SORGUM'DA OSCA GEN AİLESİNİN GENOM BOYU ARAŞTIRILMASI

Doç. Dr. Hülya SİPAHİ (ORCID ID: 0000-0002-7925-2766)
Eskisehir Osmangazi University, Agricultural Biotechnology Department
Email: hulya.sipahi@ogu.edu.tr

ÖZET

Bitkileri strese sokan hiperozmotik veya hipoozmotik ortamlarda, hücreler sensör ve sinyal ağlarının aktivitesini tetikler. Kalsiyum iyonu, hiperozmotik basınca verilen ilk tepkilerin birincil düzenleyicisi olarak çalışır. Hiperosmolalite kapılı kalsiyum geçirgen kanal (OSCA) gen ailesi üyeleri, kuraklık ve tuzluluk gibi hipertonik strese tepkinin erken aşamalarında aktif olarak yer alan sensörlerdir. Sorgum (*Sorghum bicolor* L. Moench), tohumu gıda maddesi olarak kullanılabilen, sapından etil alkol üretilebilen, biyokütle verimi yüksek bir C4 bitkisidir. Bu çalışmada in silico yaklaşımlarla, sorgumda OSCA gen ailesi tanımlanmış ve karakterize edilmiştir. Sorgum genomunda beş kromozoma dağılmış on bir *OSCA* geni (*SbOSCA*) tanımlanmıştır. Filogenetik analiz, *Arabidopsis thaliana*, *Sorghum bicolor*, *Oryza sativa*, *Zea mays* ve *Hordeum vulgare*'nin OSCA proteinlerinin, tüm kladlarda yakın kümelendiğini ve bu genlerin dikotlar ile monokotlar ayrılmadan önce evrimleştiğini göstermiştir. SbOSCA'ların amino asit sayısı ve molekül ağırlığı sırasıyla 699 ila 808 a.a ve 79.12 ila 93.63 kDa aralığındadır. Test tüplerinde, kararsızlık indeksi 40'tan büyük olan 11 SbWAK/SbWAKL'dan 9'unun kararsız olduğu tespit edilmiştir. SbOSCA proteinleri, her kümede korunmuş motif desenleri içerir ve aynı kümedeki *SbOSCA* genlerinin, intron uzunluklarındaki farklılıklar dışında benzer olduğu bulunmuştur. Sinteni analizlerine göre, sorgum ve üç monokotiledon (cin darı, mısır ve pirinç) arasında çok sayıda, Arabidopsis ve sorgum arasında ise yalnızca bir çift OSCA geninin ortolog olduğu tespit edilmiştir. Işığa, gelişime, çevresel strese ve hormona tepki ile ilgili *SbOSCA* promotörlerinde cis elementlerinin varlığı, bu genlerin bitki büyümesinde, gelişmesinde ve stres tepkisinde fonksiyonel rollerini göstermektedir. Bu çalışmada, sorgumda hiperozmotik yanıt ile ilişkili *OSCA* gen ailesi üyelerinin olası moleküler rollerini aydınlatmak için yapılan evrimsel ilişkilerin, cis elementlerinin, protein-protein ve miRNA etkileşimlerinin analiz sonuçları, gelecekteki ozmotik strese karşı direnç açısından bitkilerin genetik düzenlenmesi ve ıslahı için bir temel oluşturacaktır.

Anahtar Kelimeler: Sorgum, OSCA gen ailesi, biyoinformatik

GENOME-WIDE SURVEY OF OSCA GENE FAMILY IN SORGHUM

ABSTRACT

In hyperosmotic or hypoosmotic environments that distress plants, cells trigger the activity of sensors and signaling networks. The calcium ion acts as the primary regulator of the initial responses to osmotic pressure. Hyperosmolality-gated calcium-permeable channel (OSCA) gene family members are sensors actively involved in the early stages of the response to hypertonic stress, such as drought and salinity. Sorghum (*Sorghum bicolor* L. Moench) is a C4 plant with a high biomass yield, the seed of which can be used as a foodstuff, and ethyl alcohol can be produced from the stem. In this study, the OSCA gene family in sorghumwas identified and characterized by *in silico* approaches. Eleven OSCA genes (SbOSCA) in the sorghum genome have been identified, scattered over five chromosomes. Phylogenetic analysis demonstrates that the OSCA proteins of *Arabidopsis thaliana, Sorghum bicolor, Oryza sativa, Zea mays,* and *Hordeum vulgare* are closely clustered in all clades, indicating that they evolved before the divergence of dicots and monocots. SbOSCAs ranged in amino acid count and molecular weight from 699 to 808 a.a and 79.12 to 93.63 kDa, respectively. In test tubes, 9 out of 11 SbOSCAs with an instability index greater than 40 were detected to be unstable. SbOSCA proteins contain conserved motif patterns in each cluster, and SbOSCA genes in the same cluster were found to be similar except for differences in intron lengths. According to synteni analyzes, it was determined that there were many orthologous OSCA genes between sorghum and three monocotyledons (gin millet, maize and rice) and only one pair of OSCA genes between Arabidopsis and sorghum. The presence of light, development, environmental stress, and hormone responsiveness-related cis-elements in *SbOSCA* promoters implied their functional roles in plant growth and development, and stress response. In this study, the analysis results of the evolutionary relationships, cis-elements, protein-protein, and miRNA interactions to elucidate the possible molecular roles of OSCA gene family members associated with the hyperosmotic response in sorghum will provide a basis for genetic regulation and breeding of plants in terms of resistance to future osmotic stress.

Keywords: Sorghum, OSCA gene family, bioinformatics

INTRODUCTION

The hyperosmolality-gated calcium-permeable channels (OSCA) family proteins are located in the plasma membrane and function as an osmosensor for external hyperosmotic stress. These channels are opened by hyperosmolality stimuli, leading to an increase in the intracellular concentration of Ca2+ ions. OSCA proteins contain conserved domains namely RSN1_TM, PHM7_cyt, and RSN1_7TM. RSN1_TM and RSN1_7TM domains are in the N-terminal transmembrane and the C-terminal of OSCA, respectively. PHM7_cyt may have been in 7TM (transmembrane). Ca^{2}+ is an essential second messenger which activates serial signal transduction (Klimecka et. al. 2007). Thus Ca^{2}+ contributes to the initial response to hyperosmotic stress (Liu et al. 2018). Various studies have demonstrated that certain OSCA proteins are closely related to plant growth and development as well as to osmotic adjustment. qRT-PCR analysis validated that osmotic stress induced by the drought or salinity treatment led to differential expression of OSCA genes in several species. For example, *HvOSCA03* and *HvOSCA05* genes in barley (Cai et. al. (2022), OsOSCA1.1, -1.2, -2.1, -2.4, -2.5, -4.1 in rice (Li et al., 2015), ZmOSCA1.1 ZmOSCA1.1b, -1.2, -1.4, -2.1, -2.4, -4.1 genes in maize (Ding et. al. 2019) were found to be upregulated after PEG treatment. Also, the expression of 13 *GmOSC*A genes in soybean was affected by alkali stress and drought (Zeng et. al.2020). In the expression analysis of OSCA genes in chickpea (*Cicer arietinum*) under desiccation, salinity stress in both roots and shoot, *CaOSCA2.6* had high expression. Also, the *CaOSCA1.1* gene and *CaOSCA2.3* are upregulated in desiccation stress in the shoot and salt stress in root tissue, respectively. And, the *CaOSCA2.1, -2.2, -2.5,* and *-3.1* gene is upregulated under desiccation stress. The upregulation of *PvOSCA2.5, -2.6, and -2.1* in drought-tolerant common bean genotypes were also detected under drought stress. The number of OSCA gene family members varies in species. Fifteen OSCA genes were found in Arabidopsis (Liu et. al. 2018), 11 in rice (Li et. al. 2015), 12 in maize (Ding et. al. 2019), 13 in four leguminous species (pigeon pea, mung bean, common bean, chickpea) (Chakraborty et. al. 2023), 14 in barley (Cai et. al. 2022), 21 in soybean (Zeng et. al. 2020), 35 in cotton (Yang et. al. 2019), and 42 in bread wheat (Kaur et. al. 2022). Sorghum is a high-energy plant that is widely grown in the world, consumed as human food, and used to meet the feed needs of animals. In this plant, whose biomass is also used as a biofuel, the OSCA gene family was the genome-wide identified for the first time using *in silico* approaches. The physicochemical properties, phylogeny, and gene structure of sorghum OSCA gene family

members were detected. This theoretical study will be the basis for editing sorghum OSCA genes in sorghum to detect their functions.

MATERIAL AND METHODS

Identification and Chromosomal Localization of OSCA Genes

To identify the OSCA genes, *Arabidopsis thaliana* OSCA protein sequences derived from TAIR https://www.arabidopsis.org/) were used as query sequences and blasted (BlastP) against the sorghum genome with an E-value of 1e-5 using NCBI (https://www.ncbi.nlm.nih.gov/). Redundant sequences were removed. The NCBI database (https://www.ncbi.nlm.nih.gov/) was used for thriving chromosomal locations, CDS sequences and gene sequences of OSCA genes. TBtools software (Chen et. al. 2020) was applied to map the chromosomal distribution of OSCA genes. The physical and chemical parameters of OSCA proteins were analysed with Protparam (https://web.expasy.org/protparam/).

Phylogenetic, Protein Conserved Motif, Gene Structure and Synteny Analysis

The OSCA protein sequences of *A. thaliana*, *S. bicolor*, *Zea mays*, *Hordeum vulgare*, and *Oryza sativa* were downloaded from NCBI and aligned using CLUSTALW (https://www.genome.jp/toolsbin/ clustalw) using default parameters (Larkin et al. 2007). The phylogenetic trees were constructed based on the neighbour-joining method of MEGA-11 software (Saitou and Nei 1987; Tamura et. al. 2021) with a bootstrap consensus tree inferred from 1000 replicates (Felsenstein 1985). The online MEME tool (http://memesuite.org/tools/meme) was used to detect the conserved motifs of OSCA proteins. The Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/) was applied to analyse the intron–exon pattern (Hu et al. 2015). The cis-acting elements of the promoters were detected using plantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). TBtools software was used for synteny analysis using genome files of *H. vulgare*, *Foxtile millet*, *O. sativa* and *S. bicolor*.

Identification of Mirna Target Sites and Protein-Protein Interactions

Using the online STRING platform V11.5, protein-protein interactions was investigated (Szklarczyk et al., 2019). Disconnected nodes in the network were concealed using a medium

confidence value of 0.400 and no more than 10 interactions to display. The target genes of S. bicolor miRNAs were predicted using the default settings on psRNATarget (https,//www.zhaolab.org/psRNATarget) (Dai et al., 2018). Cytoscape 3.9.1 was used to display the miRNA interaction network (Shannon et. al., 2003).

RESULTS AND DISCUSSION

Eleven OSCA proteins were identified in sorghum by *in silico* analysis, named SbOSCA1-SbOSCA11 (Table 1). All 11 SbOSCA proteins contained characteristic domains, namely RSN1-TM Late exocytosis, associated with Golgi transport (PF13967), PHM7-cyt Cytosolic domain of 10TM putative phosphate transporter (PF14703), Calcium-dependent channel, 7TM region, putative phosphate (PF02714). Also, these proteins had a transmembrane domain (TM) and signal peptide (SP) domain. SbOSCAs ranged in amino acid count and molecular weight from 699 to 808 a.a and 79.12 to 93.63 kDa, respectively (Table 1). The isoelectric point (pI) also varied from 8.26 to 9.17. The hydrophilic indices of the SbOSCA were all more than 0 (0.030-0.290 GRAVY value), suggesting that they are hydrophobic. The aliphatic index of all SbOSCA proteins ranged from 98.21 to 108.34, which may be regarded as thermally stable. In test tubes, 9 out of 11 SbOSCAs with an instability index greater than 40 were unstable.

Table 1. Characteristics of the OSCA proteins in the sorghum.

Proteins	Protein accession number	Length of amino acids	Molecular weight kDa	pI	Grand average of hydropathicity (GRAVY)	Aliphatic index	Instability index
SbOSCA1.1	XP_002457968.1	768	87.55	8.67	0.115	100.66	46.03
SbOSCA1.2	XP_002440380.1	766	88.00	8.84	0.088	100.77	41.70
SbOSCA1.3	XP_002441130.1	768	87.67	8.78	0.120	103.05	37.44
SbOSCA1.4	XP_002464863.1	808	93.63	9.04	0.030	98.21	44.17
SbOSCA2.1	XP_002443649.1	772	87.44	8.94	0.231	106.53	42.39
SbOSCA2.2	XP_021311202.1	760	86.44	9.17	0.164	102.2	49.4
SbOSCA2.3	XP_021318180.1	747	85.73	8.82	0.163	99.37	48.50
SbOSCA2.4	XP_021301378.1	699	79.13	8.93	0.290	108.34	44.27
SbOSCA2.5	XP_002456934.1	706	79.92	8.97	0.219	105.23	41.57
SbOSCA3.1	XP_002461460.1	732	81.91	8.90	0.260	106.12	38.24
SbOSCA4.1	XP_002468551.1	795	89.44	8.26	0.205	102.49	40.80

To demonstrate the evolutionary relationship among OSCA genes of different species, phylogenetic analysis was carried out. The phylogenetic tree showed that the OSCA proteins of *Arabidopsis thaliana*, *S. bicolor*, *Oryza sativa*, *Zea mays*, and *Hordeum vulgare* were divided into four groups (Figure 1) and closely clustered in all clades, indicating that OSCA genes evolved before the divergence of dicots and monocots.

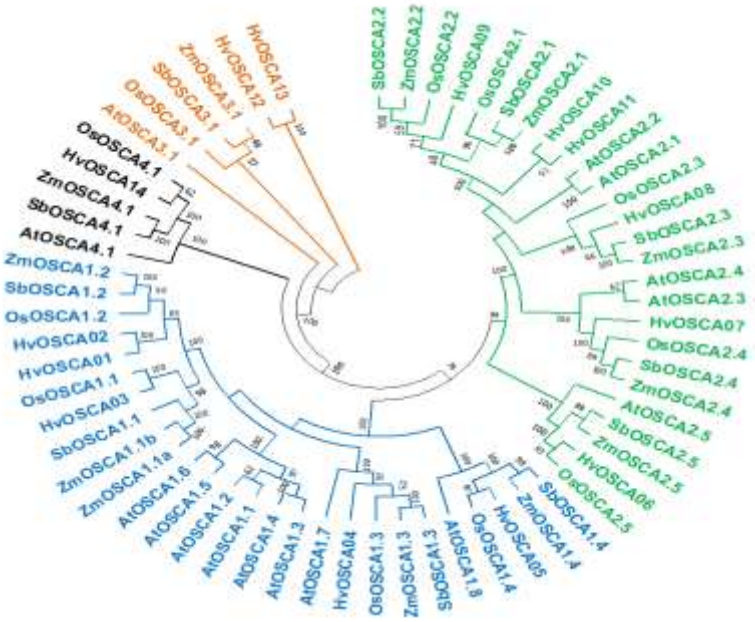


Figure 1. Phylogenetic relationship of OSCA gene family from *Arabidopsis thaliana*, *S. bicolor*, *Oryza sativa*, *Zea mays*, and *Hordeum vulgare*

Eleven *SbOSCA* genes were unevenly distributed over chromosomes 1,2 3, 8 and 9 (Figure 2).

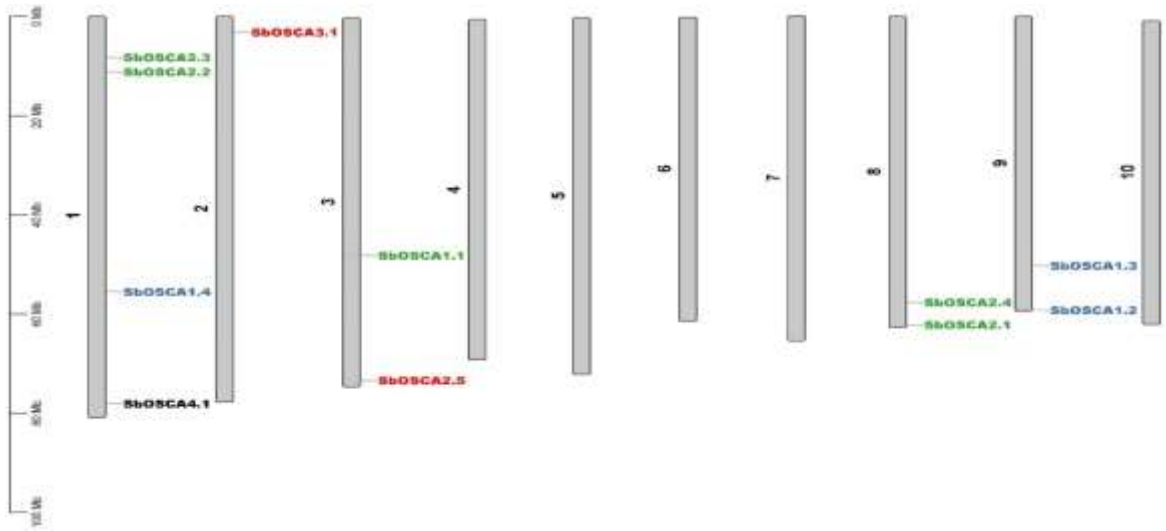


Figure 2. Chromosomal localization of the *SbOSCA* genes in *S. bicolor* chromosomes. Chromosome numbers are shown beside each chromosome. Colours of genes were obtained from the phylogenetic tree.

While SbOSCA proteins were mostly found in the plasma membrane of the cell, they were also detected in the endoplasmic reticulum, vacuole, and chloroplast, respectively (Figure 3). Only the SbOSCA2.5 was found in mitochondria.

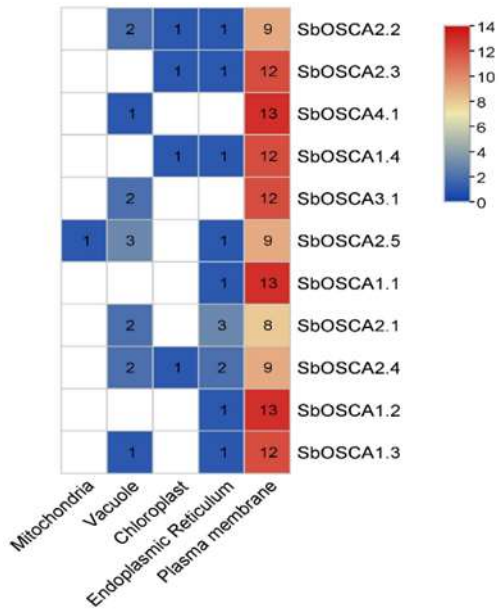


Figure 3. The subcellular location of *SbOSCA* genes through WoLF PSORT.

To investigate the potential functions of *SbOSCA* genes, cis-acting elements in 2000 bp upstream of promotor sequences in *SbOSCA* genes were analyzed (Figure 4A-D). Considering hormone-responsive elements (Figure 4A), abscisic-acid-responsive cis-elements, including ABRE, ABRE3a, and ABRE4, were found in all *SbOSCA* gene promoters except *SbOSCA2.1*. Five *SbOSCA* genes (*SbOSCA 2.2., -2.4, -2.5, 1.2, -1.3*) had MeJA-responsive elements (TGACG-motif and/or CGTCA-motif). The salicylic-acid-responsive elements (TCA, TCA-element) were found in *SbOSCA2.2* and *SbOSCA4.1*. The abscisic acid, MeJA, and salicylic acid hormones are mainly interested in several stress responses and cell signal transduction. The ethylene-responsive elements (ERE) were found to be only in *SbOSCA2.3* and *SbOSCA3.1* promotors. Ethylene controls both growth and senescence. The gibberellin-responsive elements (GARE-motif, P-box) and the auxin-responsive element (TGA-element) were observed in *SbOSCA2.1*, *SbOSCA4.1*, *SbOSCA1.4*, and *SbOSCA2.5* genes, respectively. The auxin and gibberellin phytohormones have functions in cell elongation. The expression level of *SbOSCA* genes having cis-acting elements might be stimulated after these hormone treatments.

Thirteen types of environmental stress-related elements were discovered in *SbOSCA* promotors (Figure 4B). Among them, MYB (MYB binding site involved in drought-inducibility), MYC ((drought, salt, and stress response), STRE (stress response) were the most predominant. Three *SbOSCA* genes had LTR (low-temperature responsiveness), and five *SbOSCA* genes contained ARE (anaerobic induction). box S (elicitation, wounding, and pathogen responsiveness element (box S) was found only *SbOSCA1.1* gene. Development-related elements such as AAGAA-motif (involved in the endosperm-specific negative expression), O2-site (involved in zein metabolism), as-1 (root-specific expression), CAT-box (meristem expression), CCAAT-box (meristem expression), GCN4_motif (endosperm expression), RY-element (seed-specific regulation) were also found in nine *SbOSCA* genes (Figure 4C). The root-specific elements (as-1) were the prominent found, while four of them (AAGAA-motif, O2-site, GCN4_motif, and MSNA-like) were found only in one *SbOSCA* gene. In addition, 17 light-responsive elements were observed in the promoters (Figure 4D). Numerous Box 4 and G-Box, G-box cis-acting elements were especially the most abundant light response elements found in many *SbOSCA* genes.

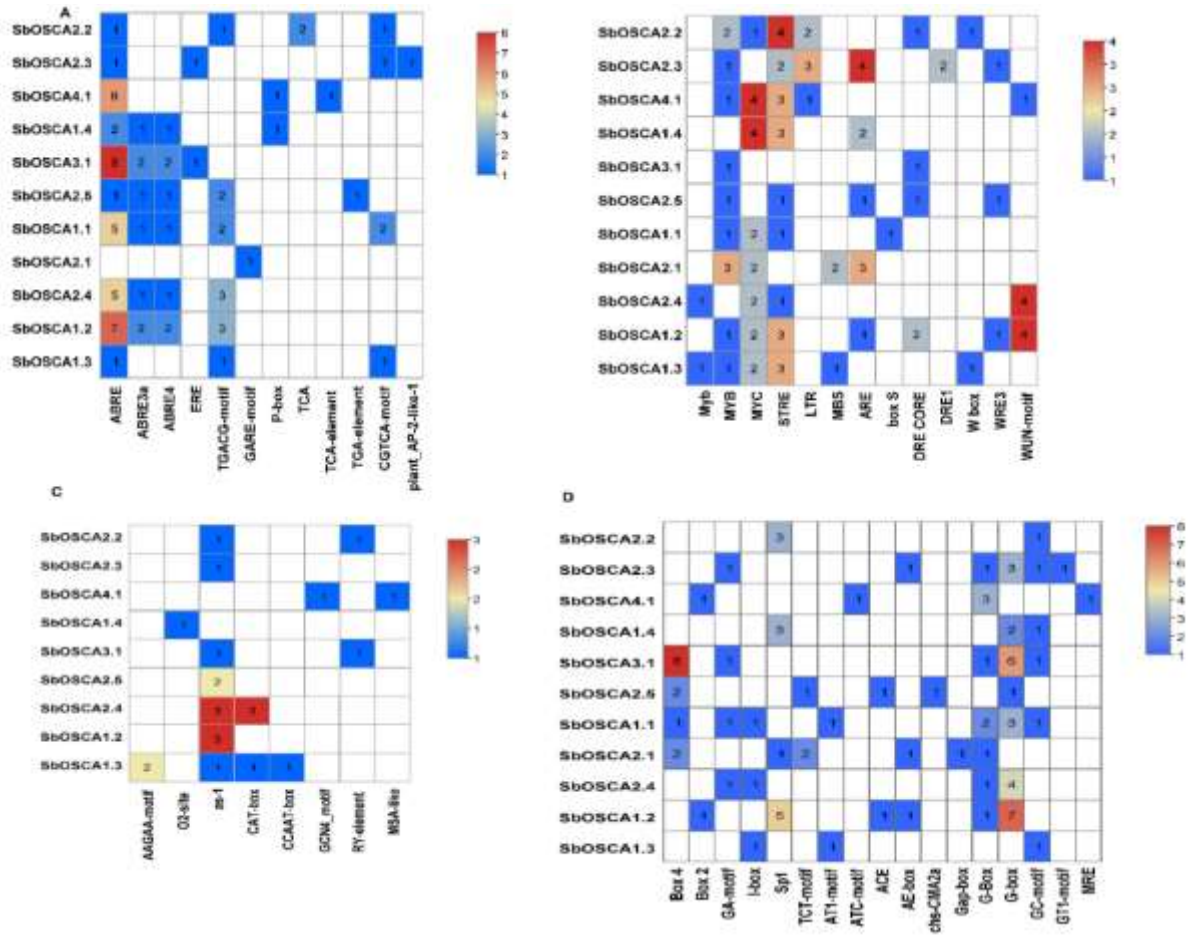


Figure 4. Cis-acting elements in promoter regions of *SbOSCA* genes. A) hormone-responsive elements, B) environmental-stress-related elements, C) development-related elements and D) light-responsive elements. The numbers in the boxes represent the number of cis-acting elements.

In order to understand the functional diversity and motif distribution among the SbOSCA family, the conserved motifs of these proteins were detected using MEME tool (Figure 5b). SbOSCA1.1, -1.2, -1.3, -1.4 and SbOSCA3.1 had all ten motifs suggesting a functional similarity. Also, SbOSCA2.1, -2.2, -2.3, -2.4 and -2.5 contained all motifs except motif 7 which is a calcium-dependent channel and related to putative phosphate domain. Interestingly, SbOSCA4.1 had only one motif (motif 3) which corresponds to CSC1-like protein having calcium activated cation channel. The distribution of the conserved motif was found to be consistent with those of the phylogenetic analysis.

According to gene structures, the *SbOSCA* genes with similar intron and exon numbers have been clustered into the same subgroups (Figure 5c). Subgroup I including *SbOSCA2.1—2.5* had 10 exons and 9 introns. But there are significant differences in the length of introns among them (Figure 5c). The other subgroups II including *SbOSCA1.1—1.4* had 11 exons and 10 different introns in length like subgroup I. Interestingly, *SbOSCA4.1* had only one long exon and no intron. In addition, *SbOSCA3.1* had a unique gene structure with 6 exons and 5 introns.

Similarly, it has been reported that in four Leguminous species, this gene consists only of a single exon without introns, and therefore may differ functionally from other genes (Chakraborty et. al. 2023).

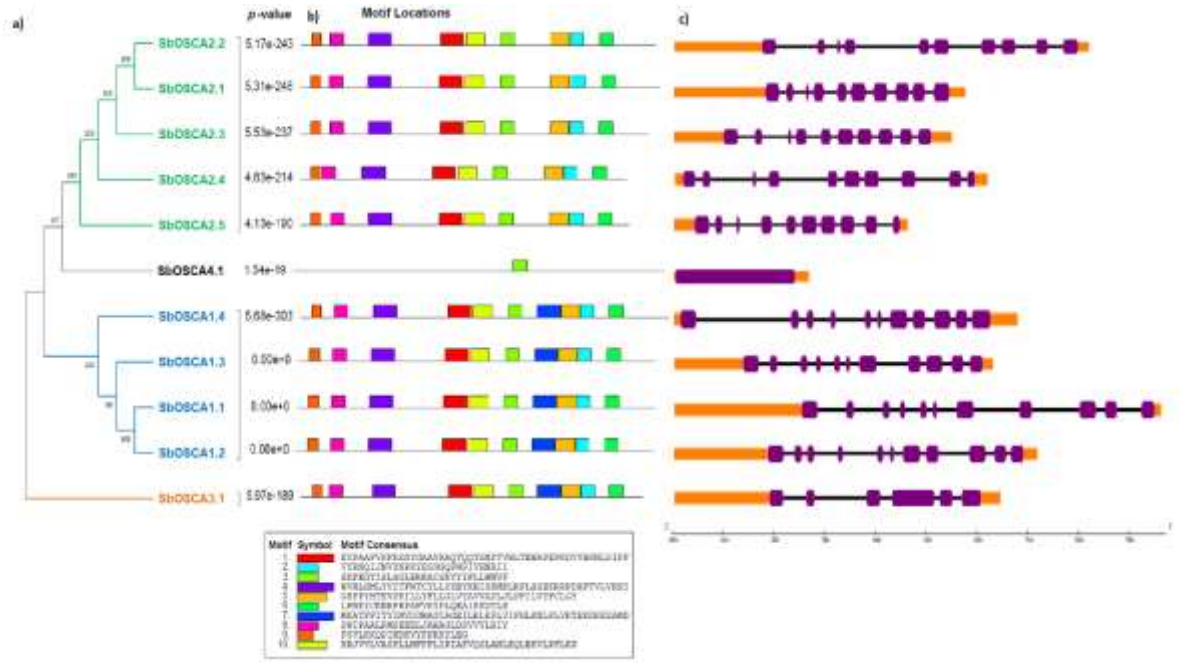


Figure 5. (a) The phylogenetic tree of SbOSCA gene family was constructed with 1000 bootstrap using the neighbor-joining algorithm, (b) the motifs of the SbOSCA protein sequences (c) The gene structure of SbOSCAs. The orange boxes, violet boxes, and black lines represent upstream/downstream regions, exons, and introns, respectively

The evolutionary process of the OSCA genes is further searched by collinearity analysis between one dicotyledons species (*A. thaliana*), three monocotyledons species (*Z. mays*, *H. vulgare*, and *O. sativa*), and *S. bicolor* (Figure 6). Ten gene pairs of *S. bicolor* had a collinear relationship with *O. sativa* and *F. millet*, and 9 gene pairs with *Z. mays*. Only two genes (*SbOSCA2.5*, *SbOSCA3.1*) revealed a collinear relationship with *A. thaliana*.

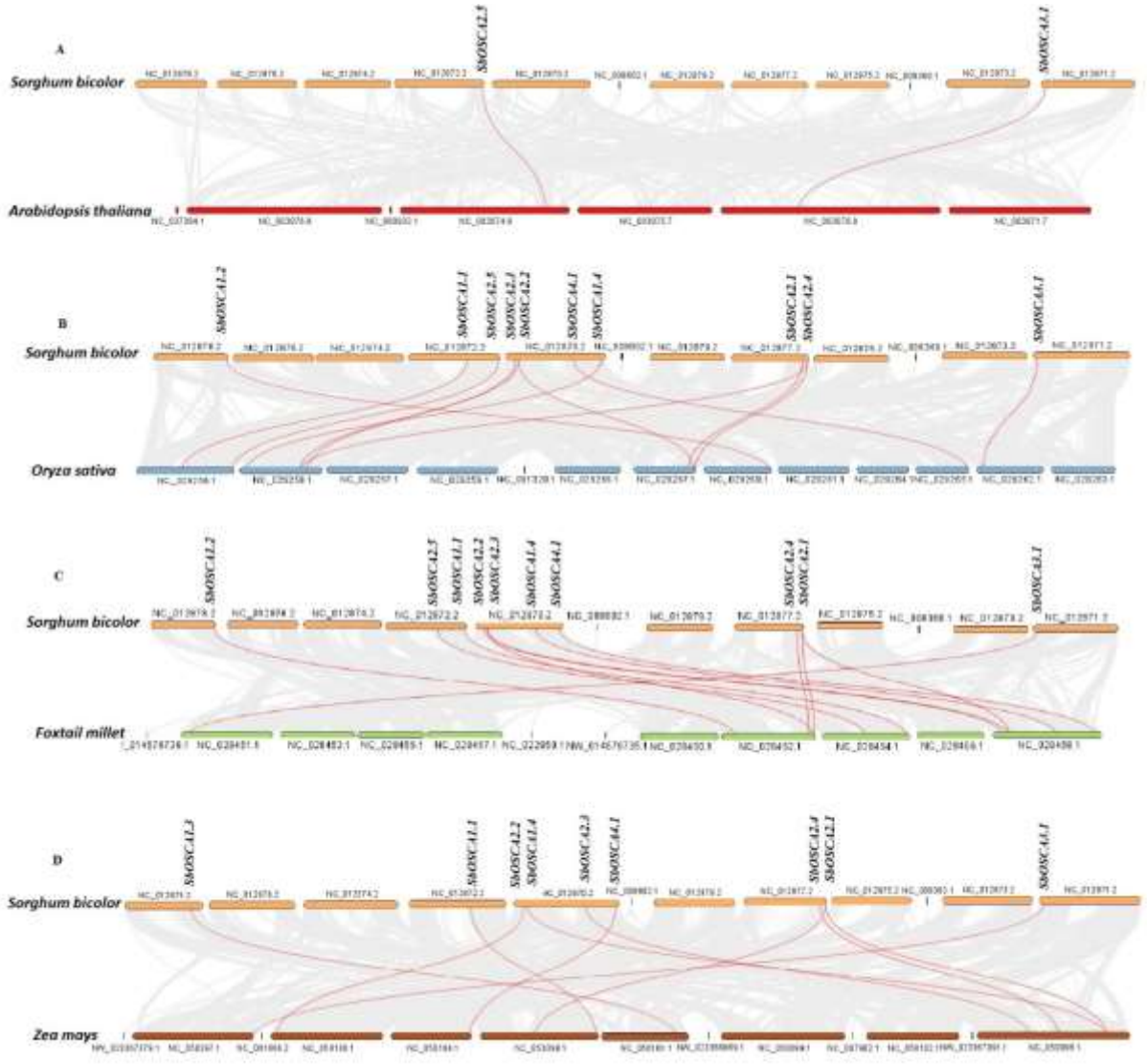


Figure 6. Collinearity analysis of *SbOSCA* genes with *A. thaliana* (a); *O. sativa* (b); and *F. millet* (c)

The probable functions of SbOSCA proteins were predicted by protein–protein interaction analysis (Figure 7). Eight SbOSCA proteins (SbOSCA2.4, SbOSCA1.4, SbOSCA1.2, SbOSCA2.3, SbOSCA4.1, SbOSCA2.5, SbOSCA1.1, and SbOSCA1.3) interacted with Sb09g011890.1 protein (Rhodanese-like domain-containing protein 7, alternative name in Arabidopsis, Sulfur transferase 7). A rhodanese-like protein domain is constituted of wide diverse proteins (Burroughs and Aravind 2023). For example, proteins with single rhodanese domains are involved in the process of leaf senescence in *Arabidopsis, Nicotiana tabacum and Raphanus sativus* (Azumi et. al. 1991; Oh et. al. 1996). In addition, certain enzymes which regulate redox and thiotransfer activities have also rhodanese-like domains. And, the calcium-sensing receptors in chloroplast have a rhodanese-like domain and these receptors induce stomatal closure in presence of external Ca2+ ions (Han et. al. 2003).

SbOSCA4.1 was the most interactive protein. It interacts with Sb05g022850.1(Adp-ribosylation factor-like protein 5b, ARFs), Sb01g028220.1 (Dnaj homolog subfamily c member 13), and Sb10g025180.1 (Px domain-containing protein erel1). ARFs confer tolerance to biotic and abiotic stresses (Muthamilarasan et. al. 2016). DNAJ (Hsp40; heat shock protein40) are molecular chaperons interacting with Hspa70. They together are responsible for protein folding on ribosomes, transport across membranes, and degradation of unfoldable proteins to protect against cellular damage or refolding them after exposure to various stressful situations (Li et. al. 2009). The PX domain is involved in the targeting of proteins to cell membranes.

SbOSCA1.3 interacts with Sb01g027390.1(Uncharacterized protein).

All these protein-protein interactions show that OSCA proteins play roles in different cellular processes, especially signaling and stress response.

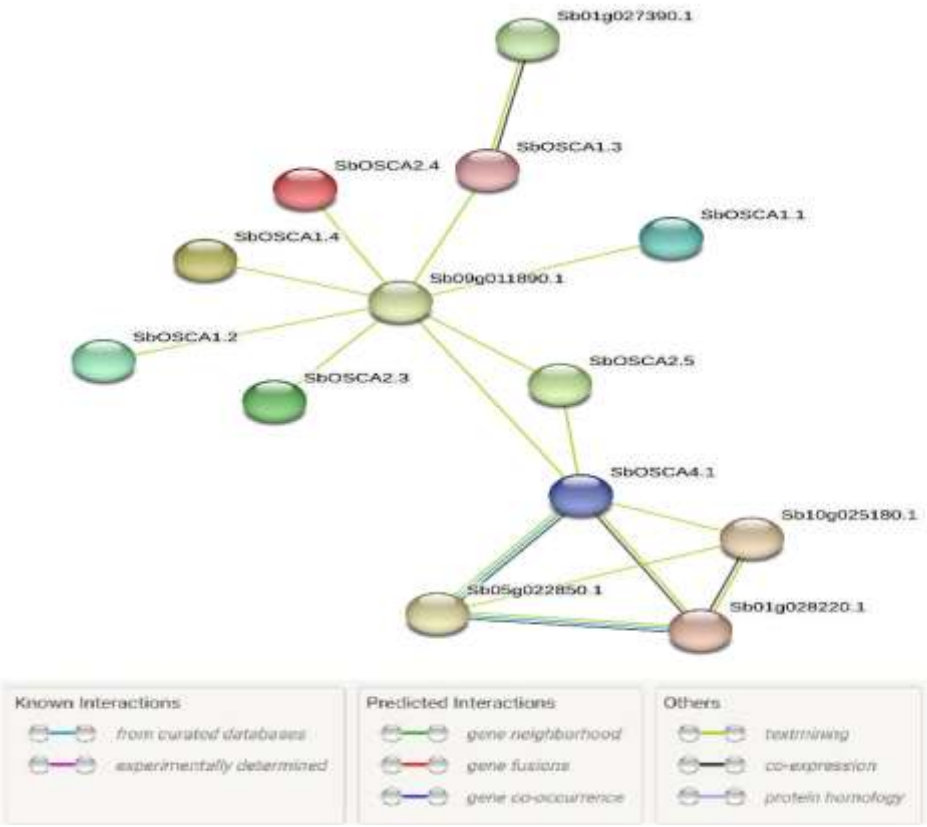


Figure 7. Protein–protein interaction network of SbOSCA

Micro-RNAs (miRNAs) with 21–24 nucleotides play an important role in post transcriptional regulation of gene expression by cleavage and translational events. Therefore, in order to find out regulatory effect of miRNAs on *SbOSCA* genes, the potential miRNA target sites were detected in *SbOSCA* genes (Figure 8). Nine *SbOSCA* genes were predicted to be potential targets of miRNAs. However, there was no miRNA was predicted for *SbOSCA1.4 and SbOSCA2.3*. The largest number of miRNAs (twelve sbi-miR437(a-w) and one sbi-miR5564a) were predicted for *SbOSCA4.1* gene. In the previous study, sbi-miR437a-w were found to target ribonucleoprotein and DGRE_CAEEL DGCR14 protein homolog genes which are responsible for RNA processing (Banerjee et. al. 2022). A total number of 9 and 7 miRNAs were predicted for *SbOSCA3.1* and *SbOSCA2.1*, respectively. *SbOSCA3.1* gene were targeted by sbi-miR395a-j. In another study, it was demonstrated that ATP sulphurylases (APS1, APS4) that catalyze the sulphate assimilation pathway was targeted by miR395 (Jones-Rhoades et al. 2006).

sbi-miR5568c-5p, sbi-miR5565e, sbi-miR821b, d, and sbi-miR395l targeted *SbOSCA2.1* gene.

In another study, sbi-miR-5568 targeted glycosyl hydrolase (Sb03g007230.1) (Yu et. al. 2015). sbi-miR5565e was identified to function in the regulation of Cd accumulation and tolerance (Jia et. al. 2021). Also, Banerjee et. al. (2022) indicated that sbi-miR5565e targeted Alanine-tRNA ligase (chloroplastic/mitochondrial) and Serine/threonine-protein kinase.

SbOSCA2.4 gene was targeted by sbi-miR156a, b, c, d, e, f, g, h, I, sbi-miR159a, sbi-miR5388, sbi-miR6222-3p, sbi-miR6223-3p. In Arabidopsis, miR156 was up regulated in response to salt stress (Liu et. al. 2008) and found to be involved in response to drought and salt stresses. In a study in cowpea, when miR159 expression was suppressed in drought, its target phospholipase D accumulated and ABA-induced stomatal movement and regulation of antioxidant defense were initiated (Maarouf et al. 1999). Also, sbi-miR6223-3p with target of sucrose-phosphate synthase (Sb03g043900.1), was found to be related to sucrose synthesis. (Yu et al. 2015).

SbOSCA1.1, SbOSCA1.2, SbOSCA1.3, SbOSCA2.2 and *SbOSCA2.5* genes were found to be potential the targets for sbi-miR390, sbi-miR408, sbi-miR6231-3p, sbi-miR528 and sbi-miR5385, respectively. Du et. al. 2010 detected that sbi-miR528 targeted heat shock protein 70 which is responsible stress response. In addition, miR408 is found to be involved in response to copper stress (Axtell and Bowman 2008) and the regulation of Cd accumulation and tolerance (Jia et. al. 2021). Finally, Akbudak et. al. (2018) predicted that sbi-miR5385 targeted sorghum *SbDREB2C1* (dehydration-responsive element-binding protein) gene. As a result, it could be deduced that sb-miRNAs might cause the changes of SbOSCA genes expression to regulate response to stress.

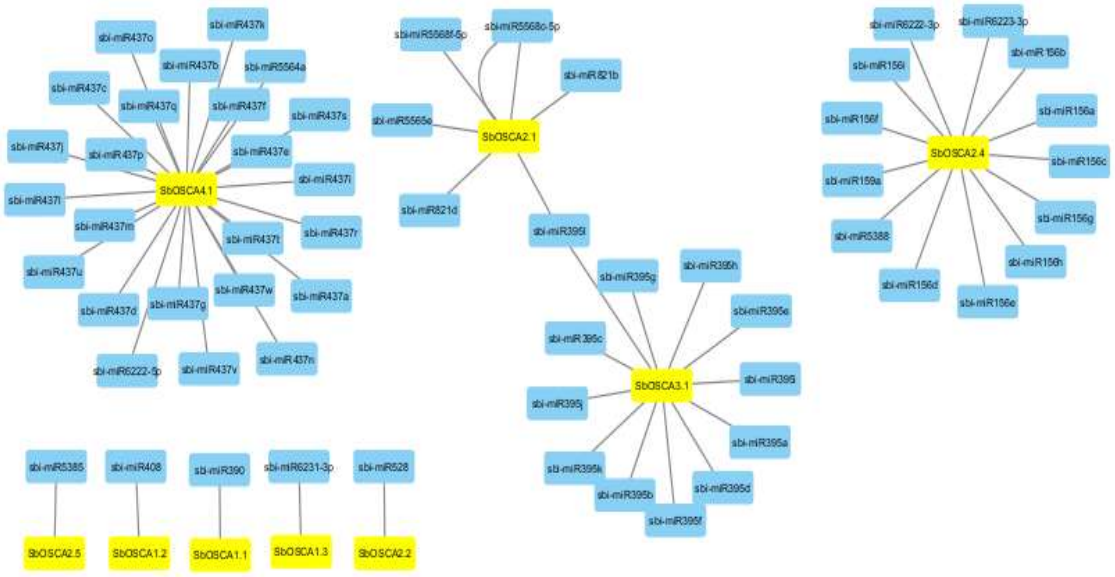


Figure 8. miRNA interaction network of *SbOSCA* genes.

CONCLUSION

In this study, 11 genes belonging to the OSCA gene family in Sorghum bicolor were identified using bioinformatics tools. The enlightening information gained about these gene family members, which are associated with hyperosmolality, will be especially useful in combating osmotic stress caused by drought and salinity. In addition, under conditions of osmotic stress, the determination of the expression levels of OSCA gene family members and gene silencing or over-expression experiments will provide further information about the functions of these genes, and this study will be the basic source for such studies.

REFERENCES

Akbudak, M.A., Filiz, E., Kontbay, K. (2018) DREB2 (dehydration-responsive element-binding protein 2) type transcription factor in sorghum (Sorghum bicolor): genome-wide identification, characterization and expression profiles under cadmium and salt stresses. 3 Biotech 8, 426. https://doi.org/10.1007/s13205-018-1454-1

Azumi, Y., Watanabe, A. (1991) Evidence for a senescence-associated gene induced by darkness. Plant Physiology 95:577–583. doi: 10.1104/pp.95.2.577.

Axtell, M.J., Bowman J.L. (2008) Evolution of plant microRNAs and their targets. Trends Plant Sci 13(7):343–349. https://doi.org/10.1016/j.tplants.2008.03.009

Banerjee, N., Kumar, S., Singh, A., Annadurai, A., & Thirugnanasambandam, P. P. (2022) Identification of microRNAs involved in sucrose accumulation in sugarcane (Saccharum species hybrid). Plant Gene 29, 100352.

Burroughs, A.M., L Aravind, (2023) New biochemistry in the Rhodanese-phosphatase superfamily: emerging roles in diverse metabolic processes, nucleic acid modifications, and biological conflicts, *NAR Genomics and Bioinformatics*, 5 (1), lqad029, https://doi.org/10.1093/nargab/lqad029

Cai, Qian, Wang, Y., Ni, S., Mu, J., Liu, M., Wang, Y., & Zhao, Y. (2022) Genome wide identification and analysis of the OSCA gene family in barley (Hordeum vulgare L.). Journal of Genetics 101.2: 34.

Chakraborty, S., Gangwar, R., Zahra, S., Poddar, N., Singh, A.,Kumar, S. (2023). Genome-wide characterization and comparative analysis of the OSCA gene family and identification of its potential stress-responsive members in legumes. Scientific Reports, 13(1), 5914.

Chen, C., Chen, H., Zhang, Y., Thomas, H.R., Frank, M.H., He, Y., Xia, R. (2020) TBtools: An integrative toolkit developed for interactive analyses of big biological data. Mol. Plant, 13, 1194–1202.

Dai, X., Zhuang, Z., & Zhao, P. X. (2018) PsRNATarget: A plant small RNA target analysis server (2017 release) Nucleic Acids Research, 46(W1), W49–W54. https://doi.org/10.1093/nar/gky316

Ding, S., Feng, X., Du, H., Wang, H. (2019) Genome-wide analysis of maize OSCA family members and their involvement in drought stress. PeerJ, e6765.

Du, J., Wu, Y., Fang, X. et al. (2010) Prediction of sorghum miRNAs and their targets with computational methods. Chin. Sci. Bull. 55, 1263–1270. https://doi.org/10.1007/s11434-010-0035-4

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39, 783–791.

Han, S., Tang, R., Anderson, L. K., Woerner, T. E., and Pei, Z. M. (2003) A cell surface receptor mediates extracellular Ca2þsensing inguard cells. Nature 425, 196 – 200.

Hu B., Jin J., Guo A. Y., Zhang H., Luo J., Gao G. (2015) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31, 1296–1297.

Jia, W., Lin, K., Lou, T. et al. (2021) Comparative analysis of sRNAs, degradome and transcriptomics in sweet sorghum reveals the regulatory roles of miRNAs in Cd accumulation and tolerance. Planta 254, 16 https://doi.org/10.1007/s00425-021-03669-2

Jones-Rhoades, M.W., Bartel, D.P., Bartel, B. (2006) MicroRNAS and their regulatory roles in plants. Annual Review Plant Biology 57, 19-53

Kaur, A., Sharma, A., Madhu, Dixit, S., Singh, K., & Upadhyay, S. K. (2022). OSCA genes in bread wheat: Molecular characterization, expression profiling, and interaction analyses indicated their diverse roles during development and stress response. International Journal of Molecular Sciences, 23(23), 14867.

Klimecka, Maria, and Grażyna Muszyńska (2007) Structure and functions of plant calcium-dependent protein kinases. Acta Biochimica Polonica 54.2: 219-233.

Larkin M. A., Blackshields G., Brown N. P., Chenna R., McGettigan P. A., McWilliam H. et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Li, Y., Yuan, F., Wen, Z., Li, Y., Wang, F., Zhu, T., Han, S. (2015) Genome-wide survey and expression analysis of the OSCA gene family in rice. BMC plant biology, 15(1), 1-13.

Li, J., Qian, X, Sha, B. (2009) Heat shock protein 40: structural studies and their functional implications. Protein Pept Lett. 16(6):606-12. doi: 10.2174/092986609788490159. PMID: 19519518; PMCID: PMC2755554.

Liu, H.H., Tian, X., Li, Y.J., Wu, C.A. and Zheng, C.C. (2008) Microarray-Based Analysis of Stress-Regulated MicroRNAs in Arabidopsis thaliana. RNA, 14, 836-843. http://dx.doi.org/10.1261/rna.895308

Liu, X., Wang, J., Sun, L. (2018) Structure of the hyperosmolality gated calcium-permeable channel OSCA1.2. Nat. Commun. 9, 5060–5068.

Maarouf, H. E., Zuily-Fodil, Y., Gareil, M., d'Arcy-Lameta, A., & Thu Pham-Thi, A. (1999). Enzymatic activity and gene expression under water stress of phospholipase D in two cultivars of Vigna unguiculata L. Walp. differing in drought tolerance. Plant molecular biology, 39, 1257-1265.

Muthamilarasan M, Mangu VR, Zandkarimi H, Prasad M, Baisakh N. (2016) Structure, organization and evolution of ADP-ribosylation factors in rice and foxtail millet, and their expression in rice. Sci Rep. Apr 21; 6:24008. doi: 10.1038/srep24008. PMID: 27097755; PMCID: PMC4838888.

Oh S.A., Lee S.Y., Chung I.K., Lee C.H., Nam H.G. (1996) A senescence-associated gene of *Arabidopsis thaliana* is distinctively regulated during natural and artificially induced leaf senescence. Plant Mol. Biol. 30:739–754. doi: 10.1007/BF00019008.

Saitou, N.; Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003) Cytoscape: A software Environment for integrated models of biomolecular interaction networks. Genome Research, 13(11), 2498–2504. https://doi.org/10.1101/gr.1239303

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & Von Mering, C. (2019) STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Research, 47(D1), D607–D613. https://doi.org/10.1093/nar/gky1131

Tamura, K.; Stecher, G.; Kumar, S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology Evolution 38, 3022–3027.

Yang, X., Xu, Y., Yang, F., Magwanga, R.O., Cai, X., Wang, X., Wang, Y., Hou, Y., Wang, K., Liu, F., Zhou, Z. (2019) Genome-wide identification of OSCA gene family and their potential function in the regulation of dehydration and salt stress in Gossypium hirsutum. J. Cotton Res. 2, 1–18.

Yu, H., Cong, L., Zhu, Z., Wang, C., Zou, J., Tao, C., Zhensheng, S., Lu, X (2015) Identification of differentially expressed microRNA in the stems and leaves during sugar accumulation in sweet sorghum, Gene, 571, 2, 221-230, https://doi.org/10.1016/j.gene.2015.06.056.

Zeng, H.; Zhao, B.; Wu, H.; Zhu, Y.; Chen, H. (2020) Comprehensive in silico characterization and expression profiling of nine gene families associated with calcium transport in soybean. Agronomy 10, 1539.