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Insights into the gene and protein structures of the *CaSWEET* family members in chickpea (*Cicer arietinum*), and their gene expression patterns in different organs under various stress and abscisic acid treatments

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ABSTRACT

'Sugars Will Eventually be Exported Transporters' (SWEETs) are a group of sugar transporters that play crucial roles in various biological processes, particularly plant stress responses. However, no information is available yet for the *CaSWEET* family in chickpea. Here, we identified all putative *CaSWEET* members in chickpea, and obtained their major characteristics, including physicochemical patterns, chromosomal distribution, subcellular localization, gene organization, conserved motifs and three-dimensional protein structures. Subsequently, we explored available transcriptome data to compare spatiotemporal transcript abundance of *CaSWEET* genes in various major organs. Finally, we studied the changes in their transcript levels in leaves and/or roots following dehydration and exogenous abscisic acid treatments using RT-qPCR to obtain valuable information underlying their potential roles in chickpea responses to water-stress conditions. Our results provide the first insights into the characteristics of the *CaSWEET* family members and a foundation for further functional characterizations of selected candidate genes for genetic engineering of chickpea.

Abbreviations: 3-D, Three-dimensional; aa, amino acid; ABA, abscisic acid; ABRE, ABA-responsive element; CDS, coding DNA sequence; CE3, coupling element 3; DRE, dehydration-responsive element; gDNA, genomic DNA sequence; EE, evening element; ERE, ethylene-responsive element; FB, flower bud; FL, flower; GbRE, gibberellin-responsive element; GRAVY, grand average of hydropathicity; HSE, heat stress element; JARE, jasmonic acid-responsive element; Ka, non-synonymous substitutions per non-synonymous site; kDa, kilo Dalton; Ks, synonymous substitutions per synonymous site; LTRE, low temperature-responsive element; MBS, MYB-binding site; MEGA, Molecular Evolutionary Genetics Analysis; MEME, Multiple EM for Motif Elicitation; ML, mature leaf; MYC, MYC recognition site; NACR, NAC recognition site; NCBI, National Center for Biotechnology Information; pI, isoelectric point; PIECE, Plant Intron Exon Comparison and Evolution; R, root; RPKM, reads per kilobase of transcript per million mapped reads; RT-qPCR, real-time quantitative polymerase chain reaction; S, shoot; SARE, salicylic acid-responsive element; SWEET, Sugars Will Eventually be Exported Transporter; YP, young pod.

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1. Introduction

Chickpea (*Cicer arietinum*) has been recognized as the second most important grain legume crop in the world, which is cultivated in many countries in the arid and semi-arid regions (de Camargo et al., 2019). Chickpea grains serve as high-nutrient food and high-quality feed for humans and animals, respectively (Margier et al., 2018). Additionally, cultivation of chickpea also supplies a major source of nitrogen to the soil through atmospheric nitrogen fixation (Nasr Esfahani et al., 2017; Roy et al., 2020). However, adverse environmental conditions, especially abiotic stresses like drought, salinity, nutrient deficiency and extreme temperatures, severely affect the global chickpea productivity (Jha, 2018; Nadeem et al., 2019; Nasr Esfahani et al., 2021). Drought has been regarded as the most serious abiotic stresses, which could be responsible for 40-45% of chickpea yield losses (Devasirvatham and Tan, 2018).

To deal with the problems resulted from drought, various studies have concentrated on functional characterizations of genes encoding metabolic and regulatory proteins, because understanding the functions of these genes may provide an option for development of improved drought-tolerant cultivars by genetic engineering (Li et al., 2019; Mostofa et al., 2018; Mahdavi Mashaki et al., 2018; Sen et al., 2017). Sucrose transporters, namely 'Sugars Will Eventually be Exported Transporters' (SWEETs), are involved in translocation of monosaccharides and disaccharides (Julius et al., 2017; Baker et al., 2012); and thus, in modulating various biological processes in plants (Baker et al., 2012; Chen, 2014). Being osmotic regulators (Daloso et al., 2016), the concentrations of soluble sugars (predominantly sucrose) increase in plants in responses to abiotic stresses (Rahman et al., 2021; Mostofa et al., 2020). Sucrose transport from source to sink organs is associated with the expression levels of *SWEET* genes that plays an important role in plant development (Baker et al., 2012; Yang et al., 2018), particularly under stressful conditions (Chandran, 2015; Li et al., 2017b). To gain an insight into their functions, a number of studies have been carried out to identify and characterize the *SWEET* gene families in many major crops, such as rice (*Oryza sativa*) (Yuan and Wang, 2013), grapevine (*Vitis vinifera*) (Chong et al., 2014); tomato (*Solanum lycopersicum*) (Feng et al., 2015), soybean (*Glycine max*) (Patil et al., 2015); sorghum (*Sorghum bicolor*) (Mizuno et al., 2016), oilseed rape (*Brassica napus*) (Jian et al., 2016), banana (*Musa acuminata*) (Miao et al., 2017); cucumber (*Cucumis sativus*) (Hu et al., 2017), Chinese white pear (*Pyrus bretschneideri*) (Li et al., 2017a), cotton (*Gossypium* spp.) (Zhao et al., 2018), apple (*Malus domestica*) (Zhen et al., 2018); *Phalaenopsis equestris* and *Dendrobium officinale* (Wang et al., 2018), Chinese cabbage (*B. rapa*) (Li et al., 2018; Miao et al., 2018), wheat (*Triticum aestivum*) (Gao et al., 2018; Gautam et al., 2019), tea plant (*Camellia sinensis*) (Wang et al., 2018); *Medicago truncatula* (Hu et al., 2019), litchi (*Litchi chinensis*) (Xie et al., 2019); cabbage (*B. oleracea* var. *capitata* L.) (Zhang et al., 2019) and three parasitic weeds (*Triphysaria versicolor*; *Phelipanche aegyptiaca* and *Striga hermonthica*) (Misra et al., 2019). However, no information is available yet for this important gene family in chickpea. Identification and characterization of all *CaSWEET* members in chickpea at genome-wide level will, therefore, be required for advancing their functional analyses in this important grain legume crop.

Thus, the aim of this present study was to systematically identify and characterize the *CaSWEET* gene family in chickpea. Specifically, we conducted a genome-scale survey to identify all putative *CaSWEET* members, and obtain their major characteristics, including physicochemical patterns, chromosomal distribution, subcellular localization, gene organization, conserved motifs and three-dimensional (3D) structure of the corresponding CaSWEET proteins. Subsequently, we explored available transcriptome data to attain expression profiles of *CaSWEET* genes in various major organs to compare their spatiotemporal transcript abundance. Finally, we studied the changes in their transcript levels in leaves and/or roots following dehydration and exogenous abscisic acid (ABA) treatments to obtain valuable information underlying their potential roles in chickpea responses to water-stress conditions.

2. Materials and methods

2.1. Web-based identification and annotation of CaSWEET members in chickpea

To comprehensively identify CaSWEETs in chickpea (*Cicer arietinum*), the conserved Pfam domain (https://pfam.xfam.org/) (El-Gebali et al., 2019) of SWEET, namely 'PF03083' (Julius et al., 2017; Baker et al., 2012), was used to search against the currently available proteomes of the 'Kabuli' (RefSeq assembly accession: GCF_000331145.1) (Varshney et al., 2013) and the 'Desi' (GenBank assembly accession: GCA_000347275.4) (Parween et al., 2015; Jain et al., 2013) chickpea databases available in the Phytozome version 12.0 (https://phytozome.jgi.doe.gov/pz/portal.html) (Goodstein et al., 2012) and National Center for Biotechnology Information (NCBI, https://ncbi.nlm.nih.gov). The E-value $\leq$ 1e−10 was selected as the best cut-off value for identification of CaSWEET proteins based on the results of a previous study (Feng et al., 2015). The basic annotated information of the identified *CaSWEET* members, including coding DNA sequence (CDS), genomic DNA sequence (gDNA), identifier code and chromosomal localization for each gene, were then obtained for further *in silico* analyses.

2.2. Protein features, conserved motif identification and 3D structure

The physicochemical parameters of CaSWEET proteins, including molecular mass (kilo Dalton, kDa), size (amino acid residues, aa-s), instability index, isoelectric point (pI) and grand average of hydropathicity (GRAVY) were retrieved by using the ExPASy Protparam (https://web.expasy.org/protparam) (Gasteiger et al., 2003). Instability indices <40 and >40 indicate potential stability and instability, respectively (Guruprasad et al., 1990). GRAVY values <0 and >0 suggest hydrophilic and hydrophobic characteristics, respectively (Kyte and Doolittle, 1982). Cellular localization of CaSWEET proteins was predicted using the TargetP server (http://cbs.dtu.dk/services/TargetP/) (Emanuelsson et al., 2007). The online Multiple EM for Motif Elicitation (MEME) analysis (http://meme.ebi.edu.au/meme/intro.html) (Bailey et al., 2015) and TMHMM server version 2.0 (http://cbs.dtu.dk/services/TMHMM) (Chaturvedi et al., 2011) were explored to predict the unknown conserved motifs and transmembrane helices in the full-length CaSWEETs, respectively. Two well-modeled SWEET proteins, OsSWEET2b from rice (Tao et al., 2015) and AtSWEET13 from *A. thaliana* (Han et al., 2017) were used for homology modeling of the 3D structures of the identified CaSWEETs with the aid of the Phyre2 server (http://sbg.bio.ic.ac.uk/~phyre2/html) (Kelley et al., 2015).

2.3. Phylogenetic analysis and prediction of gene duplication events

A Neighbor-Joining phylogenetic tree comprising the complete aa sequences of all identified CaSWEETs was constructed with the aid of the Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 (Kumar et al., 2016) as previously reported with 1000 bootstrapped replications (Chu et al., 2018; Niu et al., 2020). To analyze the duplication events occurred among all the putative *CaSWEET* genes, their identity matrix was constructed and used with the criterion of >70% identity at nucleotide level. The segmental or tandem duplication events were subsequently defined on the basis of the location of duplicated *CaSWEET* genes on different chromosomes or on the same chromosome within a region of 20 kb, respectively (Chu et al., 2018). The 'non-synonymous substitutions per non-synonymous site (Ka)' and 'synonymous substitutions per synonymous site (Ks)' values of duplicated *CaSWEET* genes were used to assess the selection history of this family using the DnaSP version 6.0 (Rozas et al., 2017). The exon/intron structure of each *CaSWEET* gene was analyzed by subjecting the CDS and

corresponding gDNA to the Plant Intron Exon Comparison and Evolution (PIECE) version 2.0 tool (https://wheat.pw.usda.gov/piece/) (Wang et al., 2013).

2.4. Expression patterns of CaSWEET genes in various organs and under various abiotic stress conditions from re-analysis of available RNA-sequencing data

To analyze spatiotemporal transcript abundance of *CaSWEET* genes in chickpea, we firstly downloaded two gene expression datasets of *C. arietinum* genotype ICC4958 (ecotype 'Desi'), which were obtained under normal growth conditions (Singh et al., 2013; Garg et al., 2011), from the Legume Information System (https://legumeinfo.org) (Dash et al., 2016). More specifically, the first dataset contained expression data in five organs [including shoot (S), root (R), mature leaf (ML), flower bud (FB) and young pod (YP)], which were generated based on the provided RPKM values (reads per kilobase of transcript per million mapped reads) (Garg et al., 2011). The second dataset contained expression data during various periods of flower development, including flower buds at sizes 4 mm (FB1), 6 mm (FB2), 8 mm (FB3) and 8–10 mm (FB4), and flowers with closed petals (FL1), partially opened petals (FL2), opened and faded petals (FL3) and senescing petals (FL4), which were presented in terms of provided $\log_2$ basemean values (Singh et al., 2013). A gene is declared differentially expressed if the RPKM value is >100 (Garg et al., 2011) or $\log_2$ basemean value is >10 (Singh et al., 2013) as previously described (Chu et al., 2018; Ha et al., 2014; Tran et al., 2018).

The following RNA-sequencing datasets were also used in expression analysis of the *CaSWEET* genes under various stress conditions: (i) four RNA-sequencing datasets of chickpea obtained under cold stress (GSE53711, root and shoot tissues of 10-day-old seedlings subjected to cold water at 4 ± 1 °C for 5 h) (Garg et al., 2015), drought [GSE70274, root tissues of chickpea plants subjected to drought treatment at early (flowering) and late (podding) reproductive stages using a 'dry-down' approach], salinity [GSE70377, root tissues from vegetative and reproductive stages of chickpea plants subjected to salinity at vegetative (40 mM NaCl applied before sowing and 40 mM after 8 days of sowing) and late reproductive stages (two doses of 40 mM NaCl applied by 5-day interval at the start of flowering)] (Garg et al., 2016), and heavy metal stress [GSE86807, leaf tissues of chickpea plants subjected to 150 μM concentration of arsenic (As), chromium (Cr) and cadmium (Cd) for 48 h] (Yadav et al., 2019) from the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) (Clough and Barrett, 2016), and (ii) two RNA-sequencing datasets of chickpea obtained under nutrient stress, including nodule tissues of 30-day-old chickpea plants (late vegetative stage) subjected to phosphate (Pi) deficiency (accession number in the DNA Data Bank of Japan: DRA005219) (Nasr Esfahani et al., 2017), and root and leaf tissues of 30-day-old chickpea plants subjected to nitrate (NO_3^-) and/or Pi deficiencies (DRA009618) (Nasr Esfahani et al., 2021). Differential gene expression was defined by a fold-change cut-off (|fold-change| ≥ 2.0). The cluster heatmap for the expression abundances of the *CaSWEET* genes under various stress conditions was visualized in R software with the gplots package (Liao et al., 2019).

2.5. Prediction of the cis-motifs

The 2-kb sequence upstream from the start codon site ('ATG') of each identified *CaSWEET* gene was obtained from the published chickpea genome (Varshney et al., 2013). The putative stress-responsive [e.g., low temperature-responsive element (LTRE) related to cold stress responsiveness, heat stress element (HSE) related to heat stress responsiveness and TC-rich repeats related to the defense and stress responsiveness] and phytohormone-responsive [e.g., ABA-responsive element (ABRE, i.e. 'ACGTG'), jasmonic acid-responsive elements (JAREs), including CGTCA-motif and TGACG-motif, gibberellin-responsive elements (GbREs), including GARE-motif ('TCTGTTG') and P-box ('CCTTTTG'), salicylic acid-responsive element (SARE, i.e. the TCA-element), auxin-responsive elements (AuREs), including TGA-element ('AACGAC') and AuxRR-core ('GGTCCAT'), and ethylene-responsive element (ERE, i.e. 'ATTTTAAA')] *cis*-motifs located in these sequences were predicted using the PlantCARE tool (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) (Lescot et al., 2002). In addition, seven drought/dehydration-responsive *cis*-motifs, including dehydration-responsive element (DRE, i.e. 'GCCGAC' or 'ACCGAC'), MYB-binding site (MBS, i.e. 'CAACTG' or 'TAACTG'), MYC recognition site (MYCR, i.e. 'CACATG'), coupling element 3 (CE3, i.e. ' CACGCG'), T/G Box ('CACGTT'), evening element (EE, i.e. 'AATATC') and NAC recognition site (NACR, i.e. 'CACGCA') (Mathiyalagan et al., 2010; Maruyama et al., 2012; Abe et al., 2003; Yamaguchi-Shinozaki and Shinozaki, 2005), were also manually searched in the promoter regions as previously described (Chu et al., 2018).

2.6. Plant materials and plant treatments with dehydration and ABA

Seedlings of chickpea Hashem cultivar (ecotype 'Kabuli'), were used for expression analysis of the identified *CaSWEET* genes following dehydration and abscisic acid (ABA) treatments. Chickpea seeds were germinated and seedlings were grown in vermiculite in greenhouse under the growth conditions as described previously (continuous 30 °C temperature, 12/12 h light/dark cycle, 60% relative humidity and 150 μmol $m^{-2}s^{-1}$ photon flux density) (Ha et al., 2014). Nine-day-old plants were carefully lifted from the pots and gently washed by distilled water to remove soil before exposing them to dehydration, and ABA and water control treatments (Ha et al., 2014). For dehydration treatment, plants were dried onto a 3 M filter paper (Toyo Roshi Kaisha, Japan) for 2 and 5 h on bench under the laboratory conditions (Ha et al., 2014). The intensity of the time-course dehydration treatment was estimated by the relative water contents of the treated samples (2-h- and 5-h-dehydrated plants were 55% and 33%, respectively) (Ha et al., 2014). For water control and ABA treatments, the root part of each plant was submerged in water or an ABA solution of 100 μM, respectively, for 2 and 5 h under the laboratory conditions (Ha et al., 2014). Subsequently, roots and leaves were separately collected from treated plants ($n = 3$ biological replicates, one plant/replicate), immediately frozen in liquid nitrogen, and stored at −80 °C until further analyses.

2.7. Primer designing, real-time quantitative PCR and statistical analyses

The primers used for real-time quantitative polymerase chain reaction (RT-qPCR) were designed with the aid of the Primer-BLAST tool from NCBI (https:// ncbi.nlm.nih.gov/tools/primer-blast) (Ye et al., 2012) and listed in Table S1. For primer design, the following parameters were set: melting temperature between 55 and 65 °C, primer length between 19 and 24 bp, and amplicon lengths between 100 and 255 bp. The primer specificity was determined by analyzing the respective melting curves and amplicon fragments. The amplification efficiency of each primer pair was analyzed based on linear regression of the exponential section of the curve using LinRegPCR software (Ruijter et al., 2009) (Table S1). The *Initiation factor 4a* (*IF4a*, GenBank accession number: FL512356) was explored as a reference gene (Garg et al., 2010). RT-qPCR was carried out in the 96-well plates using a PCR cycler (Mx3000P, Stratagene, Germany). The ΔCT method was initially used to determine the transcript levels of *CaSWEET* genes as previously described (Silver et al., 2006). Significant differences in expression changes at one-time point was assessed using a Student's *t*-test (|fold-change| ≥ 2.0; *P*-value < 0.05).

3. Results and discussion

3.1. Identification and chromosomal distribution of the CaSWEET genes

To identify all potential members of the CaSWEET family in chickpea, a broad search for the Pfam domain of SWEETs (Julius et al., 2017; Baker et al., 2012) was performed on the currently available chickpea proteome (Varshney et al., 2013) found on the Phytozome database (Goodstein et al., 2012). Our genome-scale analysis revealed a total of 21 potential CaSWEET proteins in chickpea with the recommended cut-off E-value $\leq$ 1e−10 (Table 1). Subsequently, the aa sequence of each CaSWEET was used to BlastP against the publically available chickpea assembly and annotation (Varshney et al., 2013) to obtain the corresponding gene identification code, nucleotide sequences (both CDS and gDNA) and chromosomal distribution. All the detailed relevant information obtained for each CaSWEET member is provided in Table 1 and Dataset 1.

We noted that the number of *SWEET* genes is greatly variable among the green plants (Viridiplantae) (Jia et al., 2017) (Table S2). Briefly, one to four copies of *SWEET* genes have been identified in unicellular and green algae, while approximately 13–108 *SWEET* genes have been found in plants (Jia et al., 2017) (Table S2). More specifically, genome-wide analyses of the *SWEET* gene family in several monocotyledonous plants revealed that *P. equestris*, rice, *D. officinale*, sorghum, banana and wheat have 16 (Wang et al., 2018); 21 (Yuan and Wang, 2013); 22 (Wang et al., 2018); 23 (Mizuno et al., 2016); 25 (Miao et al., 2017) and 108 (59 in the earlier report) (Gao et al., 2018; Gautam et al., 2019), respectively (Table S2). Results of genome surveys of dicotyledonous plants revealed that the members of *SWEET* gene family are also highly variable, ranging from 13 in both *C. sinensis* (Wang et al., 2018) and *T. versicolor* (Misra et al., 2019), to 17 in both of grapevine and cucumber (Chong et al., 2014; Hu et al., 2017), 52 in soybean (Patil et al., 2015), and 68 in oilseed rape (Jian et al., 2016) (Table S2). Our study demonstrated that the number (21) of *CaSWEET* genes found in chickpea is higher than in several dicotyledonous plants, such as *C. sinensis* (13) (Wang et al., 2018); *T. versicolor* (13) (Misra et al., 2019), litchi (16) (Xie et al., 2019), grapevine (17) (Chong et al., 2014), cucumber (17) (Hu et al., 2017) and *P. bretschneideri* (Li et al., 2017a), while being lower than in *G. arboreum* (22) (Zhao et al., 2018); *M. truncatula* (25) (Hu et al., 2019), apple (25) (Chen et al., 2017); *S. hermonthica* (25) (Misra et al., 2019) and tomato (29) (Feng et al., 2015), and less than that in cabbage (30) (Zhang et al., 2019); *G. raimondii* (31) (Zhao et al., 2018), Chinese cabbage (32) (Miao et al., 2018), soybean (52) (Patil et al., 2015); *G. hirsutum* (55) (Zhao et al., 2018) and oilseed rape (68) (Jian et al., 2016) (Table S2).

In chickpea genome, 20 *CaSWEET* genes, excluding *CaSWEET21* that was localized to unassembled genomic sequence scaffolds, were plotted on seven out of eight chromosomes (except chromosome Ca8) (Fig. 1). Chromosome Ca5 contains five, i.e. the maximum number of *CaSWEET* genes per chromosome (Fig. 1). Chromosomes Ca1, Ca2, Ca3, Ca4, and Ca6 also possess multiple CaSWEET genes, namely three, four, two, two and three members, respectively, while Ca7 has only one member (*CaSWEET20*) (Fig. 1). Interestingly, several *CaSWEET* genes like *CaSWEET01*, *03*, *04*, *08* and *09* were noted to be localized on the region near the chromosome ends (Fig. 1). More specifically, four genes, *CaSWEET01* and *03*, and *CaSWEET08* and *09* were found in the subtelomeric region of chromosomes Ca1 and Ca3, respectively, while only *CaSWEET04* was localized near the end of the Ca2 (Fig. 1). Previously, this similar phenomenon was observed in all identified *SWEET* gene families in plant genome, such as in tomato (Feng et al., 2015), soybean (Patil et al., 2015); oilseed rape (Jian et al., 2016), sorghum (Mizuno et al., 2016), Chinese white pear (Li et al., 2017a), cucumber (Hu et al., 2017), cotton species (Zhao et al., 2018), apple (Zhen et al., 2018), Chinese cabbage (Miao et al., 2018); wheat (Gao et al., 2018; Gautam et al., 2019), litchi (Xie et al., 2019); *M. truncatula* (Hu et al., 2019), cabbage (Zhang et al., 2019). Our observation suggests that the distribution of several *SWEET* genes on the subtelomeric region of the chromosomes may play important roles in the chromosome recognition and pairing during meiosis in chickpea, as previously suggested in other plants (Calderón et al., 2014).

3.2. Phylogenetic relationship and physicochemical properties of the CaSWEET members

To study the phylogenetic relationship of the CaSWEET proteins, a neighbor-joining tree was created by aligning 21 identified full-length CaSWEET proteins using the MEGA software (Kumar et al., 2016). We found that four previously reported clades of SWEET proteins (Patil et al., 2015) were clearly found in the resulting tree (Fig. 2A). All CaSWEET proteins were classified into four groups (Clade I-IV) (Fig. 2A), with clade I and IV, each having seven CaSWEET members. As supported by the high bootstrap values separating the groups, we

Table 1
Overview of *Cicer arietinum SWEET* gene family.

#	Gene name	Transcript identifier	Protein identifier	Locus identifier	Size	Molecular mass	pI	Instability index	GRAVY	Subcellular localization
1	*CaSWEET01*	XM_004488926.2	XP_004488983.1	LOC101509458	239	26.75	6.80	45.94	0.85	S
2	*CaSWEET02*	XM_004487778.2	XP_004487835.1	LOC101509872	235	25.67	8.69	37.73	0.70	S
3	*CaSWEET03*	XM_012719392.1	XP_012574846.1	LOC101498095	253	28.65	5.83	37.62	0.79	S
4	*CaSWEET04*	XM_004489049.2	XP_004489106.1	LOC101497133	254	28.75	8.16	37.33	0.75	S
5	*CaSWEET05*	XM_004490445.2	XP_004490502.1	LOC101497351	270	30.28	8.95	27.39	0.78	S
6	*CaSWEET06*	XM_004489239.2	XP_004489296.1	LOC101506045	246	27.36	9.17	33.81	0.67	–
7	*CaSWEET07*	XM_004489238.2	XP_004489295.1	LOC101505723	237	26.76	8.33	39.78	0.71	–
8	*CaSWEET08*	XM_004491623.2	XP_004491680.1	LOC101511936	257	28.84	9.23	35.43	0.62	S
9	*CaSWEET09*	XM_004491624.2	XP_004491681.1	LOC101512270	259	29.05	9.40	33.26	0.67	S
10	*CaSWEET10*	XM_004498321.2	XP_004498378.1	LOC101498274	242	26.80	9.47	34.69	0.69	S
11	*CaSWEET11*	XM_004498340.2	XP_004498397.1	LOC101504169	259	28.71	9.44	36.82	0.49	–
12	*CaSWEET12*	XM_004502518.2	XP_004502575.1	LOC101515250	247	27.44	9.65	27.76	0.79	S
13	*CaSWEET13*	XM_004501669.2	XP_004501726.1	LOC101510607	296	33.47	8.35	54.85	0.33	S
14	*CaSWEET14*	XM_004501759.2	XP_004501816.1	LOC101488880	262	29.22	9.15	31.90	0.66	S
15	*CaSWEET15*	XM_004502010.1	XP_004502067.1	LOC101512545	253	28.07	9.59	41.52	0.76	S
16	*CaSWEET16*	XM_004502557.2	XP_004502614.1	LOC101499800	250	27.91	8.10	26.72	0.69	S
17	*CaSWEET17*	XM_004503532.2	XP_004503589.1	LOC101488443	251	27.79	9.25	40.29	0.71	S
18	*CaSWEET18*	XM_004503722.2	XP_004503779.1	LOC101491370	281	31.77	8.31	35.50	0.54	S
19	*CaSWEET19*	XM_004503721.1	XP_004503778.1	LOC101491054	255	28.58	9.74	40.48	0.90	S
20	*CaSWEET20*	XM_004508799.2	XP_004508856.1	LOC101491395	230	26.29	9.28	34.32	0.87	–
21	*CaSWEET21*	XM_004515143.1	XP_004515200.1	LOC101489507	232	25.93	8.96	40.75	0.93	S

The protein size (amino acid residues, aa-s), molecular mass (kilo Dalton, kDa), isoelectric point (pI), GRAVY (grand average of hydropathy), S (secretory pathway), "–" (no information).

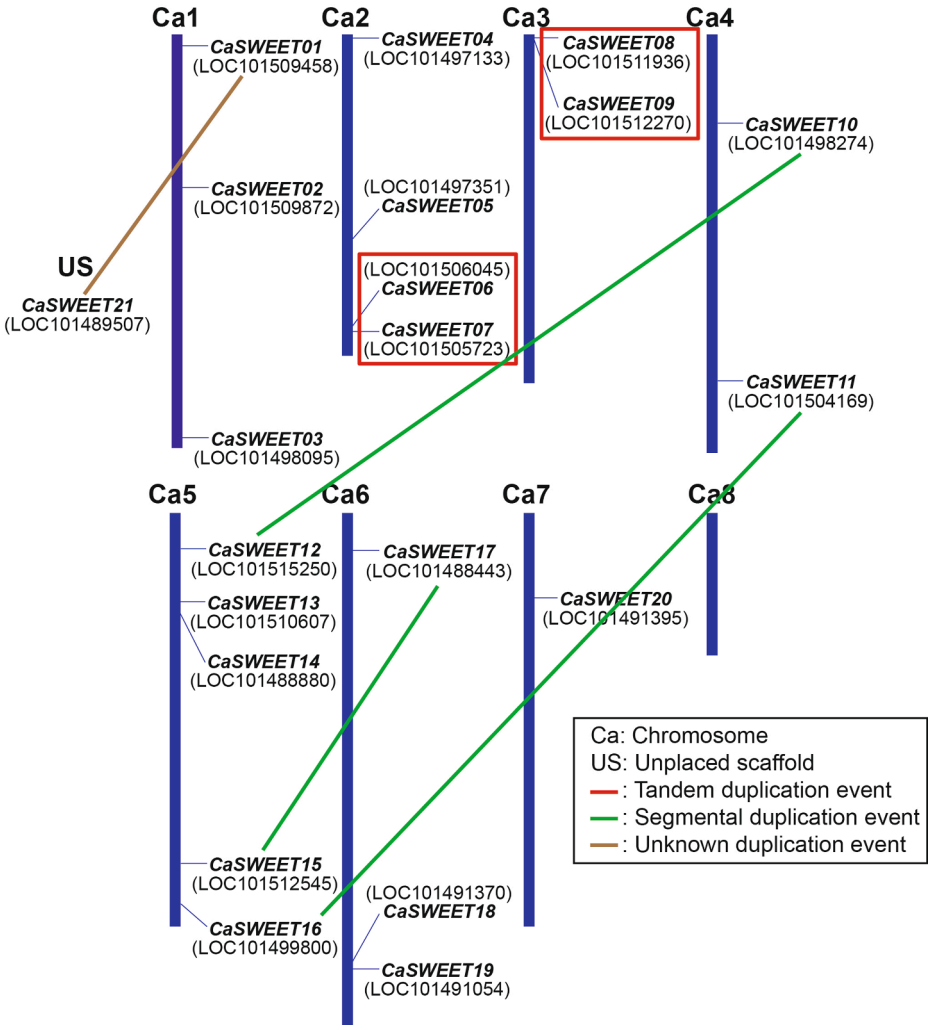

Fig. 1. Chromosomal locations and duplication events of chickpea *CaSWEET* genes. Brown line shows the unknown duplication event, while red boxes and green lines show the tandem and segmental duplication events, respectively.

suggested that the CaSWEET proteins in each clade should share the highly conserved characteristics at the aa level (Fig. S1).

To obtain the physicochemical properties of CaSWEETs, the full-length aa sequences were globally analyzed by the ExPASy Protparam online (Gasteiger et al., 2003). Based on the detailed information, the lengths of these CaSWEET proteins ranged from 230 (CaSWEET20) to 296 (CaSWEET13) aa-s, with the average of lengths being 252.76 aa-s, while the molecular masses ranged from 25.67 (CaSWEET02) to 33.47 (CaSWEET13) kDa, with the average of molecular masses being 28.29 kDa (Table 1). The predicted pI values of the CaSWEETs varied from the acidic (CaSWEET03 with pI of 5.83) to basic (CaSWEET19 with pI of 9.74), with the average pI of 8.75 (Table 1). Our findings were supported by previously published data in other plants. For example, SWEET proteins with the lengths from 233 to 308 aa-s were found in tomato (Feng et al., 2015), 56–303 aa-s (6.50–33.45 kDa) in *B. napus* (Jian et al., 2016), 171–333 aa-s (19.10–37.42 kDa) in banana (Miao et al., 2017), 215–340 aa-s in apple (Zhen et al., 2018) and 229–300 aa-s (25.6–33.6 kDa) in litchi (Xie et al., 2019). Additionally, the majority of CaSWEET proteins (15 out of 21) was predicted to be stable, with the value of instability index being smaller than 40 (Guruprasad et al., 1990) (Table 1). In addition, the scores of GRAVY were higher than 0, suggesting that all CaSWEETs are more likely membranous, i.e. hydrophobic (Table 1). Next, the cellular localization of the CaSWEET proteins was investigated by searching their peptides against the TargetP tool (Emanuelsson et al., 2007). Most CaSWEET proteins (17 out of 21) were found to target secretory pathway (Table 1). Taken together, the highly variable structure of SWEETs in plant species may indicate their divergent functional roles in various biological processes and/or under different growth conditions.

3.3. Gene structure and evolution of the CaSWEET genes

It has been suggested that gene duplication occurred during the process of chickpea evolution (Varshney et al., 2013). In the next line of our study, gene duplication was analyzed among the *CaSWEET* members based on the identity matrix at the nucleotide level of their CDS (Fig. S2). A total of six duplication events (three segmental, two tandem and an unknown duplication events) were found in the *CaSWEET* gene family (Fig. 1; Fig. S1; Table S3). Particularly, three pairs of segmental duplicated genes were found with an identity of 72.6% (*CaSWEET15* and *17* localized on chromosomes Ca5 and Ca6, respectively), 73.3% (*CaSWEET11* and *16* localized on chromosomes Ca4 and Ca5, respectively) and 74.8% (*CaSWEET10* and *12* localized on chromosomes Ca4 and chromosome Ca5, respectively) (Fig. 1; Fig. S1; Table S3). Two pairs of *CaSWEET* genes, *CaSWEET06* and *07* (75.6%), and *CaSWEET08* and *09* (77.3%) were regarded as tandem-duplicated genes on the chromosomes Ca2 and Ca3, respectively (Fig. 1; Fig. S1; Table S3), on the basis of the preset criteria of localization on the same chromosome and within a region of 20 kb (Chu et al., 2018). In addition, a duplicated pair of *CaSWEET01* (Ca1) and *CaSWEET21* (unplaced scaffold) was also found

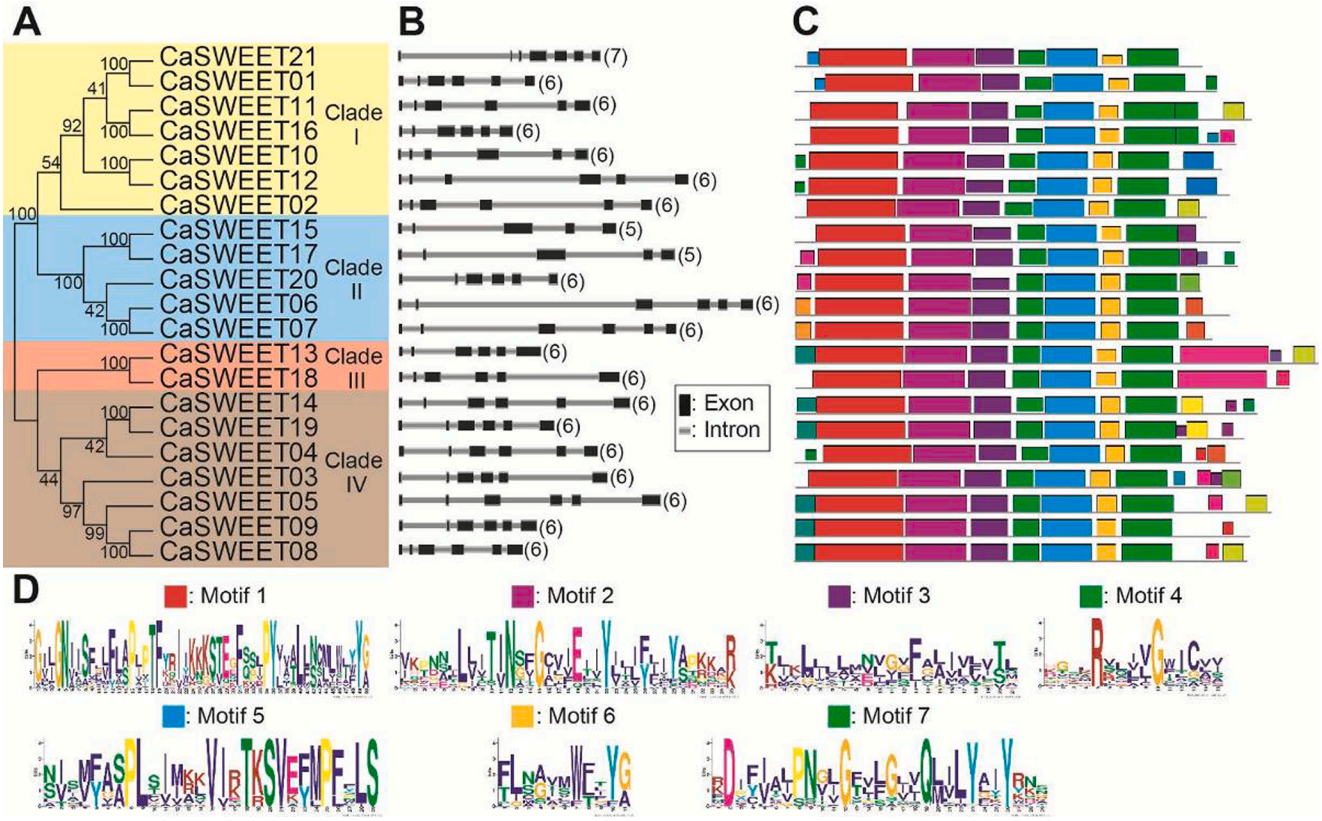

Fig. 2. Structural investigation of CaSWEET proteins in chickpea based on the neighbor-joining phylogenetic tree. (A) Phylogenetic analysis of CaSWEET proteins. (B) Gene structure of *CaSWEET* genes with the number of exons describing at the end of each gene. (C) The sequences of seven highly conserved motifs found in the CaSWEET proteins. (D) Discovery of motif compositions in the full-length CaSWEET proteins.

to have an identity of 73.5% (Fig. 1; Fig. S1; Table S3). Previously, Patil et al. (2015) have analyzed and summarized a total of 56 tandemly duplicated genes and 95 segmental duplication events (out of 411 *SWEET* genes) occurred in 23 plant genomes. Similarly, 21/2, 9/6, 11/0 and 7/0 were identified as segmental/tandem duplication events in oilseed rape (Jian et al., 2016), Chinese white pear (Li et al., 2017a), Chinese cabbage (Li et al., 2018) and cabbage (Zhang et al., 2019), respectively. On the other hand, 51 pairs of *SWEETs* (19 *GhSWEET* pairs in *G. hirsutum*, 24 *GbSWEET* pairs in *G. barbadense*, three *GaSWEET* pairs in *G. arboreum* and five *GrSWEET* pairs in *G. raimondii*) were involved in segmental duplication events, while the tandem duplication event was not found in *Gossypium* spp. (Zhao et al., 2018). More recently, 0/2, 1/3 and 8/12 were assigned as segmental/tandem duplication events the *SWEET* gene family in cucumber (Hu et al., 2017), litchi (Xie et al., 2019) and *M. truncatula* (Hu et al., 2019), respectively. These results suggest that the contribution of segmental duplication events to the expansion of the *CaSWEET* gene family in chickpea was greater than that of tandem duplications, as it was also observed in oilseed rape (Jian et al., 2016), Chinese white pear (Li et al., 2017a), Chinese cabbage (Li et al., 2018), cabbage (Zhang et al., 2019) and *Gossypium* spp. (Zhao et al., 2018). Additionally, these duplication events of the *CaSWEET* genes in chickpea, and perhaps also in other plant species, might contribute to their reproductive processes (Li et al., 2017a; Patil et al., 2015), as well as their ability to adapt to diverse environmental conditions (Chandran, 2015; Li et al., 2017b).

Next, to estimate the selective pressure of natural selection on the duplicated *CaSWEET* genes, the Ka/Ks ratios were calculated (Rozas et al., 2017). Principally, Ka/Ks values more than one indicate Darwinian (positive) selection, while those less than one provide evidence of purifying (stabilizing) selection, and Ka/Ks = 1 supports the hypothesis of neutral evolution (Li et al., 2009). The Ka/Ks values of all duplicated *CaSWEET* gene pairs were less than one (Table S3), indicating that their evolution occurred under the influence of strong purifying selection. Previously, the Ka/Ks values of the duplicated *SWEET* genes in many other plant species, such as soybean (Patil et al., 2015), banana (Miao et al., 2017) and cabbage (Zhang et al., 2019) were far less than one, indicating the occurrence of strongly purifying selection during the evolution of these *SWEET* gene families. Taken together, these findings suggested that purifying selection was the main force driving the evolution of the *SWEET* genes in chickpea, and perhaps in some other higher plant species.

In addition, the evolution of the *CaSWEET* gene family was strongly supported by gene structure and conserved motif analyses. First, the structure of *CaSWEET* genes was investigated using the PIECE tool (Wang et al., 2013). We found that all *CaSWEET* genes from chickpea contain intron regions in their gDNA sequences (Fig. 2B), and a greater number of *CaSWEET* genes (18 out of 21) contain six exons, while only one (*CaSWEET21*) and two *CaSWEET* genes (*CaSWEET15* and *17*) contain seven and five exons, respectively (Fig. 2B). Such common structure of six exons in the *CaSWEET* genes was also found in many members of the *SWEET* gene family of many other plant species, including tomato (23 out of 29) (Feng et al., 2015), oilseed rape (51 out of 68) (Jian et al., 2016), cucumber (12 out of 17) (Hu et al., 2017), Chinese white pear (12 out of 18) (Li et al., 2017a), Chinese cabbage (26 out of 34) (Miao et al., 2018), tea plant (11 out of 13) (Wang et al., 2018), cabbage (15 out of 30) (Zhang et al., 2019) and litchi (14 out of 16) (Xie et al., 2019). These findings together indicate that the majority of the *SWEET* genes in plants might have the conserved structure of six exons.

3.4. Motif compositions and homology modeling of the CaSWEETs

Typically, SWEET proteins in plant species contain seven transmembrane (TM) helices, possessing two conserved MtN3/saliva domains (PF03083), each of which is a triple helix bundle consisted of three TM helices (Julius et al., 2017; Baker et al., 2012). Our alignment of the full-length CaSWEET protein sequences showed that the CaSWEET members share seven highly conserved TM helices, including two repeats of three TM sub-domains ('TM1-TM2-TM3′ and 'TM5-TM6-TM7′) divided by a single TM unit (TM4) (Figs. S2 and S3), which was in agreement with the presence of seven putative motifs in the CaSWEET proteins (Fig. 2C, D) as also demonstrated earlier in other plant species (Xuan et al., 2013). Moreover, the existence of the seven TM regions in all CaSWEET members suggests that CaSWEET proteins are membrane receptors, as previously reported in *B. rapa* (Miao et al., 2018), litchi (Xie et al., 2019) and some other plant species (Patil et al., 2015).

Next, we investigated the 3D structures of CaSWEET proteins. Briefly, using the Phyre2 (Kelley et al., 2015), predicted models for the 3D structures of CaSWEET proteins were generated based on the reported templates 'c5cthB' and 'c5xpdA' from the structures of OsSWEET2b of rice (Tao et al., 2015) and AtSWEET13 of *Arabidopsis* (Han et al., 2017), respectively, to maximize the alignment coverage, percentage identity and confidence scores of the full-length CaSWEET proteins. The high percentage values of structural coverage between the full-length sequences and the corresponding predicted 3D models clearly demonstrated that the 3D structure prediction of CaSWEETs is highly reliable (Fig. S4). Data obtained from Phyre2 revealed both the 2D and 3D structures. We found that the α-helix was the major secondary structure in each of the CaSWEET proteins (67–85%), whereas β-strand was only detected in CaSWEET12 (1%) (Fig. S4). As for the 3D structure, template 'c5xpdA' from the structure of AtSWEET13 (Han et al., 2017) could be used to predict the structure of most CaSWEET proteins (17 out of 21), while template 'c5cthB' from the structure of OsSWEET2b (Tao et al., 2015) could be used for the modeling of only CaSWEET01, 07, 20 and 21 (Fig. S4). Taken together, the modeling results revealed that the CaSWEET proteins identified in this study shared the similar tertiary structures, implying that the majority of these proteins might have been evolved from the same ancestor sequence and/or under putifying selection process to maintain stabilization during the long-term acclimatization after their initial divergence. Furthermore, our findings may also suggest the conserved 3D structures among the SWEET proteins of dicot plant species.

3.5. Enrichment analysis of cis-motifs in promoters of the CaSWEET genes

The occurrence of various well-characterized stress- and phytohormone-responsive *cis*-acting elements in the promoter of a gene may indicate its possible response patterns to different environmental stresses (Chu et al., 2018; Mochida et al., 2011). Fig. S5 illustrated various types and number of stress- and phytohormone-responsive *cis*-motifs analyzed in the 2-kb upstream sequence of each *CaSWEET* gene along with their occurrence. These *cis*-elements included those responsive to drought/dehydration, heat stress and low temperature stress like MBS, MYCR, DRE, CE3, T/G Box, EE, NACR, TC-rich repeats, HSE and LTRE, and those responsive to phytohormones like ABRE, JAREs (CGTCA-motif and TGACG-motif), GbREs (GARE-motif and P-box), SARE (TCA-element), AuREs (TGA-element and AuxRR-core) and ERE (Fig. S5). Results indicated that most of the 21 *CaSWEET* promoter regions, except that of the *CaSWEET10*, contained one or more phytohormone-responsive *cis*-elements (Fig. S5). For example, the promoter regions of 14 (out of 21) *CaSWEET* genes have two types of JAREs (CGTCA-motif and TGACG-motif) (Nakashima and Yamaguchi-Shinozaki, 2013), while only those of *CaSWEET04*, *11* and *19* contain the GbREs (GARE-motif and P-box) (Nakashima and Yamaguchi-Shinozaki, 2013) (Fig. S5). Two types of AuREs, TGA-element and AuxRR-core (Nakashima and Yamaguchi-Shinozaki, 2013), were found in the promoter regions of *CaSWEET04* and *20*, while SARE (TCA-element) and ERE (Nakashima and Yamaguchi-Shinozaki, 2013) were found in 10 and 11 *CaSWEET* promoter regions, respectively (Fig. S5). Interestingly, we found the high occurrence frequency of ABRE) in the promoter regions of the *CaSWEET* members (17 out of 21) (Fig. S5). The number of ABRE ranged from 1 (*CaSWEET04*, *07*, *08*, *09*, *15*, *17* and *20*) to 4 (*CaSWEET02*, *03*, *05* and *06*), with the average of 1.81 ABREs/gene (Fig. S5). These findings suggest that the majority of *CaSWEET* genes might be involved in the signal transduction mediated by ABA.

Since identification of *CaSWEET* genes involved in environmental stress responses is our main interest, we focused on the search for the presence(s) of well-known stress-responsive *cis*-motifs, including MBS, LTRE, HSE, TC-rich repeats, MYCR, DRE, CE3, T/G Box, EE and NACR, in the *CaSWEET* genes. A total of five LTREs and four TC-rich repeats were found in the promoter sequences of four (*CaSWEET07*, *09*, *16* and *21*) and four *CaSWEET* genes (*CaSWEET08*, *10*, *15* and *18*), respectively (Fig. S5), while 41 HSEs were enriched in the majority of *CaSWEET* genes (18 out of 21), with the average of 1.95 HSEs/gene (Fig. S5). Interestingly, all members in the *CaSWEET* gene family contained at least one type of dehydration/drought-responsive *cis*-motifs (Fig. S5). Among them, MBS, EE and MYCR were found as the three most enriched dehydration/drought-responsive *cis*-motifs, with the total occurrence of 27, 25 and 23 in 11, 16 and 14 *CaSWEET* genes, respectively (Fig. S5). Taken together, our promoter analysis implied that the *CaSWEET* genes might be involved in chickpea responses to different types of environmental stress, particularly dehydration/drought.

3.6. Tissue-specific expression patterns of CaSWEET genes in chickpea

To analyze the tissue-specific expression patterns of 21 *CaSWEET* genes, two transcriptome datasets of *C. arietinum* genotype ICC4958 (Singh et al., 2013; Garg et al., 2011) from the LIS website were explored (Dash et al., 2016). According to the heatmaps shown in Fig. 3, expression data were available for only 14 *CaSWEET* genes, including *CaSWEET01*, *03*, *05*, *06*, *07*, *10*, *11*, *12*, *13*, *15*, *17*, *18*, *19* and *21*. Based on the first expression dataset in five major tissues (Garg et al., 2011); five *CaSWEET* genes, *CaSWEET01*, *02*, *05*, *06* and *19* showed relatively low transcript levels in all examined tissues (Fig. 3A). Six other *CaSWEET* genes, *CaSWEET07*, *11*, *12*, *13*, *17* and *18*, displayed expression in at least one organ (Fig. 3A). Interestingly, three genes *CaSWEET03*, *10* and *15* were noted to exclusively express in FB (Fig. 3A).

Furthermore, we also found that 13 out of 14 explored *CaSWEET* genes, excluding *CaSWEET15*, were strongly expressed in three vegetative tissues and/or eight stages of flower development based on the dataset produced by Singh et al. (2013) (Fig. 3B). Among them, *CaSWEET10* was exclusively expressed in all FB and FL tissues, while *CaSWEET13* was found to be a highly expressed gene in four stages of FL tissues (Fig. 3B), suggesting their potential roles in the development of reproductive organs (Lin et al., 2014). Similarly, *AnmSWEET5* and *11* were reported to highly express during the early stages of fruit development of *Ananas comosus* (Guo et al., 2018), while nine *MdSWEET* genes were highly expressed during the fruit development of apple plant, of which *MdSWEET9b* and *15a* were found to participate in regulation of sugar accumulation in apple (Zhen et al., 2018). During the flower developmental stages of *Petunia axillaris*, transcript levels of *PaSWEET1d*, *5a*, *9a*, *13c* and *14a* were found to increase with the maturation of the flower and reach their maximum in the fully open flowers (Iftikhar et al., 2020). In *Jasminum sambac*, the transcripts of *JsSWEET2* and *9* were shown to significantly accumulated in fully-opened flower tissues, while those of *JsSWEET1*, *5*, *10* and *17* slightly accumulated at stage associated with fragrance release, suggesting their specific function in sugar transport and allocation during flowering and reproductive processes (Iftikhar et al., 2020). Therefore, it will be interesting to further delineate the functions of flower-specific *CaSWEET* genes during reproductive development of chickpea plants.

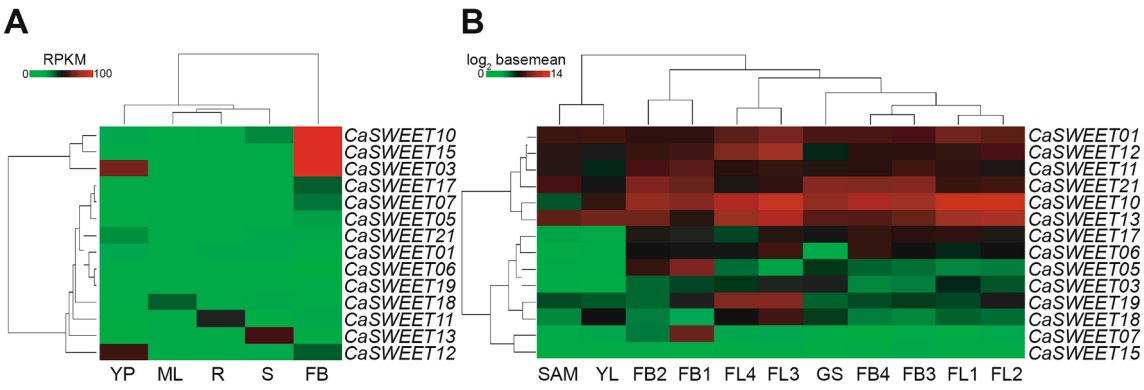

Fig. 3. Tissue-specific expression patterns of *CaSWEETs* in various organs. (A) Heatmap showing expression levels of the *CaSWEET* genes in different tissues. The expression of *CaSWEET* genes in five major organs, including shoot (S), root (R), mature leaf (ML), flower bud (FB) and young pod (YP) were analyzed using the RPKM (reads per kilobase of transcript, per million mapped reads) values according to Garg et al. (2011). (B) Heatmap showing expression levels of the *CaSWEET* genes at numerous stages of flower development. The expression of *CaSWEET* genes in shoot apical meristem (SAM), germinating seedling (GS), young leaf (YL), four stages of flower bud development (FB1-4), and four stages of flowering (FL1-4) were analyzed using the $\log_2$ basemean values according to Singh et al. (2013). The green and red colors indicated the low and high levels of transcript abundance of the *CaSWEET* genes, respectively.

Next, we re-analyzed the expression patterns of the *CaSWEET* genes in six transcriptome datasets of chickpea plants under diverse abiotic stresses, including exposure to heavy metal stress (Yadav et al., 2019) (Fig. 4A), cold stress (Garg et al., 2015) (Fig. 4B), drought and salinity (Garg et al., 2016) (Fig. 4C, D), and NO_3^- and/or Pi deficiencies (Nasr Esfahani et al., 2017; Nasr Esfahani et al., 2021) (Fig. 4E). Under heavy metal stress, only *CaSWEET01* and *10* were induced in Cd- and As-treated leaves, respectively, while *CaSWEET12* was up-regulated in both Cd- and As-treated leaves (Fig. 4A). However, no *CaSWEET* genes were found to be responsive to Cr treatment (Fig. 4A). Under cold stress, only *CaSWEET04* was up-regulated in shoots, whereas three genes, namely *CaSWEET02*, *13* and *15* were down-regulated in roots (Fig. 4B). With respect to drought, five *CaSWEET* genes, namely *CaSWEET12*, *13*, *17*, *19* and *20*, were found to be differentially expressed in roots at early

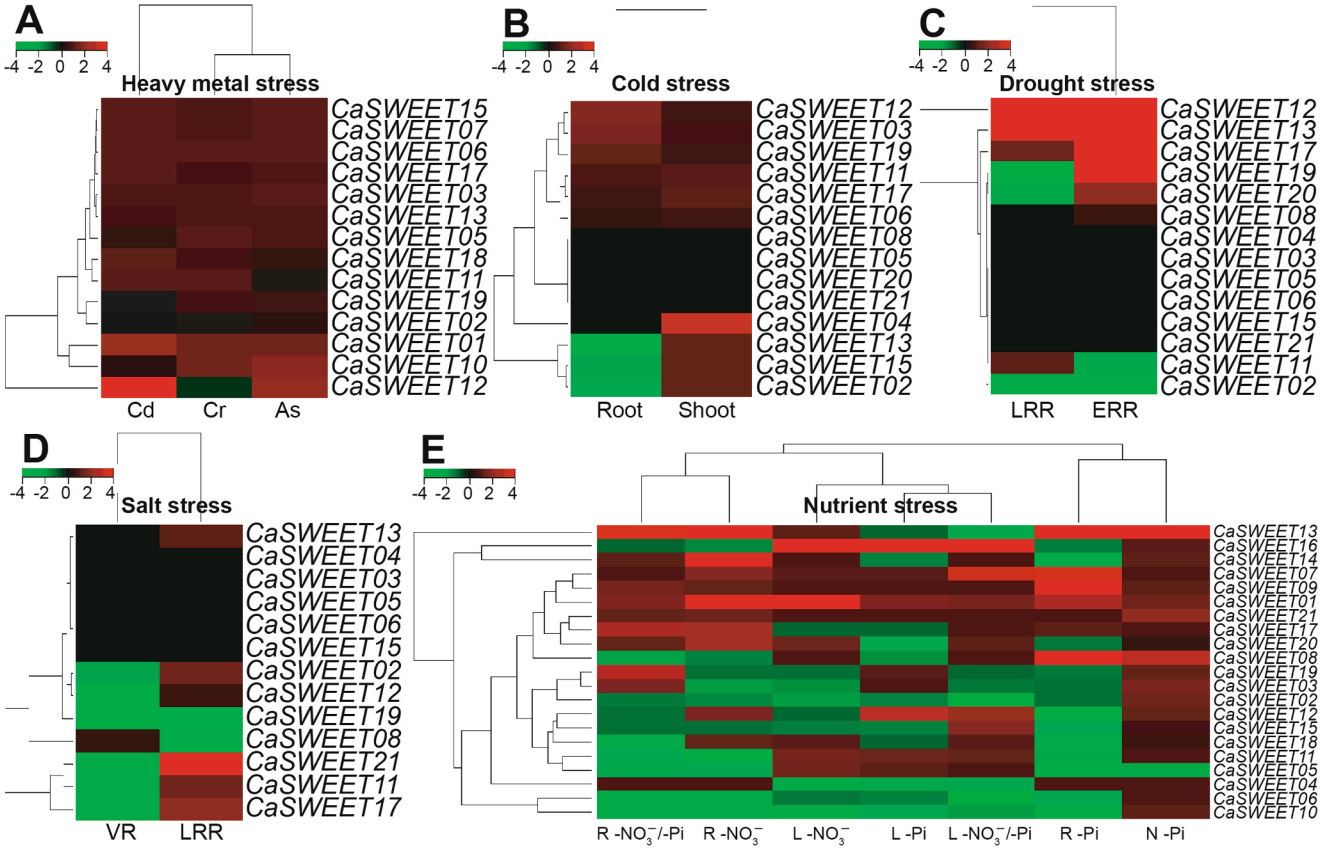

Fig. 4. Heat map of the expression of *CaSWEET* genes under different abiotic stressors. The abiotic stressors include (A) heavy metals [arsenic (As), chromium (Cr) or cadmium (Cd)] according to the GSE86807 dataset, (B) cold according to the GSE53711 dataset, (C) drought according to the GSE70274 dataset, (D) salt according to the GSE70377 dataset, (E) nitrate and/or phosphate deficiencies according to the DRA005219 and DRA009618 datasets. Color scales indicate expression changes where green and red colors indicate down-regulation and up-regulation, respectively. VR, ERR and LRR represent root tissues at vegetative, early and late reproductive stages, respectively. R, root tissues; N, nodule tissues; L, leaf tissues; —NO_3^-, nitrate deficiency; —Pi, phosphate deficiency; —NO_3^-/—Pi, combined nitrate and phosphate deficiency.

and/or late reproductive stages (Fig. 4C). Among them, two genes, *CaSWEET12* and *13*, and *CaSWEET02* were strongly induced and reduced, respectively, in roots under drought (Fig. 4C). In response to salinity, the expression of seven *CaSWEET* genes, including *CaSWEET02*, *08*, *11*, *12*, *17*, *19* and *21* was down-regulated in roots at early and/or late reproductive stages (Fig. 4D). Under NO_3^- and/or Pi deficiencies, 12 (*CaSWEET01*, *07*, *08*, *09*, *12*, *13*, *14*, *16*, *17*, *19*, *20* and *21*) and 11 *CaSWEET* genes (*CaSWEET02*, *04*, *05*, *06*, *10*, *11*, *12*, *14*, *15*, *18* and *20*) were up-regulated and down-regulated, respectively, in at least one organ among nodule, roots and leaves (Fig. 4E). Among them, *CaSWEET05* and *13* were down-regulated and up-regulated, respectively, in both roots and nodules, while *CaSWEET04* and *16* were down-regulated and up-regulated, respectively, in leaves under all tested nutrient deficiency conditions (Fig. 4E). Taken together, our detailed analysis suggested that the *CaSWEET* genes are expressed differentially in different organs, during different developmental stages and under various abiotic stress conditions, which may suggest their versatile functions during plant growth and development, and responses to environmental stimuli.

3.7. Transcriptional profiling of chickpea CaSWEET genes in responses to dehydration and ABA treatments

Chickpea, like other legumes species (Thao and Tran, 2012), is frequently threatened by drought during growth and development (Devasirvatham and Tan, 2018). Of our interest, we carried out a comprehensive analysis of all 21 identified *CaSWEET* genes using RT-qPCR to monitor their expression patterns in leaves and roots of the chickpea seedlings exposed to dehydration and ABA treatments. The amplification efficiencies of all primer pairs (21 designed *CaSWEET* and *IF4a* reference gene) were provided in Table S1. Eight (*CaSWEET01*, *04*, *05*, *09*, *10*, *13*, *18* and *20*) *CaSWEET* genes were induced (fold change $\geq$ 2, *P*-value < 0.05) in leaves and/or roots under dehydration (Figs. 5, 6; Table S4). Among them, *CaSWEET20* was noted to be the most induced gene in both dehydrated leaves (~20.02-fold) and roots (~35.26-fold), while *CaSWEET13* was the most highly up-regulated gene in dehydrated roots (~185.25-fold) (Figs. 5, 6; Table S4). Furthermore, under drought, *CaSWEET13* shared similar expression trends in roots at both early and late reproductive stages, while *CaSWEET20* showed up- and down-regulated patterns in the same organ at early and late reproductive stages, respectively (Fig. 4C). These results suggest that these two genes might play a crucial role in chickpea adaptation to water-deficit stress during reproductive stage. Additionally, out of seven (*CaSWEET02*, *09*, *10*, *11*, *13*, *16* and *19*) down-regulated *CaSWEET* genes, *CaSWEET11* and *19* were the most highly down-regulated *CaSWEET* genes in dehydrated leaves (~14.29-fold) and dehydrated roots (~4.00-fold), respectively (Figs. 5, 6; Table S4). *CaSWEET11* was also down-regulated in roots under drought (at early reproductive stage) and salinity treatments (at

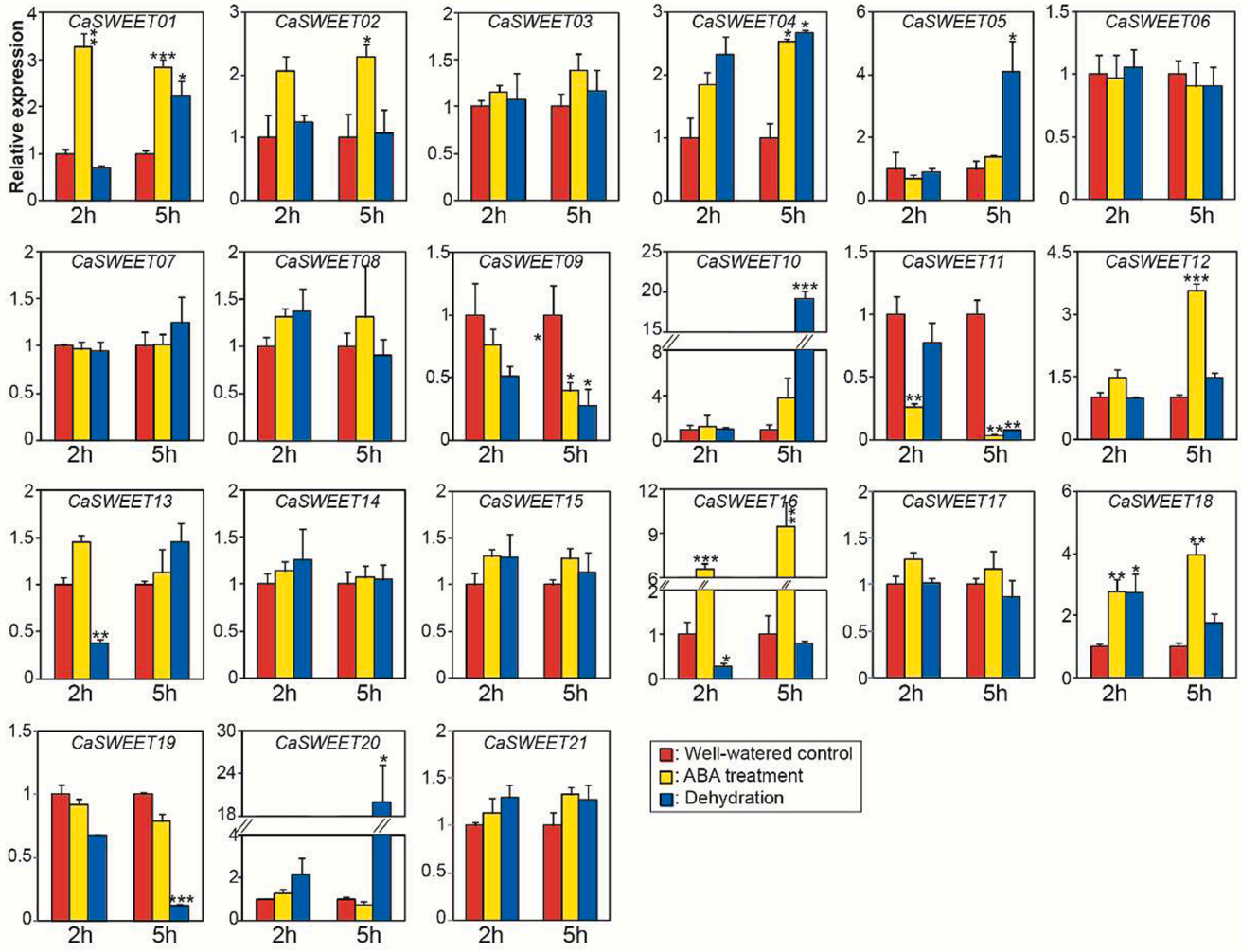

Fig. 5. Transcriptional profiling of *CaSWEET* genes in chickpea leaves under dehydration and ABA treatments. Mean relative expression levels of each *CaSWEET* gene were normalized to a value of 1 in the respective well-watered control. Error bars indicate the standard errors of three biological replicates ($n = 3$). Asterisks show significant differences in expression changes as assessed by a Student's *t*-test (|fold-change| $\geq$ 2.0; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

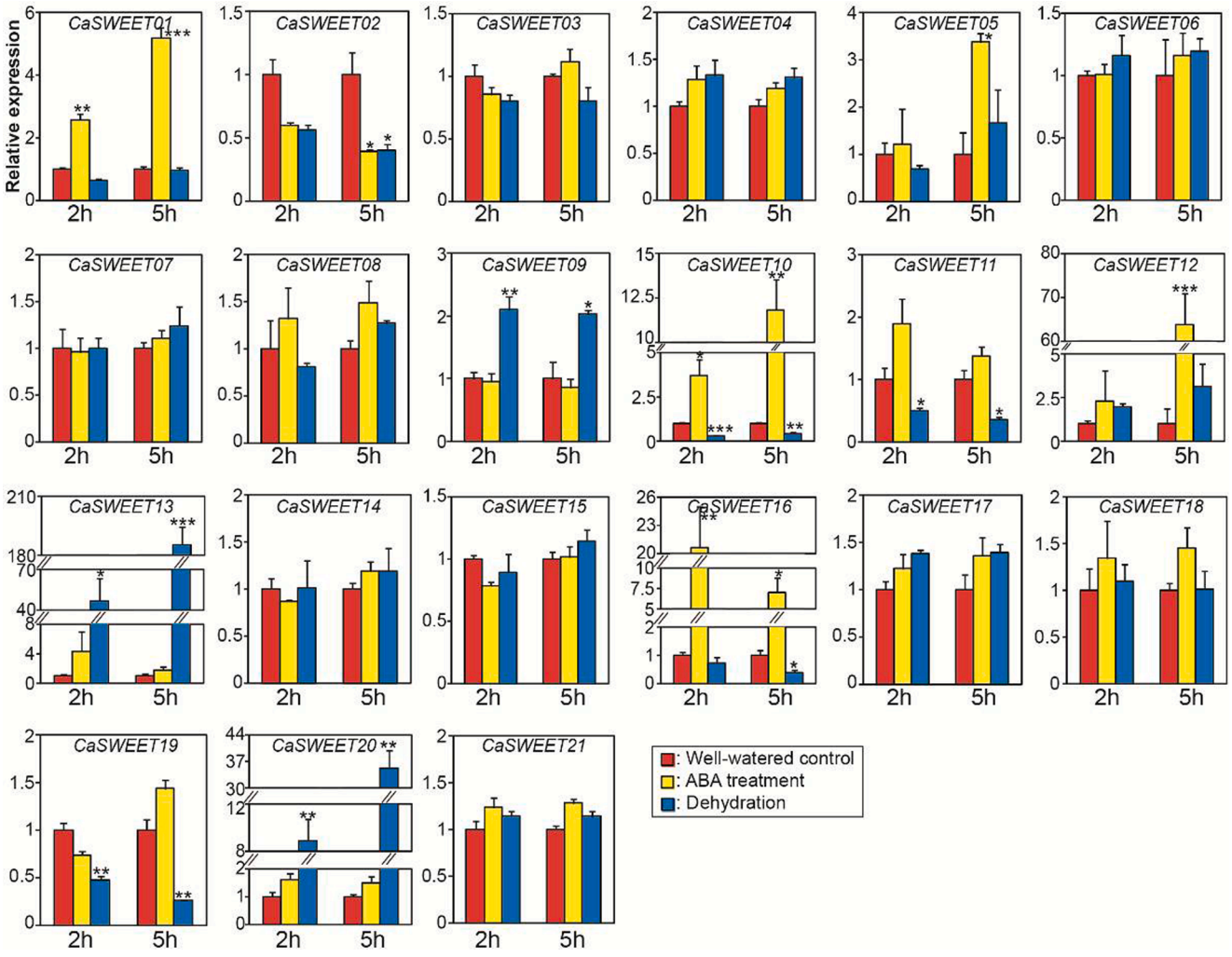

Fig. 6. Transcriptional profiling of *CaSWEET* genes in chickpea roots under dehydration and ABA treatments. Mean relative expression levels of each *CaSWEET* gene were normalized to a value of 1 in the respective well-watered control. Error bars indicate the standard errors of three biological replicates ($n = 3$). Asterisks show significant differences in expression changes as assessed by a Student's *t*-test (|fold-change| $\geq$ 2.0; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

vegetative stage) (Fig. 4C, D), and under the deficiency of NO_3^- and/or Pi (at late vegetative stage) (Fig. 4E). These data collectively suggest that *CaSWEET11* may play a role in chickpea responses to multiple stresses, particularly in roots. *CaSWEET19* was noted to reduce in root tissues subjected to drought at late reproductive stage (Fig. 4C) and root tissues at reproductive stages under salinity treatment (Fig. 4D). Additionally, the expression of *CaSWEET02* was repressed in roots under both of cold stress (Fig. 4B), drought (Fig. 4C) and salinity (Fig. 4D). Previously, *SWEET* genes were reported to be up-regulated under multiple abiotic stresses in many plant species, such as banana (Miao et al., 2017), cotton (Zhao et al., 2018); *P. equestris* (Wang et al., 2018) and *M. truncatula* (Hu et al., 2019). Particularly, five, five and four (out of 25) *MaSWEET* genes were up-regulated in five-leaf stage banana seedlings subjected to osmotic (200 mM mannitol for 7 d), salt (300 mM NaCl for 7 d) and cold (4 °C for 22 h) stresses, respectively (Miao et al., 2017). Nine (out of 55) *GhSWEET* genes were validated to be highly induced in leaves of *G. hirsutum* seedlings subjected to salinity (300 mmol/L NaCl for 12 h) or osmotic (10% PEG 6000 for 12 h) stress (Zhao et al., 2018). Three and one (out of 16) *PeSWEET* genes were significantly up-regulated in floral organs of two-year-old *P. equestris* seedlings subjected to heat (42 °C for 12 h) and cold (4 °C for 12 h) stresses, respectively (Wang et al., 2018). The stress-induced expression of *SWEETs* is thought to promote sugar accumulation in plant vacuoles, facilitating adaptation to various abiotic stresses (Chandran, 2015). Taken together, it is reasonable to propose that these dehydration-responsive *CaSWEET* genes in leaves and/or roots of chickpea plants subjected to dehydration could be attributable to chickpea responses to water-stress conditions.

ABA has been well characterized as one of the key hormones regulating water-stress responses in many plant species (Gupta et al., 2020; Osakabe et al., 2014), including chickpea (Rani et al., 2020; Pang et al., 2017). ABA-dependent pathway can regulate the transcription of many genes encoding transporters under water stress (Osakabe et al., 2014; Saddhe et al., 2021). For example, two members of sucrose transporters in *Arabidopsis*, AtSUT2 and AtSUT4, which participate in sucrose phloem loading, were found to be responsive to salt and drought stresses via ABA-signaling and sucrose-signaling pathways (Gong et al., 2015). In *M. domestica*, MdAREB2, a transcription factor involved in ABA-signaling, induces the expression of *MdSUT2* through direct binding to the ABRE element present in its promoter region to promote the accumulation of soluble sugars (Ma et al., 2017), suggesting that ABA mediates sugar accumulation in plants (Saddhe et al., 2021). Therefore, we investigated the ABA-mediated regulation of expression of *CaSWEET* genes in chickpea. Under the exogenous ABA treatment, eight (*CaSWEET01*, *02*, *04*, *05*, *10*, *12*, *16* and *18*) and three (*CaSWEET02*, *09* and *11*) *CaSWEET* genes were up-regulated and down-regulated, respectively, in leaves and/or roots (Figs. 5, 6; Table S4). Interestingly, we found that various phytohormone-responsive and/or stress-responsive *cis*- regulatory elements were remarkably enriched in the 2-

kb upstream sequences of these ABA-responsive and/or dehydration-responsive *CaSWEET* genes (Figs. 5, 6; Table S4; Fig. S5). Thus, the results of promoter and expression analyses showed a good correlation (Figs. 5, 6; Table S4; Fig. S5), suggesting that *cis*-motif enrichment analysis may serve as a reliable method to predict or validate the expression data. Moreover, nine (*CaSWEET01*, *02*, *04*, *05*, *09*, *10*, *11*, *16* and *18*) ABA-responsive *CaSWEET* genes were also responsive to dehydration treatment in leaves and/or roots (Figs. 5, 6; Table S4), suggesting that the ABA-dependent pathway might be responsible for the expression of these dehydration-responsive *CaSWEET* genes.

4. Conclusion

In this study, we carried out a comprehensive analysis of 21 *CaSWEET* genes with regard to their chromosomal distribution, gene structure, gene duplication, protein features, subcellular localization, conserved motifs, 2D and 3D modeling, promoter analysis and expression profiling. Using available RNA-sequencing data, we found that the expression of the *CaSWEET* genes showed remarkable expression changes in various organs, during diverse developmental stages and under various abiotic stress conditions. Furthermore, using RT-qPCR, we demonstrated that the expression of nine dehydration-responsive *CaSWEET* genes was also responsive to exogenous ABA treatment in leaves and/or roots. Under dehydration, *CaSWEET13* was the most highly induced gene in roots, while *CaSWEET20* was the most highly up-regulated gene in two organs. These three dehydration-responsive *CaSWEET* genes could be used as the candidates for further functional characterization aiming at developing chickpea varieties with improved drought tolerance. Taken together, our study could provide a solid foundation for further functional characterization of the *CaSWEET* gene members, ultimately lead to better understanding of their functions in growth and development, as well as responses of chickpea plants to various environmental stresses.

Author contributions

TDL, HDC and L-SPT designed the methods and conceived the study. HVL, HDC, CDT, KHN, QTNL, CMH, BPC, ATCP, TQN, LVN, CVH, HTL and HHL implemented the entire method and performed the experiments. HVL, HDC, CDT and KHN analyzed the data with the input from L-SPT. HVL, HDC and L-SPT wrote the manuscript. All authors read and approved the final manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2022.146210.

References

Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K., 2003. *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell 15 (1), 63–78. https://doi.org/10.1105/tpc.006130.

Bailey, T.L., Johnson, J., Grant, C.E., Noble, W.S., 2015. The MEME suite. Nucleic Acids Res. 43 (W1), W39–W49. https://doi.org/10.1093/nar/gkv416.

Baker, R.F., Leach, K.A., Braun, D.M., 2012. SWEET as sugar: new sucrose effluxers in plants. Mol. Plant 5 (4), 766–768. https://doi.org/10.1093/mp/sss054.

Calderón, M., Rey, M.D., Cabrera, A., Prieto, P., 2014. The subtelomeric region is important for chromosome recognition and pairing during meiosis. Sci. Rep. 4 (6488), 1–6. https://doi.org/10.1038/srep06488.

Chandran, D., 2015. Co-option of developmentally regulated plant SWEET transporters for pathogen nutrition and abiotic stress tolerance. IUBMB Life 67 (7), 461–471. https://doi.org/10.1002/iub.1394.

Chaturvedi, N., Shanker, S., Singh, V.K., Sinha, D., Pandey, P.N., 2011. Hidden markov model for the prediction of transmembrane proteins using MATLAB. Bioinformation 7 (8), 418–421. https://doi.org/10.6026/97320630007418.

Chen, L.Q., 2014. SWEET sugar transporters for phloem transport and pathogen nutrition. New Phytol. 201 (4), 1150–1155. https://doi.org/10.1111/nph.12445.

Chen, H., Shao, H., Li, K., Zhang, D., Fan, S., Li, Y., Han, M., 2017. Genome-wide identification, evolution, and expression analysis of GATA transcription factors in apple (*Malus* x *domestica* Borkh.). Gene 627, 460–472. https://doi.org/10.1016/j.gene.2017.06.049.

Chong, J., Piron, M.C., Meyer, S., Merdinoglu, D., Bertsch, C., Mestre, P., 2014. The SWEET family of sugar transporters in grapevine: VvSWEET4 is involved in the interaction with *Botrytis cinerea*. J. Exp. Bot. 65 (22), 6589–6601. https://doi.org/10.1093/jxb/eru375.

Chu, H.D., Nguyen, K.H., Watanabe, Y., Le, D.T., Pham, T.L.T., Mochida, K., Tran, L.P., 2018. Identification, structural characterization and gene expression analysis of members of the nuclear factor-Y family in chickpea (*Cicer arietinum* L.) under dehydration and abscisic acid treatments. Int. J. Mol. Sci. 19 (11), E3290. https://doi.org/10.3390/ijms19113290.

Clough, E., Barrett, T., 2016. The gene expression omnibus database. Methods Mol. Biol. 1418, 93–110. https://doi.org/10.1007/978-1-4939-3578-9_5.

Daloso, D.M., Anjos, L., Fernie, A.R., 2016. Roles of sucrose in guard cell regulation. New Phytol. 211 (3), 809–818. https://doi.org/10.1111/nph.13950.

Dash, S., Campbell, J.D., Cannon, E.K., Cleary, A.M., Huang, W., Kalberer, S.R., Karingula, V., Rice, A.G., Singh, J., Umale, P.E., Weeks, N.T., Wilkey, A.P., Farmer, A.D., Cannon, S.B., 2016. Legume information system (LegumeInfo.org): a key component of a set of federated data resources for the legume family. Nucleic Acids Res. 44 (D1), D1181–D1188. https://doi.org/10.1093/nar/gkv1159.

de Camargo, A.C., Favero, B.T., Morzelle, M.C., Franchin, M., Alvarez-Parrilla, E., de la Rosa, L.A., Geraldi, M.V., Marostica Junior, M.R., Shahidi, F., Schwember, A.R., 2019. Is chickpea a potential substitute for soybean? Phenolic bioactives and potential health benefits. Int. J. Mol. Sci. 20 (11), E2644. https://doi.org/10.3390/ijms20112644.

Devasirvatham, V., Tan, D.K.Y., 2018. Impact of high temperature and drought stresses on chickpea production. Agronomy 8 (8), 145. https://doi.org/10.3390/agronomy8080145.

El-Gebali, S., Mistry, J., Bateman, A., Eddy, S.R., Luciani, A., Potter, S.C., Qureshi, M., Richardson, L.J., Salazar, G.A., Smart, A., Sonnhammer, E.L.L., Hirsh, L., Paladin, L., Piovesan, D., Tosatto, S.C.E., Finn, R.D., 2019. The Pfam protein families database in 2019. Nucleic Acids Res. 47 (D1), D427–D432. https://doi.org/10.1093/nar/gky995.

Emanuelsson, O., Brunak, S., von Heijne, G., Nielsen, H., 2007. Locating proteins in the cell using TargetP, SignalP and related tools. Nat. Protoc. 2 (4), 953–971. https://doi.org/10.1038/nprot.2007.131.

Feng, C.Y., Han, J.X., Han, X.X., Jiang, J., 2015. Genome-wide identification, phylogeny, and expression analysis of the *SWEET* gene family in tomato. Gene 573 (2), 261–272. https://doi.org/10.1016/j.gene.2015.07.055.

Gao, Y., Wang, Z.Y., Kumar, V., Xu, X.F., Yuan, P., Zhu, X.F., Li, T.Y., Jia, B., Xuan, Y.H., 2018. Genome-wide identification of the *SWEET* gene family in wheat. Gene 642, 284–292. https://doi.org/10.1016/j.gene.2017.11.044.

Garg, R., Sahoo, A., Tyagi, A.K., Jain, M., 2010. Validation of internal control genes for quantitative gene expression studies in chickpea (*Cicer arietinum* L.). Biochem. Biophys. Res. Commun. 396 (2), 283–288. https://doi.org/10.1016/j.bbrc.2010.04.079.

Garg, R., Patel, R.K., Jhanwar, S., Priya, P., Bhattacharjee, A., Yadav, G., Bhatia, S., Chattopadhyay, D., Tyagi, A.K., Jain, M., 2011. Gene discovery and tissue-specific transcriptome analysis in chickpea with massively parallel pyrosequencing and web resource development. Plant Physiol. 156 (4), 1661–1678. https://doi.org/10.1104/pp.111.178616.

Garg, R., Bhattacharjee, A., Jain, M., 2015. Genome-scale transcriptomic insights into molecular aspects of abiotic stress responses in chickpea. Plant Mol. Biol. Rep. 33 (3), 388–400. https://doi.org/10.1007/s11105-014-0753-x.

Garg, R., Shankar, R., Thakkar, B., Kudapa, H., Krishnamurthy, L., Mantri, N., Varshney, R.K., Bhatia, S., Jain, M., 2016. Transcriptome analyses reveal genotype- and developmental stage-specific molecular responses to drought and salinity stresses in chickpea. Sci. Rep. 6, 19228. https://doi.org/10.1038/srep19228.

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R.D., Bairoch, A., 2003. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res. 31 (13), 3784–3788. https://doi.org/10.1093/nar/gkg563.

Gautam, T., Saripalli, G., Gahlaut, V., Kumar, A., Sharma, P.K., Balyan, H.S., Gupta, P.K., 2019. Further studies on sugar transporter *(SWEET*) genes in wheat (*Triticum aestivum* L.). Mol. Biol. Rep. 46 (2), 2327–2353. https://doi.org/10.1007/s11033-019-04691-0.

Gong, X., Liu, M., Zhang, L., Ruan, Y., Ding, R., Ji, Y., Zhang, N., Zhang, S., Farmer, J., Wang, C., 2015. Arabidopsis *AtSUC2* and *AtSUC4*, encoding sucrose transporters, are required for abiotic stress tolerance in an ABA-dependent pathway. Physiol. Plant. 153 (1), 119–136. https://doi.org/10.1111/ppl.12225.

Goodstein, D.M., Shu, S., Howson, R., Neupane, R., Hayes, R.D., Fazo, J., Mitros, T., Dirks, W., Hellsten, U., Putnam, N., Rokhsar, D.S., 2012. Phytozome: a comparative

platform for green plant genomics. Nucleic Acids Res. 40, D1178–D1186. https://doi.org/10.1093/nar/gkr944.
Guo, C., Li, H., Xia, X., Liu, X., Yang, L., 2018. Functional and evolution characterization of SWEET sugar transporters in *Ananas comosus*. Biochem. Biophys. Res. Commun. 496 (2), 407–414. https://doi.org/10.1016/j.bbrc.2018.01.024.
Gupta, A., Sinha, R., Fernandes, J.L., Abdelrahman, M., Burritt, D.J., Tran, L.P., 2020. Phytohormones regulate convergent and divergent responses between individual and combined drought and pathogen infection. Crit. Rev. Biotechnol. 40 (3), 320–340. https://doi.org/10.1080/07388551.2019.1710459.
Guruprasad, K., Reddy, B.V., Pandit, M.W., 1990. Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting *in vivo* stability of a protein from its primary sequence. Protein Eng. 4 (2), 155–161. https://doi.org/10.1093/protein/4.2.155.
Ha, C.V., Esfahani, M.N., Watanabe, Y., Tran, U.T., Sulieman, S., Mochida, K., Nguyen, D. V., Tran, L.S., 2014. Genome-wide identification and expression analysis of the *CaNAC* family members in chickpea during development, dehydration and ABA treatments. PLoS ONE 9 (12), e114107. https://doi.org/10.1371/journal.pone.0114107.
Han, L., Zhu, Y., Liu, M., Zhou, Y., Lu, G., Lan, L., Wang, X., Zhao, Y., Zhang, X.C., 2017. Molecular mechanism of substrate recognition and transport by the AtSWEET13 sugar transporter. Proc. Natl. Acad. Sci. U S A 114 (38), 10089. https://doi.org/10.1073/pnas.1709241114.
Hu, B., Wu, H., Huang, W., Song, J., Zhou, Y., Lin, Y., 2019. *SWEET* gene family in *Medicago truncatula*: Genome-wide identification, expression and substrate specificity analysis. Plants 8 (9), 338. https://doi.org/10.3390/plants8090338.
Hu, L.-P., Zhang, F., Song, S.-H., Tang, X.-W., Xu, H., Liu, G.-M., Wang, Y., He, H.-J., 2017. Genome-wide identification, characterization, and expression analysis of the *SWEET* gene family in cucumber. J. Integr. Agric. 16 (7), 1486–1501. https://doi.org/10.1016/s2095-3119(16)61501-0.
Iftikhar, J., Lyu, M., Liu, Z., Mehmood, N., Munir, N., Ahmed, M.A.A., Batool, W., Aslam, M.M., Yuan, Y., Wu, B., 2020. Sugar and hormone dynamics and the expression profiles of SUT/SUC and SWEET sweet sugar transporters during flower development in *Petunia axillaris*. Plants 9 (12), 1770. https://doi.org/10.3390/plants9121770.
Jain, M., Misra, G., Patel, R.K., Priya, P., Jhanwar, S., Khan, A.W., Shah, N., Singh, V.K., Garg, R., Jeena, G., Yadav, M., Kant, C., Sharma, P., Yadav, G., Bhatia, S., Tyagi, A. K., Chattopadhyay, D., 2013. A draft genome sequence of the pulse crop chickpea (*Cicer arietinum* L.). Plant J. 74 (5), 715–729. https://doi.org/10.1111/tpj.12173.
Jha, U.C., 2018. Current advances in chickpea genomics: applications and future perspectives. Plant Cell Rep. 37 (7), 947–965. https://doi.org/10.1007/s00299-018-2305-6.
Jia, B., Zhu, X.F., Pu, Z.J., Duan, Y.X., Hao, L.J., Zhang, J., Chen, L.-Q., Jeon, C.O., Xuan, Y.H., 2017. Integrative view of the diversity and evolution of SWEET and SemiSWEET sugar transporters. Front. Plant Sci. 8, 2178. https://doi.org/10.3389/fpls.2017.02178.
Jian, H., Lu, K., Yang, B., Wang, T., Zhang, L., Zhang, A., Wang, J., Liu, L., Qu, C., Li, J., 2016. Genome-wide analysis and expression profiling of the SUC and SWEET gene families of sucrose transporters in oilseed rape (*Brassica napus* L.). Front. Plant Sci. 7, 1464. https://doi.org/10.3389/fpls.2016.01464.
Julius, B.T., Leach, K.A., Tran, T.M., Mertz, R.A., Braun, D.M., 2017. Sugar transporters in plants: new insights and discoveries. Plant Cell Physiol. 58 (9), 1442–1460. https://doi.org/10.1093/pcp/pcx090.
Kelley, L.A., Mezulis, S., Yates, C.M., Wass, M.N., Sternberg, M.J.E., 2015. The Phyre2 web portal for protein modeling, prediction and analysis. Nat. Prot. 10 (6), 845–858. https://doi.org/10.1038/nprot.2015.053.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33 (7), 1870–1874. https://doi.org/10.1093/molbev/msw054.
Kyte, J., Doolittle, R.F., 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157 (1), 105–132. https://doi.org/10.1016/0022-2836(82)90515-0.
Lescot, M., Déhais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., Rouzé, P., Rombauts, S., 2002. PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. Nucleic Acids Res. 30 (1), 325–327. https://doi.org/10.1093/nar/30.1.325.
Li, W., Herrera-Estrella, L., Tran, L.-S.-P., 2019. Do cytokinins and strigolactones crosstalk during drought adaptation? Trends Plant Sci. 24 (8), 669–672. https://doi.org/10.1016/j.tplants.2019.06.007.
Li, H., Li, X., Xuan, Y., Jiang, J., Wei, Y., Piao, Z., 2018. Genome wide identification and expression profiling of *SWEET* genes family reveals its role during *Plasmodiophora brassicae*-induced formation of clubroot in *Brassica rapa*. Front. Plant Sci. 9, 207. https://doi.org/10.3389/fpls.2018.00207.
Li, J., Qin, M., Qiao, X., Cheng, Y., Li, X., Zhang, H., Wu, J., 2017a. A new insight into the evolution and functional divergence of SWEET transporters in Chinese white pear (*Pyrus bretschneideri*). Plant Cell Physiol. 58 (4), 839–850. https://doi.org/10.1093/pcp/pcx025.
Li, Y., Wang, Y., Zhang, H., Zhang, Q., Zhai, H., Liu, Q., He, S., 2017b. The plasma membrane-localized sucrose transporter IbSWEET10 contributes to the resistance of sweet potato to *Fusarium oxysporum*. Front. Plant Sci. 8, 197. https://doi.org/10.3389/fpls.2017.00197.
Li, J., Zhang, Z., Vang, S., Yu, J., Wong, G.-K.-S., Wang, J., 2009. Correlation between Ka/Ks and Ks is related to substitution model and evolutionary lineage. J. Mol. Evol. 68 (4), 414–423. https://doi.org/10.1007/s00239-009-9222-9.
Liao, Y., Smyth, G.K., Shi, W., 2019. The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. Nucleic Acids Res. 47 (8), e47. https://doi.org/10.1093/nar/gkz114.
Lin, I.W., Sosso, D., Chen, L.Q., Gase, K., Kim, S.G., Kessler, D., Klinkenberg, P.M., Gorder, M.K., Hou, B.H., Qu, X.Q., Carter, C.J., Baldwin, I.T., Frommer, W.B., 2014. Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. Nature 508 (7497), 546–549. https://doi.org/10.1038/nature13082.
Ma, Q.J., Sun, M.H., Lu, J., Liu, Y.J., Hu, D.G., Hao, Y.J., 2017. Transcription factor AREB2 Is involved in soluble sugar accumulation by activating sugar transporter and amylase genes. Plant Physiol. 174 (4), 2348–2362. https://doi.org/10.1104/pp.17.00502.
Mahdavi Mashaki, K., Garg, V., Nasrollahnezhad Ghomi, A.A., Kudapa, H., Chitikineni, A., Zaynali Nezhad, K., Yamchi, A., Soltanloo, H., Varshney, R.K., Thudi, M., Prasad, M., 2018. RNA-Seq analysis revealed genes associated with drought stress response in kabuli chickpea (*Cicer arietinum* L.). PLoS ONE 13 (6), e0199774. https://doi.org/10.1371/journal.pone.0199774.
Margier, M., Georgé, S., Hafnaoui, N., Remond, D., Nowicki, M., Du Chaffaut, L., Amiot, M.-J., Reboul, E., 2018. Nutritional composition and bioactive content of legumes: characterization of pulses frequently consumed in France and effect of the cooking method. Nutrients 10 (11), 1668. https://doi.org/10.3390/nu10111668.
Maruyama, K., Todaka, D., Mizoi, J., Yoshida, T., Kidokoro, S., Matsukura, S., Takasaki, H., Sakurai, T., Yamamoto, Y.Y., Yoshiwara, K., Kojima, M., Sakakibara, H., Shinozaki, K., Yamaguchi-Shinozaki, K., 2012. Identification of *cis*-acting promoter elements in cold- and dehydration-induced transcriptional pathways in *Arabidopsis*, rice, and soybean. DNA Res. 19 (1), 37–49. https://doi.org/10.1093/dnares/dsr040.
Mathiyalagan, R., Muthurajan, R., Subramaniyam, S., Jegadeesan, R., 2010. *Insilico* analysis of drought tolerant genes in rice. Int. J. Biol. Med. Res. 1 (3), 36–40.
Miao, L., Lv, Y., Kong, L., Chen, Q., Chen, C., Li, J., Zeng, F., Wang, S., Li, J., Huang, L., Cao, J., Yu, X., 2018. Genome-wide identification, phylogeny, evolution, and expression patterns of *MtN3/saliva/SWEET* genes and functional analysis of BcNS in *Brassica rapa*. BMC Genomics 19 (1), 174. https://doi.org/10.1186/s12864-018-4554-8.
Miao, H., Sun, P., Liu, Q., Miao, Y., Liu, J., Zhang, K., Hu, W., Zhang, J., Wang, J., Wang, Z., Jia, C., Xu, B., Jin, Z., 2017. Genome-wide analyses of SWEET family proteins reveal involvement in fruit development and abiotic/biotic stress responses in banana. Sci. Rep. 7 (1), 3536. https://doi.org/10.1038/s41598-017-03872-w.
Misra, V.A., Wafula, E.K., Wang, Y.u., dePamphilis, C.W., Timko, M.P., 2019. Genome-wide identification of MST, SUT and SWEET family sugar transporters in root parasitic angiosperms and analysis of their expression during host parasitism. BMC Plant Biol. 19, 196. https://doi.org/10.1186/s12870-019-1786-y.
Mizuno, H., Kasuga, S., Kawahigashi, H., 2016. The sorghum *SWEET* gene family: stem sucrose accumulation as revealed through transcriptome profiling. Biotechnol. Biofuels 9, 127. https://doi.org/10.1186/s13068-016-0546-6.
Mochida, K., Yoshida, T., Sakurai, T., Yamaguchi-Shinozaki, K., Shinozaki, K., Tran, L.S., 2011. *In silico* analysis of transcription factor repertoires and prediction of stress-responsive transcription factors from six major gramineae plants. DNA Res. 18 (5), 321–332. https://doi.org/10.1093/dnares/dsr019.
Mostofa, M.G., Li, W., Nguyen, K.H., Fujita, M., Tran, L.-S., 2018. Strigolactones in plant adaptation to abiotic stresses: an emerging avenue of plant research. Plant Cell Environ. 41 (10), 2227–2243. https://doi.org/10.1111/pce.13364.
Mostofa, M.G., Rahman, M.M., Siddiqui, M.N., Fujita, M., Tran, L.-S., 2020. Salicylic acid antagonizes selenium phytotoxicity in rice: selenium homeostasis, oxidative stress metabolism and methylglyoxal detoxification. J. Hazard. Mater. 394, 122572. https://doi.org/10.1016/j.jhazmat.2020.122572.
Nadeem, M., Li, J., Yahya, M., Sher, A., Ma, C., Wang, X., Qiu, L., 2019. Research progress and perspective on drought stress in legumes: a review. Int. J. Mol. Sci. 20 (10), 2541. https://doi.org/10.3390/ijms20102541.
Nakashima, K., Yamaguchi-Shinozaki, K., 2013. ABA signaling in stress-response and seed development. Plant Cell Rep. 32 (7), 959–970. https://doi.org/10.1007/s00299-013-1418-1.
Nasr Esfahani, M., Inoue, K., Chu, H.D., Nguyen, K.H., Van Ha, C., Watanabe, Y., Burritt, D.J., Herrera-Estrella, L., Mochida, K., Tran, L.-S., 2017. Comparative transcriptome analysis of nodules of two *Mesorhizobium*-chickpea associations with differential symbiotic efficiency under phosphate deficiency. Plant J. 91 (5), 911–926. https://doi.org/10.1111/tpj.13616.
Nasr Esfahani, M., Inoue, K., Nguyen, K.H., Chu, H.D., Watanabe, Y., Kanatani, A., Burritt, D.J., Mochida, K., Tran, L.-S., 2021. Phosphate or nitrate imbalance induces stronger molecular responses than combined nutrient deprivation in roots and leaves of chickpea plants. Plant Cell Environ. 44 (2), 574–597. https://doi.org/10.1111/pce.13935.
Niu, L., Chu, H.D., Tran, C.D., Nguyen, K.H., Pham, H.X., Le, D.T., Li, W., Wang, W., Le, T.D., Tran, L.-S.-P., 2020. The *GATA* gene family in chickpea: Structure analysis and transcriptional responses to abscisic acid and dehydration treatments revealed potential genes involved in drought adaptation. J Plant Growth Reg. 39 (4), 1647–1660. https://doi.org/10.1007/s00344-020-10201-5.
Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.S., 2014. Response of plants to water stress. Front. Plant Sci. 5, 86. https://doi.org/10.3389/fpls.2014.00086.
Pang, J., Turner, N.C., Khan, T., Du, Y.L., Xiong, J.L., Colmer, T.D., Devilla, R., Stefanova, K., Siddique, K.H.M., 2017. Response of chickpea (*Cicer arietinum* L.) to terminal drought: leaf stomatal conductance, pod abscisic acid concentration, and seed set. J. Exp. Bot. 68 (8), 1973–1985. https://doi.org/10.1093/jxb/erw153.
Parween, S., Nawaz, K., Roy, R., Pole, A.K., Venkata Suresh, B., Misra, G., Jain, M., Yadav, G., Parida, S.K., Tyagi, A.K., Bhatia, S., Chattopadhyay, D., 2015. An advanced draft genome assembly of a desi type chickpea (*Cicer arietinum* L.). Sci. Rep. 5, 12806. https://doi.org/10.1038/srep12806.
Patil, G., Valliyodan, B., Deshmukh, R., Prince, S., Nicander, B., Zhao, M., Sonah, H., Song, L., Lin, L., Chaudhary, J., Liu, Y., Joshi, T., Xu, D., Nguyen, H.T., 2015. Soybean (*Glycine max*) SWEET gene family: insights through comparative genomics,

transcriptome profiling and whole genome re-sequence analysis. BMC Genomics 16, 520. https://doi.org/10.1186/s12864-015-1730-y.

Rahman, M., Mostofa, M.G., Keya, S.S., Rahman, A., Das, A.K., Islam, R., Abdelrahman, M., Bhuiyan, S.U., Naznin, T., Ansary, M.U., Tran, L.-S., 2021. Acetic acid improves drought acclimation in soybean: an integrative response of photosynthesis, osmoregulation, mineral uptake and antioxidant defense. Physiol. Plant. 172 (2), 334–350. https://doi.org/10.1111/ppl.13191.

Rani, A., Devi, P., Jha, U.C., Sharma, K.D., Siddique, K.H.M., Nayyar, H., 2020. Developing climate-resilient chickpea involving physiological and molecular approaches with a focus on temperature and drought stresses. Front. Plant Sci. 10, 1759. https://doi.org/10.3389/fpls.2019.01759.

Roy, S., Liu, W., Nandety, R.S., Crook, A., Mysore, K.S., Pislariu, C.I., Frugoli, J., Dickstein, R., Udvardi, M.K., 2020. Celebrating 20 years of genetic discoveries in legume nodulation and symbiotic nitrogen fixation. Plant Cell 32 (1), 15–41. https://doi.org/10.1105/tpc.19.00279.

Rozas, J., Ferrer-Mata, A., Sanchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sanchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 34 (12), 3299–3302. https://doi.org/10.1093/molbev/msx248.

Ruijter, J.M., Ramakers, C., Hoogaars, W.M., Karlen, Y., Bakker, O., van den Hoff, M.J., Moorman, A.F., 2009. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res. 37 (6), e45. https://doi.org/10.1093/nar/gkp045.

Saddhe, A.A., Manuka, R., Penna, S., 2021. Plant sugars: Homeostasis and transport under abiotic stress in plants. Physiol. Plant. 171 (4), 739–755. https://doi.org/10.1111/ppl.13283.

Sen, S., Chakraborty, J., Ghosh, P., Basu, D., Das, S., 2017. Chickpea WRKY70 regulates the expression of a homeodomain-leucine zipper (HD-Zip) I transcription factor *CaHDZ12*, which confers abiotic stress tolerance in transgenic tobacco and chickpea. Plant Cell Physiol. 58 (11), 1934–1952. https://doi.org/10.1093/pcp/pcx126.

Silver, N., Best, S., Jiang, J., Thein, S.L., 2006. Selection of housekeeping genes for gene expression studies in human reticulocytes using real-time PCR. BMC Mol. Biol. 7, 33. https://doi.org/10.1186/1471-2199-7-33.

Singh, V.K., Garg, R., Jain, M., 2013. A global view of transcriptome dynamics during flower development in chickpea by deep sequencing. Plant Biotechnol. J. 11 (6), 691–701. https://doi.org/10.1111/pbi.12059.

Tao, Y., Cheung, L.S., Li, S., Eom, J.-S., Chen, L.-Q., Xu, Y., Perry, K., Frommer, W.B., Feng, L., 2015. Structure of a eukaryotic SWEET transporter in a homotrimeric complex. Nature 527 (7577), 259–263. https://doi.org/10.1038/nature15391.

Thao, N.P., Tran, L.-S.-P., 2012. Potentials toward genetic engineering of drought-tolerant soybean. Crit. Rev. Biotechnol. 32 (4), 349–362. https://doi.org/10.3109/07388551.2011.643463.

Tran, C.D., Chu, H.D., Nguyen, K.H., Watanabe, Y., La, H.V., Tran, K.D., Tran, L.-S.P., 2018. Genome-wide identification of the TCP transcription factor family in chickpea (*Cicer arietinum* L.) and their transcriptional responses to dehydration and exogenous abscisic acid treatments. J Plant Growth Reg. 37 (4), 1286–1299. https://doi.org/10.1007/s00344-018-9859-y.

Varshney, R.K., Song, C., Saxena, R.K., Azam, S., Yu, S., Sharpe, A.G., Cannon, S., Baek, J., Rosen, B.D., Tar'an, B., Millan, T., Zhang, X., Ramsay, L.D., Iwata, A., Wang, Y., Nelson, W., Farmer, A.D., Gaur, P.M., Soderlund, C., Penmetsa, R.V., Xu, C., Bharti, A.K., He, W., Winter, P., Zhao, S., Hane, J.K., Carrasquilla-Garcia, N., Condie, J.A., Upadhyaya, H.D., Luo, M.-C., Thudi, M., Gowda, C.L.L., Singh, N.P., Lichtenzveig, J., Gali, K.K., Rubio, J., Nadarajan, N., Dolezel, J., Bansal, K.C., Xu, X., Edwards, D., Zhang, G., Kahl, G., Gil, J., Singh, K.B., Datta, S.K., Jackson, S.A., Wang, J., Cook, D.R., 2013. Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nat. Biotech. 31 (3), 240–246. https://doi.org/10.1038/nbt.2491.

Wang, T., Song, Z., Meng, W.L., Li, L.B., 2018. Identification, characterization, and expression of the *SWEET* gene family in *Phalaenopsis equestris* and *Dendrobium officinale*. Biol. Plant. 62 (1), 24–32. https://doi.org/10.1007/s10535-017-0750-7.

Wang, L., Yao, L., Hao, X., Li, N., Qian, W., Yue, C., Ding, C., Zeng, J., Yang, Y., Wang, X., 2018. Tea plant SWEET transporters: expression profiling, sugar transport, and the involvement of CsSWEET16 in modifying cold tolerance in *Arabidopsis*. Plant Mol. Biol. 96 (6), 577–592. https://doi.org/10.1007/s11103-018-0716-y.

Wang, Y., You, F.M., Lazo, G.R., Luo, M.-C., Thilmony, R., Gordon, S., Kianian, S.F., Gu, Y.Q., 2013. PIECE: a database for plant gene structure comparison and evolution. Nucleic Acids Res. 41 (D1), D1159–D1166. https://doi.org/10.1093/nar/gks1109.

Xie, H., Wang, D., Qin, Y., Ma, A., Fu, J., Qin, Y., Hu, G., Zhao, J., 2019. Genome-wide identification and expression analysis of *SWEET* gene family in *Litchi chinensis* reveal the involvement of *LcSWEET2a/3b* in early seed development. BMC Plant Biol. 19, 499. https://doi.org/10.1186/s12870-019-2120-4.

Xuan, Y.H., Hu, Y.B., Chen, L.Q., Sosso, D., Ducat, D.C., Hou, B.H., Frommer, W.B., 2013. Functional role of oligomerization for bacterial and plant SWEET sugar transporter family. Proc. Natl. Acad. Sci. U S A 110 (39), E3685–E3694. https://doi.org/10.1073/pnas.1311244110.

Yadav, B.S., Singh, S., Srivastava, S., Singh, N.K., Mani, A., 2019. Whole transcriptome expression profiling and biological network analysis of chickpea during heavy metal stress. J. Plant Biochem. Biotechnol. 28 (3), 345–352. https://doi.org/10.1007/s13562-019-00486-3.

Yamaguchi-Shinozaki, K., Shinozaki, K., 2005. Organization of *cis*-acting regulatory elements in osmotic- and cold-stress-responsive promoters. Trends Plant Sci. 10 (2), 88–94. https://doi.org/10.1016/j.tplants.2004.12.012.

Yang, J., Luo, D., Yang, B., Frommer, W.B., Eom, J.-S., 2018. SWEET11 and 15 as key players in seed filling in rice. New Phytol. 218 (2), 604–615. https://doi.org/10.1111/nph.15004.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., Madden, T.L., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinf. 13, 134. https://doi.org/10.1186/1471-2105-13-134.

Yuan, M., Wang, S., 2013. Rice *MtN3/saliva/SWEET* family genes and their homologs in cellular organisms. Mol. Plant 6 (3), 665–674. https://doi.org/10.1093/mp/sst035.

Zhang, W., Wang, S., Yu, F., Tang, J., Shan, X.i., Bao, K., Yu, L.i., Wang, H., Fei, Z., Li, J., 2019. Genome-wide characterization and expression profiling of *SWEET* genes in cabbage (*Brassica oleracea* var. *capitata* L.) reveal their roles in chilling and clubroot disease responses. BMC Genomics 20, 93. https://doi.org/10.1186/s12864-019-5454-2.

Zhao, L., Yao, J., Chen, W., Li, Y., Lü, Y., Guo, Y., Wang, J., Yuan, L.i., Liu, Z., Zhang, Y., 2018. A genome-wide analysis of *SWEET* gene family in cotton and their expressions under different stresses. J Cotton Res 1, 7. https://doi.org/10.1186/s42397-018-0007-9.

Zhen, Q., Fang, T., Peng, Q., Liao, L., Zhao, L., Owiti, A., Han, Y., 2018. Developing gene-tagged molecular markers for evaluation of genetic association of apple *SWEET* genes with fruit sugar accumulation. Hortic. Res. 5, 14. https://doi.org/10.1038/s41438-018-0024-3.