agronomy

MDPI

Article

Genome-Wide Identification and Expression Analysis of *SnRK* Gene Family under Abiotic Stress in Cucumber (*Cucumis sativus* L.)

Yanyan Luo, Yuan Niu, Rong Gao, Chunlei Wang and Weibiao Liao *

College of Horticulture, Gansu Agricultural University, Lanzhou 730070, China; luoyy@gsau.edu.cn (Y.L.); niuy@st.gsau.edu.cn (Y.N.); gaor@st.gsau.edu.cn (R.G.); wangchunlei@gsau.edu.cn (C.W.)
* Correspondence: liaowb@gsau.edu.cn; Tel.: +86-138-9328-7942

Abstract: Sucrose non-fermenting 1-related protein kinases (*SnRKs*) are a kind of plant-specific serine/threonine (Ser/Thr) protein kinase, which play an important role in plant stress resistance. However, the scale analysis of *SnRK* in the cucumber genome is currently unclear. In the study, a total of 30 *CsSnRK* genes were identified from genomic data. They were distributed on six chromosomes, including 1 *CsSnRK1*, 10 *CsSnRK2s* and 19 *CsSnRK3s*. According to the analysis of gene structure and motif composition, *CsSnRKs* showed obvious differences among the three subfamilies. The ratio of synonymous (Ks) and nonsynonymous (Ka) nucleotide substitutions (Ka/Ks) of three paralogues indicates that the *CsSnRK* gene family undergoes a purifying selection. The analysis of cis-acting elements shows that the promoter region of each *CsSnRK* gene contained different classes of hormone and stress-related cis-acting elements. Furthermore, based on RNA-sequencing data from the Short Read Archive (SRA) database of NCBI, the expression patterns of *CsSnRK* genes in six tissues were investigated, indicating that the expression of multiple *CsSnRK* genes was prevalent in these tissues. Transcription levels of *CsSnRK* genes after drought, methyl jasmonate (MeJA) and abscisic acid (ABA) treatments were analyzed by quantitative RT-PCR, and the results show that most of the *CsSnRK* genes responded to these stresses. However, under different treatments, individual genes played a major role. For example, under ABA treatment, *CsSnRK2.2* and *CsSnRK2.3* played a major role in the response to ABA. These results provide clear evidence that *CsSnRKs* may be involved in cucumber growth, development and stress response, and provide valuable information for future functional studies of *CsSnRKs*.

Keywords: cucumber; *SnRK*; structure; hormones; stress responses; expression patterns

Citation: Luo, Y.; Niu, Y.; Gao, R.; Wang, C.; Liao, W. Genome-Wide Identification and Expression Analysis of *SnRK* Gene Family under Abiotic Stress in Cucumber (*Cucumis sativus* L.). *Agronomy* **2022**, *12*, 1550. https://doi.org/10.3390/agronomy12071550

Academic Editor: Antonio Lupini

Received: 18 April 2022
Accepted: 26 June 2022
Published: 28 June 2022

1. Introduction

Plants are constantly exposed to various unfavorable conditions in the natural environment, including salt, drought and low temperature stresses [1]. Plants form a variety of molecular defense mechanisms to deal with abiotic stresses, which helps plant to maintain optimal growth and development conditions [2]. Protein kinases and phosphatases are the main components of intracellular signal transduction and play an important role in stress response [3]. In recent years, protein kinases, such as calcium-dependent protein kinases (*CDPK)* [4], mitogen-activated protein kinase (*MAPK)* [5] and sucrose non-fermenting 1 related protein kinases (*SnRKs*) [6–8], have been extensively researched. Among them, *SnRKs* play important roles in regulating metabolism. In plants, *SnRKs* proteins can be divided into three subfamilies according to their sequence and structure, including *SnRK1*, *SnRK2* and *SnRK3* [9]. *SnRKs* are highly conserved in the organism and not only participate in plant metabolism regulation, but also in stress response [10–12]. All members of the *SnRKs* gene family have similar lengths and sequences and similar kinase domains. The kinase domain is located near the N-terminus, the middle is the junction domain and the C-terminus is the regulatory domain [9]. *SnRKs* is a key kinase in plant sugar signal

transduction, stress response, seedling growth and seed germination and other biological processes. *SnRK* genes induced by drought, salt and cold stress are also activated by abscisic acid (ABA).

SnRK1 exists not only in plants, but also in yeast and mammals. Its subfamily has a highly conserved catalytic domain, which is a homolog of *SNF1* in yeast and *AMPKs* in mammals [10]. *SnRK1* is first found in rye (*Secale graine* L.) with a total length of 502aa [13]. Study has shown that *SnRK1* is related to sugar and ABA signaling pathways and plays a central role in metabolic regulation [14]. The decreased expression of *SnRK1* increases sucrose accumulation and reduces maturity in pea seeds [15]. *SnRK1* also regulates gene activity and causes the change of gene expression to nutrient starvation [16]. Plants also contain two other *SnRK* protease subfamilies, *SnRK2* and *SnRK3*, which also contain catalytic domains, but these two subfamilies are far less similar to *SNF1* and *AMPK* than *SnRK1* 9. The *SnRK2* and *SnRK3* subfamily are unique in plants and are more diverse than the *SnRK1* subfamily. *SnRK2* subfamily members have kinase domain, ATP binding domain, serine/threonine active site and four N-fourteen sites [17]. *SnRK2* mainly participates in the signal transduction process of plant abiotic stress, and *SnRK2* kinase plays a role in an ABA-dependent or independent manner in plants [18]. In *Arabidopsis*, high osmotic pressure and salt stress can induce the expression of 8 *SnRK2* genes, and ABA can activate the expression of 5 *SnRK2* genes [19]. The *SnRK2* subfamily also plays a key role in the regulation of gene expression by activating the basic region leucine zipper (bZIP) transcription factor. The bZIP transcription factor is related to the epigenetic mechanism that controls gene activation or suppression [20]. *SnRK3* kinases are named *CIPKs* (CBL-interacting protein kinases), which interact with the calcium sensor calcineurin b-like protein (CBLs) to mediate the calcium signaling pathway. In general, *CIPKs* are composed of the Pkinase domain conserved at the n-terminal, the NAF domain and the PPI domain of the C-terminal regulatory region [21]. The first *SnRK3* gene identified is also from wheat and is originally named wpk4. The *SnRK3* gene family is even larger than the *SnRK2* family 9. *SnRK3* protein can interact with *CBL* protein to form a calcium signal complex, which plays an important role in responding to various stresses such as salt, drought, cold and ABA [22]. For example, the overexpression of *BnCBL1-BnCIPK6* can enhance high salt tolerance and low potassium tolerance in *Brassica napus* [23]. Thus, more and more evidence indicates the importance of *SnRKs* function in nutrient utilization and stress response, but the specific function may vary with different plant species. Therefore, it is necessary to study its role in different types of plants.

Cucumber (*Cucumis sativus* L.) is a widely cultivated crop species and occupies an extremely important economic position in China's agricultural production [24]. It belongs to the Cucurbitaceae family. The function of the *SnRKs* family has been widely studied in various plants. However, genome-wide information on members of the *CsSnRKs* family has not been reported. In the light of the important role of *SnRKs* in regulating metabolism and stress signals, we identified *SnRK* members in cucumber. A total of 30 *CsSnRK* members were identified and then their physicochemical properties, evolutionary relationship, gene structure, chromosomal location and cis-acting element distributions of *SnRK* genes in cucumber were analyzed. Members of the *SnRK2* subfamily are major players in plant responses to osmotic stress and abscisic acid (ABA)-dependent developmental processes [25]. The best known signaling in which *SnRK3s* are involved is the Salt Overly Sensitive (SOS) pathway, which regulates plant salt tolerance [26]. More importantly, through cis-acting elements analysis, we found that cucumber *SnRKs* mainly were enriched in drought stress response, so *CsSnRK2s* were chosen to analyze their expression pattern. We determined the tissue-specific expression patterns, and the expression patterns under drought stress and hormonal conditions. Therefore, our results provide important information on the *CsSnRK* genes family and lay the foundation for the study on the abiotic stress tolerance in cucumber.

2. Materials and Methods

2.1. Identification and Phylogenetic Analysis of the CsSnRK Gene Family in Cucumber

The genome and protein sequence information file of *Arabidopsis* and cucumber were obtained from the National Center for Biotechnology Information (NCBI). Based on the third-generation high-throughput sequencing genome of cucumber, the protein sequences of three subgroup members in the *Arabidopsis SnRK* family were used as probes. Local BLASTP (E-value-20) searches were performed based on Hidden Markov Model (HMM) profiles of *SnRK* gene domains from the Pfam database (http://pfam.janelia.org/; accessed on 4 January 2022). Redundant and incomplete sequences were removed, and the remaining sequences containing the protein kinase domain (PF00069) were considered as candidate *SnRK* members. The candidate genes were obtained and used to construct a phylogenetic tree with *SnRK* of *Arabidopsis* and rice. The candidate genes clustered with *Arabidopsis* and rice *SnRK* were identified as putative *CsSnRK*. To further ensure the existence of *SnRK* conserved structures, we used the online software SMART (http://smart.embl-heidelberg.de/; accessed on 4 January 2022), HMMER (https://www.ebi.ac.uk/Tools/hmmer/, accessed on 4 January 2022) and Pfam (http://pfam.xfam.org/; accessed on 4 January 2022) on the structure of candidate members (Pkinase/PF00069 and Pkinase_Tyr/PF07714) for reconfirmation. The members that contain Pkinas (PF00069), UBA (PF00627) and KA1 (PF02149) domains were grouped into subgroup I, and Pkinase (the members of the PF00069) domain were classified into subgroup II, and the members containing both the Pkinase (PF00069) and NAF (PF03822) domains were classified into the third subgroup. Finally, we obtained the members of the cucumber *SnRK* genes family, and used ExPASy (https://web.expasy.org/protparam/, accessed on 11 January 2022) to analyze the physical and chemical properties of these genes. The subcellular localization of CsSnRK protein was predicted using the online software PlantmPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/; accessed on 11 January 2022).

The ClustalX software was used for multiple sequence alignment of 30 CsSnRK proteins [27]. Based on the alignments, the phylogenetic tree of CsSnRK between cucumber, *Arabidopsis*, rice and barley was constructed using MEGA 11 software [28] with the neighbor-joining method with 1000 replicates of bootstrap values [29]. The EvolView tool (http://www.evolgenius.info, accessed on 12 January 2022) was used to draw the phylogenetic tree.

2.2. Gene Structure and Protein Motif Analysis

The online Gene Structure Display Server (GSDS; http://gsds.cbi.pku.edu.cn; accessed on 11 January 2022) was used to assess the distribution of introns and exons and intron phase patterns [30]. The online software Multiple Expectation Maximization for Motif Elicitation (MEME; http://meme-suite.org/tools/meme; accessed on 11 January 2022) was used to analyze the conserved motifs. The optimized parameters were as follows: number of repetitions, any; maximum number of motifs, 20; and optimum width of each motif, between 6 and 100 residues.

2.3. Chromosomal Location and Gene Duplication Analyses

The chromosomal location of the *CsSnRK* genes information was extracted by BLAST (Basic Local Alignment Search Tool; https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on 8 January 2022), and the distribution of the geneson chromosomes was drawn using the MG2C v.2 program (http://mg2c.iask.in/mg2c_v2.0/; accessed on 8 January 2022). Gene duplication, including segmental and tandem duplication, was analyzed using the MCScanX program [31]. Tandem duplication events were defined as $\geq$2 adjacent genes on the same chromosome. A segmental duplication event was defined as duplicate pairs located on different chromosomes [32]. The ratio of synonymous (Ks) and nonsynonymous (Ka) nucleotide substitutions between paralogues gene pairs was calculated by using the KaKs Calculator 2.0.

2.4. Cis-Acting Elements Analysis CsSnRK Genes

In order to analyze the cis-acting elements of *CsSnRK* family, the up-stream 2000 bp DNA sequences of *CsSnRK* genes were submitted to PlantCARE Database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/; accessed on 13 January 2022). Excel was used to count the number of the cis-acting elements and made the stacked chart.

2.5. Expression Analysis of CsSnRK2 Genes in Different Plant Tissues

The expression data of cucumber *CsSnRK2* genes in six different tissues was obtained from the Short Read Archive (SRA) database of NCBI (accession number SRP071224). The differential expression analysis of *CsSnRK2* subfamily was performed according to the method of Wei et al. [33]. Gene expression levels were calculated with Log (RPKM + 1). The heat map of the expression profile *CsSnRK2s* was created by TBtools software.

2.6. Expression Analysis of CsSnRK Genes under Different Abiotic Stresses

2.6.1. Plant Materials and Treatments

Cucumber seeds ("China Long" inbred line 9930) were provided by Shenzhen Institute of Genomics, Chinese Academy of Agricultural Sciences. We soaked the seeds in water at 55 °C for 15 min, then placed them on damp filter paper and germinated overnight in a 25 °C incubator. After the cotyledons were fully expanded, they were cultivated in a hydroponic box. The environment of the growth room was controlled to have a photoperiod of 14/10 h (light/dark), an air temperature of 28/18 °C (day/night), and a light intensity of 200 μmol·m^{-2}·s^{-1}. The nutrient solution was changed every three days. As for ABA, methyl jasmonate (MeJA) and drought stress treatments, the selected seedlings were transferred to nutrient solution containing 15% (*w*/*v*) PEG6000 [34], 100 μM MeJA or 100 μM ABA [35]. Plant of the control group was grown in the nutrient solution without adding other reagents. All the seedlings were grown in the same conditions of a growth chamber. Then leaves and roots were collected at 0, 3, 12, 24 and 48 h and immediately frozen in liquid nitrogen. Finally, samples were stored at −80 °C for qRT-PCR experiment [36]. There were three biological replicates per treatment.

Meanwhile, the roots, stems, young leaves, old leaves, female flowers and male flowers of untreated seedlings were collected for *CsSnRK2* genes expression analysis. There were three biological replications collected under the identical experimental condition. Then the collections were frozen with liquid nitrogen and stored at −80 °C.

2.6.2. RNA Extraction and Quantitative RT-PCR

Total RNA was isolated from cucumber leaves with TRIzol reagent. Then, according to the manufacturer's instructions, total RNA was reverse-transcribed to cDNA using cDNA Synthesis Kit (Tiangen, Beijing, China). The primers were designed with Primer 5 and their specificities were confirmed by a BLAST search. The cucumber *ACTIN* gene [37] was used to normalize relative expression levels, as shown in Table S1. RT-PCR was performed using the SuperReal PreMix Plus kit (Tiangen, Beijing, China) and Roche LightCycler instrument. There were three biological replicates per gene. The $2^{-\Delta\Delta Ct}$ method [38] was used to analyze the relative expression level of each *CsSnRK* gene.

3. Results

3.1. Identification of CsSnRK Gene Family in Cucumber

Using a Hidden Markov model, according to the sequence alignment of *Arabidopsis* and rice, a total of 30 *CsSnRK* members were finally identified from the cucumber genome database, including 1 *CsSnRK1*, 10 *CsSnRK2s* and 19 *CsSnRK3s*. According to the conserved domains and nomenclature in *Arabidopsis*, they were named *CsSnRK1.1*, *CsSnRK2.1* to *CsSnRK2.10* and *CsSnRK3.1* to *CsSnRK3.19*. The physical parameters of these genes were summarized in Table 1. 30 *CsSnRK* members encoded 340 to 673 amino acids, with a molecular weight (MW) of 38.38 to 77.01 kDa. The isoelectric point (pI) of *CsSnRKs* was from 4.44 to 9.64. The predicted subcellular localization showed that *CsSnRK1.1* and

CsSnRK2s were localized to the cytoplasm and nucleus, whereas most *CsSnRK3s* were localized to cytoplasm or chloroplast. Among them, only *CsSnRK3.15* was at the nucleus.

Table 1. The characteristics of the *CsSnRK* gene family in cucumber.

Gene Name	Gene ID	Amino Acid (aa)	Exons	MW (kDa)	pI	Subcellular Localization
CsSnRK1.1	CsaV3_6G006250.1	515	10	58.81	8.59	cytoplasm
CsSnRK2.1	CsaV3_2G014200.1	361	9	41.24	4.44	nucleus
CsSnRK2.2	CsaV3_2G016930.1	363	10	41.07	4.45	nucleus
CsSnRK2.3	CsaV3_3G002220.1	340	9	38.38	5.95	cytoplasm
CsSnRK2.4	CsaV3_4G030050.1	355	9	40.90	6.38	nucleus
CsSnRK2.5	CsaV3_4G030080.1	673	18	77.01	7.02	cytoplasm
CsSnRK2.6	CsaV3_4G030100.1	372	12	42.44	8.78	nucleus
CsSnRK2.7	CsaV3_4G030120.1	486	13	55.73	5.53	nucleus
CsSnRK2.8	CsaV3_4G030260.1	355	10	40.94	5.83	nucleus
CsSnRK2.9	CsaV3_6G045260.1	344	9	38.95	5.07	cytoplasm
CsSnRK2.10	CsaV3_7G008840.1	365	9	41.22	4.52	cytoplasm
CsSnRK3.1	CsaV3_1G028820.1	430	1	48.51	9.44	cytoplasm
CsSnRK3.2	CsaV3_1G030280.1	356	15	40.56	5.74	chloroplast
CsSnRK3.3	CsaV3_1G033260.1	463	2	52.40	9.63	chloroplast
CsSnRK3.4	CsaV3_1G036820.1	464	16	52.05	9.55	cytoplasm
CsSnRK3.5	CsaV3_2G003670.1	433	2	48.69	9.52	cytoplasm
CsSnRK3.6	CsaV3_2G013250.1	430	1	47.88	9.45	chloroplast
CsSnRK3.7	CsaV3_2G030480.1	446	14	50.85	7.79	cytoplasm
CsSnRK3.8	CsaV3_3G007670.1	441	14	50.08	8.42	cytoplasm
CsSnRK3.9	CsaV3_3G012720.1	433	1	48.60	9.64	chloroplast
CsSnRK3.10	CsaV3_3G015590.1	442	12	49.58	6.05	cytoplasm
CsSnRK3.11	CsaV3_4G017300.1	639	20	72.87	7.7	cytoplasm
CsSnRK3.12	CsaV3_4G036630.1	443	1	50.29	8.61	cytoplasm
CsSnRK3.13	CsaV3_6G000230.1	436	12	49.38	6.45	cytoplasm
CsSnRK3.14	CsaV3_6G000240.1	431	12	49.03	8.34	chloroplast
CsSnRK3.15	CsaV3_6G019060.1	408	4	46.14	8.41	nucleus
CsSnRK3.16	CsaV3_6G019070.1	424	1	48.14	8.86	cytoplasm
CsSnRK3.17	CsaV3_6G050960.1	467	1	53.01	8.06	cytoplasm
CsSnRK3.18	CsaV3_6G051970.1	454	15	51.17	9.22	chloroplast
CsSnRK3.19	CsaV3_UNG100750.1	432	1	48.94	6.63	cytoplasm

Note: pI, isoelectric point; MW, molecular weight.

3.2. Phylogeny Analysis of CsSnRK Gene Family

We constructed a phylogenetic tree using the amino acid sequences of *Arabidopsis* (39), rice (50), barley (50) and cucumber (30) in order to analyze their evolutionary relationships (Figure 1). Based on the phylogenetic tree, all SnRK proteins were divided into three groups: SnRK1, SnRK2, SnRK3. In details, the SnRK1 subfamily had the fewest members and all family members contained Pkinase (PF00069 of Pfam), UBA (PF00627) and KA1 (PF02149) domains, while SnRK2 proteins contained pkinase domain. The SnRK3 subfamily had the largest number of members, and all family members contain Pkinase and NAF (PF03822) domains. The assignation of each cucumber SnRK to the groups confirmed the Pfams analysis. The study found that most of the CsSnRK proteins had high homology with *Arabidopsis*. Furthermore, some SnRK members within species clustered together, implying expansion after speciation.

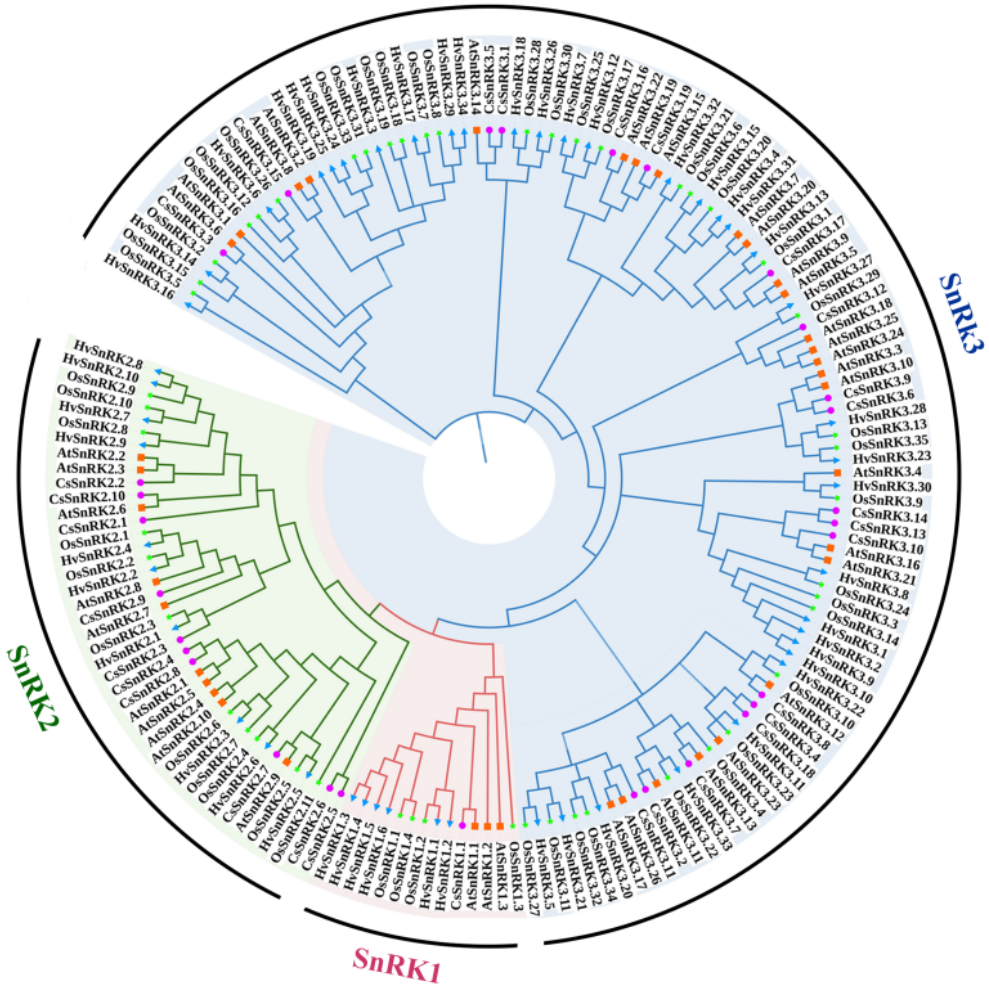

Figure 1. Phylogenetic relationship of the SnRK homologs in different species. The four differently-colored shapes represent SnRK proteins from four species. The orange rectangle, green pentacle, blue triangle and purple round represent *Arabidopsis*, rice, barley and cucumber SnRK proteins, respectively. Different color sectors represent SnRKs from SnRK1 subfamilies (blue), SnRK2 (pink) and SnRK3 (green).

3.3. Gene Structure and Conserved Motifs Analysis of CsSnRK Gene Family

We analyzed 10 conserved motifs of 30 CsSnRK proteins using an online MEME program. In this study, except for CsSnRK3.2, which lacked motif 3, all other genes contained 3 motifs (1, 2, 3) (Figure 2B). In detail, the motif of the CsSnRK1 subfamily was 1, 2, 3, 5, 6 and 9; CsSnRK2 subfamily had motif 1, 2, 3, 4 and 7; the motif of the CsSnRK3 subfamily was 1, 2, 3, 5, 6, 8, 9 and 10, except that the motif of CsSnRK3.2 was 1, 2, 5, 8, 9 and 10. Taken together, CsSnRK proteins had a conserved motif composition and similar gene structure within the same subfamily, which strongly supported the reliability of phylogenetic analysis to classify this subfamily.

To further understand the gene structure, we combined phylogenetic tree and the Gene Structure Display server (GSDS) analysis to determine the intron/exon structure of the *CsSnRK* genes (Figure 2A,C). *CsSnRK1.1* had 10 exons, while *CsSnRK2* subfamily had 9–18 exons. However, the number of exons in the *CsSnRK3* subfamily varies. The 10 *CsSnRK3* genes contained 1 exon, and the 9 *CsSnRK3* genes contained 12 to 20 exons. Therefore, *CsSnRK3* genes can be divided into two subgroups, exon-rich and exon-poor subgroups, based on the number of exons. *CsSnRK* genes exhibited similar exon-intron structures in each subfamily, confirming their close evolutionary relationship and subfamily classification.

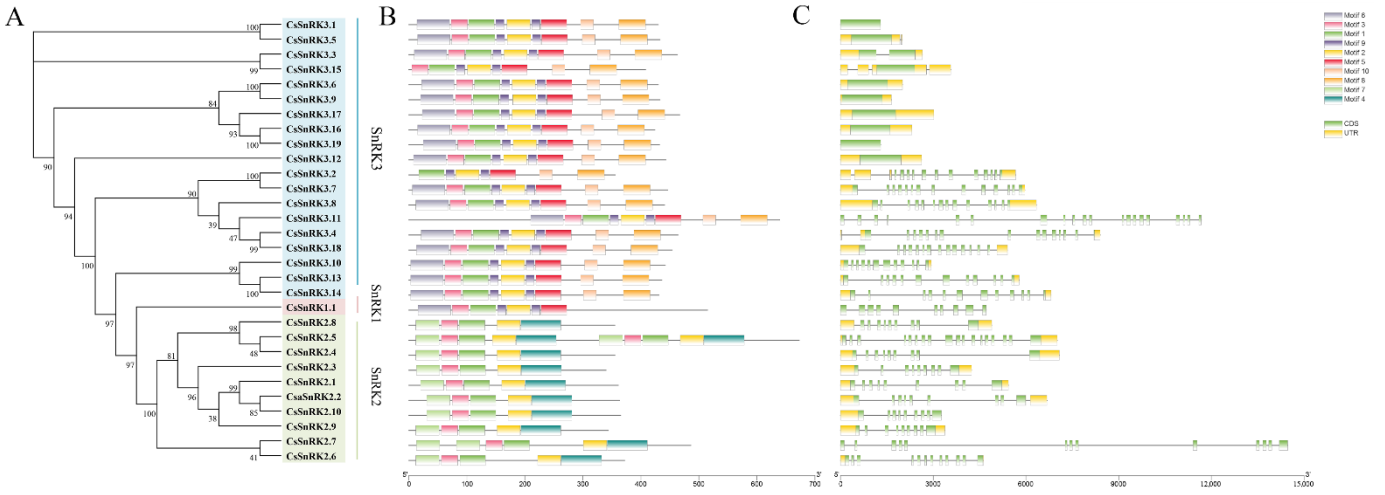

Figure 2. Phylogenetic relationships, architecture of conserved protein motifs and gene structure of *CsSnRK* genes. (**A**) The phylogenetic tree of 30 *CsSnRK* genes was built with MEGA7 choosing the NJ method. Pink, green and blue background color represents *SnRK1*, *SnRK2* and *SnRK3* subgroups, respectively. (**B**) The motif compositions of 30 CsSnRK proteins. The motifs were identified using the MEME program. Boxes of different colors represent motif 1 to 10, respectively. The length of amino acid sequences can be estimated by the scale at the bottom. (**C**) Gene structures of 30 *CsSnRK* genes. Green boxes represent exons (CDS), yellow boxes represent the upstream/downstream region (UTR), and black lines represent introns. The length of nucleotide sequences of exons/introns/UTRs can be inferred by the scale at the bottom.

3.4. Chromosomal Location and Gene Duplication Analyses

In order to clarify the distribution of *CsSnRK* genes on the chromosomes, we used the cucumber genome annotation information and TBtools software to visualize the distribution of chromosomes (Figure 3). Among 30 *CsSnRK* genes, the 29 *CsSnRK* genes were distributed on 6 out of 7 chromosomes and the number of genes on each chromosome was irrelevant of the size of the chromosome. *CsSnRK2.9* gene, which could not be conclusively mapped to any chromosome, was mapped to scaffold94. Chromosome 6 (Chr 6) contained the largest number of cucumber genes (8 *CsSnRK* genes) whereas Chr7 contained only one. No *CsSnRK* genes were detected on Chr5. The *CsSnRK2* genes were mainly distributed on Chr4, while the *CsSnRK3* genes were mainly distributed on Chr 6. *CsSnRK1.1* was distributed on Chr 6.

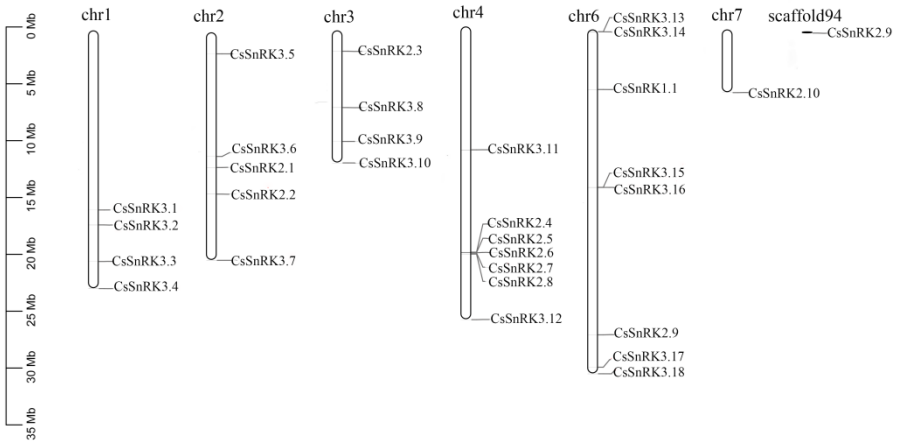

Figure 3. Chromosomal locations of *CsSnRK* genes. The length of chromosomes can be estimated using the scale on the left.

To identify duplications of the *CsSnRK* genes, we analyzed the segmental duplications and tandem duplications of the cucumber genome during evolution. As shown in Figure 4, there were three segmental duplication events (*CsSnRK3.1*/*CsSnRK3.12*, *CsSnRK3.3*/*CsSnRK3.17* and *CsSnRK3.6*/*CsSnRK3.9*) between different chromosomes. Simultaneously, the Ka/Ks ratio between these duplicated gene pairs were calculated. The Ka/Ks values of the three gene paralogs were less than1 (Table 2), which indicates that they had undergone purifying selection during evolution. We also calculated the KaKs values of the segmental duplicated *CsSnRK* genes in other species of Cucurbitaceae (Table S2). The Ka/Ks values were less than 1, which indicates that the similar segmental duplication happened in other closely related cucumber species.

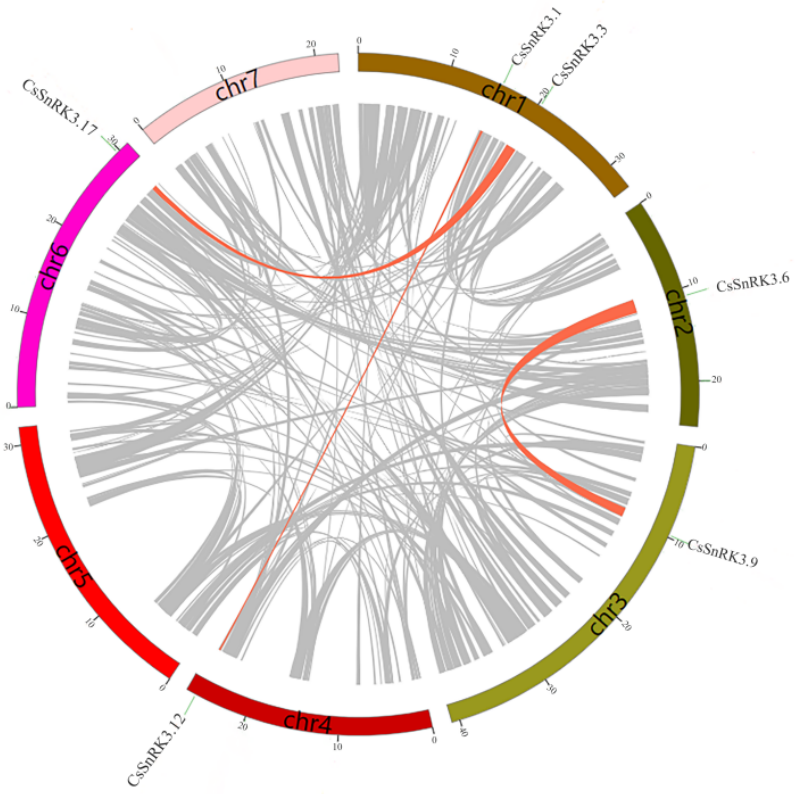

Figure 4. The duplication analysis of *CsSnRK* family in cucumber. Gray lines indicated all synteny blocks in the cucumber genome, and the orange lines indicated duplicated *CsSnRK* gene pairs. The chromosome number is indicated on each chromosome. Scale bar marked on the chromosome indicates chromosome lengths (Mb). Chr, Chromosome.

Table 2. The KaKs values of the paired duplicated *CsSnRK* genes.

Duplicated Genes	Ka	Ks	Ka/Ks
CsSnRK3.1/*CsSnRK3.12*	0.313425	4.27516	0.073313
CsSnRK3.3/*CsSnRK3.17*	0.322712	4.26853	0.075603
CsSnRK3.6/*CsSnRK3.9*	0.23843	1.75127	0.136146

3.5. Cis-Acting Regulatory Elements Analysis in the Promoter Region of CsSnRK Genes

To investigate the potential functions and regulatory mechanisms of CsSnRK genes, we analyzed cis-acting regulatory elements in the 2000 bp promoter regions using the Plant CARE database. The results show that cis-acting regulatory elements were mainly divided into two categories: hormone-responsive and abiotic stress-responsive (Figure 5 and Table S2). Among them, hormone response elements included ABRE, TGA-element, AuxRR-core, P-box, ERE, TCA-element and CGTCA-motif. Almost all CsSnRK genes contained the

ABA-responsive element (ABRE) and the ethylene-responsive element (ERE), indicating that it may be sensitive to hormones. Abiotic stress response elements included MBS, MYC and ARE. MYC was a cis-expression element that regulated drought stress and was contained in all genes, indicating that CsSnRKs played an important regulatory role in drought stress.

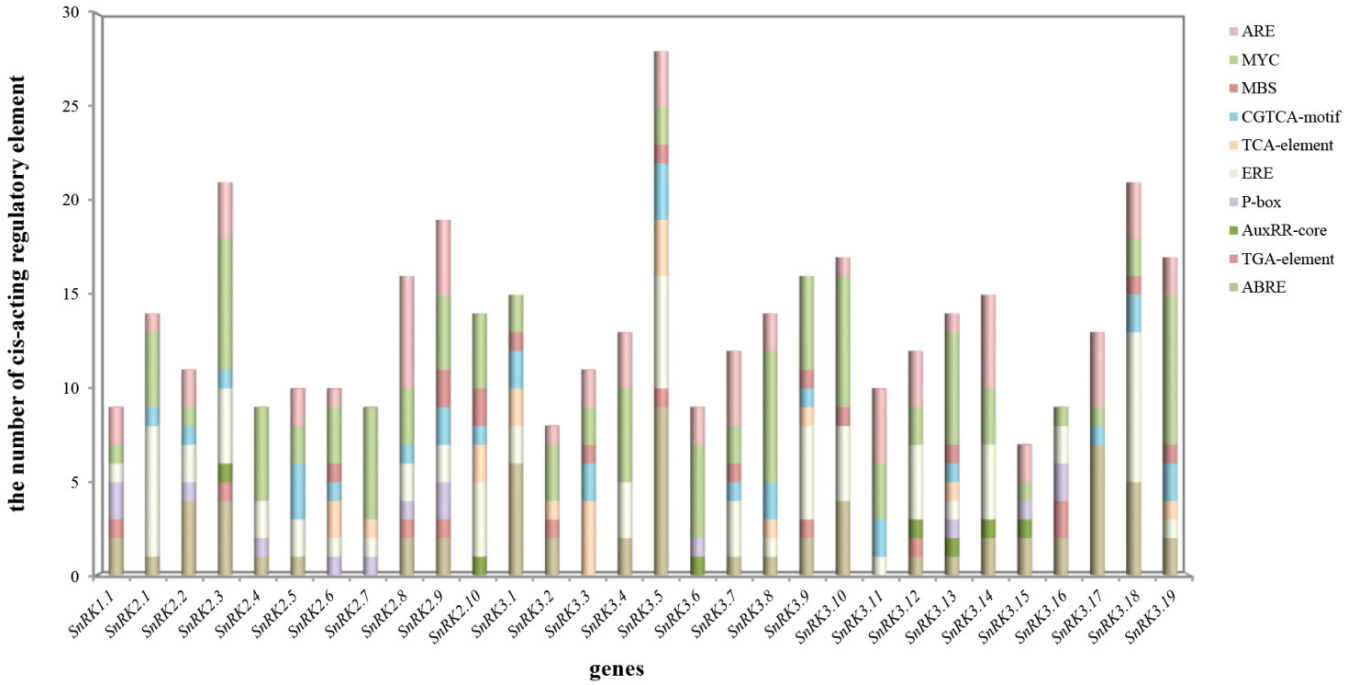

Figure 5. Predicted cis-acting regulatory elements in *CsSnRK* promoters. Different colors represented different cis-elements.

3.6. Expression Profiles Analysis of CsSnRK2 Genes

3.6.1. Expression Patterns of CsSnRK2 Gene Family in Different Tissues

To understand the roles of *CsSnRK2* subfamily members in osmotic stress and hormone response, we analyzed the expression of *CsSnRK2* genes in different tissues (leaf, root, stem, and flower) (Figure 6). The expression level was based on the RNA sequencing data from SRA database of NCBI (accession number SRP071224) (Figure 6A). *CsSnRK2.2*, *CsSnRK2.3*, *CsSnRK2.4*, *CsSnRK2.8*, *CsSnRK2.9* and *CsSnRK2.10* showed high expression in all tissues, whereas the expression of *CsSnRK2.5* and *CsSnRK2.6* were very low in roots, stems and leaves. *CsSnRK2.7* was specifically expressed in roots at very low levels. More significantly, *CsSnRK2.1* was not expressed in roots and stems. *CsSnRK2.3* and *CsSnRK2.4* were highly expressed in female and male flowers. Meanwhile, *CsSnRK2.5*, *CsSnRK2.6* and *CsSnRK2.7* were not expressed in male flower tissues. These results suggest that *CsSnRK2* genes displayed diverse expression patterns and *CsSnRK2* genes might play special roles in different growth stages and different plant organs. To validate the RNA-seq data, qRT-PCR (quantitative real-time polymerase chain reaction) was performed. Total RNA from different tissues collected at developmental stage (Figure 6B), was used to measure the transcription levels of *CsSnRK2* in different tissues. The qRT-PCR data were consistent with the RNA-seq data. The close correlation of these results indicated that the RNA-seq results were accurate in representing the gene expression levels.

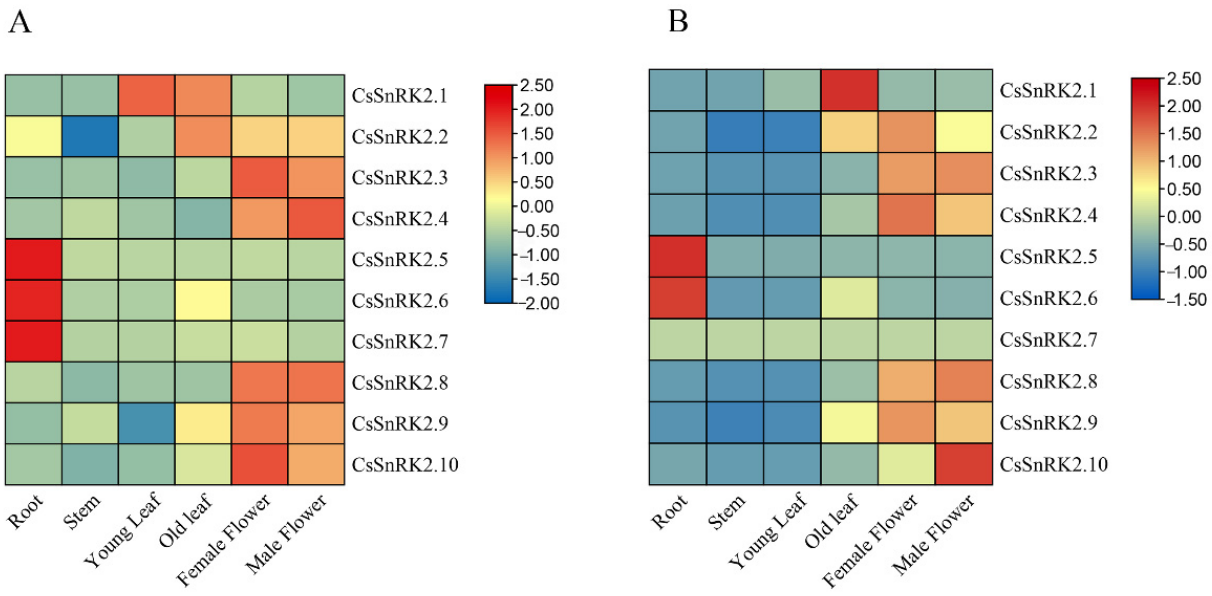

Figure 6. Expression patterns of 10 *CsSnRK2* genes in six tissues of cucumber. (**A**) The expression level based on the RNA sequencing data from SRA database of NCBI (accession number SRP071224). (**B**) The relative expression levels of *CsSnRK2* genes in different tissues from qRT-PCR analysis. Color bars represent expression values: red and blue indicate high and low expression levels, respectively.

To validate the RNA-seq data, qRT-PCR was performed. Total RNA from different tissues collected at developmental stage (Figure 6B), was used to measure the transcription levels of *CsSnRK2* in different tissues. The qRT-PCR data was consistent with the RNA-seq data. The close correlation of these results indicated that the RNA-seq results were accurate in representing the gene expression levels.

3.6.2. Expression Profiles of CsSnRK2 Gene Family under Different Abiotic Stresses

SnRK2s was confirmed to participate widely in response to various environmental elicitors. To investigate the potential function of *CsSnRK2s* at seedling stage in response to exogenous factors, the expression patterns of 10 *CsSnRK2* genes in roots and leaves under PEG, ABA and MeJA treatments were examined using qRT-PCR assays (Figures 7 and 8). In leaves (Figure 7), except for *CsSnRK2.7*, all *CsSnRK2* genes could be induced by various abiotic stresses. All genes were up-regulated after ABA treatment and reached the highest value after 24 h of treatment. Among them, the expression level of *CsSnRK2.2* and *CsSnRK2.3* had the most significant change. After PEG treatment, the expression level of *CsSnRK2.6* was down-regulated. However, other 8 *CsSnRK2* genes reached their highest values and were significantly up-regulated after 12 and 48 h of treatment. Under MeJA treatment, the transcription level of 9 *CsSnRK2* genes almost had no change after 3 and 12 h treatment, but it was significantly up-regulated and reached the highest expression level at 24 or 48 h. Among them, the expression levels of *CsSnRK2.3* and *CsSnRK2.8* were significantly increased by more than 16 times. Taken together, the three abiotic stress treatments induced changes in the abundance of most *CsSnRK2* genes transcripts, especially for the expression of *CsSnRK2.3* and *CsSnRK2.8*, and the expression patterns of *CsSnRK2* genes suggest that *CsSnRK2* genes may play a role in abiotic stress responses in cucumber.

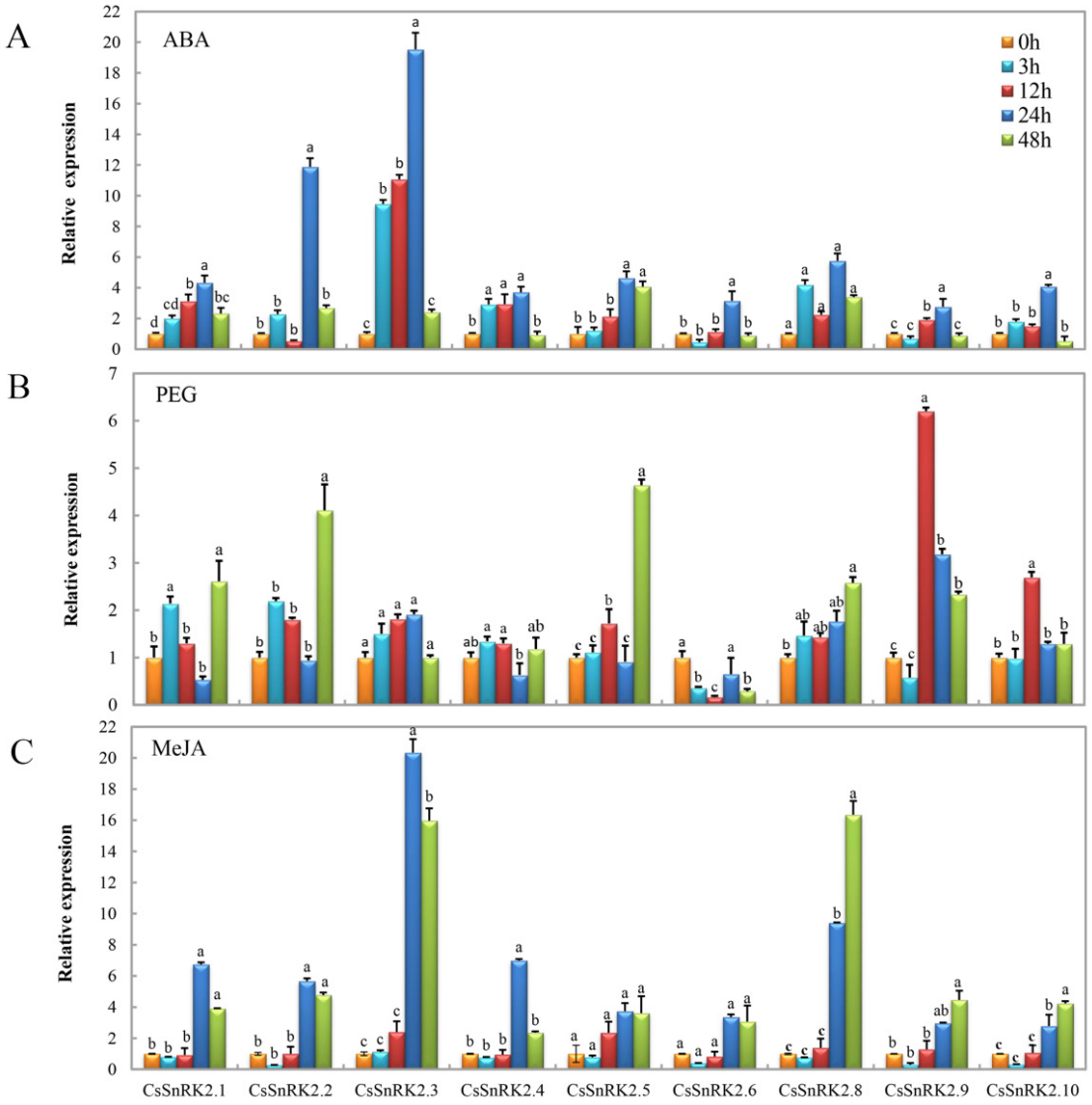

Figure 7. The expression pattern of *CsSnRK* genes in leaves treated with (**A**) abscisic acid (ABA), (**B**) PEG and (**C**) methyl jasmonate (MeJA). The sampling time points were 0, 3, 12, 24 and 48 h. Error bars represent the standard error (SE) of three replicates. The relative expression of each gene in different treatment is expressed as mean ± SE (n = 3). Bars with different lowercase letters were significantly different by Duncan's multiple range tests ($p < 0.05$).

CsSnRK2.1 and *CsSnRK2.7* were not expressed in roots (Figure 8) under different treatments, which was consistent with the results of tissue expression. It also further illustrates that the RNA-seq results are accurate in representing gene expression levels. The expression of *CsSnRK2.2*, *CsSnRK2.3* and *CsSnRK2.9* genes was up-regulated by ABA treatment and reached the highest value after 24 h of treatment. The expression of other genes was down-regulated by ABA stress. In PEG treatment, except for *CsSnRK2.5*, the expression level of all genes decreased after 3 h of treatment and reached the highest value after 12 h. The expression pattern of each gene was different in MeJA treatment. Among them, *CsSnRK2.9* changed most significantly after 12 h of treatment. In addition, we also observed the phenotype of cucumber seedlings after 48 h of different treatments (Figure 9). Drought stress caused the seedlings to wilt and dwarf, while ABA and MeJA treatments inhibited the growth of the seedlings compared to the control. The expression patterns of *CsSnRK2* genes in roots and leaves were different, indicating that *CsSnRK2*s play different roles in different tissues at seedling stage under abiotic stress.

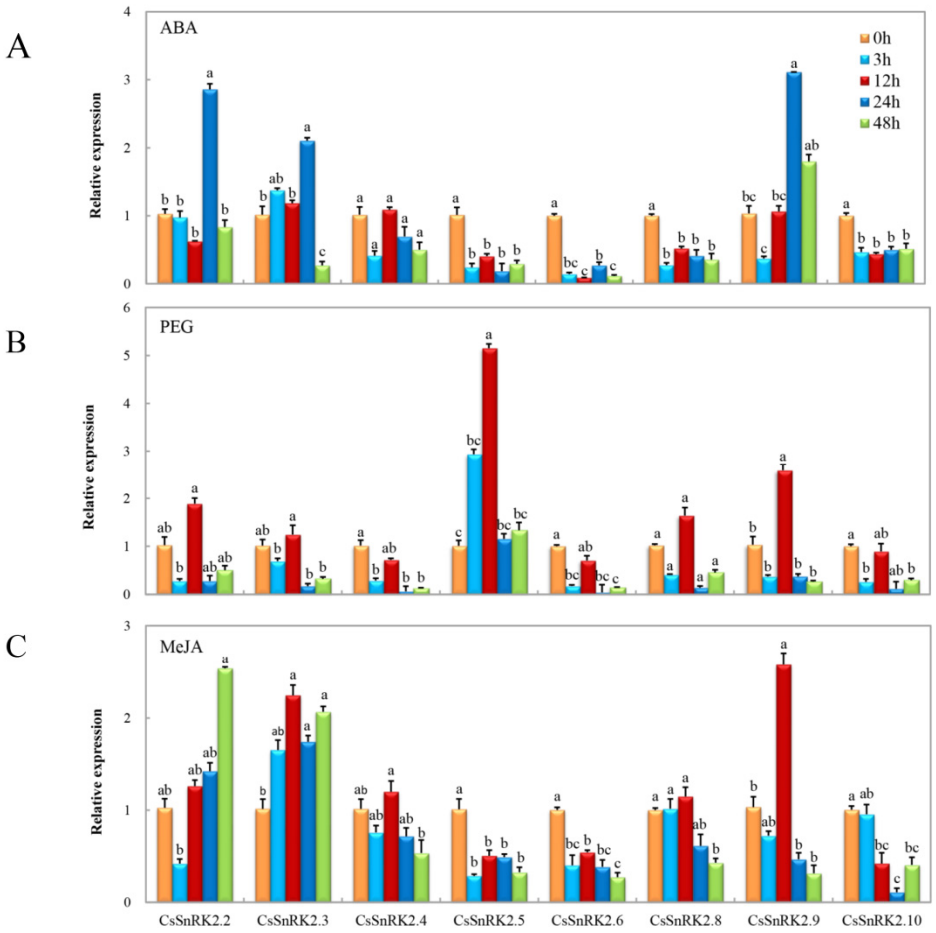

Figure 8. The expression pattern of *CsSnRK2* genes in roots treated with (**A**) abscisic acid (ABA), (**B**) PEG and (**C**) methyl jasmonate (MeJA). The sampling time points were 0, 3, 12, 24 and 48 h. Error bars represent the standard error (SE) of three replicates. The relative expression of each gene in different treatment is expressed as mean ± SE (n = 3). Bars with different lowercase letters were significantly different by Duncan's multiple range tests ($p < 0.05$).

Figure 9. Plant morphology of cucumber seedlings under different treatments. Cucumber seedlings at the two-leaf stage were treated with 15% (w/v) PEG6000, 100 μM methyl jasmonate (MeJA), 100 μM ABA, and photographed 48 h after treatments.

4. Discussion

Phosphorylation of plant protein kinases plays a very important role in the mechanism of response to stress. However, *SnRKs* as a type of Ser/Thr protein kinase represent an interface between stress and ABA signal transduction [39]. It has been shown that 39, 48, 50 and 44 *SnRK* genes were identified in *Oryza sativa*, *Brachypodium distachyon* [3], *Arabidopsis thaliana* [40] and *Hordeum vulgare* [41], respectively. In this study, we were the first to comprehensively identified *SnRK* members of cucumber genome, which will provide crucial information to research their functions in the future. Bioinformatic analysis of *SnRK* family has isolated a total of 30 *CsSnRKs* including 1 *CsSnRK1*, 10 *CsSnRK2s* and 19 *CsSnRK3s* in cucumber. *SnRK2* and *SnRK3* subfamilies were larger and relatively more diverse than *SnRK1*. These results support the previous view that the *SnRK2* and *SnRK3* subfamilies evolved from the *SnRK1* subfamily and that their numbers have increased during evolution [8].

To explore the evolutionary relationship between *CsSnRK* members and other species, we analyzed the phylogenetic tree containing *SnRK* members of cucumber, *Arabidopsis*, rice and barley. *CsSnRKs* were clearly divided into three subgroups and clustered with the corresponding subgroups in rice, *Arabidopsis* and barley, suggesting that the structure and function of *CsSnRK* are highly conserved during plant evolution. *CsSnRKs* from the same subgroup had similar conserved domains, motif composition and gene structure, with differences among different subgroups. Different numbers of exons/introns played important roles in the evolution and function of different gene families. The *CsSnRK1.1* had 10 exons, which was the same as that reported for *EgrSnRK1s* and *BdSnRK1s* [3,7]. *CsSnRK2* subfamily had 9–18 exons. Previous study showed that *SnRK3* subfamily can be divided into two clades according to the number of exons [42]. The *CsSnRK3* subfamily was also subdivided into the exon-rich and exon-poor type. Study has also shown that the *SnRK3* subfamily originated from green algae, and the intron-poor group first appeared in seed plants [6]. These results were consistent with phylogenetic tree studies showing that *SnRKs* exon-intron numbers were highly conserved during the evolution of each subfamily. Different subfamilies of *SnRK* genes contained different conserved domains, but all *SnRK* genes in cucumber contained an N-terminal protein kinase domain. Each domain had its own unique function, for example, *CsSnRK3* subfamily genes contained NAF domains and have been reported to interact with CBLs in a calcium-dependent manner. Furthermore, the NAF domain as a target of the CBL calcium sensor protein was defined a group of heterologous kinases involved in a variety of signal transduction processes [21]. This study also found that different *CsSnRKs* subfamily genes shared different types of conserved domains. This may indicate that there was functional diversity in the *CsSnRK* gene family according to their domains.

In our current research, the identified *CsSnRK* genes were unevenly distributed across the six chromosomes of the cucumber genome. In addition, based on the distribution of *CsSnRKs* in chromosomes, we identified gene duplication events. Gene duplication plays an irreplaceable role in the evolution of plant genomes and genetic systems [43]. Duplicated genes can generate new genes and corresponding new functions in the form of segmental duplications, tandem duplications and translocation events. Segmental and tandem duplications can lead to the expansion of gene families [44]. Our research results demonstrate that in the cucumber genome, three duplication events were observed between chromosomes, which evolved from segment duplication. These segmental duplications might have contributed greatly to the expansion and evolution of the *CsSnRK* gene family.

When plants are stimulated by external factors, some transcription factors are activated, and the activated factors combine with the cis-acting elements of the downstream target gene promoters to change the expression pattern of genes. The study of gene cis-acting elements is particularly important for mining the potential function of gene. To date, many cis-acting elements have been well characterized and divided into distinct groups [45]. Many cis-acting elements responsive to hormone responses were found in the promoter sequence of the *CsSnRK* genes, such as ABRE, ERE, CGTCA-motif and TGA-element. These

results indicate that the *CsSnRK* genes might respond to a variety of hormone signals and interact with other metabolic pathways. A large body of evidence indicates that the *SnRK* family was widely involved in the response to various biotic and abiotic stresses, including drought, salt and temperature stresses. The cis-acting element in response to drought stress, such as MBS and MYC, were also found in *CsSnRK* genes. The widespread presence of these cis-acting elements suggests a critical role for *CsSnRK* members in responding to abiotic stress and hormonal stimuli. For instance, under ABA treatment, *CsSnRKs* was not expressed, which was consistent with the distribution of ABA-responsive elements. However, some conflicting results also emerged in our study. The promoter region of *CsSnRK2.6* did not contain an AREB binding site, but it responded to ABA treatment at a low level. Therefore, gene activity is often associated with differences in cis-acting elements in the promoter region [46].

Numerous studies demonstrated that *SnRK2s* were involved in multiple abiotic stress responses. In *Arabidopsis*, *SnRK2* genes (*SRK2C*, *SRK2D*, *SRK2E*, *SRK2F* and *SRK2I*) were activated by ABA [47,48]. In cotton, *GhSnRK2.3*, *GhSnRK*2.7, *GhSnRK2.8*, *GhSnRK*2.9 and *GhSnRK*2.10 were notably up-regulated under salt and PEG treatment [49]. In pepper, *CaSnRK2.1*, *CaSnRK2.3* and *CaSnRK2.8* were significantly induced by PEG and NaCl treatment [50]. In this study, in order to verify the response of *CsSnRK* genes to abiotic stress, we used qRT-PCR to detect the expression of 10 *CsSnRK2* genes in leaves and roots. All *CsSnRK2s* were found to respond to PEG, ABA and MeJA stress except for *CsSnRK2.7* of leaves and *CsSnRK2.1 and CsSnRK2.7* of roots. In cucumber seedling leaves, *CsSnRK2.3* was significantly up-regulated under ABA and MeJA treatments. *CsSnRK2.2*, *CsSnRK2.5* and *CsSnRK2.9* were significantly up-regulated under PEG treatment. In roots, *CsSnRK2.2*, *CsSnRK2.3* and *CsSnRK2.9* were significantly up-regulated by ABA and MeJA treatments. *CsSnRK2.5* was significantly up-regulated by PEG treatment. The above results indicate that *CsSnRK2.3* might regulate ABA and MeJA synthesis in leaves, while *CsSnRK2.2*, *CsSnRK2.5* and *CsSnRK2.9* might play a major role in regulating drought stress in leaves. Meanwhile, *CsSnRK2.5* might play a major role in the regulation of drought in roots, *CsSnRK2.2*, *CsSnRK2.3* and *CsSnRK2.9* regulate ABA and MeJA synthesis in roots. In *Arabidopsis*, *AtSnRK2.2* also responded to drought [51]. *CsSnRKs* showed different expression patterns for different treatments, suggesting that *CsSnRKs* may be specific during evolution. Therefore, further studies on the interacting proteins of the *CsSnRK2* family and phenotypic changes in transgenic plants will help to clarify the precise biological role of each member of the *CsSnRK2* family in vivo, and, thus, determine the involvement of the ABA pathway on *CsSnRK2s*.

In plants, *SnRK* genes have been widely transcribed in many tissues, but different *SnRK* members have different expression levels at different growth and development stages [3]. The extensive expression of *SnRK* genes indicates that *SnRKs* could be necessary for plants to grow and develop different tissues. For example, there were eight *SnRK2* genes that were widely transcribed in a variety of tissues in *Brachypodium distachyon* [3]. In apples, *MdCIPK4*, *MdCIPK*9, *MdCIPK*15 and *MdCIPK*32 were highly expressed in flowers, and *MdCIPK*29 was highly expressed in fruits, indicating that they had different biological functions in their respective tissues [52]. In this study, transcriptome data from different tissues of *CsSnRK2* genes were used to study the expression patterns. Six genes were highly expressed in flower tissues, while *CsSnRK2.5*, *CsSnRK2.6* and *CsSnRK2.7* were very poorly expressed. All genes were expressed in roots except *CsSnRK2.1*. The above results indicate that *CsSnRK* genes had tissue-specific and developmental stage-specific expression in different tissues.

5. Conclusions

In this study, the identified 30 cucumber *SnRK* gene families were comprehensively studied and divided into 3 subfamilies with high similarity in gene structure and motif composition. The evolutionary relationship of *CsSnRK* genes in cucumber and other species was established, showing that these subfamilies were evolutionarily conserved in both

structure and function. Moreover, the cis-acting elements, tissue-specific analysis of *CsSnRK* family were also determined. Last but not least, we provided evidence that cucumber *SnRKs* were involved in alleviating drought stress and hormonal responses. The present genomic and bioinformatics analyses of *CsSnRK* genes provided a solid foundation for further investigation of *CsSnRK* gene functions. In addition, these results will also help to expand our knowledge to identify candidate genes that improve plant architecture under stressful conditions, and open up possibilities for breeding and genetic improvement of other agricultural crops.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12071550/s1. Table S1: Primer sequence of *CsSnRK2* for real-time RT-PCR. Table S2: cis-acting regulatory elements in *CsSnRK* promoters of cucumber.

Author Contributions: Formal analysis, Y.L. and W.L.; Funding acquisition, C.W. and W.L.; Software, Y.N.; Visualization, R.G. and W.L.; Writing—original draft, Y.L.; Writing—review and editing, Y.L. and W.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key Research and Development Program (2018YFD1000800); the National Natural Science Foundation of China (Nos. 31860568, 31560563 and 31160398); the Research Fund of Higher Education of Gansu, China (No. 2018C-14); the Post-Doctoral Foundation of China (Nos. 20100470887 and 2012T50828) and the Natural Science Foundation of Gansu Province, China (Nos. 1606RJZA073 and 1606RJZA077).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All relevant data are contained within the article.

Acknowledgments: We would like to thank Wei Li (Shenzhen Institute of Genomics, Chinese Academy of Agricultural Sciences, China) for providing us with cucumber seeds.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Demidchik, V.; Maathuis, F.; Voitsekhovskaja, O. Unravelling the plant signalling machinery: An update on the cellular and genetic basis of plant signal transduction. *Funct. Plant Boil.* **2018**, *45*, 1–8. [CrossRef] [PubMed]
2. Bohnert, H.J.; Gong, Q.Q.; Li, P.H.; Ma, S.S. Unraveling abiotic stress tolerance mechanisms-getting genomics going. *Curr. Opin. Plant Biol.* **2006**, *9*, 180–188. [CrossRef] [PubMed]
3. Wang, L.; Hu, W.; Sun, J.; Liang, X.; Yang, X.; Wei, S.; He, G. Genome-wide analysis of *SnRK* gene family in *Brachypodium distachyon*, and functional characterization of *BdSnRK2. 9*. *Plant Sci.* **2015**, *237*, 33–45. [CrossRef] [PubMed]
4. Baba, A.I.; Rigó, G.; Ayaydin, F.; Rehman, A.U.; Andrási, N.; Zsigmond, L.; Cséplő, Á. Functional analysis of the *Arabidopsis thaliana*, CDPK-related kinase family: AtCRK1 regulates responses to continuous light. *Int. J. Mol. Sci.* **2018**, *19*, 1282. [CrossRef]
5. Xu, J.; Zhang, S. Mitogen-activated protein kinase cascades in signaling plant growth and development. *Trends Plant Sci.* **2015**, *20*, 56–64. [CrossRef]
6. Colina, F.; Amaral, J.; Carbó, M.; Pinto, G.; Soares, A.; Cañal, M.J.; Valledor, L. Genome-wide identification and characterization of CKIN/SnRK gene family in *Chlamydomonas reinhardtii*. *Sci. Rep.* **2019**, *9*, 350. [CrossRef]
7. Wang, Y.; Yan, H.; Qiu, Z.; Hu, B.; Zeng, B.; Zhong, C.; Fan, C. Comprehensive analysis of *SnRK* gene family and their responses to salt stress in *Eucalyptus grandis*. *Int. J. Mol. Sci.* **2019**, *20*, 2786. [CrossRef]
8. Zhang, Y.; Ye, Y.; Jiang, L.; Lin, Y.; Gu, X.; Chen, Q.; Sun, B.; Zhang, Y.; Luo, Y.; Wang, Y.; et al. Genome-wide characterization of Snf1-Related Protein Kinases (SnRKs) and expression analysis of SnRK1. 1 in strawberry. *Genes* **2020**, *11*, 427. [CrossRef]
9. Hrabak, E.M.; Chan, C.W.; Gribskov, M.; Harper, J.F.; Choi, J.H.; Halford, N.; Harmon, A.C. The Arabidopsis CDPK-SnRK superfamily of protein kinases. *Plant Physiol.* **2003**, *132*, 666–680. [CrossRef]
10. Coello, P.; Hey, S.J.; Halford, N.G. The sucrose non-fermenting-1-related (SnRK) family of protein kinases: Potential for manipulation to improve stress tolerance and increase yield. *J. Exp. Bot.* **2011**, *62*, 883–893. [CrossRef]
11. Halford, N.G.; Hey, S.J. Snf1-related protein kinases (SnRKs) act within an intricate network that links metabolic and stress signalling in plants. *Biochem. J.* **2009**, *419*, 247–259. [CrossRef] [PubMed]
12. Hey, S.J.; Byrne, E.; Halford, N.G. The interface between metabolic and stress signalling. *Ann. Bot.* **2009**, *105*, 197–203. [CrossRef] [PubMed]

13. Alderson, A.; Sabelli, P.A.; Dickinson, J.R.; Cole, D.; Richardson, M.; Kreis, M.; Halford, N.G. Complementation of snf1, a mutation affecting global regulation of carbon metabolism in yeast, by a plant protein kinase cDNA. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 8602–8605. [CrossRef] [PubMed]
14. Jossier, M.; Bouly, J.P.; Meimoun, P.; Arjmand, A.; Lessard, P.; Hawley, S.; Thomas, M. SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in *Arabidopsis thaliana*. *Plant J.* **2009**, *59*, 316–328. [CrossRef] [PubMed]
15. Radchuk, R.; Radchuk, V.; Weschke, W.; Borisjuk, L.; Weber, H. Repressing the expression of the SUCROSE NONFERMENTING-1-RELATED PROTEIN KINASE gene in pea embryo causes pleiotropic defects of maturation similar to an abscisic acid-insensitive phenotype. *Plant Physiol.* **2006**, *140*, 263–278. [CrossRef]
16. Baena-González, E.; Rolland, F.; Thevelein, J.M.; Sheen, J. A central integrator of transcription networks in plant stress and energy signalling. *Nature* **2007**, *448*, 938–942. [CrossRef]
17. Saha, J.; Chatterjee, C.; Sengupta, A.; Gupta, K.; Gupta, B. Genome-wide analysis and evolutionary study of sucrose non-fermenting 1-related protein kinase 2 (*SnRK2*) gene family members in *Arabidopsis* and *Oryza*. *Comput. Boil. Chem.* **2014**, *49*, 59–70. [CrossRef]
18. Kulik, A.; Wawer, I.; Krzywińska, E.; Bucholc, M.; Dobrowolska, G. SnRK2 protein kinases-key regulators of plant response to abiotic stresses. *Omics* **2011**, *15*, 859–872. [CrossRef]
19. Boudsocq, M. Barbier-Brygoo, H., Lauriere, C. Identification of nine sucrose non-fermenting 1-related protein kinases 2 activated by hyperosmotic and saline stresses in *Arabidopsis thaliana*. *J. Biol. Chem.* **2004**, *279*, 41758–41766. [CrossRef]
20. Fujii, H.; Verslues, P.E.; Zhu, J.K. *Arabidopsis* decuple mutant reveals the importance of SnRK2 kinases in osmotic stress responses in vivo. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 1717–1722. [CrossRef]
21. Albrecht, V.; Ritz, O.; Linder, S.; Harter, K.; Kudla, J. The NAF domain defines a novel protein-protein interaction module conserved in Ca^{2+}-regulated kinases. *EMBO J.* **2001**, *20*, 1051–1063. [CrossRef] [PubMed]
22. Xiang, Y.; Huang, Y.; Xiong, L. Characterization of stress-responsive *CIPK* genes in rice for stress tolerance improvement. *Plant Physiol.* **2007**, *144*, 1416–1428. [CrossRef] [PubMed]
23. Chen, L.; Ren, F.; Zhou, L.; Wang, Q.Q.; Zhong, H.; Li, X.B. The *Brassica napus* calcineurin B-Like 1/CBL-interacting protein kinase 6 (CBL1/CIPK6) component is involved in the plant response to abiotic stress and ABA signalling. *J. Exp. Bot.* **2012**, *63*, 6211–6222. [CrossRef] [PubMed]
24. Li, J.; Yang, X.L.; Zhao, Y.Z. Selection for cold-resistance cucumber varieties in solar greenhouse in Beijing and Tianjin. *Tianjin Agric. Sci.* **2019**, *25*, 26–28+33.
25. Fujii, H.; Zhu, J.K. Osmotic stress signaling via protein kinases. *Cell. Mol. Life Sci.* **2012**, *69*, 3165–3173. [CrossRef]
26. Shao, Y.; Qin, Y.; Zou, Y.; Ma, F. Genome-wide identification and expression profiling of the *SnRK2* gene family in *Malus prunifolia*. *Gene* **2014**, *552*, 87–97. [CrossRef]
27. McWilliam, H.; Li, W.; Uludag, M.; Squizzato, S.; Park, Y.M.; Buso, N.; Lopez, R. Analysis tool web services from the EMBL-EBI. *Nucleic Acids Res.* **2013**, *41*, W597–W600. [CrossRef]
28. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]
29. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef]
30. Hu, B.; Jin, J.; Guo, A.Y.; Zhang, H.; Luo, J.; Gao, G. GSDS 2.0: An upgraded gene feature visualization server. *Bioinformatics* **2015**, *31*, 1296–1297. [CrossRef]
31. Wang, Y.; Tang, H.; DeBarry, J.D.; Tan, X.; Li, J.; Wang, X.; Paterson, A.H. MCScanX: A toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res.* **2012**, *40*, e49. [CrossRef]
32. Cannon, S.B.; Mitra, A.; Baumgarten, A.; Young, N.D.; May, G. The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. *BMC Plant Biol.* **2004**, *4*, 10. [CrossRef]
33. Wei, G.; Tian, P.; Zhang, F.; Qin, H.; Miao, H.; Chen, Q.; Hu, Z.; Cao, L.; Wang, M.; Gu, X.; et al. Integrative analyses of nontargeted volatile profiling and transcriptome data provide molecular insight into VOC diversity in cucumber plants (*Cucumis sativus*). *Plant Physiol.* **2016**, *172*, 603–618. [CrossRef] [PubMed]
34. Gao, R.; Luo, Y.; Yun, F.; Wu, X.; Wang, P.; Liao, W. Genome-wide identification, expression profile, and alternative splicing analysis of CAMTA family genes in Cucumber (*Cucumis sativus* L.). *Agronomy* **2021**, *11*, 1827. [CrossRef]
35. He, M.W.; Wang, Y.; Wu, J.Q.; Shu, S.; Sun, J.; Guo, S.R. Isolation and characterization of *S*-Adenosylmethionine synthase gene from cucumber and responsive to abiotic stress. *Plant Physiol. Biochem.* **2019**, *141*, 431–445. [CrossRef] [PubMed]
36. Noman, M.; Jameel, A.; Qiang, W.D.; Ahmad, N.; Liu, W.C.; Wang, F.W.; Li, H.Y. Overexpression of *GmCAMTA12* enhanced drought tolerance in *Arabidopsisand soybean*. *Int. J. Mol. Sci.* **2019**, *20*, 4849. [CrossRef]
37. Zhou, Y.; Hu, L.; Wu, H.; Jiang, L.; Liu, S. Genome-wide identification and transcriptional expression analysis of cucumber superoxide dismutase (SOD) family in response to various abiotic stresses. *Int. J. Genom.* **2017**, *2017*, 7243973. [CrossRef] [PubMed]
38. Han, L.M.; Hua, W.P.; Cao, X.Y.; Yan, J.A.; Chen, C.; Wang, Z.Z. Genome-wide identification and expression analysis of the superoxide dismutase (SOD) gene family in *Salvia miltiorrhiza*. *Gene* **2020**, *742*, 144603. [CrossRef]
39. Emanuelle, S.; Doblin, M.S.; Stapleton, D.I.; Bacic, A.; Gooley, P.R. Molecular insights into the Enigmatic Metabolic Regulator, SnRK1. *Trends Plant Sci.* **2016**, *21*, 341–353. [CrossRef] [PubMed]

40. Lyzenga, W.J.; Liu, H.; Schofield, A.; Muise-Hennessey, A.; Stone, S.L. Arabidopsis CIPK26 interacts with KEG, components of the ABA signalling network and is degraded by the ubiquitin-proteasome system. *J. Exp. Bot.* **2013**, *64*, 2779–2791. [CrossRef]
41. Chen, Z.; Zhou, L.; Jiang, P.; Lu, R.; Halford, N.G.; Liu, C. Genome-wide identification of sucrose nonfermenting-1-related protein kinase (SnRK) genes in barley and RNA-seq analyses of their expression in response to abscisic acid treatment. *BMC Genom.* **2021**, *22*, 300. [CrossRef] [PubMed]
42. Tang, J.; Lin, J.; Li, H.; Li, X.; Yang, Q.; Cheng, Z.M.; Chang, Y. Characterization of CIPK family in Asian pear (*Pyrus bretschneideri* Rehd) and co-expression analysis related to salt and osmotic stress responses. *Front. Plant Sci.* **2016**, *7*, 1361. [CrossRef] [PubMed]
43. Moore, R.C.; Purugganan, M.D. The early stages of duplicate gene evolution. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15682–15687. [CrossRef] [PubMed]
44. Kong, H.; Landherr, L.L.; Frohlich, M.W.; Leebens-Mack, J.; Ma, H.; DePamphilis, C.W. Patterns of gene duplication in the plant *SKP1* gene family in angiosperms: Evidence for multiple mechanisms of rapid gene birth. *Plant J.* **2007**, *50*, 873–885. [CrossRef]
45. Hadiarto, T.; Tran, L.S.P. Progress studies of drought-responsive genes in rice. *Plant Cell Rep.* **2011**, *30*, 297–310. [CrossRef]
46. Xue, T.; Wang, D.; Zhang, S.; Ehlting, J.; Ni, F.; Jakab, S.; Zheng, C.; Zhong, Y. Genome-wide and expression analysis of protein phosphatase 2C in rice and Arabidopsis. *BMC Genom.* **2008**, *9*, 550. [CrossRef]
47. Fujii, H.; Zhu, J.K. Arabidopsis mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction, and stress. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 8380–8385. [CrossRef]
48. Wang, P.; Xue, L.; Batelli, G.; Lee, S.; Hou, Y.J.; van Oosten, M.J.; Zhang, H.; Tao, W.A.; Zhu, J.K. Quantitative phosphoproteomics identifies SnRK2 protein kinase substrates and reveals the effectors of abscisic acid action. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 11205–11210. [CrossRef]
49. Liu, Z.; Ge, X.; Yang, Z.; Zhang, C.; Zhao, G.; Chen, E.; Liu, J.; Zhang, X.; Li, F. Genome-wide identification and characterization of *SnRK2* gene family in cotton (*Gossypium hirsutum* L.). *BMC Genet.* **2017**, *18*, 54. [CrossRef]
50. Wu, Z.; Cheng, J.; Hu, F.; Qin, C.; Xu, X.; Hu, K. The SnRK2 family in pepper (*Capsicum annuum* L.): Genome-wide identification and expression analyses during fruit development and under abiotic stress. *Genes Genom.* **2020**, *42*, 1117–1130. [CrossRef]
51. Yoshida, T.; Fujita, Y.; Maruyama, K.; Mogami, J.; Todaka, D.; Shinozaki, K.; Yamaguchi-Shinozaki, K.A.Z.U.K.O. Four *Arabidopsis* AREB/ABF transcription factors function predominantly in gene expression downstream of SnRK2 kinases in abscisic acid signalling in response to osmotic stress. *Plant Cell Environ.* **2015**, *38*, 35–49. [CrossRef] [PubMed]
52. Niu, L.; Dong, B.; Song, Z.; Meng, D.; Fu, Y. Genome-wide identification and characterization of *CIPK* family and analysis responses to various stresses in apple (*Malus domestica*). *Int. J. Mol. Sci.* **2018**, *19*, 2131. [CrossRef] [PubMed]