Genome-Wide Identification and Expression Analysis of the *CmHAK* Gene Family in Melon (*Cucumis melo* L.)

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Abstract: (1) Background: As the largest family of potassium transporters in plants, KT/HAK/KUP plays an important function in plant growth, development, and stress, especially for potassium-loving plants such as melon. (2) Methods: The members of the KT/HAK/KUP gene family in the melon genome were identified by bioinformatics technology. The gene structure, chromosome location, phylogeny, and expression analysis were comprehensively and systematically analyzed. (3) Results: The results showed that there are 14 members of the KT/HAK/KUP gene family in melon, which are distributed on seven chromosomes. Each member contains 3–11 introns and 4–12 exons, and could be divided into three distinct branches in phylogeny. The number of amino acid residues encoded by each member varies between 610 and 878. In terms of expression, after 12 h of chilling stress, most of the *CmHAK* genes were expressed in two melon varieties with different chilling resistances. The expression levels of *CmHAK6* and *CmHAK8* were downregulated in chilling-resistant varieties, but there was no significant change in chilling-sensitive varieties, indicating that *CmHAK6* and *CmHAK8* genes may play a negative regulatory role in chilling-resistant varieties. (4) Conclusions: The study provides a theoretical basis for in-depth analysis of the functions of KT/HAK/KUP gene family and cultivation of high-potassium stress-resistant melon varieties.

Keywords: melon; KT/HAK/KUP; CmHAK; bioinformatics; chilling stress

1. Introduction

Melon (*Cucumis melo* L.) is an important horticultural crop that belongs to the *Cucurbitaceae* family [1]. Melon is native to Persia (Iran) and its eastern and western neighbors, and is widely cultivated in temperate to tropical regions of the world [2]. Due to its fresh, tender, juicy flesh and sweet taste, it is deeply loved by widespread consumers [3]. Potassium plays an important role in the growth and stress responses of melon [2,4], and is one of the essential nutrients for plant growth, accounting for 2% to 7% of the total dry matter of plants [5]. Potassium can regulate the osmotic pressure of cells, balance the charge inside and outside the membrane, participate in the enzyme activity reaction, improve the photosynthetic reaction rate, and promote the transportation of sugar and protein synthesis in the plant. Potassium plays an important regulatory role in plant root growth, photosynthesis, stress resistance, water balance, and acid–base balance [6]. Research has shown that using cultivation techniques and other means can improve the efficiency of potassium utilization in melon, thereby promoting the improvement of melon quality and yield [7]. However, there have been no reports on the molecular mechanism of potassium

ion absorption in melon [8]. Thus, studying the absorption and transport of potassium in melon has become a current research direction.

Plants absorb and transport potassium by two systems of potassium ion channel and transporters [9]. According to previous studies in Arabidopsis and rice, it has been determined that potassium transporters are mainly divided into four families: KEA, KT/HAK/KUP, Trk/HKT, and CHX. As the largest family of potassium transporters in plants, the KT/HAK/KUP gene family was first identified in *Escherichia coli*, known as the KUP transporter, and is responsible for potassium ion absorption. If found in soil yeast, it is called the HAK1 transporter, which has a high affinity for potassium ion [10]. The KT/HAK/KUP gene family generally contains 10–15 transmembrane regions with conserved potassium transport domains, mainly located in the cell membrane system and expressed in organs such as roots, stems, leaves, and fruits of plants [11]. The KT/HAK/KUP gene family consists of four subfamilies (I–IV), each with a relatively conservative structure and function. Subfamily I is mainly expressed in roots and participates in the absorption and transportation of potassium in the environment by plant roots. Subfamily II is mainly involved in physiological processes such as plant growth regulation. There are few reports on the functions of subfamilies III and IV [12].

The HAK gene family has a large number of members, mainly involved in the absorption and transportation of potassium in plants and the transportation of root auxin [13]. The functions of the HAK gene family in plants are highly conserved and play an important role in coping with stress [14]. The *OsHAK5* gene in rice, when overexpressed, can increase its salt tolerance by transporting potassium from the underground to the aboveground parts. The *AtHAK5* gene in Arabidopsis can respond to salt stress by regulating potassium transport [15]. The *IbHAK8* gene in sweet potatoes is significantly upregulated under drought and salt stress, and it is speculated that this gene plays an important role in sweet potatoes' resistance to drought and salt stress [16]. When wheat seedlings are exposed to potassium deficiency, high salt stress, and dehydration conditions, the expression level of the TaHAK gene family in wheat seedlings significantly increases or decreases [17]. Under salt and drought treatments, the expression of *MtHAK2/6/7/12* in alfalfa was highly induced, indicating that the HAK gene plays a key role in alfalfa's response to various abiotic stresses [18]. Thus far, the HAK gene family has been reported in Arabidopsis, rice, corn, wheat, soybean, potato, tomato, rapeseed, pear, cassava, chili pepper, pomegranate, banana, and apple, and some HAK genes have been cloned and the functions of gene products verified [19,20], but relevant research in melon has not yet been carried out.

In this study, according to the latest melon reference genome, the members of the KT/HAK/KUP gene family in the melon genome were identified through bioinformatics technology, and the gene structure, chromosome location, phylogeny, and expression analysis were comprehensively and systematically analyzed, aiming to provide a theoretical basis for in-depth analysis of the function of KT/HAK/KUP gene family and cultivation of high-potassium stress-resistant melon varieties.

2. Materials and Methods

2.1. Genome Wide Identification of CmHAK in Melon

To identify the HAK proteins of melon, the HAK protein sequences of *Arabidopsis*, *Oryza sativa*, *Fragaria vesca*, *Vitis vinifera*, and *Capsicum annuum* were obtained from the Phytozome (12.1; https://phytozome.jgi.doe.gov/pz/portal.html, accessed on 14 September 2022) database, which were taken as the construction of a hidden Markov model with HMMER 3.0 (this tool can be used to create probability models for protein and DNA sequence domain families, as well as annotate new sequences) (https://www.ebi.ac.uk/Tools/hmmer/search/phmmer/, accessed on 10 December 2022). Using this model, all putative HAK protein sequences from the melon genome database (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_025177605.1/, accessed on 14 September 2022) were identified. Next, the HAK family reference sequence was created using blastp (version: ncbi blast 2.10.1+) by aligning the sequences of these

proteins (the e-value was set to 1×10^{-5}). The alignment sequence was used as the candidate HAK family sequence. These sequences were combined as candidate HAK protein sequences. Pfamscan (version: v1.6) and the Pfam A database (version: v33.1) were used to annotate the sequence domain obtained [21]. The 14 candidate *CmHAK* genes were numbered according to the ANSI (ANSI encoding is a character encoding standard used to map characters to corresponding integer values.) encoding method [22,23].

Subcellular localization was predicted using Softberry (http://linux1.softberry.com/berry.phtml?topic=protcomppl&group=programs&subgroup=proloc, accessed on 10 May 2022). The ExPASy website (http://web.expasy.org/protparam/, accessed on 10 May 2022) was used to determine the chemical and physical characteristics of the CmHAK proteins, including their molecular weight, amino acid count, isoelectric point (pI), GRAVY, instability index, and aliphatic index. The CmHAK members' chromosomal positions were investigated using an easy-to-use web tool (http://mg2c.iask.in/mg2c_v2.1/, accessed on 10 May 2022, Chinese Academy of Agricultural Sciences, Qingdao, China). Based on various artificial neural network algorithms, SignalP 4.0 [24] software was used to predict the hidden position of the CmHAK protein in the given amino acid sequence and the cutting site of the signal peptide. The transmembrane signal of PbrLhc was predicted using the transmembrane prediction tool DeepTMHMM (version: v1.0.8, https://dtu.biolib.com/DeepTMHMM, accessed on 10 May 2022).

2.2. Classification, Structural, and Cis-Element of the Promoter Region Analysis of CmHAK Genes

The Gene Structure Display Server (http://gsds.gao-lab.org/, accessed on 10 May 2022, Peking University, Beijing, China) was utilized to analyze the *CmHAK* gene structures. The MEME software (v5.0.5, http://meme.nbcr.net/meme, accessed on 10 May 2022, University of Nevada, Reno, NV, USA), was used to find the common motifs, with the maximum number of motifs set to 15 and the ideal motif width set between 6 and 20. The PlantRegMap database was utilized to analyze putative TF binding sites in the 2 kb promoter regulatory region sequences upstream of the *CmHAK* beginning point [25]. The species chosen was melon, and the e-value was set at 1×10^{-4}.

2.3. Phylogenetic Tree of CmHAK Genes

Multiple alignments of amino acid sequences were performed using ClustalW [26]. With the use of MAFFT (version: v1.6) software, the protein sequences of HAK family genes from six species (melon, Arabidopsis, rice, chili peppers, grapes, and strawberries) were aligned using the default settings. Finally, these HAK protein sequences were used to create a phylogenetic tree, which was then visualized by MEGA7.0 using the neighbor-joining method with 1000 bootstrap replicates.

2.4. Collinearity, Ka/Ks, and Expression Pattern Analysis

MCScanX (version: v2) software was used to analyze the collinearity of the *CmHAK* gene [27], and Circos (version: v2) software was used to visualize the obtained data and information. Utilizing KaKs_Calculator2.0 [28], we predicted the selection pressure of the PbrLhc protein. The RNA-seq data of *CmHAK* genes under different chilling stress conditions were obtained from the melon transcriptome database (https://peerj.com/articles/12921/#supp-4, accessed on 14 January 2023) [29], the data were analyzed using MAV (version: v1.1) software, and a clustering heat map of expression was generated using the hierarchical clustering (HCL) method.

3. Results

3.1. Identification of CmHAK Genes

A total of 14 *CmHAK* genes were identified. The genes were named from *CmHAK1* to *CmHAK14*. All of the 14 CmHAK gene and protein sequences are shown in Supplemental Table S1. As shown in Table 1, these results contain the gene identifier in the genome database, chromosomal location, and some basic genetic properties. The amino acid lengths of these CmHAKs were determined to be between 610 and 878 aa. CmHAK13 was identified to be the smallest protein, with 610 aa, whereas the largest one was CmHAK12 (878 aa). The pIs were between 5.48 and 9.15, and the molecular weights ranged from 68,685.45 to 99,060.22 Da. Among these results, the pI of *CmHAK10* was the lowest, whereas the pI of *CmHAK5* was the highest. All CmHAK proteins were predicted to have no signal peptides and only have one transcript. Further analyses indicated that most of CmHAK proteins contain 12 transmembrane regions (TMRs), but CmHAK5 and CmHAK13 were predicted to have 10 and 8 TMRs, respectively (Table 1; Figure S1).

Table 1. Statistical table of family member information.

Name	Gene ID	Protein Number	Number of Amino Acid	Molecular Weight (Da)	PI	Instability Index	Aliphatic Index	Gravy	Number of Predicted TMRs
CmHAK1	MELO3C002408.2	MELO3C002408.2.1	790	88,244.04	6.93	38.39	109.38	0.40	12
CmHAK2	MELO3C002409.2	MELO3C002409.2.1	791	88,627.52	8.65	36.49	109.20	0.35	12
CmHAK3	MELO3C009941.2	MELO3C009941.2.1	772	86,450.17	8.69	37.60	105.76	0.285	12
CmHAK4	MELO3C012009.2	MELO3C012009.2.1	767	86,166.87	8.06	39.72	108.23	0.308	12
CmHAK5	MELO3C014763.2	MELO3C014763.2.1	618	68,789.20	9.15	45.90	115.74	0.532	10
CmHAK6	MELO3C016040.2	MELO3C016040.2.1	750	84,726.73	6.39	36.08	113.67	0.286	12
CmHAK7	MELO3C016041.2	MELO3C016041.2.1	758	85,178.40	8.94	41.16	107.10	0.250	12
CmHAK8	MELO3C017828.2	MELO3C017828.2.1	787	86,897.46	8.92	27.54	109.43	0.393	12
CmHAK9	MELO3C017974.2	MELO3C017974.2.1	784	88,060.25	6.96	33.00	103.38	0.197	12
CmHAK10	MELO3C019015.2	MELO3C019015.2.1	851	94,840.64	5.48	35.32	108.57	0.341	12
CmHAK11	MELO3C020132.2	MELO3C020132.2.1	790	88,054.72	6.97	38.73	110.53	0.381	12
CmHAK12	MELO3C020532.2	MELO3C020532.2.1	878	99,060.22	7.94	37.14	108.42	0.393	12
CmHAK13	MELO3C021533.2	MELO3C021533.2.1	610	68,685.45	8.68	34.01	106.89	0.259	8
CmHAK14	MELO3C026470.2	MELO3C026470.2.1	838	93,447.33	6.24	39.95	106.78	0.36	12

In addition, subcellular localization of all CmHAK proteins were predicted in plasma membrane. The genetic mapping of *CmHAK* genes to the chromosomes was carried out according to the genome data (Figure 1). These 14 *CmHAKs* were randomly located on seven different chromosomes. *CmHAK8–10* were located on chromosome 07 and *CmHAK4, 10, 11* were located on chromosome 10. Meanwhile, there were three genes located on chromosome 12: *CmHAK1, 2,* and *12*. Two genes, *CmHAK6* and *CmHAK7*, were located in close proximity on chromosome 01, indicating that the two genes were a pair of tandem duplication genes. *CmHAK3, 5,* and *13* were located on chromosomes 04, 06, and 09, respectively.
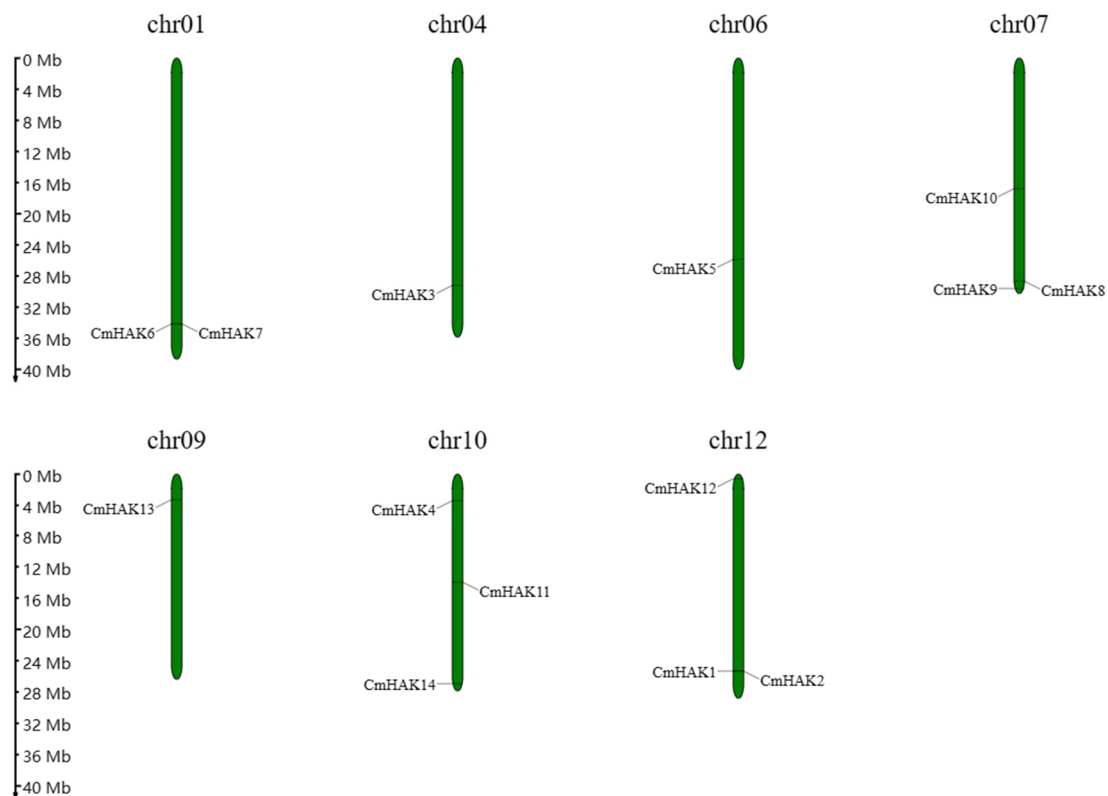
Figure 1. Chromosome locations of *CmHAK* genes in the melon genome. The physical location map was drawn based on the location of the *CmHAK* genes on the chromosomes by MG2G.

3.2. Classification and Structural Analysis of CmHAK Genes

To analyze the gene structure of *CmHAKs*, the exon–intron organizations of all the identified *CmHAK* genes were examined to gain more insight into the evolution of the CmHAK family in melon. The results revealed that differences in the exon/intron structures of the different *CmHAK* genes were apparent (Figure 2). Among these, *CmHAK1–3*, *CmHAK7*, and *CmHAK8* had eight exons. There were seven exons in four genes, including *CmHAK4–6* and *CmHAK9*. *CmHAK11* and *CmHAK14* had nine exons. Additionally, *CmHAK13* contained four exons; *CmHAK10* had ten exons, and the number of exons in *CmHAK12* was twelve. Genes with only one exon were not observed.
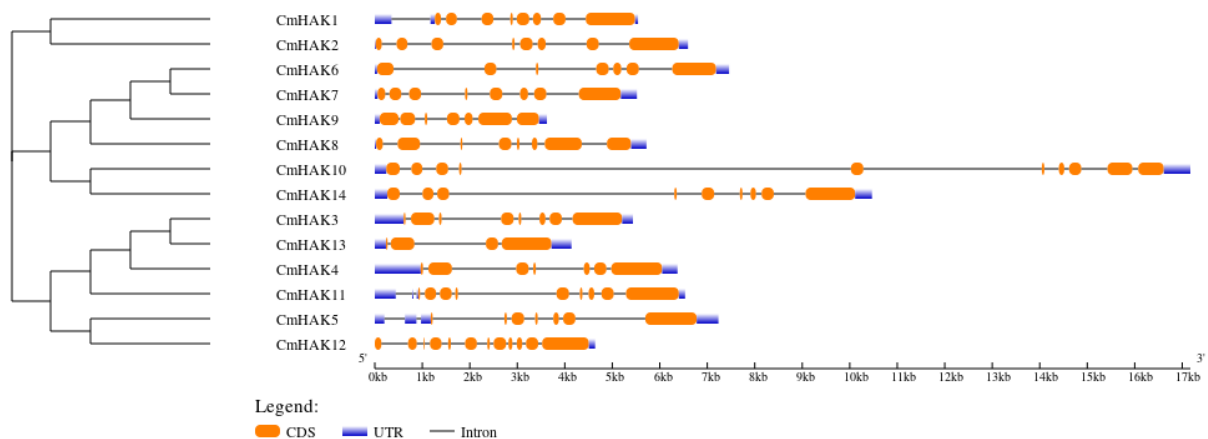
Figure 2. Structure of *CmHAK* genes. Introns, exons, and untranslated regions (UTRs) are represented by black lines, deep orange boxes, and blue boxes, respectively.

To further study the conserved domain of CmHAK proteins in melon, multiple alignment of amino acid sequences of the CmHAK proteins were used to identify the conserved protein motifs (Figures 3 and 4). As shown in Figure 4, the sequences in the CmHAK domain were highly conserved. A total of 15 conserved motifs were found in *CmHAK* genes. Different members had different conserved motifs. There were twelve *CmHAK* genes that contained motifs 1–15. However, CmHAK13 had only eleven motifs, excluding motifs 4, 7, 12, and 14. Additionally, except for motifs 3, 9, and 11, the rest were included in CmHAK5 proteins.
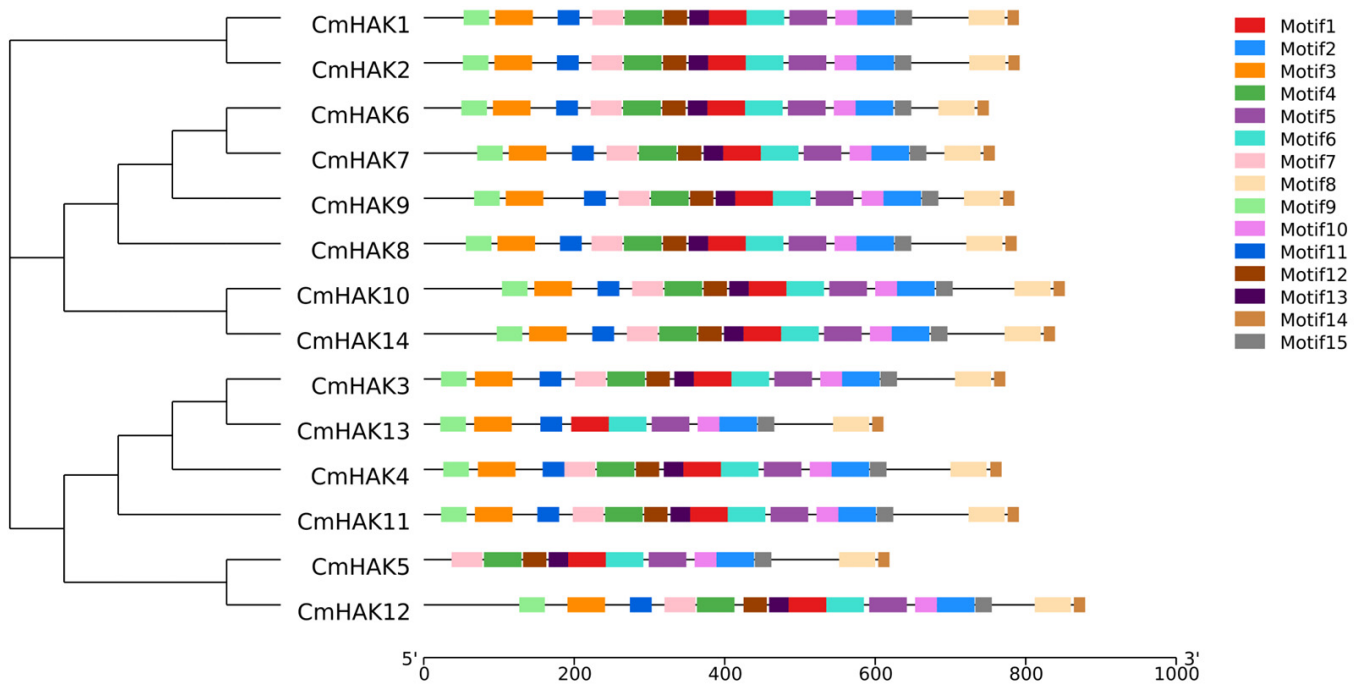
Figure 3. *CmHAK* genes family motifs in melon. The various conserved motifs are labeled with colored boxes.

To further understand the transcriptional regulation of *CmHAK* genes, 2000 bp upstream of their coding regions were predicted by PlantCARE. As shown in Figure 5, a total of 12 cis-effects were identified in the promoter region of *CmHAK* genes. In general, the elements of plant promoters that respond to external stimuli can be roughly divided into four categories: plant growth and development elements, light response elements, stress response elements, and hormone response elements. All of the 14 *CmHAK* genes contained TATA and CAAT elements related to plant growth and development as well as light-responsive elements. Several TF-binding sites were found in their promoter regions, such as ARE, MYB, MYC, ERE, etc. (Figures S2 and 5), and all of the 14 *CmHAK* genes contained at least one stress-responsive element. The results indicated that the expression of given genes might be regulated by these TFs.
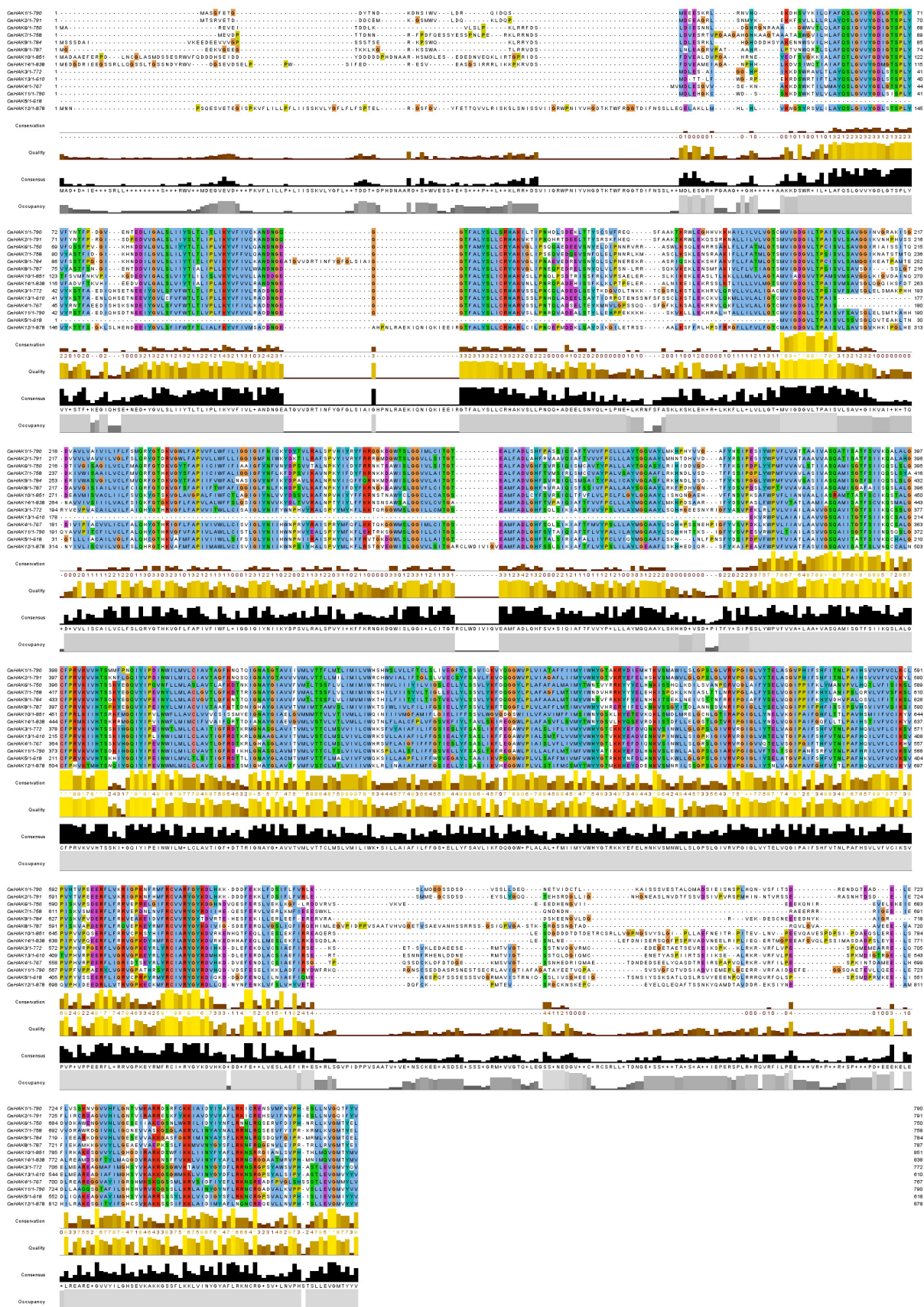
Figure 4. Multiple sequence alignment of *CmHAK* genes family in melon.

Figure 5. Promoter element prediction analysis of *CmHAK* genes. Transcription factor binding sites within a region 2000 bp upstream of *CmHAKs* start site was predicted. Only the top 12 transcription factor families are shown. The number of putative TF sites in the promoters of *CmHAK* genes are marked.

3.3. Phylogenetic Analysis of CmHAK Genes

In order to analyze the evolutionary relationships between CmHAK proteins, the HAK proteins of *Arabidopsis thaliana* and *Cucumis melo* were used to construct a phylogenetic tree using MEGA 7.0 software. The phylogenetic analysis indicated that the HAK domains between *Arabidopsis thaliana* and *Cucumis melo* could be divided into three large groups corresponding to groups I, II, and III (Figure 6). Groups II and III were further divided into two categories. Among the 14 CmHAK proteins, CmHAK6–9 and AtHAK5 belonged to group IA, and AtHAK5 plays a physiological role in plants at very low K+ concentrations and provides a cesium uptake pathway in Arabidopsis [30]. CmHAK5, 12 and AtHAK1, 3, 4 belonged to group IIA; CmHAK3, 4, 11, and 14 were divided into group IIB and contained AtHAK2, 6, and 8. *AtHAK4* mutations lead to impaired Arabidopsis auxin transport, and *AtKUP2* (*shy3-1*) mutations reduce bud cell expansion [31,32]. While the

remaining CmHAK proteins were in group III, such as CmHAK1 and 2 belonging to group IIIA, CmHAK10 and 14 were divided into group IIIB.
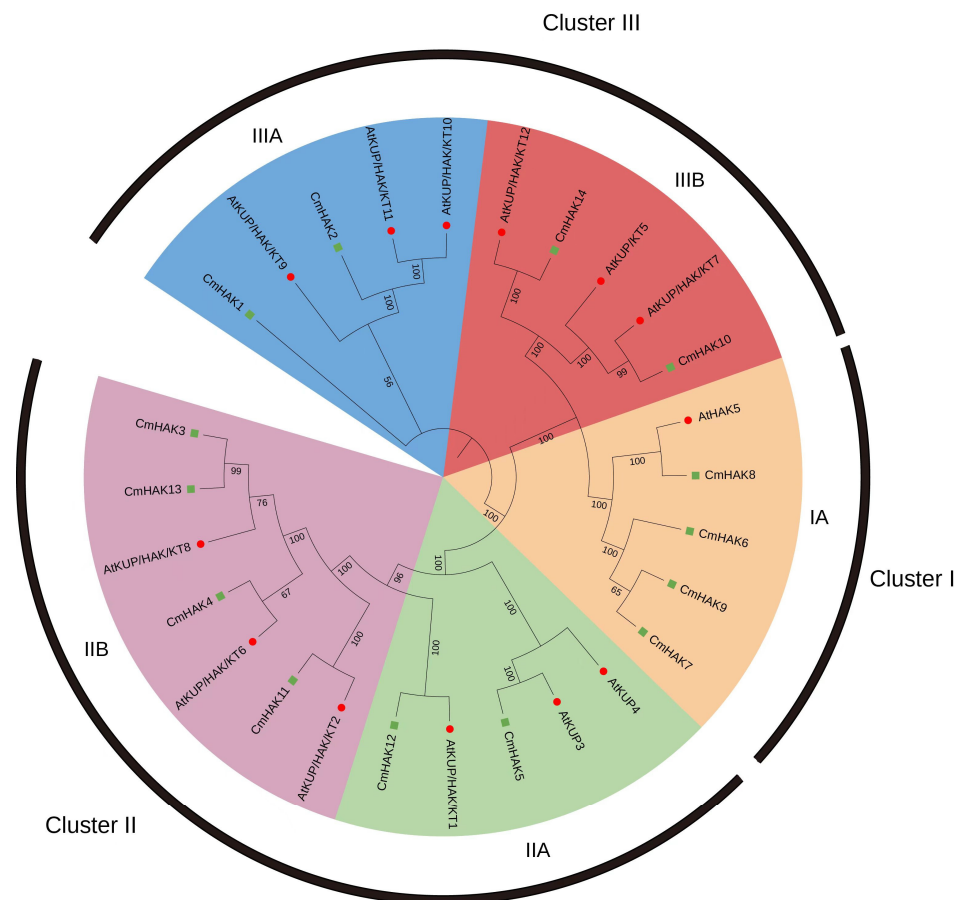
Figure 6. Phylogenetic tree of the HAK gene family between *Arabidopsis thaliana* and *Cucumis melo*.

To further analyze the evolutionary relationships of CmHAK proteins among different species, the CmHAK proteins of *Arabidopsis thaliana*, *Oryza sativa*, *Fragaria vesca*, *Vitis vinifera*, *Capsicum annuum,* and *Cucumis melo* were used to construct a phylogenetic tree. The results indicated that the HAK domains in five species could be divided into four large groups corresponding to groups I, II, III, and IV (Figure S3). Each group was further divided into two categories. However, the 14 CmHAK proteins in *Cucumis melo* still belonged to groups I, II, and III. The results of the phylogenetic analysis showed that most of the CmHAK proteins were similar in sequence and origin. Therefore, we conclude that the CmHAK proteins were highly evolutionarily conserved. These results will provide important support for the further analysis of *CmHAK* genes.

3.4. Collinearity and Ka/Ks Analysis of CmHAK

Gene replication is a basic process of gene family evolution that plays an important role in species differentiation and diversity. A collinearity analysis of the 14 identified CmHAK family members was performed between different *CmHAK* genes, and the results demonstrated that there was a collinearity relationship between some *CmHAK* genes, such as between *CmHAK3* and *CmHAK4*, *CmHAK3* and *CmHAK13*, and *CmHAK4* and *CmHAK13* (Figure 7). This indicates that some *CmHAK* genes may be generated by gene replication, and fragment replication events may be one of the main driving forces for the evolution of the *CmHAK* gene family.
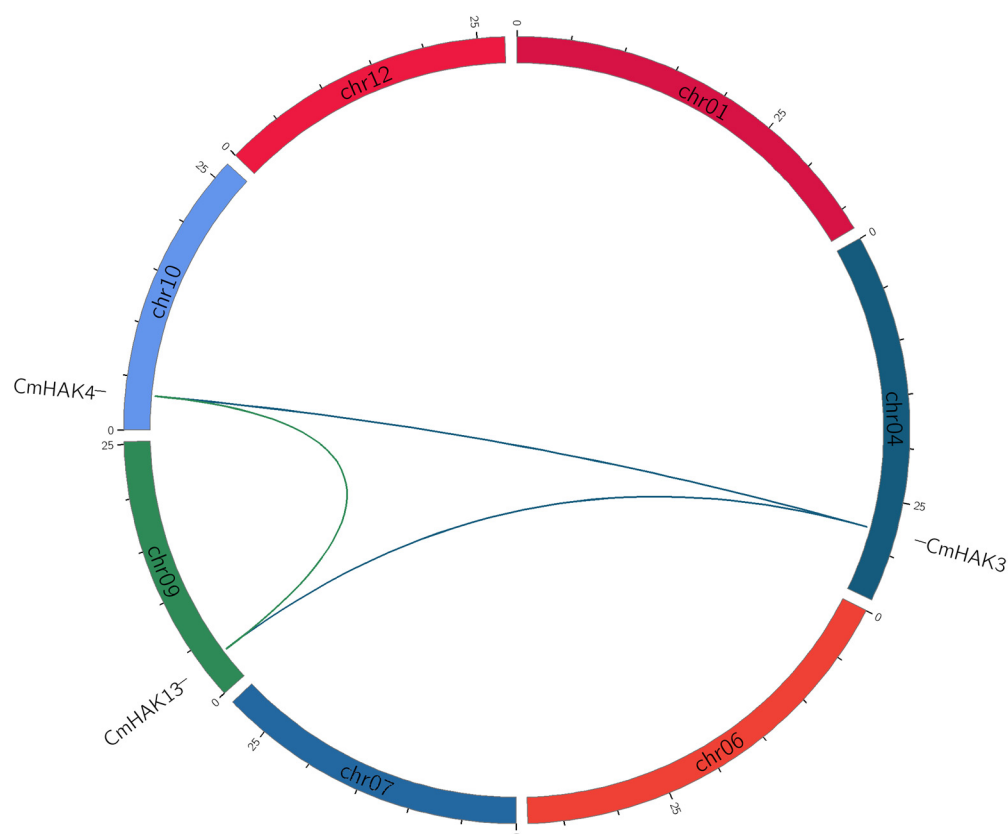
Figure 7. Collinearity analysis of the 14 identified *CmHAK* genes.

Ka/Ks represents the ratio between the nonsynonymous substitution rate (Ka) and synonymous substitution rate (Ks) of two protein-coding genes. If the Ka/Ks value is equal to 1, it indicates that the gene has mainly experienced neutral selection after replication. If the Ka/Ks value is greater than 1, it indicates that the gene mainly undergoes positive selection after replication. If the Ka/Ks value is less than 1, it indicates that the gene mainly undergoes purification selection after replication [33]. The results showed that the Ka/Ks values of these three pairs of genes (*CmHAK3/CmHAK4*, *CmHAK3/CmHAK13*, and *CmHAK4/CmHAK13*) were all less than 1, and they mainly experienced purification selection after replication events (Table 2).

Table 2. Characteristics of *CmHAK* genes in melon.

Sequence	Ka	Ks	Ka/Ks	*p*-Value (Fisher)	Length
CmHAK13-CmHAK3	0.142624	1.83892	0.077559	5.69E-225	1821
CmHAK13-CmHAK4	0.201892	3.51955	0.0573628	0	1824
CmHAK3-CmHAK4	0.171156	2.35486	0.0726821	0	2262

3.5. Gene Expression Analysis

Based on the previous chilling stress transcriptome data of melon [21], the potential function of the CmHAK protein was explored. The expression heat maps of 14 *CmHAK* genes were constructed under 0 h and 12 h of chilling stress in two varieties, chilling-tolerant (162) and chilling-sensitive (13-5A). The results showed that after 12 h of chilling treatment, the expression levels of *CmHAK1*, *CmHAK2*, *CmHAK3*, and *CmHAK4* in the tolerant varieties (162) were upregulated, while the remaining *CmHAK* genes were downregulated (Figure 8). The expression levels of *CmHAK1*, *CmHAK2*, *CmHAK3*, *CmHAK4*, *CmHAK5*, *CmHAK7*, and *CmHAK12* were upregulated in the sensitive varieties, while *CmHAK6* and *CmHAK8* did not significantly change, and the remaining *CmHAK* genes were downregulated. In

summary, the expression levels of most *CmHAK* genes in "162" and "13-5A" changed after 12 h of cold storage treatment, indicating that some of these *CmHAK* genes may be involved in regulating the response of melon to chilling stress. CmHAK6 and CmHAK8 were downregulated in chilling-tolerant materials and remained unchanged in chilling-sensitive materials, and the different expression levels of these two genes in different cold-resistant materials may affect the tolerance of muskmelon to chilling pressure and have a negative regulatory effect.
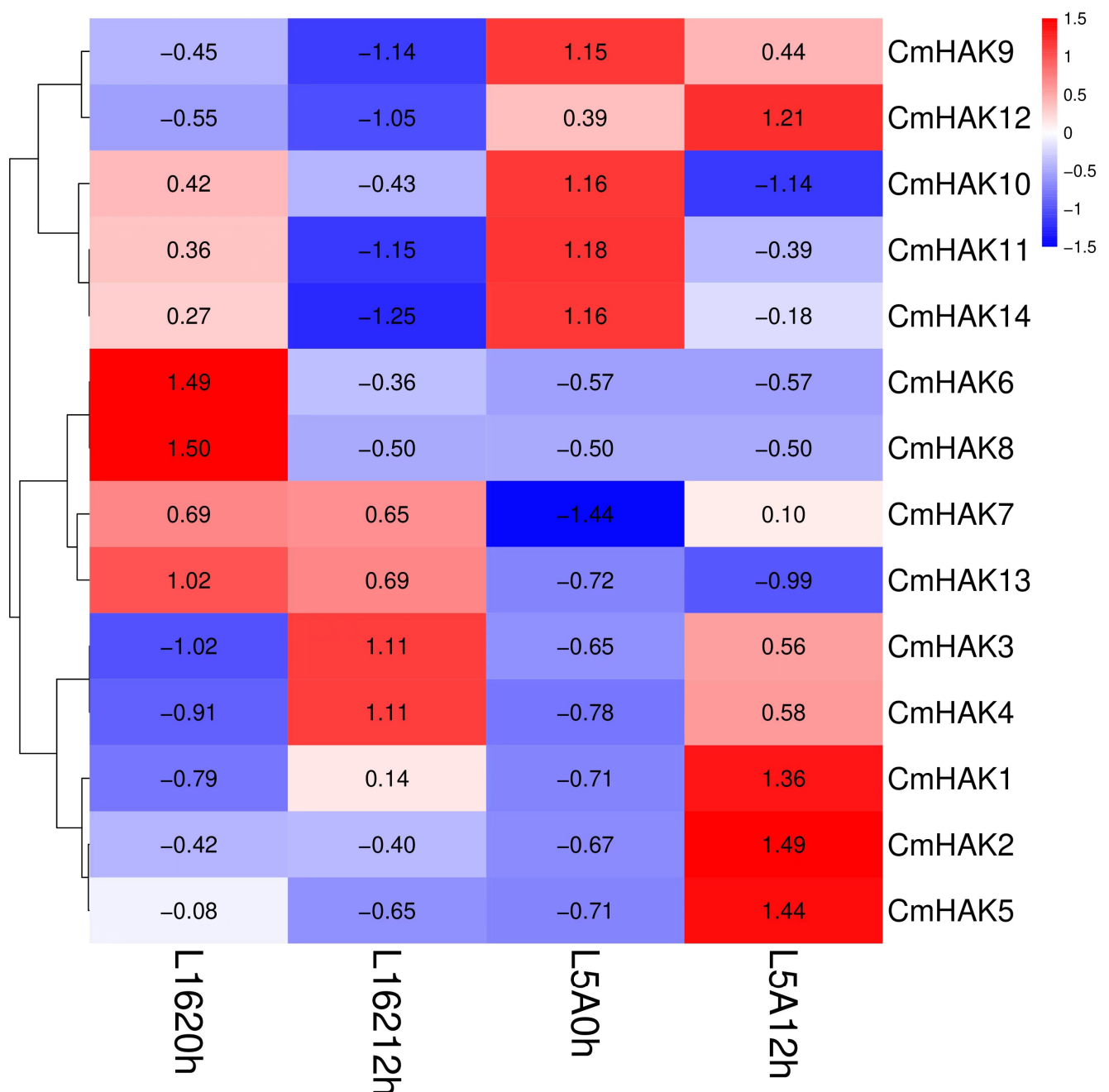
Figure 8. Heatmap cluster analysis of the FPKM of *CmHAK* genes during the chilling stress of melon. L162—chilling-tolerant varieties (162); L5A—chilling-sensitive varieties (13-5A) [29].

4. Discussion

Melon has a short cultivation cycle, simple cultivation techniques, the ability to be intercropped with other crops, a relatively high land use efficiency and multiple cropping index, strong production adaptability, a large market demand, and high economic benefits,

making it an efficient and economic fruit crop [2]. As one of the three major chemical elements necessary for plant growth, potassium plays an important role in regulating plant growth and development and participating in plant stress responses [6]. At present, KUP/HAK/KT family genes have been identified or cloned in the whole genome of many species. However, there is little research on the potassium transporter gene family in melon, and there is no report on the whole genome identification and analysis of large HAK family genes [11]. In this study, we identified 14 *CmHAK* genes from the whole melon genome data. The number of *CmHAK* genes in melon was similar to that in Arabidopsis, tomatoes, and peaches, but lower than that in rice, corn, soybeans, etc. [34–38].

The localization of all CmHAK proteins was predicted in the plasma membrane, which is consistent with previous research on soybean KUP/HAK/KT, indicating that CmHAK proteins may play a role in potassium ion transport upstream of the plasma membrane [36]. The gene family of melon CmHAKs encodes 610–878 amino acids; the relative molecular weight of protein is 68,685–94,840 Da; and the theoretical isoelectric point is 5.48–9.15. The gene coding length, the number of introns and exons, the type and number of conserved order types, the transmembrane domain, and the signal peptide are highly conserved, which may be the reason why HAKs of melon are highly conserved. In this study, we found that 14 members of the *CmHAK* gene family were evenly distributed on 7 chromosomes through chromosome mapping, and 3 of them had gene collinearity, which may be caused by gene replication. Gene replication events can lead to the formation of a large number of duplicate genes in the plant genome, and the presence of duplicate genes can promote the evolution of new gene functions and enhance the adaptability of plants to environmental changes [39]. The three collinear genes may be an evolutionary mechanism for muskmelon to adapt to high potassium demands.

The phylogenetic analysis of HAK/KUP/KT in melon and other species (Arabidopsis, rice, strawberries, chili peppers, and grapes) revealed that the HAK/KUP/KT family can be divided into four groups, each of which can be divided into four subgroups. Among them, group II has the most members, group IV contains the fewest members, and group IV does not contain the HAK family genes in melon. The evolutionary relationship between HAK/KUP/KT proteins in melon and these plants was relatively close [12]. The predictive analysis of the promoter elements of the *CmHAK* gene includes elements related to plant growth and stress response elements, such as TATA and MYB elements, indicating that some growth regulatory or biotic and abiotic stress transcription factors can bind to the promoter of *CmHAK*, driving or inhibiting the expression of *CmHAK*.

Previous studies have shown that HAK/KUP/KT genes are involved in K$^+$ absorption, transport, salt tolerance, osmotic regulation, plant hormone responses, and abiotic stress [17]. The *AtKUP/HAK/KT2* mutation has been shown to lead to shortened hypocotyls [40], while knocking out *AtKUP3* leads to an increase in root hairs [41], indicating that *CmHAK11* and *CmHAK5*, which are homologous to *AtKUP/HAK/KT2* and *AtKUP3*, may play an important role in the growth and development of melon. The expression of *AtHAK5* is induced by low K+ stress [35], and *CmHAK8* is homologous to *AtKUP5*, indicating that *CmHAK8* may also be induced by low K+ stress. The K+ transporter protein coding genes may also be involved in salt stress regulation, such as *AtKUP/HAK/KT1*, *AtKUP/HAK/KT2*, and *AtKUP/HAK/KT6* in Arabidopsis [35], and their homologs *CmHAK12*, *CmHAK11*, and *CmHAK4* may also be involved in salt stress regulation in melon. *AtKUP/HAK/KT2* and *AtKUP/HAK/KT6* of Arabidopsis are involved in root K+ efflux [42], and *CmHAK4*, *CmHAK3*, and *CmHAK13*, which share high homology with them, may be involved in K+ transport in melon roots. *AtKUP3* is strongly induced by K+ starvation [43], and *CmHAK5* is homologous to *AtKUP3*, indicating that *CmHAK5* may also have a K+-starvation-induced expression pattern.

In addition, overexpression of the *OsHAK5* gene in tobacco can further improve salt tolerance by enhancing the transport capacity of K$^+$ from underground to aboveground parts [44]. There is a significant difference in the expression level of the *PhaHAK2* gene in reed between salt-tolerant materials and controls [45]. Multiple genes such as

AtHAK5, *AtKUP4*, and *AtKUP11* in Arabidopsis are highly expressed in plants under salt stress [46,47]. The optimal growth temperature for muskmelon is 25–32 °C, and its main production area is in the northwest region of China, which is often affected by low temperatures. Therefore, it is crucial to cultivate cold-resistant varieties of melon. In this research, transcriptome analysis of two melon varieties with different cold resistance ("162", chilling-tolerant; "13-5A", chilling-sensitive) at 0 h and 12 h of chilling stress showed that most of the *CmHAK* genes were expressed. Among them, the expression of *CmHAK6* and *CmHAK8* genes belonging to group I, subgroup IB, was downregulated in chilling-tolerant varieties, but there was no obvious change in chilling-sensitive varieties. It may be that these two genes have different structures in different resistant varieties or that the expression patterns of key cold tolerance genes are different, resulting in inconsistent functions. When melon is subjected to cold stimulation, the *CmHAK6* and *CmHAK8* genes of chilling-resistant varieties are downregulated in response to stress, negatively regulating the cold resistance of plants. Therefore, the *HAK* genes of different species in the group I and IB subgroups may play a role under cold stress.

In this study, we systematically analyzed the members of each family of melon HAK/KUP/KT from the aspects of identification, chromosome location, gene structure, phylogeny, and expression mode of members of the gene family, and screened out the members that mainly express cold stimulation. The results provide a theoretical basis for further study of the function of the KT/HAK/KUP gene family and cultivating melon varieties with high potassium tolerance.

5. Conclusions

To study the biological functions of the *Cucumis melo* potassium transferrin (KT/HAK/KUP) gene family in growth, development, and stress, 14 members of the KT/HAK/KUP gene family were identified from the melon genome using bioinformatics, and a comprehensive and systematic analysis was carried out from the aspects of gene structure, chromosome location, phylogeny, and expression analysis. The results showed that 14 members of the KT/HAK/KUP gene family were distributed on seven chromosomes, and the number of amino acid residues encoded by each member varied from 610 to 878. The molecular weight of these proteins was 68.69–94.84 kDa, and the protein products were located on the cytoplasmic membrane. There are 3–11 introns and 4–12 exons in 14 *CmHAKs*, and they are divided into four distinct branches in phylogeny. In terms of expression, after 12 h of chilling stress, most of the *CmHAK* genes were expressed in two melon varieties with different chilling resistances. The expression levels of *CmHAK6* and *CmHAK8* genes were downregulated in chilling-resistant varieties, but there was no significant change in chilling-sensitive varieties, indicating that *CmHAK6* and *CmHAK8* genes may play a negative regulatory role in chilling-resistant varieties. The results provide a theoretical basis for in-depth analysis of the functions of the KT, HAK, and KUP gene families, as well as for the cultivation of melon varieties under high potassium stress to improve yield and quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9101138/s1, Figure S1: Number of predicted TMRs of 14 CmHAK proteins in *Cucumis melo*; Figure S2. Promoter element prediction analysis of *CmHAK* genes; Figure S3. Phylogenetic tree of the HAK gene family. At—*Arabidopsis thaliana*; Os—*Oryza sativa*; Fve—*Fragaria vesca*; Vv—*Vitis vinifera*; Ca—*Capsicum annuum*; Cm—*Cucumis melo*. Table S1: CDS and protein sequence of *CmHAK* genes in melon.

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