and in salt stress and provide a theoretical basis for further studies aimed at exploring the regulation of salt stress responses in *S. australis*.

Materials and Methods

Plant materials, cDNA synthesis, and transcriptome sequencing

In the present study, the plants of *S. australis* were grown in Dinghai District, Zhoushan City, Zhejiang Province, China (E122°11', N30°02') and then transplanted to Fishery College, Zhejiang Ocean University. Voucher specimens were identified by Yinquan Qu, and deposited in Molecular Ecology Lab of Zhejiang Ocean University (Voucher code: 2022-1-ZS). In addition, we treated plants with salt treatment (ST) with two levels (ST1: the salt concentration of the soil was 10 g/kg; ST2: 18 g/kg). The leaves were sampled in reproductive development stage (24 October 2022) from *S. australis* individuals with one year old. The leaves were immediately collected and rapidly frozen in liquid nitrogen, followed by storage at − 80°C. Each leaf sample consisted of three distinct biological replicates, each of which consisted of tissues from three individual plants. Total RNA extraction was performed employing the E.Z.N.A Plant RNA Isolation Kit (OMEGA, USA). Concentration and purity of RNAs were assessed using the NanoDrop spectrophotometer 2000C (Thermo Fisher Scientific, Waltham, MA, USA), while confirmation of RNA integrity was achieved through 1.0% agarose gel electrophoresis. The RNAs with OD_{260}/OD_{280} valus within the range of 1.8–2.0 were subsequently subjected to reverse transcription into cDNA using PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa, China), following the manufacturer's protocols. The resulting cDNAs were subsequently diluted at a 1:10 ratio with RNase-free water and stored at -20°C for future qRT-PCR analyses. All cDNA libraries were sequenced using an Illumina HiSeqTM2000 system, generating reads with a length of 2 × 100 bp [34]. Any sequences of low quality (defined by bases with Q-scores < 10%), sequences below 50bp in length, primer sequences, and adapter sequences were rigorously filtered and excluded from the initial raw data.

Identification of *SabZIP* genes in *S. australis*

The whole protein sequence of *S. australis* was obtained from the Genome Warehouse (GWH) at the National Genomics Data Center, Beijing Institute of Genomics (https://ngdc.cncb.ac.cn/gwh, accessed on July 10, 2023). To identify bZIP proteins within *S. australis*, we initiated our search using *Arabidopsis* bZIP protein sequences as our query [10]. We initiated a comprehensive search for potential bZIP proteins using the local alignment search tool (BLASTP) [35] with a stringent E-value threshold of less than 1e-10. Following this preliminary screening, we acquired the HMM (hidden Markov model) profile corresponding to the Pfam bZIP domain (PF00107) from the Pfam database (http://pfam.xfam.org/, accessed on July 10, 2023). Subsequently, we employed this HMM profile to perform a search against the *S. australis* protein dataset using HMMER v3.3.2 [36] with default parameters. The acquired sequences underwent a rigorous filtration process based on two specific criteria: (1) incomplete sequences were eliminated if their lengths fell below that of the conserved motif (33 bp). (2) sequences containing more than 10 consecutive 'N' bases were excluded from further analysis. This culling process was employed to ensure data quality and conformity to research standards.

Chromosome location and synteny analysis of *SabZIP* genes

The precise chromosome locations of *S. australis bZIP* genes were determined using the MapGene2Chrom wb v2 tool (http://mg2c.iask.in/mg2c_v2.0/, accessed on 10 July 2023) on the basis of genome annotation obtained from the GWH database.

To explore the synteny relationship of homologous *bZIP* genes in *S. australis* and three other species (*Arabidopsis thaliana*, *Beta vulgaris*, and *Oryza sativa*), we employed the Multiple Collinearity Scan toolkit (MCScanX) [37].

Prediction of cis-regulatory elements of promoter region

The 2000 bp sequences located upstream of *SabZIP* genes were obtained from the whole-genome sequence. To predict a variety of cis-acting regulatory elements, these sequences were subjected to analysis using the default parameters of the PlantCARE promoter analysis tool, accessible at http://bioinformatics.psb.ugent.be/webtools/plantcare/html/ as of 10 July 2023. This analysis aimed to predict various cis-acting regulatory elements [38].

Estimation of Ka/Ks values

The gene pairs, which belonged to both tandem and segmental duplications, were extracted from whole genome genes. Subsequently, the Ka and Ks substitution rates were computed through the utilization of the KaKs_Calculator toolkit based on verified duplicated gene pairs of the bZIP family.

Expression analysis of selected *SabZIP* genes

After quality control and adapter trimming of the RNA-seq reads, the paired-end short reads were aligned to *S. australis* genome using HiSAT2 with its default parameters. Subsequently, the calculation of the expected number of FPKM fragments mapped was performed employing the StringTie program [39] with default parameters. Moreover, the DESeq2 R package [40] was applied to identify the differentially expressed genes (DEGs). In multiple analyses, the threshold of the p-value was calculated by the false discovery rate (FDR). If the FDR was less than 0.05, and the absolute value of $\log_2$(fold change) was more than 1, the gene expression differences were considered to be significant. In addition, the Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed using the clusterProfiler R package [41].

Six *SabZIP* DEGs (*Sau08107*, *Sau08911*, *Sau11415*, *Sau16575*, *Sau19276*, and *Sau21103*) related to salt tolerant were screened for qRT-PCR analysis. The online software Primer3Plus (https://www.primer3plus.com/index.html) was used to design primers, and the primers were synthesized by Beijing Qinke Xinye Biotechnology Ltd. (Beijing, China) (Table 4). qRT-PCR was conducted using SYBR qPCR Master MIX (Vazyme) [42] and performed on StepOnePlus (Applied Biosystem, USA) [43]. *18S ribosomal RNA* as an internal control was carried out. The methods for calculating relative gene expression were introduced by previous study [44].

Table 4
Primer sequences and product size of genes.

Gene	Upstream primer (5′-3′)	Upstream primer (5′-3′)	Product length/bp
Sau08107	ATATGAATGGACCACCGACGTC	TCCTCCTTCGCCTTCTTTCATC	147
Sau08911	TGCTTTCAAACCGGGAATCG	ATGCTGCGTAATGTCATCGG	137
Sau11415	AACTGCACTCACTGTACAGC	CGCATCATTTAGTGCATCGC	133
Sau16575	TCAGGAGGAGAAAGCACAGATG	TGTTGTTGTTGCTGCTGCTG	149
Sau19276	AGAAGCCTGCAAAACCTGAG	AGAAGCCTGCAAAACCTGAG	133
Sau21103	ACATCATGTCCCTCAGCCTATG	TTTCCGACCAATTGCCTGTG	133
18S rRNA	AGCAGAATAGTCGATCCGCG	GTAGTCAGCAGGATTCTTAT	149
Note: *18S rRNA* is the housekeep gene.			

Declarations

Acknowledgments

Not applicable.

Author contributions

YQ, TG, JW, and XZ planed and designed the experiments. YQ, XM, CQ, and JW collected the samples and performed experiments. YQ analyzed and interpreted the sequencing data. YQ and JW wrote the manuscript. XZ provided funding for sequencing. All authors read and approved the final manuscript.

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Availability of data and materials

A total of 6 transcriptome raw datasets produced by Illumina NovaSeq 6000 were deposited in the Genome Sequence Archive (SRA) database (https://ngdc.cncb.ac.cn/gsa/s/9B2l2maR) under the accession number CRA011892. The genome sequences of *S. australis*was from the Genome Warehouse in National Genomics Data Center Beijing Institute of Genomics (https://ngdc.cncb.ac.cn/gwh/Assembly/reviewer/OpkDWhtDoPtEZlkphyVuIjMMoJyOVfzTimXVSABQPLBMyxGeIJKtrOzfCLtdUvuL, accession number GWHDOOL00000000).

Ethics approval and consent to participate

The materials of *S. australis* in this experiment were collected from *S. australis* germplasm resources bank (E122°11', N30°02') of the Fishery College, Zhejiang Ocean University, located in Molecular Ecology Laboratory of Zhejiang Ocean University, Zhoushan, Zhejiang Province, China. The collection of plant material complies with institutional guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

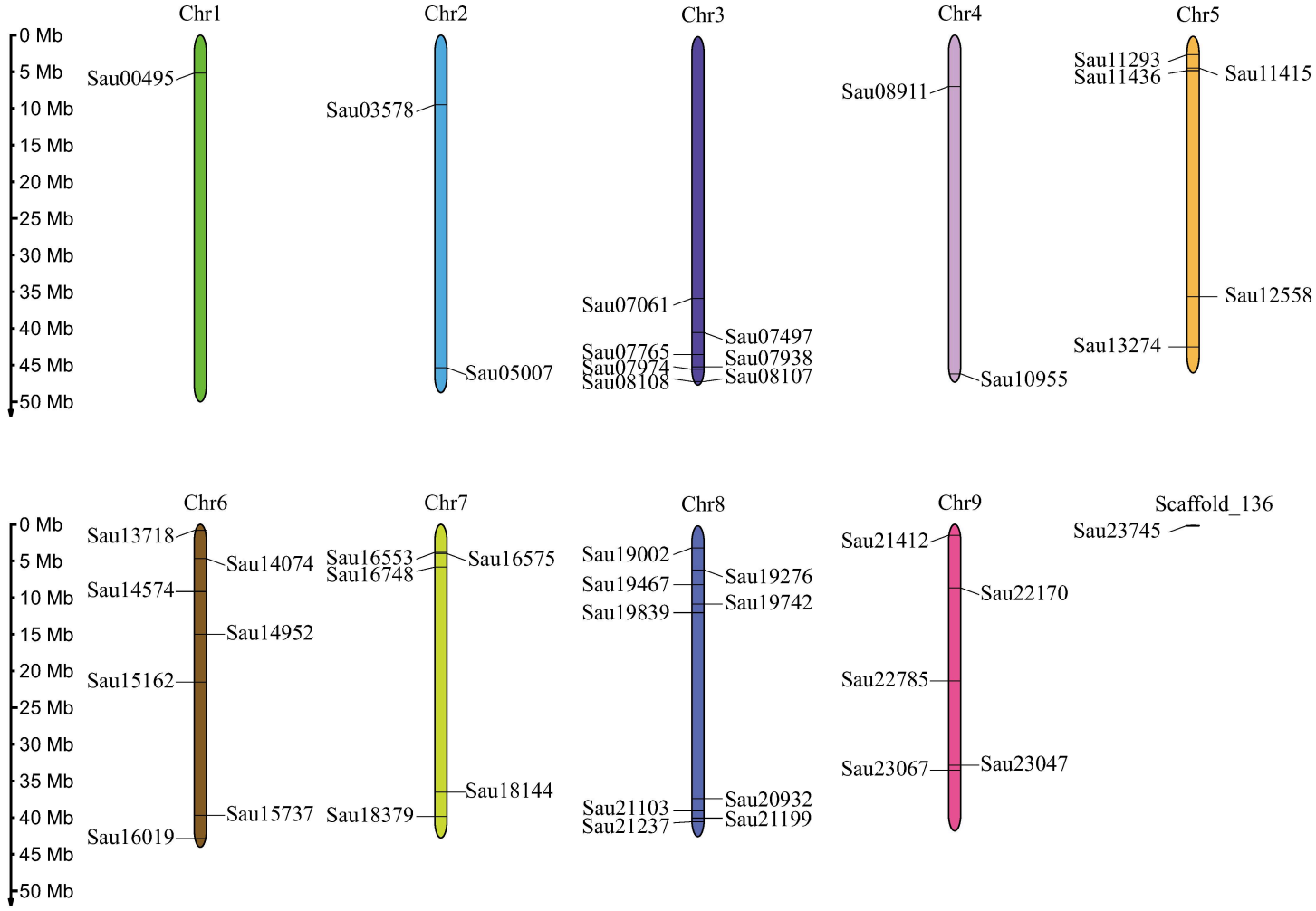

Figure 1

Chromosome distribution of *SabZIP*genes. *S. australis* chromosome number is indicated at the base of each chromosome with different colors. The *SabZIP* genes are marked at the approximate position on the chromosomes.

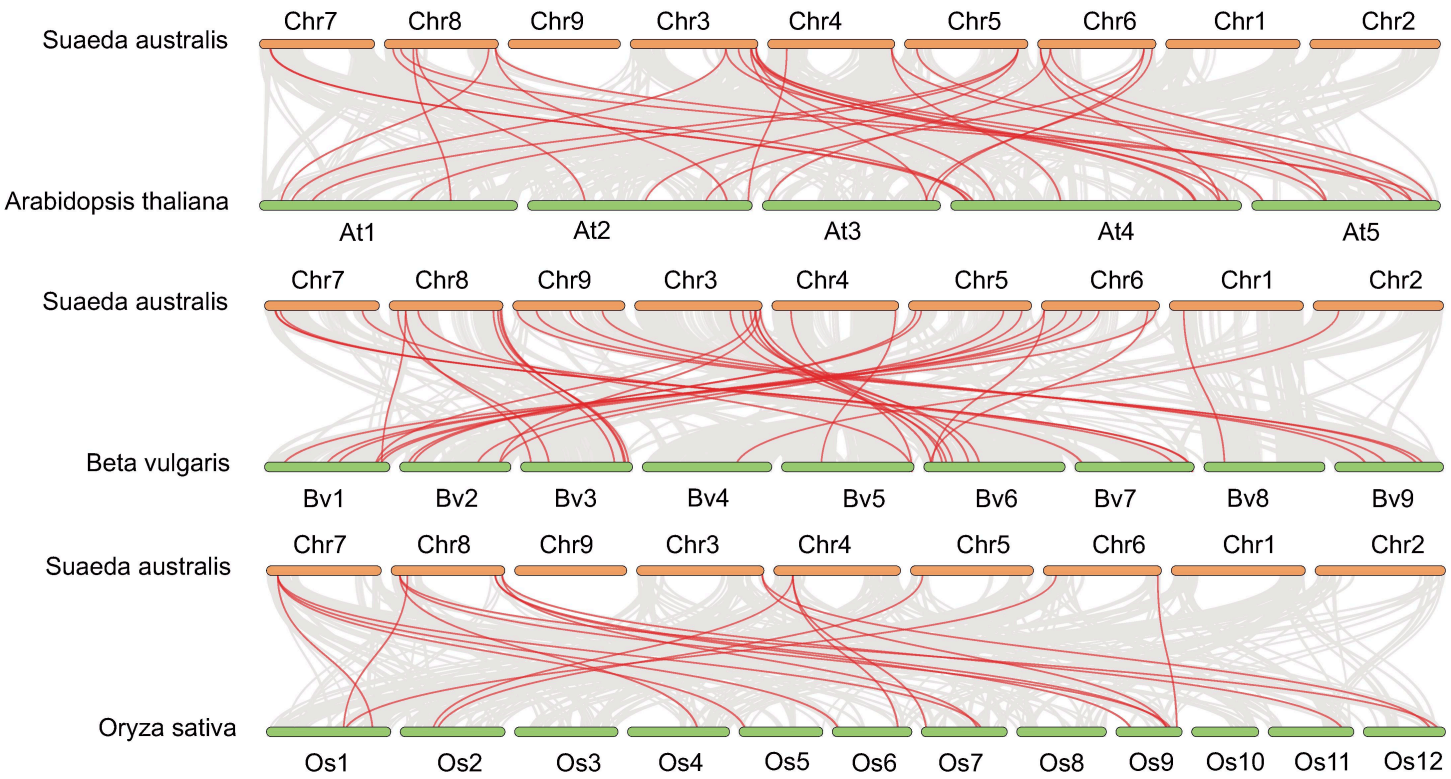

Figure 2

Synteny analysis of *SabZIP* genes between *S. australis*and three plant species. The collinear blocks between *S. australis* and other three plant genomes are shown as the gray lines in the background, while the syntenic *SabZIP* gene pairs are highlighted with red lines.

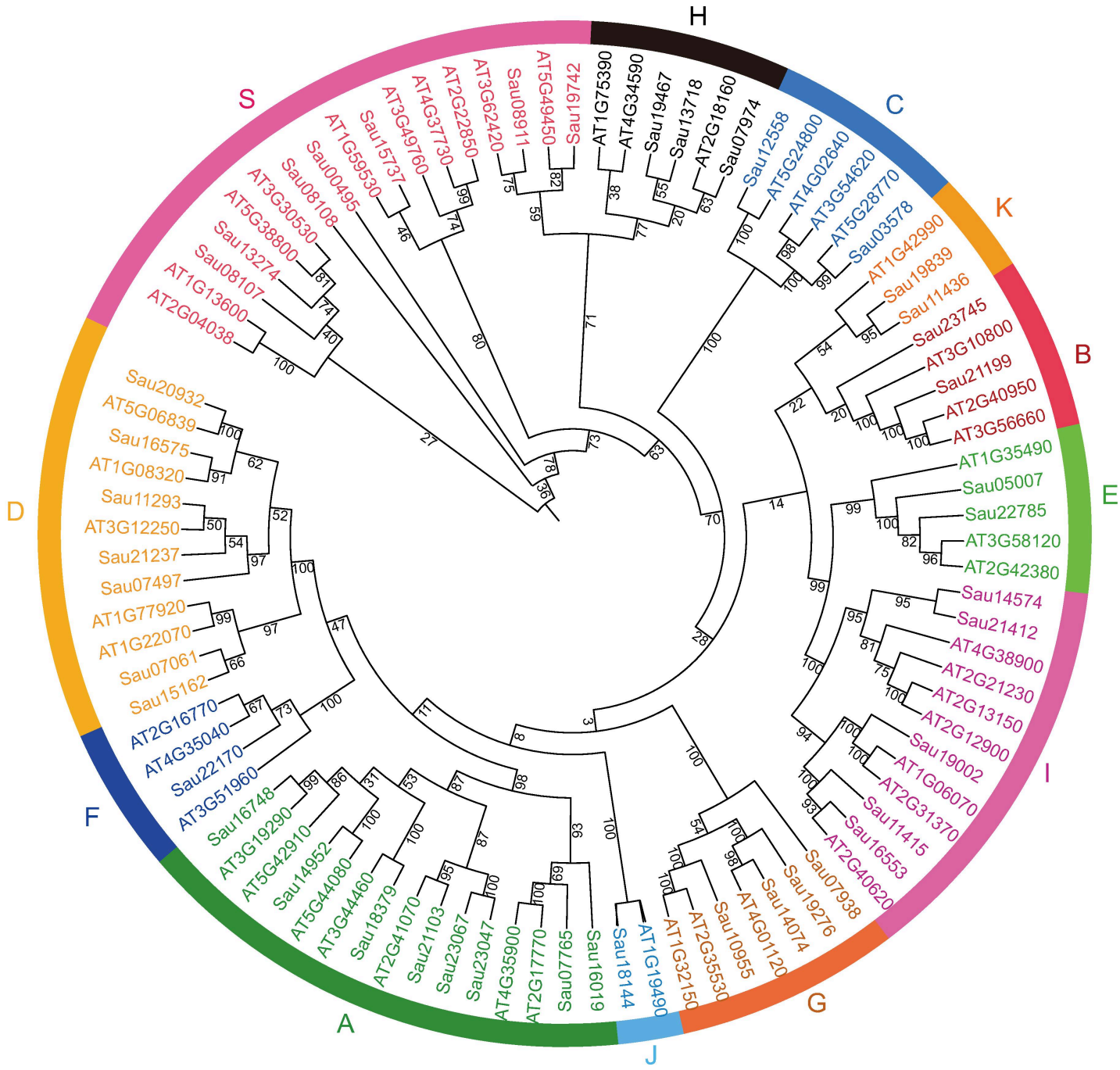

Figure 3

Phylogenetic analysis of SabZIP proteins in *S. australis*. A neighbor-joining (NJ) tree was constructed using 44 SabZIPsequences. The tree further clustered into 12 subfamilies, which are shown in different colors.

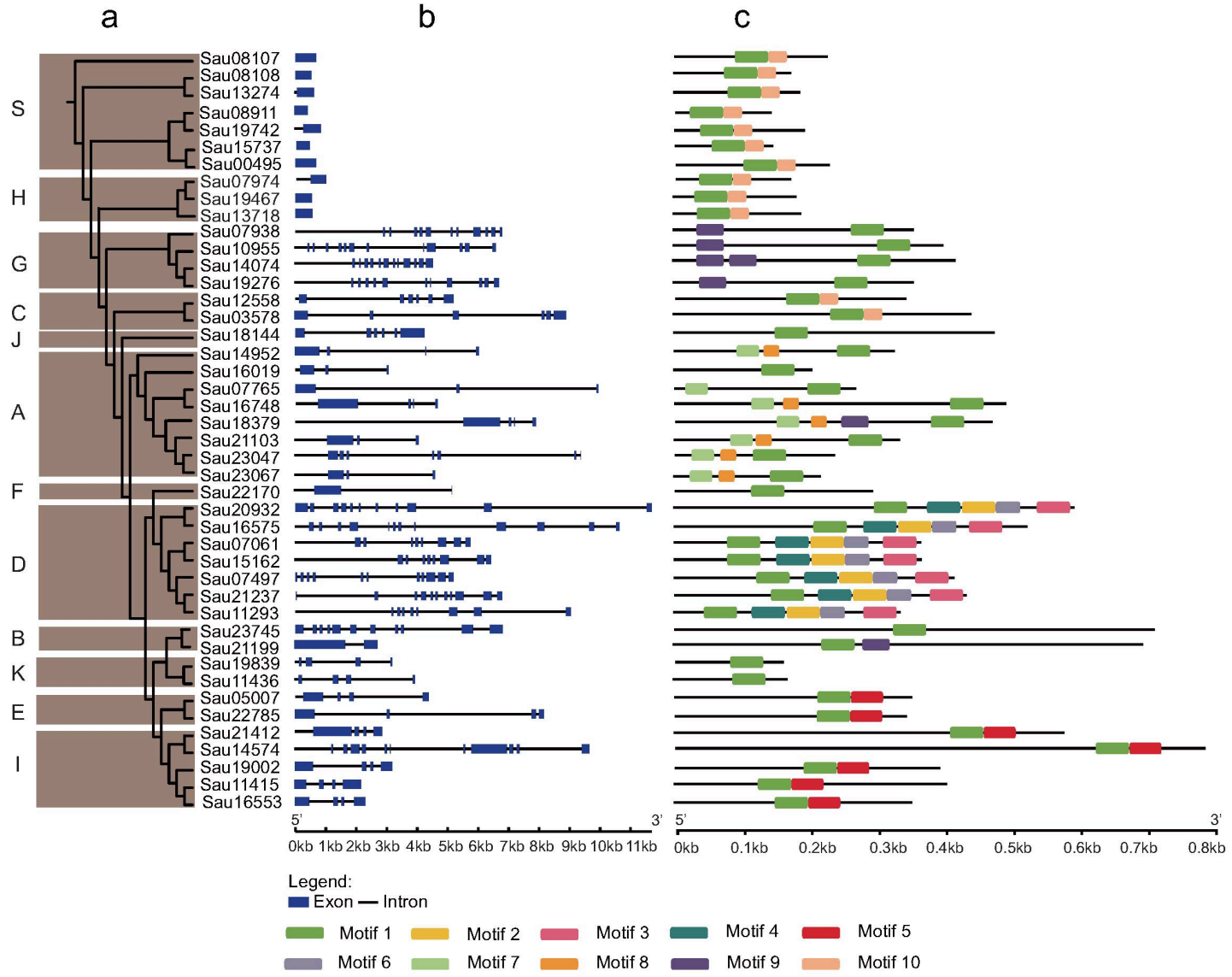

Figure 4

The phylogenetic relationship, gene structure, and motif compositions of SabZIP proteins. (**A**) The phylogenetic relationships of bZIP proteins based on the NJ method. The various colors characterize the 12 subfamilies. (**B**) Gene structure of the *SabZIP* genes family was generated on the basis of the gene Structure Display Server. Exons (blue rectangles) and introns (black lines) are marked along with their sequence length. (**C**) Motif in SabZIP proteins were predicted by MEME. The conserved motifs are represented by different colored boxes.

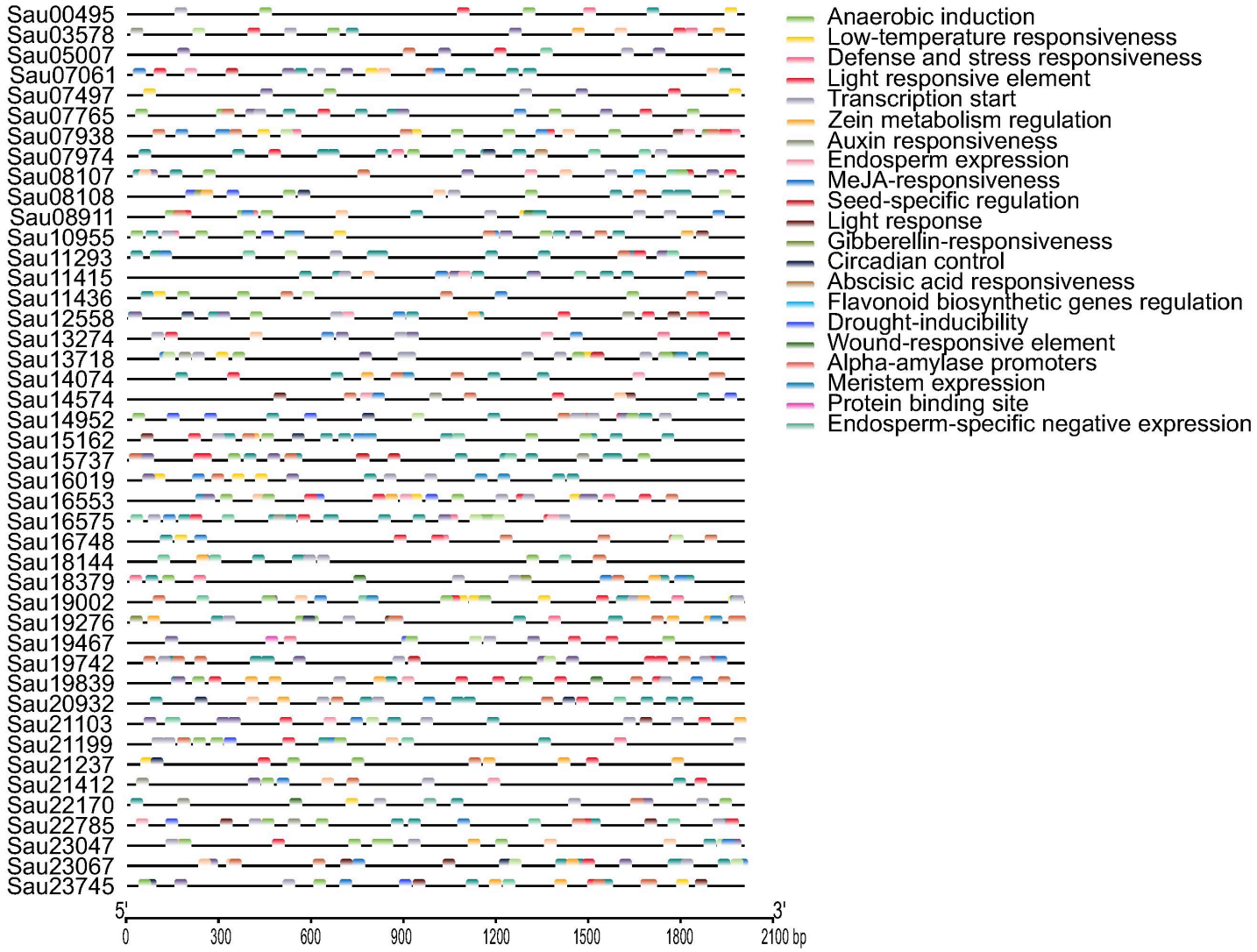

Figure 5

Cis-acting components of *SabZIP*genes in *Suaeda australis*. All promoter sequences (2000bp) were analyzed. The *SabZIP* genes are shown on the left. Scale bar at the base indicates length of promoter sequence. The functions of cis-acting element can be found in Table 3.

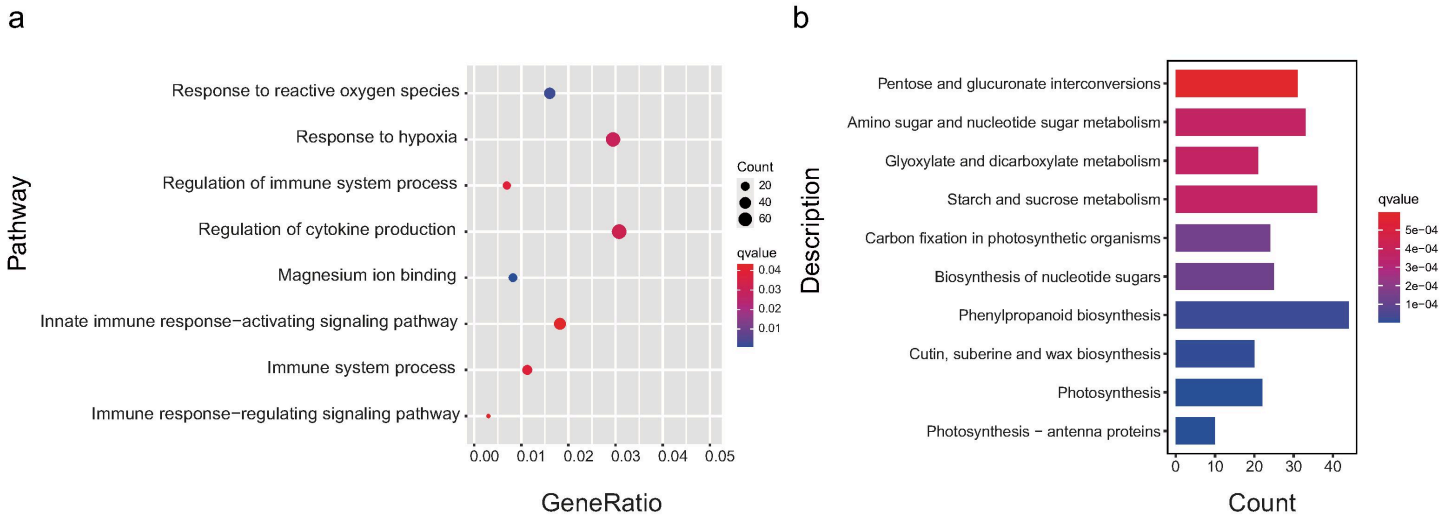

Figure 6

GO(a) and KEGG (b) enrichment of 2,434 DEGs in ST1 and ST2 samples (ST2_vs_ST1).

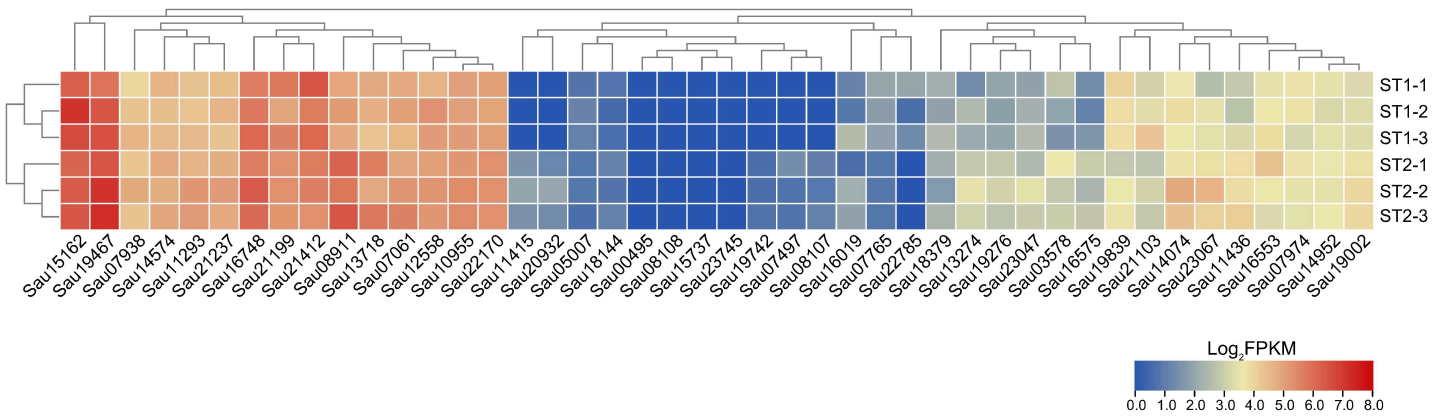

Figure 7

Heatmap showing the expression patterns of 44 *SabZIP* genes among ST1 and ST2 leaves of *Suaeda australis*. Log_2(FPKM) values were used to create the heatmap.

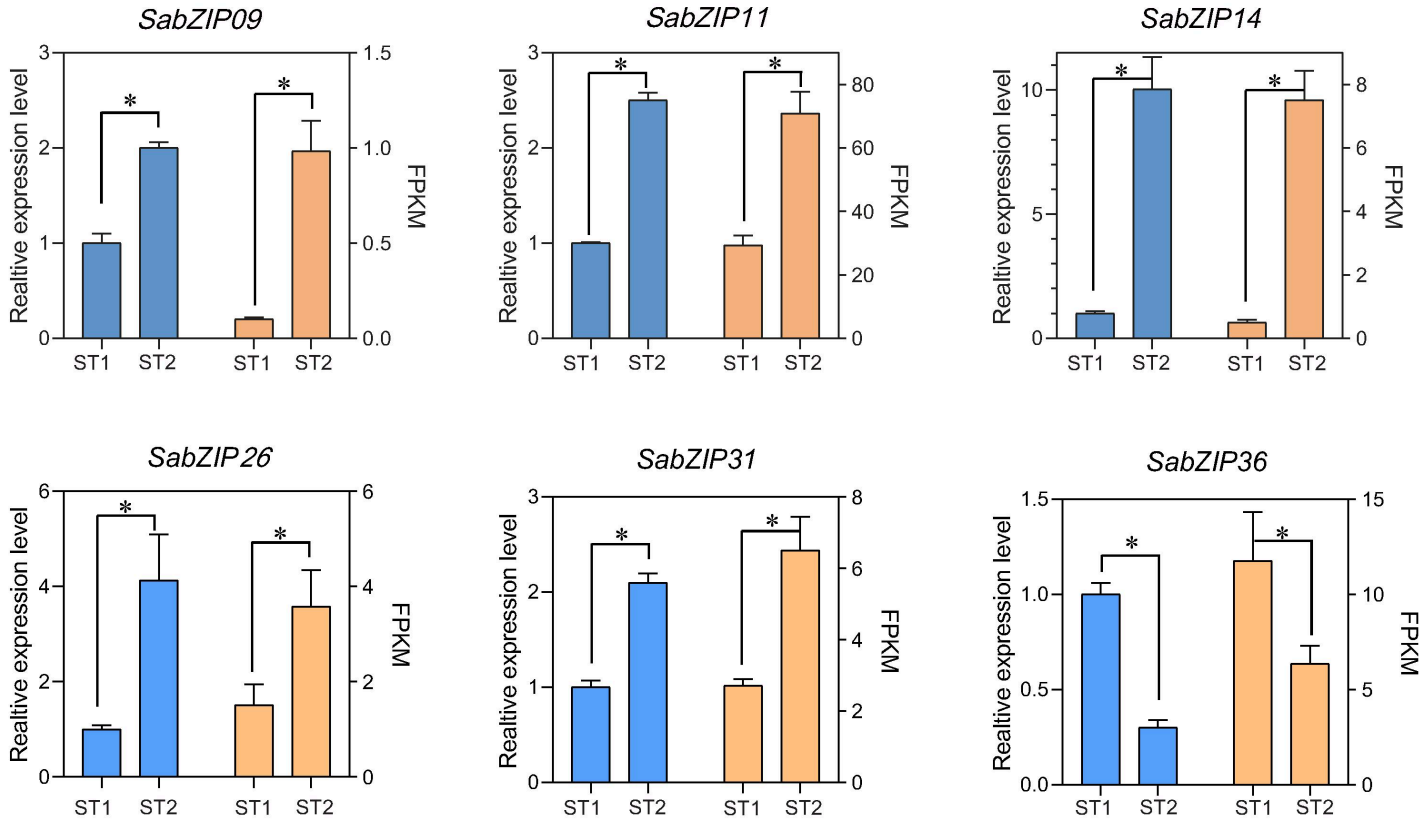

Figure 8

Comparisons of expression levels of six *SabZIP* genes obtained by qRT-PCR analysis among ST1 and ST2 leaves of *Suaeda australis*. The left Y-axis indicates the relative expression level, right Y-axis indicates the FPKM, and the X-axis represents different samples after salt stress treatment taken for expression analysis; the mean±standard error measurement (SEM) value with three replications (n=3) is displayed. * indicates $p<0.05$.