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Genome-Wide Identification of GASA Gene Family in Ten Cucurbitaceae Species and Expression Analysis in Cucumber

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Abstract: Gibberellic acid-stimulated in *Arabidopsis* (GASA), a unique small molecular protein of plants, plays an essential role in plant growth and development. The GASA family genes have been identified and studied in many plants. However, the identification of GASA gene family in Cucurbitaceae species has not been reported yet. Therefore, in this study, based on the available genome information on the Cucurbitaceae species, the GASA family genes in 10 Cucurbitaceae species including cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), pumpkin (*Cucurbita moschata*), wax gourd (*Benincasa hispida*), sponge gourd (*Luffa cylindrica*), bottle gourd (*Lagenaria siceraria*), bitter gourd (*Momordica charantia*), chayote (*Sechium edule*), and snake gourd (*Trichosanthes anguina*) were identified with bioinformatics methods. To understand the molecular functions of GASA genes, the expression pattern analysis of cucumber GASA family genes in different tissues and stress responses were also analyzed. The results showed that a total of 114 GASA genes were identified in the 10 Cucurbitaceae species, which were divided into three subfamilies. Synteny analysis of GASA genes among cucumber, *Arabidopsis* and rice showed that nine cucumber GASA genes were colinear with 12 *Arabidopsis* GASA genes, and six cucumber GASA genes were colinear with six rice GASA genes. The *cis*-acting elements analysis implied that the cucumber GASA genes contained many *cis*-elements associated with stress and hormone response. Tissue-specific expression analysis of cucumber GASA family genes revealed that only the *CsaV3_2G029490* gene was lowly or not expressed in all tissues, the *CsaV3_3G041480* gene was highly expressed in all tissues, and the other seven GASA genes showed tissue-specific expression patterns. Furthermore, nine cucumber GASA family genes exhibited different degrees of regulatory response under GA, abiotic and biotic stresses. Two cucumber GASA genes, *CsaV3_3G042060* and *CsaV3_3G041480*, were differentially expressed under multiple biotic and abiotic stresses, which indicated that these two GASA genes play important roles in the growth and development of cucumber.

Keywords: abiotic stress; bioinformatics; biotic stress; cucumber; expression analysis; GASA gene family

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1. Introduction

GASA proteins are a class of cysteine-rich peptides (CRPs) that play important roles in regulating plant growth and development [1]. GASA family genes are widely distributed in vascular plants such as ferns, gymnosperms and angiosperms [2]. The protein structures of GASA family members are relatively conservative. Generally, they have a signal peptide consisting of 18–29 amino acids at the N-terminal, a highly variable hydrophilic region consisting of 7–31 polar amino acid residues in the middle, and about 60 amino acids at the C-terminal. The GASA proteins contain a conserved region consisting of 12 cysteine residues and considered to be the critical region of GASA proteins for maintaining spatial

structure and function [1,3,4]. Most of GASA family genes are regulated by gibberellin (GA) [5,6].

GASA genes were identified in tomato as early as 1992 [7]. Subsequently, the identification of GASA family genes were reported in many important plants, such as *Arabidopsis* [8–10], maize [11], potato [12], apple [10], wheat [13], rice [14], grape [15], poplar [16], etc. GASA family genes play important regulatory roles in plant hormone signal transduction [4,17], seed germination [18], seed development [9,19], lateral root formation [11], stem growth [6,20,21], leaf growth [22], flowering time [8,21], fruit growth and development [23,24]. In addition, GASA genes also actively respond to high temperature [25,26], low temperature [27], salt damage [28], diseases [29–31] and other biological and abiotic stresses.

The Cucurbitaceae, also called cucurbits or the gourd family, are primarily herbaceous annual climbers, vines or woody perennial lianas including cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), pumpkin (*Cucurbita moschata*), wax gourd (*Benincasa hispida*), sponge gourd (*Luffa cylindrica*), bottle gourd (*Lagenaria siceraria*), bitter gourd (*Momordica charantia*), chayote (*Sechium edule*), snake gourd (*Trichosanthes anguina*) and many other important economic crops. Cucurbitaceae species play important roles in agricultural production, which is second only to *Solanaceae* in vegetables [32]. Cucurbitaceae species also have important medicinal and ornamental values [33]. In scientific research, Cucurbitaceae species are used as model plants for studying sexual differenti- ation and vascular biology [34,35]. As early as 2009, the cucumber genome sequencing project was completed [36,37]. With the rapid development of sequencing technology and genomics, whole genome sequencing projects for Cucurbitaceae species such as melon [38], watermelon [39], pumpkin [40], wax gourd [41], bottle gourd [42], sponge gourd [43], bitter gourd [44], chayote [45] and snake gourd [33] have been successively completed. By using the high-quality genome information, a large number of studies on gene family identifica- tion have been finished, such as the identification of WRKY [46–49], MADS-box [50–52], NBS [53–55], bZIP [56,57] and other gene families. However, the identification of GASA family genes in Cucurbitaceae species was still not reported, which greatly limited the biological function studies of GASA genes in Cucurbitaceae crops.

Therefore, in this study, GASA family genes were identified with the latest high-quality genome information on 10 Cucurbitaceae species, and the physicochemical characteristics, chromosome locations, gene structures, conserved motifs, phylogenetic tree, *cis*-acting elements of promoter, and synteny of GASA family genes were analyzed. Moreover, analyses of tissue-specific expression and the expression pattern in response to stresses of cucumber GASA genes were conducted to preliminarily explore the molecular biological functions of GASA family genes in cucumber. The results will lay an important foundation for the further study of molecular biological functions of GASA genes in the Cucurbitaceae species, and provide a theoretical reference for the molecular breeding of Cucurbitaceae species.

2. Materials and Methods

2.1. Identification and Chromosomal Mapping of GASA Family Genes in 10 Cucurbitaceae Species

The probable GASA family genes (E-value < 1×10^{-5}) in 10 Cucurbitaceae species genomes were scanned using HMMER 3.0 with the Hidden Markov Model (HMM) model file (PF02704). A Perl script was used to extract the sequence information of candidate GASA proteins. Pfam and SMART (http://smart.embl.de/smart/batch.pl, accessed on 17 April 2022) [58] were used to identify GASA family members. Genes containing the whole GASA domain were chosen as the final candidate GASA genes. The chromosomal locations of the GASA genes were analyzed with TBtools software [59]. The physicochemical characteristics including theoretical isoelectric point (pI), molecular weight, instability index, aliphatic index and grand average of hydropathicity of each GASA protein were calculated via the online tool ExPASy (https://web.expasy.org/protparam/, accessed on 17 April 2022). The subcellular localizations of GASA genes were predicted by the online website CELLO (http://cello.life.nctu.edu.tw/, accessed on 18 April 2022) [60].

2.2. Gene Structure Analysis and Phylogenetic Tree Construction of GASA Family Genes in 10 Cucurbitaceae Species

The General Feature Format 3 (GFF3) files of 10 Cucurbitaceae species were used to draw the structure of GASA genes with TBtools software. The online software MEME (http://meme-suite.org/, accessed on 18 April 2022) [61] was used to analyze the conserved motifs of GASA proteins with the following parameters: a maximum of 10 misfits and an optimum motif width of 6–100 amino acid residues. The upstream sequences (1.5 kb) of the coding sequences of GASA genes were extracted from the genomes of 10 Cucurbitaceae species, and submitted to PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 18 April 2022) [62] to identify the *cis*-acting elements. The phylogenetic tree was constructed using MEGA 11 software with the neighbor-joining method [63]. The parameters were pairwise deletion and 1000 bootstrap replications.

2.3. Gene Duplication and Synteny Analysis of GASA Family Genes

MCScanX [64] software was used to analyze tandem and segmental duplications of cucumber GASA family genes. The synteny analyses between the cucumber GASA family genes, *Arabidopsis* genes, and rice genes were conducted with MCScanX software; the collinearity relationships among the GASA family genes in cucumber, *Arabidopsis* and rice were then visualized by Circos software [65].

2.4. RNA-seq Reanalysis with Cucumber Transcriptome Sequencing Data

The published cucumber transcriptome sequencing data in SRA database (https://www.ncbi.nlm.nih.gov/sra, accessed on 20 April 2022) were downloaded. The downloaded SRA data were converted into Fastq data using fasterq-dump.2.11.0 (https://github.com/ncbi/sra-tools/wiki/HowTo:-fasterq-dump, accessed on 22 April 2022). The quality of Fastq data was then checked through the FastQC software [66]. The joints and low-quality sequences of the Fastq data were removed with Trimmomatic software (version = 0.39) [67], finally obtaining the filtered clean data. The index of cucumber ChineseLong_V3 genome was built with STAR software (version = 2.7.10a). The filtered clean data were compared to cucumber ChineseLong_V3 genome, generating the SAM files. Using the SAMtools software (version = 1.15) [68], the SAM files were converted into sorted BAM files. The expression data of each gene were estimated with StringTie software (v2.2.1) [69]. Finally, based on the counts data for each gene, the analysis of differentially expressed genes was performed with the DESeq2 software [70].

2.5. Tissue-Specific Expression Analysis of Cucumber GASA Family Genes

The transcriptome sequencing project performed in different cucumber tissues was retrieved from the SRA database (PRJNA80169) [71]. The tissue-specific expression analysis of cucumber GASA family genes was analyzed with the above methods. The heatmap was drawn by TBtools software.

2.6. The Expression Analysis of Cucumber GASA Family Genes under Stresses

The transcriptome sequencing data of cucumber under stress treatments such as GA (PRJNA376073) [72], high temperature (PRJNA634519) [73], low temperature (PRJNA438923), salt and silicon (PRJNA477930) [74], downy mildew (PRJNA285071) [75], powdery mildew (PRJNA321023) [76], and root-knot nematode (PRJNA419665) [77] were retrieved from the SRA database. The expression profiles of cucumber GASA family genes were analyzed using the above methods, and then the heatmaps were drawn with TBtools software.

3. Results

3.1. Genome-Wide Identification of GASA Genes in 10 Cucurbitaceae Species

Based on the published genome information of Cucurbitaceae species, 9, 9, 11, 9, 9, 8, 15, 10, 16 and 18 GASA family genes were identified in wax gourd, watermelon,

pumpkin, cucumber, sponge gourd, bottle gourd, bitter gourd, melon, chayote and snake gourd, respectively (Table 1). The chromosomal locations of GASA family genes in different Cucurbitaceae species were unevenly distributed. For all the 114 GASA proteins identified in the 10 Cucurbitaceae species, the numbers of encoded amino acids varied from 57 to 516, the molecular weight varied from 6.15 to 58.17 kDa, and the aliphatic index varied from 16.98 to 89.14. The isoelectric points (pI) of the 114 GASA proteins ranged from 5.99 to 9.9. Except for *CmoCh09G000990.1* (pI = 5.99, was acid protein), the other GASA proteins in 10 Cucurbitaceae species were basic proteins (pI > 7). Among the 114 GASA genes, 42 proteins of GASA genes were stable proteins with an instability index less than 40. The other 72 GASA proteins were unstable proteins with an instability index greater than 40 that were unevenly distributed in the 10 Cucurbitaceae species. A total of 29 GASA proteins were hydrophobic proteins whose average hydrophilic values were greater than zero. The other 85 GASA proteins were hydrophilic proteins whose average hydrophilic values were less than zero, and they were also unevenly distributed in the 10 Cucurbitaceae species. The predicted results of subcellular localization showed that 98 GASA family genes were located in the extracellular matrix, and 16 GASA family members were located in the nucleus and unevenly distributed in the 10 Cucurbitaceae species. (Table 1).

3.2. Phylogenetic Analysis of GASA Family Genes in 10 Cucurbitaceae Species

To clarify the evolutionary relationship of GASA family genes in 10 Cucurbitaceae species, all 114 Cucurbitaceae GASA proteins and 15 *Arabidopsis* GASA proteins were used to construct a phylogenetic tree by multiple sequence alignment (Figure 1). According to the classification results of *Arabidopsis* GASA family genes [10], the phylogenetic tree was divided into three subgroups, namely G1, G2 and G3. Among them, the G3 subgroup contained the largest number of GASA genes, a total of 60. The G2 subgroup had the second largest number of GASA genes, totaling 38. The G1 subgroups had the smallest number of GASA genes, at 31. The GASA family proteins in every Cucurbitaceae species could be divided into these three subfamilies.

Table 1. The physiochemical characteristics of GASA family genes in 10 Cucurbitaceae species.

Species	Gene ID	Chromosome Location	Number of Amino Acid (aa)	Molecular Weight (kDa)	Theoretical pI	Instability Index	Aliphatic Index	Grand Average of Hydropathicity	Subcellular Location
Wax gourd *(Benincasa hispida)*	*Bhi03M000750*	Chr3: 19,311,494 .. 19,311,920 (−)	80	8.77	9.04	39.58	82.88	−0.021	Extracellular
	Bhi04M000133	Chr4: 3,757,574 .. 3,758,363 (+)	97	10.33	9.21	53.79	68.45	0.073	Extracellular
	Bhi04M000374	Chr4: 10,323,755 .. 10,323,929 (−)	57	6.15	9.33	32.81	22.28	−0.749	Nuclear
	Bhi08M001209	Chr8: 42,837,600 .. 42,840,156 (+)	115	12.91	8.91	44.95	59.30	−0.355	Extracellular
	Bhi09M001786	Chr9: 59,471,263 .. 59,472,200 (+)	139	15.00	9.10	55.67	89.14	0.310	Extracellular
	Bhi09M001788	Chr9: 59,495,483 .. 59,496,419 (+)	101	11.01	7.99	43.64	65.74	0.031	Extracellular
	Bhi11M000974	Chr11: 34,037,800 .. 34,038,470 (+)	89	10.08	9.40	32.31	63.48	0.111	Extracellular
	Bhi11M001327	Chr11: 45,095,365 .. 45,097,267 (−)	133	14.80	8.97	64.51	61.58	−0.074	Extracellular
	Bhi11M002565	Chr11: 83,283,688 .. 83,287,470 (−)	232	24.48	9.84	89.32	57.93	−0.311	Nuclear
Watermelon *(Citrullus lanatus)*	*Cla97C01G013050.1*	Chr1: 26,879,226 .. 26,881,027 (−)	114	12.72	8.97	45.94	56.40	−0.393	Extracellular
	Cla97C01G016400.2	Chr1: 30,120,921 .. 30,122,856 (−)	99	10.84	9.24	52.87	70.91	−0.159	Extracellular
	Cla97C02G033610.2	Chr2: 7,155,664 .. 7,157,420 (+)	88	10.11	9.62	33.11	59.89	−0.086	Extracellular
	Cla97C05G099600.2	Chr5: 28,631,501 .. 28,637,708 (+)	216	23.23	8.76	45.58	65.51	−0.024	Extracellular
	Cla97C08G157880.1	Chr8: 25,344,837 .. 25,345,286 (+)	97	10.18	9.07	45.74	78.56	0.187	Extracellular
	Cla97C10G193530.1	Chr10: 21,997,173 .. 21,997,578 (+)	89	10.08	9.15	37.27	53.71	−0.054	Extracellular
	Cla97C10G196435.1	Chr10: 26,191,272 .. 26,194,258 (−)	141	15.55	9.07	66.45	45.67	−0.453	Extracellular
	Cla97C10G197380.1	Chr10: 27,180,180 .. 27,181,843 (+)	216	22.78	9.68	89.57	54.58	−0.385	Nuclear
	Cla97C11G215390.1	Chr11: 11,861,823 .. 11,863,862 (−)	106	11.78	9.46	50.63	71.79	−0.183	Extracellular
Pumpkin *(Cucurbita moschata)*	*CmoCh01G020290.1*	Chr1: 14,246,135 .. 14,247,088 (−)	106	10.90	8.61	54.84	81.04	0.235	Extracellular
	CmoCh05G001560.1	Chr5: 678,878 .. 679,725 (+)	85	9.18	9.19	32.68	74.59	0.054	Extracellular
	CmoCh07G006840.1	Chr7: 3,085,589 .. 3,086,076 (+)	94	10.04	8.97	43.34	71.60	−0.068	Extracellular
	CmoCh09G000990.1	Chr9: 477,271 .. 484,805 (+)	516	58.17	5.99	54.74	85.41	−0.103	Nuclear
	CmoCh11G010460.1	Chr11: 5,816,242 .. 5,817,492 (−)	108	11.89	9.45	36.93	50.65	−0.201	Extracellular
	CmoCh11G014010.1	Chr11: 9,853,936 .. 9,855,463 (−)	88	9.86	9.63	29.27	65.45	0.036	Extracellular
	CmoCh12G003610.1	Chr12: 2,236,117 .. 2,237,074 (+)	97	10.16	9.08	53.11	75.57	0.186	Extracellular
	CmoCh14G006410.1	Chr14: 3,223,478 .. 3,225,060 (−)	203	21.47	9.82	84.49	60.00	−0.342	Nuclear
	CmoCh14G011310.1	Chr14: 7,958,822 .. 7,959,972 (−)	117	12.79	8.82	50.21	49.23	−0.406	Extracellular
	CmoCh15G000310.1	Chr15: 218,843 .. 220,014 (−)	106	11.90	9.59	64.32	71.79	−0.166	Extracellular
	CmoCh18G001170.1	Chr18: 823,704 .. 825,231 (+)	115	12.22	9.36	51.06	54.26	−0.184	Extracellular

Table 1. *Cont.*

Species	Gene ID	Chromosome Location	Number of Amino Acid (aa)	Molecular Weight (kDa)	Theoretical pI	Instability Index	Aliphatic Index	Grand Average of Hydropathicity	Subcellular Location
Cucumber *(Cucumis sativus)*	*CsaV3_1G008270.1*	Chr1: 5,210,806 .. 5,212,803 (+)	115	13.05	9.19	44.35	53.39	−0.414	Extracellular
	CsaV3_2G026300.1	Chr2: 18,030,665 .. 18,031,342 (+)	85	9.47	9.20	34.97	64.24	−0.171	Extracellular
	CsaV3_2G029490.1	Chr2: 19,316,885 .. 19,317,559 (−)	61	6.59	9.11	56.39	36.89	−0.408	Nuclear
	CsaV3_3G041480.1	Chr3: 33,839,191 .. 33,840,892 (−)	104	11.46	9.64	34.12	54.52	−0.183	Extracellular
	CsaV3_3G042060.1	Chr3: 34,217,470 .. 34,219,983 (−)	231	24.24	9.77	84.05	67.06	−0.263	Nuclear
	CsaV3_3G042990.1	Chr3: 34,899,642 .. 34,900,907 (+)	117	12.94	9.12	54.69	61.62	−0.215	Extracellular
	CsaV3_3G045860.1	Chr3: 37,493,613 .. 37,494,832 (−)	102	10.59	8.57	60.59	76.57	0.249	Extracellular
	CsaV3_4G000520.1	Chr4: 298,937 .. 299,297 (+)	89	10.16	9.41	30.95	59.10	−0.115	Extracellular
	CsaV3_6G043760.1	Chr6: 25,805,953 .. 25,806,385 (−)	80	8.82	9.15	37.53	70.62	−0.148	Extracellular
Sponge gourd *(Luffa cylindrica)*	*Lcy01g018340.1*	Chr1: 39,876,992 .. 39,877,586 (−)	85	9.39	9.27	26.87	63.06	−0.111	Extracellular
	Lcy04g015090.1	Chr4: 47,422,624 .. 47,423,017 (−)	94	10.08	8.87	46.90	75.64	0.007	Extracellular
	Lcy06g003590.1	Chr6: 3,388,158 .. 3,389,543 (+)	114	12.74	8.88	54.68	55.53	−0.361	Extracellular
	Lcy07g018230.1	Chr7: 46,850,291 .. 46,852,453 (−)	109	11.92	9.57	45.40	52.94	−0.043	Extracellular
	Lcy09g000520.1	Chr9: 508,156 .. 510,023 (−)	109	12.19	9.61	50.43	68.07	−0.186	Extracellular
	Lcy09g017660.1	Chr9: 35,628,176 .. 35,629,130 (−)	106	11.81	9.67	32.92	51.60	−0.288	Extracellular
	Lcy09g018840.1	Chr9: 41,976,742 .. 41,978,205 (−)	221	23.44	9.79	80.57	58.19	−0.388	Nuclear
	Lcy12g021390.1	Chr12: 48,438,825 .. 48,439,301 (−)	101	10.80	8.29	40.87	82.08	0.156	Extracellular
	Lcy12g021440.1	Chr12: 48,464,326 .. 48,464,817 (−)	106	11.42	8.65	44.30	79.15	0.072	Extracellular
Bottle gourd *(Lagenaria siceraria)*	*Lsi01G009320.1*	Chr1: 7,534,930 .. 7,535,476 (+)	80	8.82	9.04	37.17	75.50	−0.077	Extracellular
	Lsi03G005590.1	Chr3: 6,743,934 .. 6,745,226 (−)	100	11.34	9.47	38.43	65.30	0.034	Extracellular
	Lsi03G010390.1	Chr3: 19,955,854 .. 19,956,253 (+)	89	10.18	9.33	34.28	59.10	−0.003	Extracellular
	Lsi03G014250.1	Chr3: 25,218,255 .. 25,221,595 (+)	212	22.55	9.45	92.37	51.42	−0.493	Nuclear
	Lsi04G017450.1	Chr4: 24,750,180 .. 24,751,157 (−)	111	11.86	8.81	48.82	72.07	0.142	Extracellular
	Lsi08G013250.1	Chr8: 21,566,057 .. 21,567,220 (+)	147	15.87	9.36	51.98	82.38	0.149	Extracellular
	Lsi08G015100.1	Chr8: 23,193,981 .. 23,195,157 (−)	142	15.46	8.98	23.27	66.62	−0.246	Extracellular
	Lsi09G009210.1	Chr9: 11,377,348 .. 11,377,967 (+)	82	9.37	9.50	22.47	40.37	−0.466	Extracellular

Table 1. *Cont.*

Species	Gene ID	Chromosome Location	Number of Amino Acid (aa)	Molecular Weight (kDa)	Theoretical pI	Instability Index	Aliphatic Index	Grand Average of Hydropathicity	Subcellular Location
Bitter gourd *(Momordica charantia)*	*MC01g0899.1*	Chr1: 14,806,676 .. 14,807,137 (−)	95	10.15	8.87	39.92	78.95	0.022	Extracellular
	MC02g0120.1	Chr2: 1,223,343 .. 1,224,955 (+)	108	11.64	8.89	51.46	51.67	−0.060	Extracellular
	MC03g1060.1	Chr3: 16,943,540 .. 16,943,725 (−)	62	6.65	9.32	46.78	31.45	−0.556	Nuclear
	MC04g0594.1	Chr4: 5,232,091 .. 5,232,267 (−)	59	6.78	9.84	61.72	29.83	−0.764	Nuclear
	MC04g1197.1	Chr4: 19,993,730 .. 19,993,924 (+)	65	7.30	9.00	26.91	24.00	−0.606	Extracellular
	MC04g1983.1	Chr4: 26,610,157 .. 26,612,959 (+)	109	12.18	9.75	67.07	71.65	−0.229	Extracellular
	MC04g_new0671.1	Chr4: 26,612,371 .. 26,616,386 (−)	370	41.55	8.95	44.27	74.08	−0.235	Extracellular
	MC08g0576.1	Chr8: 4,660,922 .. 4,661,179 (+)	86	9.83	9.03	31.67	62.33	−0.073	Extracellular
	MC08g0741.1	Chr8: 6,030,387 .. 6,030,572 (−)	62	6.49	8.98	43.72	44.19	−0.285	Extracellular
	MC08g1334.1	Chr8: 12,401,247 .. 12,401,444 (+)	66	7.58	9.03	36.59	56.06	−0.459	Extracellular
	MC08g1395.1	Chr8: 14,414,051 .. 14,414,239 (+)	63	7.16	9.13	45.20	16.98	−0.767	Nuclear
	MC10g0377.1	Chr10: 3,011,243 .. 3,011,419 (+)	59	6.61	9.18	34.38	24.75	−0.781	Nuclear
	MC11g0104.1	Chr11: 764,783 .. 764,971 (+)	63	6.49	8.89	75.97	43.49	−0.068	Extracellular
	MC11g0105.1	Chr11: 765,959 .. 768,132 (+)	153	16.95	8.65	54.36	85.42	0.065	Extracellular
	MC00g0794.1	scaffold142: 23,557 .. 24,251 (−)	116	12.96	9.10	55.48	47.07	−0.324	Extracellular
Melon *(Cucumis melo)*	*MELO3C018503.2.1*	Chr1: 721,831 .. 722,928 (−)	88	10.13	9.71	30.06	61.02	−0.174	Extracellular
	MELO3C011008.2.1	Chr3: 27,015,343 .. 27,016,361 (+)	97	10.47	9.34	59.97	75.46	0.080	Extracellular
	MELO3C010822.2.1	Chr3: 28,229,740 .. 28,230,991 (−)	85	9.44	9.30	33.74	67.65	−0.080	Extracellular
	MELO3C003753.2.1	Chr4: 3,727,349 .. 3,728,176 (+)	100	10.81	8.65	45.75	70.30	0.079	Extracellular
	MELO3C026601.2.1	Chr4: 25,918,328 .. 25,919,317 (−)	117	12.95	9.12	52.40	57.52	−0.302	Extracellular
	MELO3C009926.2.1	Chr4: 26,961,415 .. 26,964,335 (+)	222	23.64	9.72	93.63	55.72	−0.386	Nuclear
	MELO3C009872.2.1	Chr4: 27,411,710 .. 27,412,273 (+)	80	8.93	9.55	34.32	23.12	−0.761	Extracellular
	MELO3C017982.2.1	Chr7: 26,076,528 .. 26,081,332 (−)	89	10.21	9.36	35.50	50.34	−0.134	Extracellular
	MELO3C007600.2.1	Chr8: 3,965,613 .. 3,966,256 (−)	94	10.19	8.96	38.10	67.34	−0.077	Extracellular
	MELO3C002243.2.1	Chr12: 23,960,061 .. 23,961,357 (−)	126	14.28	9.07	52.56	58.81	−0.456	Extracellular

Table 1. *Cont.*

Species	Gene ID	Chromosome Location	Number of Amino Acid (aa)	Molecular Weight (kDa)	Theoretical pI	Instability Index	Aliphatic Index	Grand Average of Hydropathicity	Subcellular Location
Chayote (*Sechium edule*)	*Sed0003696.1*	Chr1: 12,188,492 .. 12,189,979 (−)	112	12.57	8.87	49.75	54.02	−0.271	Extracellular
	Sed0004582.1	Chr1: 18,633,871 .. 18,636,202 (−)	108	11.53	8.79	41.99	66.02	0.053	Extracellular
	Sed0003671.1	Chr1: 60,857,945 .. 60,859,823 (−)	109	11.98	8.47	62.21	57.43	−0.079	Extracellular
	Sed0018489.1	Chr3: 4,097,163 .. 4,099,736 (+)	109	12.02	9.33	50.58	64.50	−0.239	Extracellular
	Sed0001116.1	Chr3: 5,503,219 .. 5,504,432 (−)	95	10.71	9.01	26.56	61.58	0.155	Extracellular
	Sed0023008.1	Chr4: 35,040,789 .. 35,043,255 (−)	115	13.11	9.17	55.32	61.91	−0.248	Extracellular
	Sed0019149.1	Chr6: 5,108,016 .. 5,110,361 (+)	223	23.49	9.57	89.94	70.36	−0.114	Nuclear
	Sed0023122.1	Chr6: 5,770,438 .. 5,772,489 (+)	104	11.48	9.61	31.14	57.21	−0.129	Extracellular
	Sed0021074.1	Chr8: 27,782,007 .. 27,783,423 (−)	106	11.91	9.78	43.65	54.34	−0.238	Extracellular
	Sed0023101.1	Chr10: 25,350,679 .. 25,351,022 (+)	86	9.64	8.87	23.26	62.33	−0.071	Extracellular
	Sed0017754.1	Chr10: 36,389,392 .. 36,391,330 (−)	88	10.01	9.62	36.59	62.05	−0.042	Extracellular
	Sed0026538.1	Chr11: 26,467,504 .. 26,468,286 (−)	98	10.52	8.81	56.25	72.76	0.095	Extracellular
	Sed0005060.1	Chr12: 7,036,681 .. 7,038,107 (+)	111	12.46	9.16	71.39	57.93	−0.377	Extracellular
	Sed0023421.1	Chr13: 20,866,691 .. 20,868,746 (+)	107	11.16	8.24	49.56	79.25	0.396	Extracellular
	Sed0020400.1	Chr13: 20,881,190 .. 20,882,081 (+)	106	11.37	8.49	37.98	72.74	−0.007	Extracellular
	Sed0017657.1	Chr14: 17,756,540 .. 17,757,298 (−)	96	10.35	8.97	27.91	71.98	−0.072	Extracellular
Snake gourd (*Trichosanthes anguina*)	*Tan0003426.1*	Chr1: 6,253,789 .. 6,254,353 (+)	89	10.18	9.42	27.92	59.10	0.010	Extracellular
	Tan0008780.1	Chr1: 115,842,003 .. 115,843,341 (−)	96	10.50	8.64	47.66	87.40	0.329	Extracellular
	Tan0000180.1	Chr1: 115,844,096 .. 115,847,359 (−)	104	11.06	8.86	49.88	72.31	0.176	Extracellular
	Tan0005239.1	Chr1: 115,863,453 .. 115,866,091 (−)	103	10.95	8.92	52.45	83.40	0.235	Extracellular
	Tan0020894.1	Chr2: 14,079,327 .. 14,080,681 (−)	89	10.29	9.68	44.19	63.60	−0.048	Extracellular
	Tan0006783.1	Chr2: 81,798,300 .. 81,799,073 (+)	85	9.32	9.27	28.27	64.24	−0.115	Extracellular
	Tan0018763.1	Chr2: 90,173,196 .. 90,174,289 (−)	92	9.87	9.19	40.27	63.70	0.142	Extracellular
	Tan0011393.1	Chr3: 72,374,679 .. 72,375,090 (−)	80	8.73	9.17	32.55	74.25	−0.138	Extracellular
	Tan0001400.1	Chr5: 70,569,691 .. 70,571,409 (−)	111	12.50	8.60	62.49	57.03	−0.353	Extracellular
	Tan0005034.1	Chr6: 74,131,427 .. 74,134,375 (+)	116	13.00	9.29	49.14	56.47	−0.337	Extracellular
	Tan0020433.1	Chr7: 12,409,140 .. 12,411,750 (+)	114	12.98	9.90	55.96	43.68	−0.402	Extracellular
	Tan0017829.1	Chr7: 48,550,357 .. 48,551,678 (−)	106	11.70	9.61	25.70	50.66	−0.282	Extracellular
	Tan0016087.1	Chr7: 61,289,275 .. 61,291,288 (−)	234	25.13	9.75	80.79	54.49	−0.357	Nuclear
	Tan0009191.1	Chr10: 27,664,301 .. 27,664,945 (+)	103	11.45	8.87	69.36	41.65	−0.545	Extracellular
	Tan0010233.1	Chr11: 862,587 .. 863,083 (+)	95	10.55	9.09	39.86	63.68	−0.161	Extracellular
	Tan0017564.1	Chr11: 863,623 .. 865,822 (−)	109	12.17	9.48	50.35	77.80	−0.024	Extracellular
	Tan0010840.1	Chr11: 12,544,995 .. 12,546,455 (−)	95	10.88	9.30	33.22	45.26	−0.180	Extracellular
	Tan0013993.1	Contig00035: 215,038 .. 215,449 (−)	94	10.13	8.86	38.19	69.36	−0.170	Extracellular

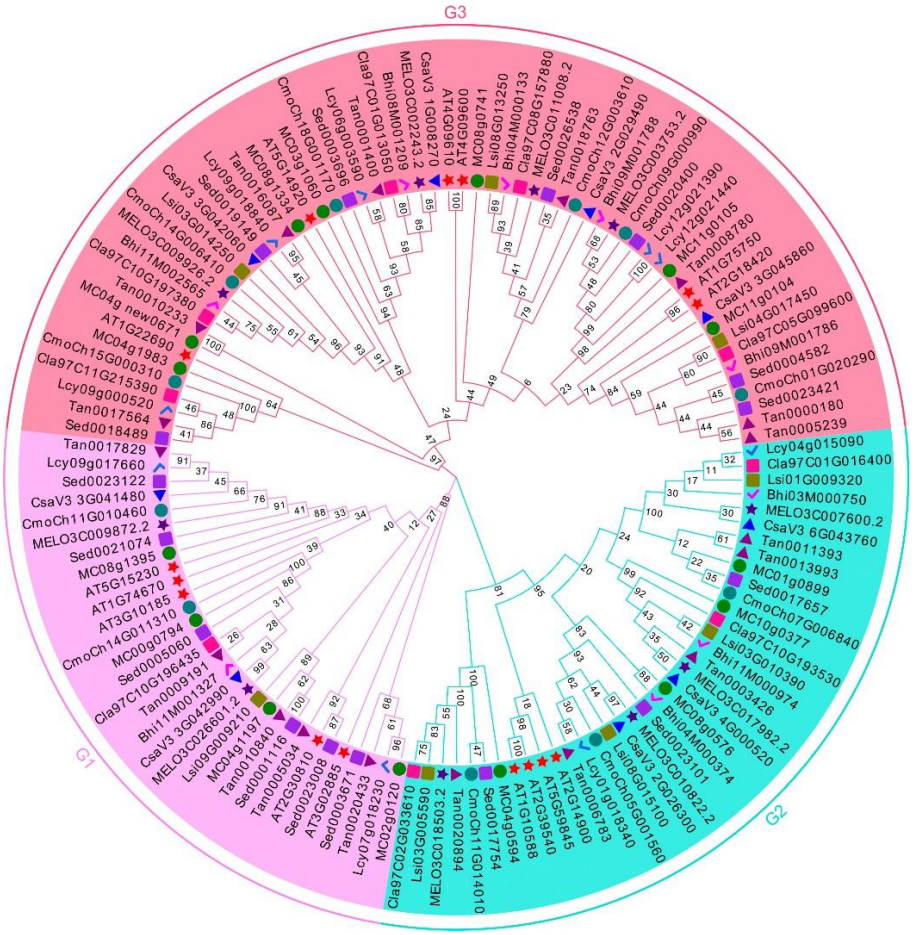

Figure 1. The phylogenetic tree of GASA proteins from *Arabidopsis* and 10 Cucurbitaceae species.

3.3. The Gene Structure and Conserved Motifs of GASA Family Genes in 10 Cucurbitaceae Species

The diagrams of phylogenetic tree and gene structures of GASA family genes in 10 Cucurbitaceae species were drawn with TBtools software. The results of phylogenetic tree analysis showed that GASA genes of 10 Cucurbitaceae species could be divided into three subgroups, namely G1, G2 and G3 subgroups (Figure 2), which was consistent with the clustering results for GASA proteins in *Arabidopsis* and 10 Cucurbitaceae species (Figure 1). Among them, there were 54 GASA genes in the G3 subgroup, 34 GASA genes in the G2 subgroup and 26 GASA genes in the G1 subgroup. The gene structure analysis showed that most of the GASA genes in the G2 subgroup were composed of two exons, while most of the GASA genes in the G1 subgroup were composed of four exons. In the G3 subgroup, most of the GASA genes were composed of three or four exons (Figure 2).

A schematic diagram of conserved motifs of GASA proteins in 10 Cucurbitaceae species was constructed with MEME analysis (Figure 2). A total of 10 motifs were revealed in the GASA proteins (Table 2). The motif compositions of GASA proteins in different subgroups were different, while GASA proteins in the same subgroup shared a similar number, type, and order of motifs. For example, most GASA proteins in the G1 subgroup contained motifs 5, 2, 1, 3, 6 and exhibited the same order. In the G3 subgroup, motifs 1 and 3 were found in all GASA proteins and showed the same order. These results indicated that the different distribution of conserved motifs in different subgroups might lead to the evolution of GASA gene functional diversity. The similar conserved motifs of GASA proteins in the same subgroup indicated that they have similar functions.

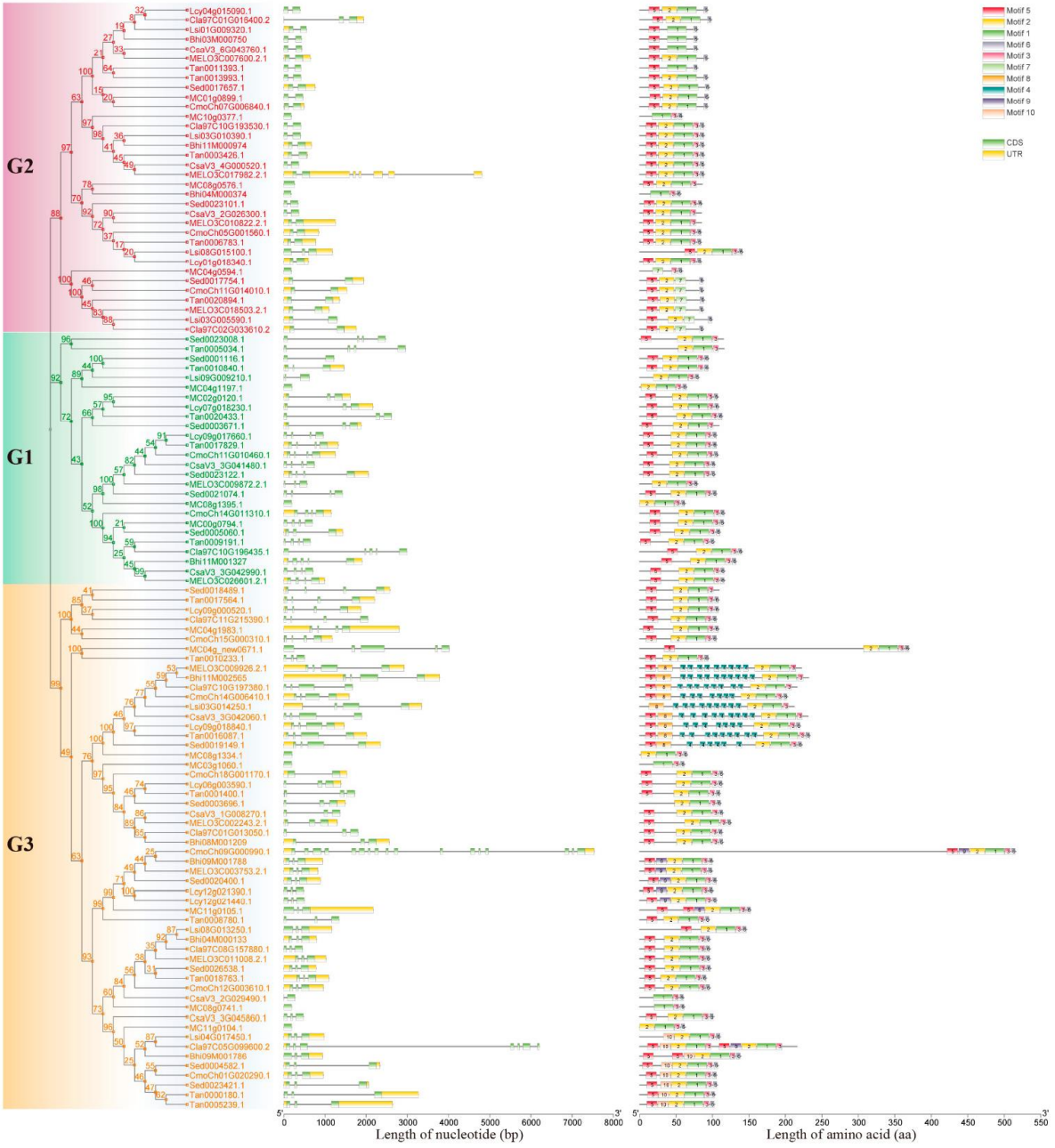

Figure 2. Exon-intron structures of GASA genes and a schematic diagram of the amino acid motifs of GASA proteins in 10 Cucurbitaceae species.

Table 2. The motif information on GASA proteins in 10 Cucurbitaceae species.

Motif	Sequence	Number of Amino Acid	Pfam Annotation
motif 1	CMRACGTCCARCKCVPPGTYGNKEVCP	27	GASA (Significant)
motif 2	QPIDCGGACARRCSKASRKKR	21	GASA (Insignificant)
motif 3	CYABMTTH	8	-
motif 4	PPVKPPYT	8	-
motif 5	LLSSLLLLLSFLDSS	15	-
motif 6	GRPKCP	6	-
motif 7	MKYCRICCSKCKCVP	15	GASA (Insignificant)
motif 8	ANGLSQEKDAVYPHVPVPVPA	21	-
motif 9	SGQMVITTQVDNPLP	15	-
motif 10	MVNSIDGVAABPVKI	15	-

3.4. Synteny Analysis of GASA Genes among Cucumber, Arabidopsis and Rice

To better understand the molecular functions of cucumber GASA family genes, a synteny analysis of GASA family genes among cucumber, *Arabidopsis* and rice was conducted. The results showed 23 syntenic relationships between all nine cucumber GASA family genes and 12 *Arabidopsis* GASA genes (*AT5G15230*, *AT3G10185*, *AT1G74670*, *AT3G02885*, *AT4G09610*, *AT1G75750*, *AT2G39540*, *AT5G59845*, *AT2G14900*, *AT2G18420*, *AT1G10588*, *AT5G14920*). There were eight syntenic relationships between six cucumber GASA genes (*CsaV3_3G042990*, *CsaV3_2G029490*, *CsaV3_3G041480*, *CsaV3_3G045860*, *CsaV3_2G026300*, *CsaV3_1G008270*) and six rice GASA genes (*Os05g31280*, *Os05g35690*, *Os06g15620*, *Os09g24840*, *Os03g14550*, *Os04g39110*). The synteny analysis of GASA family genes in cucumber showed that there were two pairs of cucumber GASA family genes (*CsaV3_2G026300*/*CsaV3_6G043760*, *CsaV3_2G029490*/*CsaV3_3G045860*) with syntenic relationships, which were segmental duplication gene pairs (Figure 3).

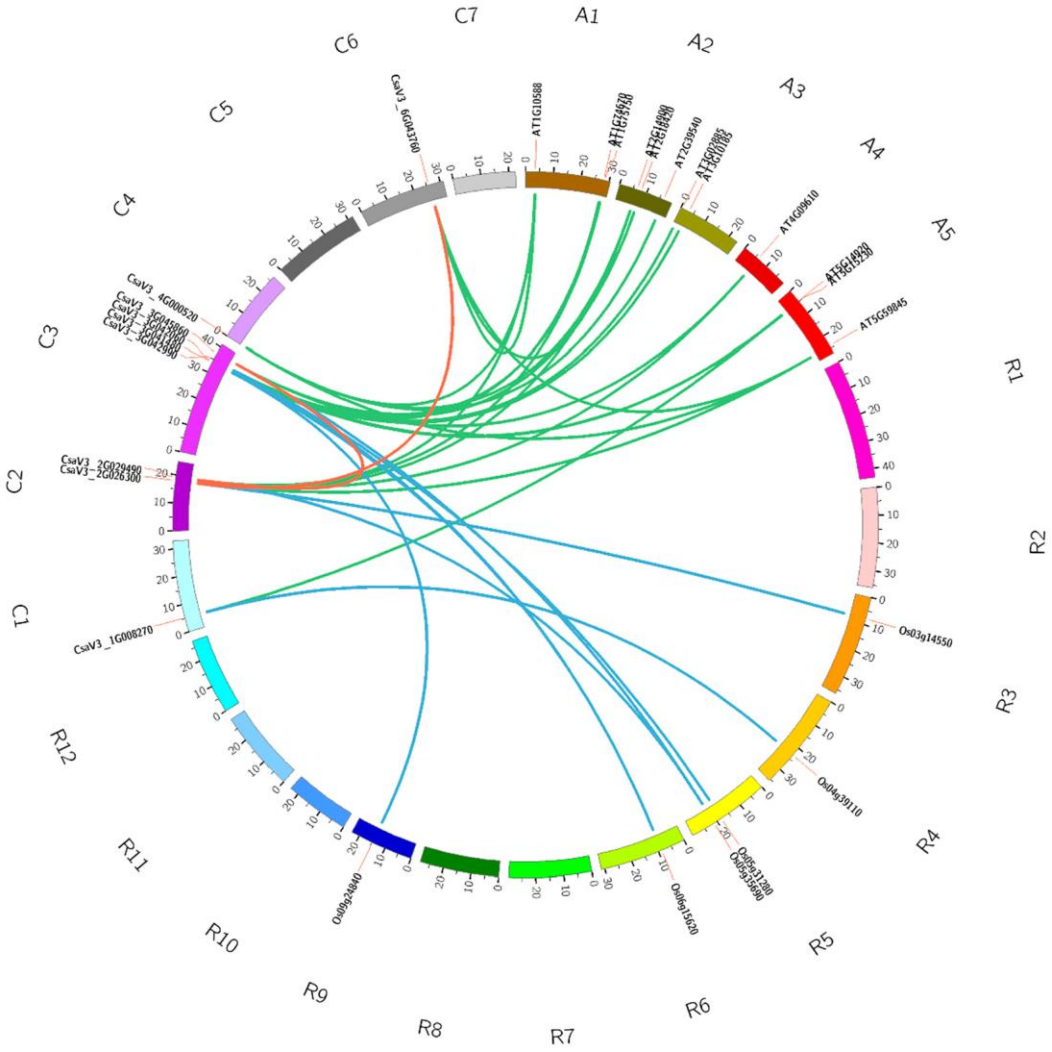

Figure 3. Syntenic relationships of GASA gene family in cucumber, *Arabidopsis* and rice.

3.5. Analysis of Cis-Acting Elements in Cucumber GASA Genes

The 1.5-kb upstream sequences from the transcription start sites of cucumber GASA family genes were selected to analyze their *cis*-acting elements in the promoters. The results showed that 14 types of *cis*-elements were identified (Figure 4). Among them, *cis*-elements related to light responsiveness were the major type, including ACE, AE-box, ATCT-motif, Box 4, Box II, G-box, GT1-motif, I-box, LAMP-element, etc., accounting for 55% of total *cis*-elements. In addition, some other *cis*-elements were also identified, including

cis-elements related to hormone response (auxin, gibberellin, salicylic acid, abscisic acid, MeJA), stress response (drought, low temperature, resistance), photoperiod regulation, endosperm expression and meristem expression. The results indicated that cucumber GASA genes play vital roles in plant growth and development.

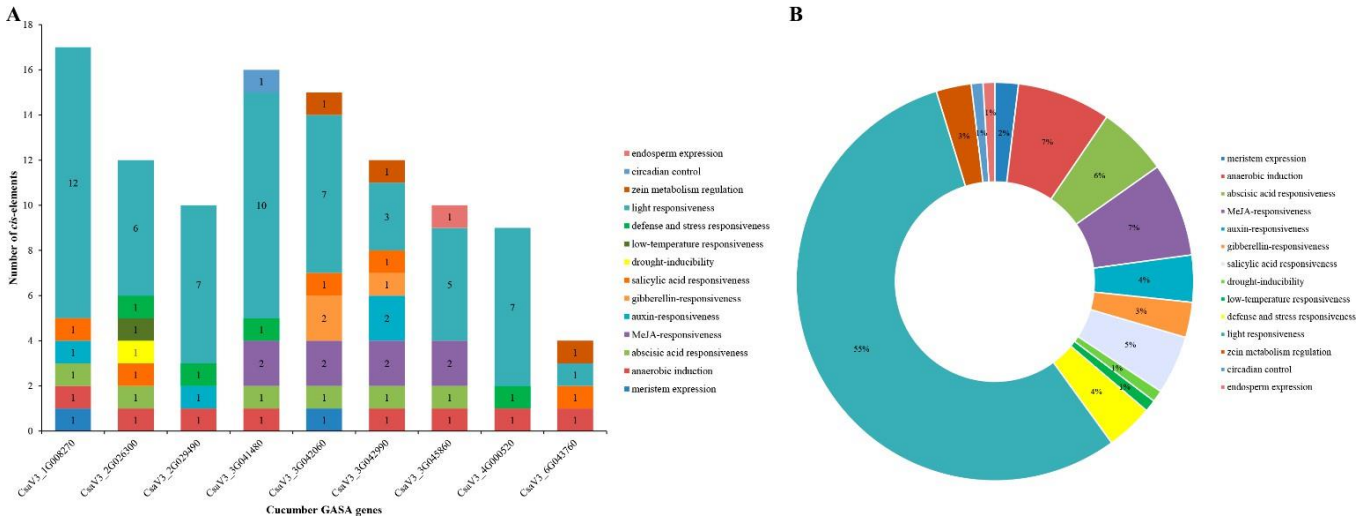

Figure 4. *Cis*-elements analysis of the promoters of cucumber GASA family genes. (**A**) The types and numbers of various *cis*-elements in the promoters of each cucumber GASA gene. (**B**) The relative proportions of different types of *cis*-elements in the promoters of cucumber GASA genes are displayed by doughnut chart.

3.6. Tissue-Specific Expression Analysis of Cucumber GASA Genes

To analyze the tissue-specific expression patterns of GASA genes in cucumber, the transcriptome sequencing data of 10 types of cucumber tissues (obtained from the published SRA data PRJNA80169) [71] were reanalyzed with ChineseLong_V3 genome. The results indicated that the GASA gene *CsaV3_2G029490* was lowly or not expressed in the cucumber tissues. The *CsaV3_3G041480* gene was highly expressed in all of the cucumber tissues. The *CsaV3_3G042990* gene was expressed in all of the cucumber tissues, with the highest expression levels in the male and female flowers, followed by the ovaries, and was lowly expressed in the root, stem, leaf and tendril. The *CsaV3_3G045860* gene was mainly expressed in the male and female flowers, but was not expressed in stem and tendril, and lowly expressed in the other tissues. The *CsaV3_4G000520* gene was highly expressed in the tendrils and flowers, but was not expressed in the ovaries, and lowly expressed in the root, stem and leaf. The *CsaV3_6G043760* gene was lowly expressed in all tissues of cucumber, with a relatively high expression level in the root, and was not expressed in the fertilized ovary. The *CsaV3_2G026300* gene was not expressed in the male flower, but was lowly expressed in the root and highly expressed in the other tissues. The *CsaV3_1G008270* gene was highly expressed in all cucumber tissues except the root. The *CsaV3_3G042060* gene was expressed in all tissues, with lower expression levels in the male and female flowers, and higher expression levels in the other tissues (Figure 5).

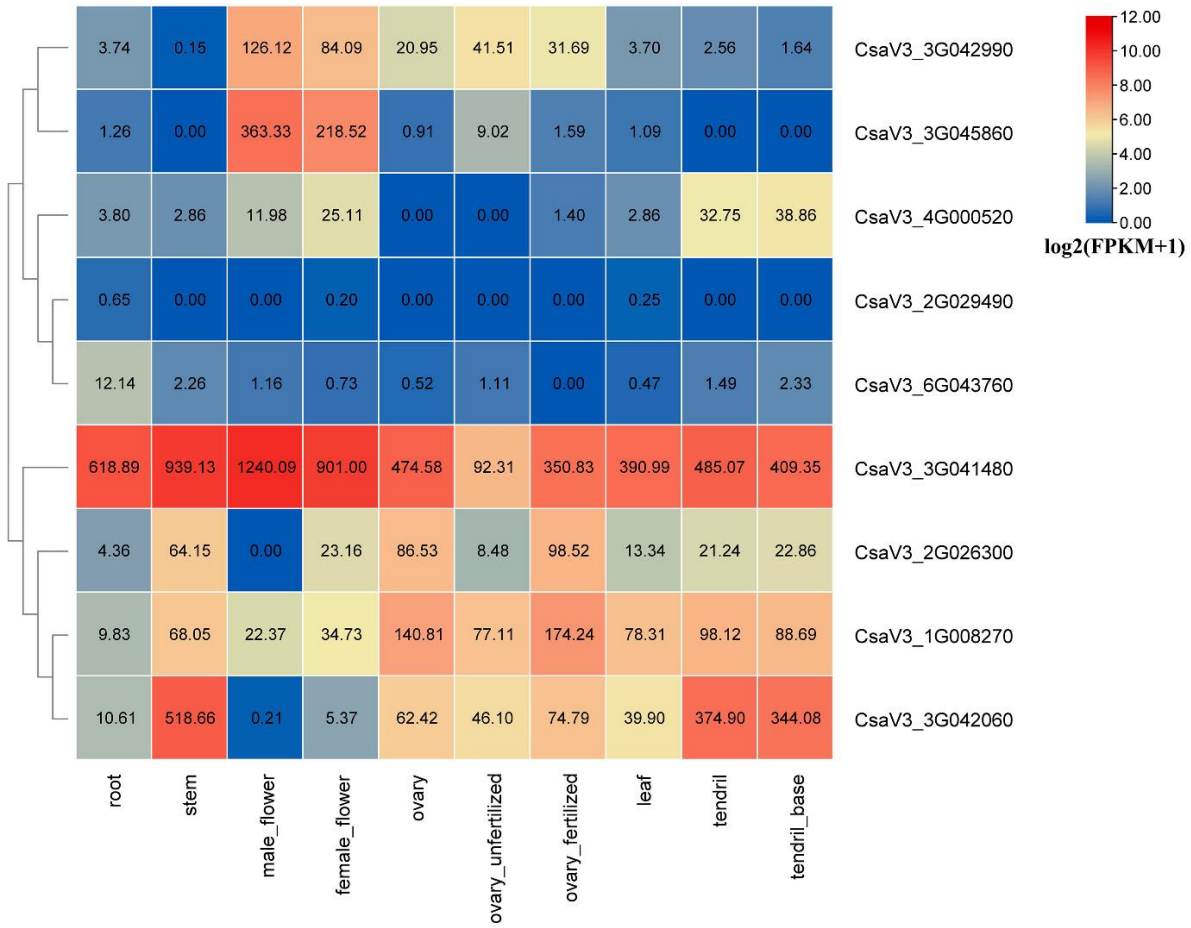

Figure 5. Expression heatmap of cucumber GASA gene family in different tissues. The data in the boxes indicate the original FPKM values.

3.7. Expression Profiles of Cucumber GASA Genes under GA Treatment

After reanalyzing the transcriptome sequencing data of cucumber under GA treatment (PRJNA376073) [72], an expression heatmap of cucumber GASA family genes in the shoot tip was drawn (Figure 6). The results showed that the expression levels of *CsaV3_2G029490*, *CsaV3_3G042990*, *CsaV3_4G000520*, *CsaV3_3G045860* and *CsaV3_6G043760* were low in the shoot tip, and did not change under GA treatment. The *CsaV3_1G008270* gene was highly expressed in the shoot tip, and not regulated by GA. However, compared with the control, the *CsaV3_2G026300* gene was significantly down-regulated after GA treatment for 12 h; the *CsaV3_3G042060* gene was significantly down-regulated after GA treatments for 6 h and 12 h; the *CsaV3_3G041480* gene was up-regulated after GA treatment for 6 h, but the expression levels were not significantly different.

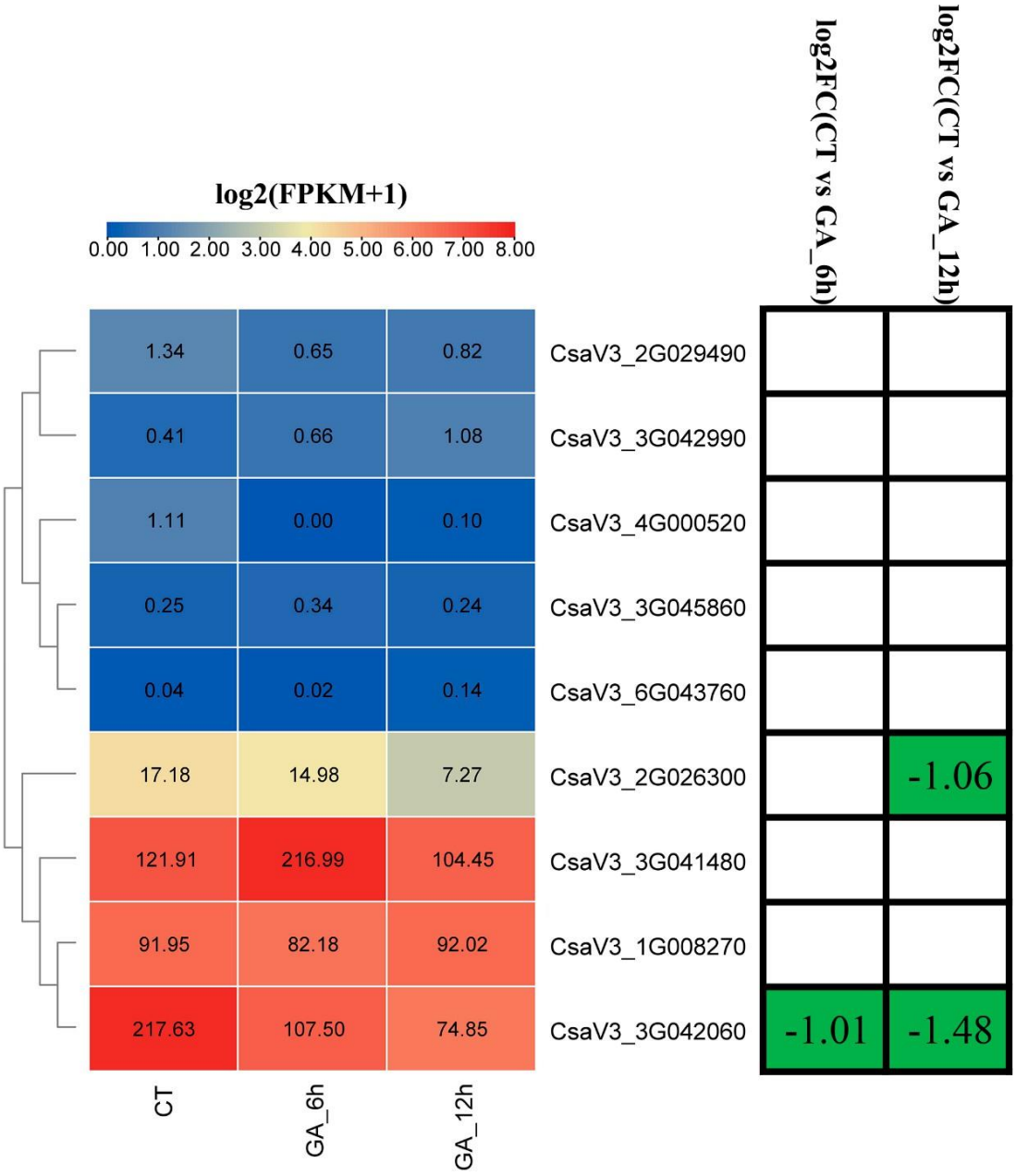

Figure 6. Expression heatmap of cucumber GASA family genes under GA treatment. CT: control treatment; GA_6 h: GA treatment for 6 h; GA_12 h: GA treatment for 12 h. The data in the left boxes indicate the original FPKM values. The data in the right boxes are log2(fold-change) values highlighted by red (up-regulation) and green (down-regulation) colors.

3.8. Expression Profiles of Cucumber GASA Genes under Abiotic Stresses

To determine the expression profiles of cucumber GASA family genes under high temperature, low temperature, salt and silicon stresses, the published transcriptome sequencing data (PRJNA634519; PRJNA438923; PRJNA477930) [73,74] were re-analyzed with the cucumber ChineseLong_V3 genome. Compared with the control, *CsaV3_3G041480* and *CsaV3_3G042990* genes were significantly down-regulated under high temperature stress. The other cucumber GASA genes were not significantly affected by high temperature treatment (Figure 7A). Cucumber GASA genes *CsaV3_3G041480*, *CsaV3_1G008270* and *CsaV3_3G042990* were significantly down-regulated under low temperature stress (Figure 7B). Under salt stress, *CsaV3_3G041480* and *CsaV3_3G042060* genes were significantly down-regulated (Figure 7C). Under silicon stress, the *CsaV3_3G042060* gene was

significantly down-regulated, and the *CsaV3_3G045860* gene was significantly up-regulated (Figure 7C).

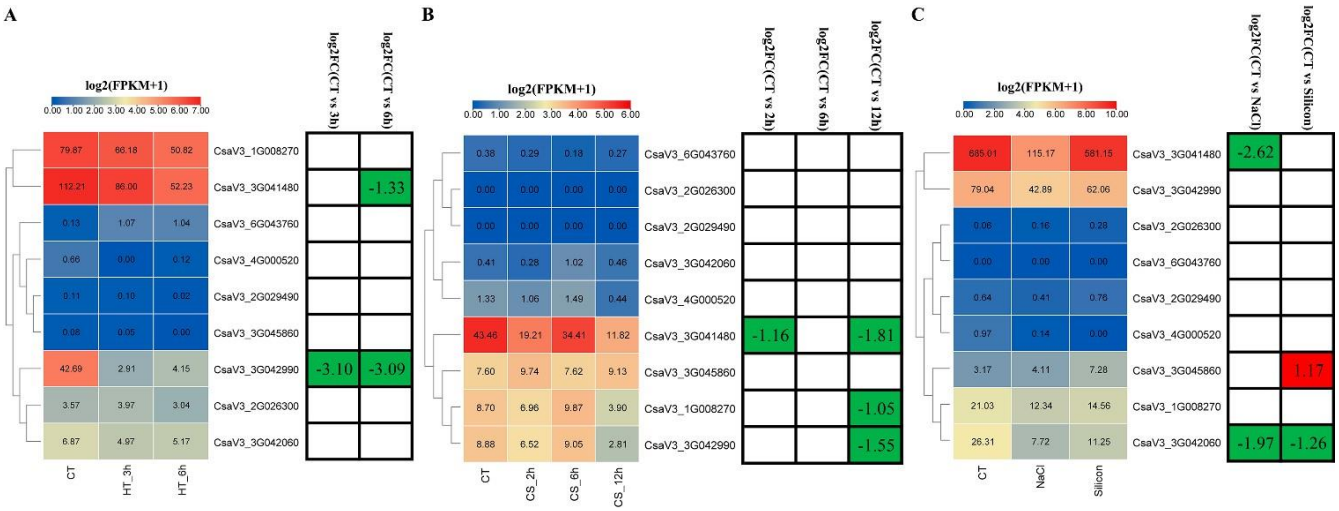

Figure 7. Expression heatmaps of cucumber GASA family genes under abiotic stresses. (**A**) Expression patterns of cucumber GASA family genes under high temperature stress. (**B**) Expression patterns of cucumber GASA family genes under low temperature stress. (**C**) Expression patterns of cucumber GASA family genes under salt and silicon stresses. CT: control treatment; HT_3 h: high temperature treatment for 3 h; HT_6 h: high temperature treatment for 6 h; CS_2 h: low temperature treatment for 2 h; CS_6 h: low temperature treatment for 6 h; CS_12 h: low temperature treatment for 12 h; NaCl: salt stress treatment. Silicon: silicon stress treatment. In each figure, the data in the left boxes indicate the original FPKM values. The data in the right boxes are log2(fold-change) values highlighted by red (up-regulation) and green (down-regulation) colors.

3.9. Expression Profiles of Cucumber GASA Genes under Biotic Stresses

To determine the expression profiles of cucumber GASA family genes under downy mildew, powdery mildew and root-knot nematode stresses, the published transcriptome sequencing data (PRJNA285071; PRJNA321023; PRJNA419665) [75–77] were re-analyzed with the cucumber ChineseLong_V3 genome. Under downy mildew stress, only the *CsaV3_2G029490* gene was significantly down-regulated in both resistant and susceptible materials. The *CsaV3_3G041480* gene was significantly up-regulated only in the resistant material (Figure 8A). Under powdery mildew stress, the *CsaV3_3G041480* and *CsaV3_3G042990* genes were significantly down-regulated in both resistant and susceptible materials, while the *CsaV3_1G008270*, *CsaV3_2G029490* and *CsaV3_3G042060* genes were only significantly up-regulated in the susceptible material (Figure 8B). Under root-knot nematode stress, the *CsaV3_2G029490* and *CsaV3_4G000520* genes were significantly up-regulated in both resistant and susceptible materials, but with different expression patterns. The *CsaV3_2G029490* gene was significantly up-regulated at 1 day post inoculation (dpi) in the susceptible material, and then the expression level decreased slowly. In the resistant material, the expression level of the *CsaV3_2G029490* gene was significantly up-regulated at 3 dpi. The expression patterns of the *CsaV3_4G000520* gene in resistant and susceptible materials were also different. The *CsaV3_4G000520* gene was only significantly differentially expressed at 3 dpi in susceptible material, but was significantly differentially expressed at 1 dpi in resistant material. The *CsaV3_3G042060* gene was significantly down-regulated in both resistant and susceptible materials, but with different expression patterns. The *CsaV3_3G042060* gene was significantly down-regulated at 1 dpi in susceptible material, but was significantly down-regulated at 2 dpi in resistant material. The *CsaV3_6G043760* gene was significantly down-regulated only in susceptible material (Figure 8C).

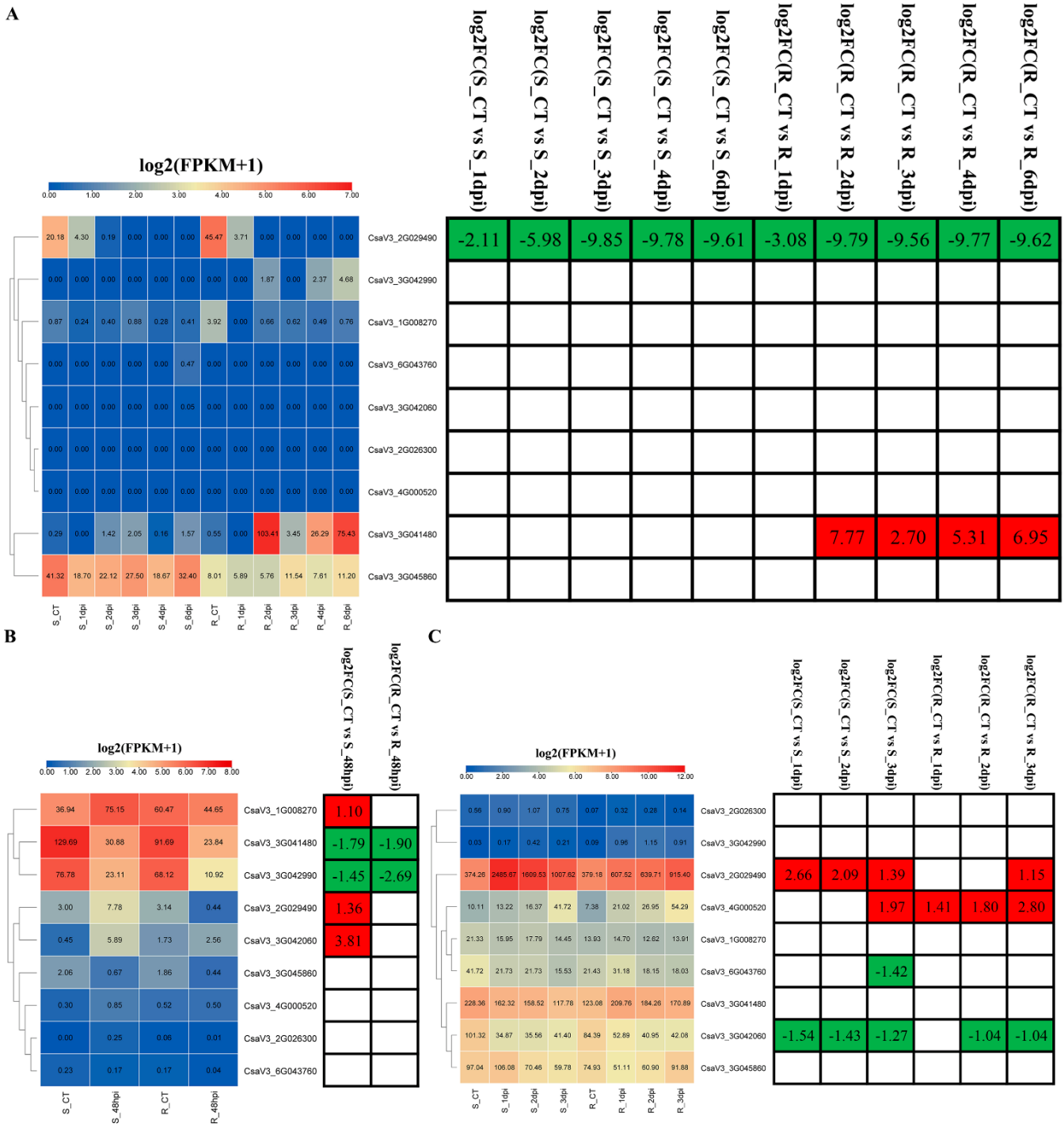

Figure 8. Expression heatmaps of cucumber GASA family genes under biotic stresses. (**A**) Expression patterns of cucumber GASA family genes under downy mildew stress. (**B**) Expression patterns of cucumber GASA family genes under powdery mildew stress. (**C**) Expression patterns of cucumber GASA family genes under root-knot nematode stress. S: susceptible plants; R: resistant plants; 1 dpi, 2 dpi, 3 dpi, 4 dpi and 6 dpi are 1, 2, 3, 4 and 6 days post inoculation, respectively; CT: control; 48 hpi: 48 h post inoculation. In each figure, the data in the left boxes indicate the original FPKM values. The data in the right boxes are log2(fold-change) values highlighted by red (up-regulation) and green (down-regulation) colors.

3.10. Regulation Patterns of Cucumber GASA Genes under Stresses

To analyze the expression patterns of cucumber GASA family genes under GA treatment and stress responses, the differentially expressed genes were labeled and drawn into

a heatmap (Figure 9). The results showed that nine cucumber GASA family genes were all differentially expressed under different stress responses. The *CsaV3_3G042060* and *CsaV3_3G041480* genes were differentially expressed under multiple types of abiotic and biotic stresses, indicating that these two GASA genes actively participate in stress responses, which indicates they could be considered as key candidate genes for further studies. The *CsaV3_1G008270* and *CsaV3_3G042990* genes were differentially expressed under a few abiotic and biotic stresses. The *CsaV3_2G026300* gene was differentially expressed only under GA treatment. The *CsaV3_3G045860* gene was up-regulated only under salt stress. Additionally, some cucumber GASA family genes, such as *CsaV3_4G000520*, *CsaV3_6G043760* and *CsaV3_2G029490*, were only involved in response to biotic stresses. The analysis of regulation patterns of cucumber GASA family genes could provide a theoretical reference for further research on molecular biological functions of cucumber GASA genes.

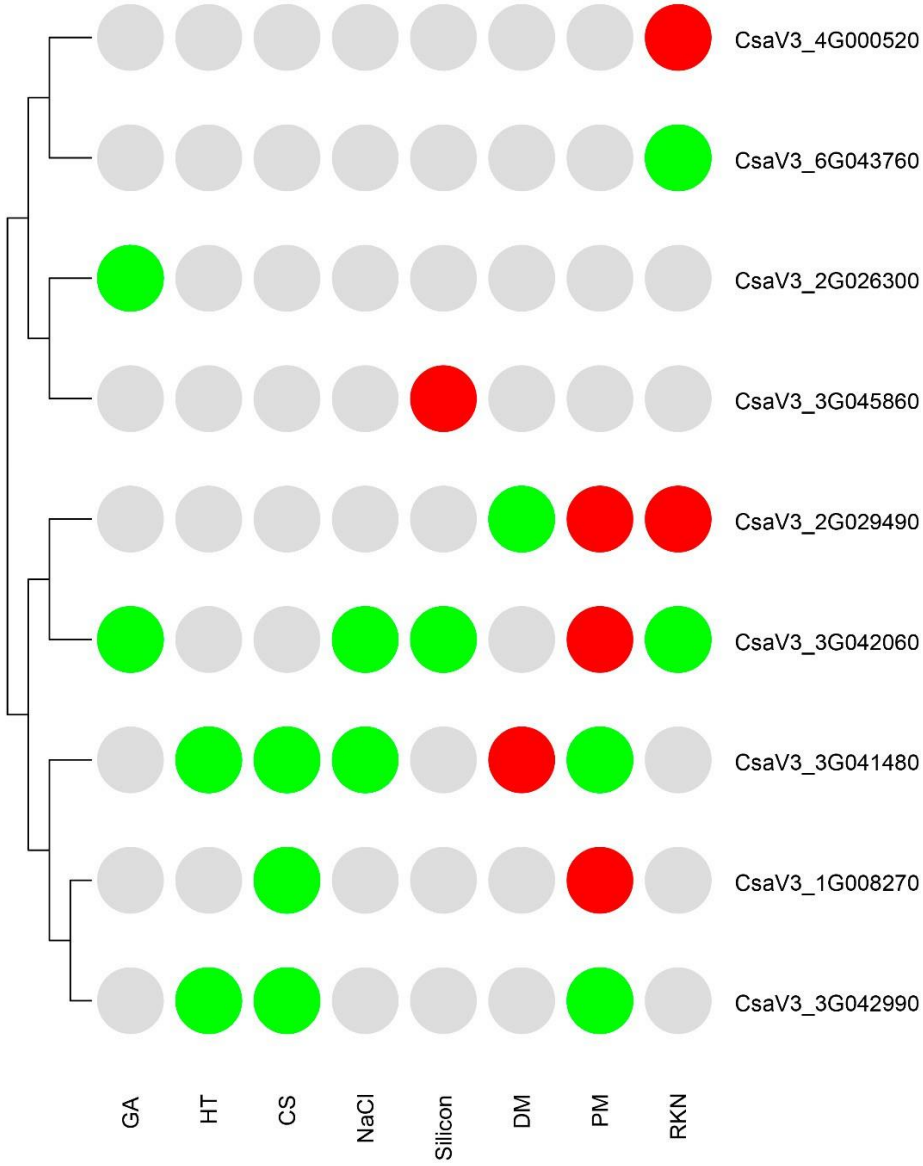

Figure 9. Expression patterns heatmap of cucumber GASA family genes under abiotic and biotic stresses. GA: gibberellin treatment; HT: high temperature stress; CS: low temperature stress; NaCl: salt stress; Silicon: silicon stress; DM: downy mildew stress; PM: powdery mildew stress; RKN: root-knot nematode stress. Gray color represents expression level that was not changed, red color represents up-regulated expression, green color represents down-regulated expression.

4. Discussion

In recent years, with the rapid development of genome sequencing technology, more and more plant genome information has been published [78]. The identification of many important gene families has gradually been carried out in different plants. GASA is a type of gene family induced by gibberellin, encoding a small molecular polypeptide. Its N-terminal contains a signal peptide, and its C-terminal contains 12 conserved cysteines [79]. The GASA gene family has been successively identified in model plants including *Arabidopsis thaliana* [8–10] and rice [14], and subsequently reported in some important field crops such as maize [11] and wheat [13]. The identification of GASA gene family has also been reported in fruit trees such as apple [10] and grape [15]. However, there were few studies on the identification of GASA gene family in vegetable crops; only the identification of GASA gene family in tomato was reported [14]. As important vegetable crops widely cultivated in the world, Cucurbitaceae is the second largest fruit and vegetable family, and its members are among the most important edible plants in the world, next only to Solanaceae [66]. With the genome resolution of a large number of Cucurbitaceae crops, annotation research on gene function has become a current hotspot [32]. However, research on GASA gene family identification and function in Cucurbitaceae crops has not been performed, which greatly limits the molecular biological function research on GASA genes in Cucurbitaceae species. Therefore, using the genome information on Cucurbitaceae crops, the GASA gene families were identified in 10 Cucurbitaceae crops. Moreover, the expression profiles of GASA family genes in different tissues and stress responses were analyzed in cucumber, which provided a theoretical reference for further study on molecular functions of GASA genes and laid a theoretical basis for cucumber resistance molecular breeding.

In this study, a total of 114 members of the GASA gene family in 10 Cucurbitaceae crops were identified. There were 9, 9, 11, 9, 9, 8, 15, 10, 16 and 18 GASA members in wax gourd, watermelon, pumpkin, cucumber, sponge gourd, bottle gourd, bitter gourd, melon, chayote and snake gourd, respectively. The GASA family gene numbers in *Arabidopsis*, maize, potato, apple, wheat, grape, *Populus Tomentosa*, *Populus euphratica*, and rice were 15, 10, 16, 26, 37, 14, 21, 19 and 9 [10–13,15,16,79,80], respectively, most of which outnumbered the GASA genes in Cucurbitaceae crops. The analysis of physicochemical characteristics showed that all GASA proteins except *CmoCh09G000990.1* in Cucurbitaceae crops were basic proteins, which was similar to the physicochemical characteristics of cocoa GASA proteins (except that one GASA protein was acidic, while the other GASA proteins were basic proteins) [31]. In *Arabidopsis*, rice, grape and tomato, all of the GASA proteins were basic proteins. Phylogenetic tree analysis of 114 GASA genes divided these GASA genes into three subgroups, namely G1, G2 and G3, which was similar to the clustering results of phylogenetic analysis of GASA family genes in *Arabidopsis* [10], grape [15] and poplar [16]. There were significant differences in gene structure among the GASA genes within three different subgroups, and the gene structures and conserved motifs of GASA genes in the same subgroup were similar. The analysis of gene duplication events for cucumber GASA family genes found that there were no tandem duplication genes in cucumber. However,
there were two segmental duplications in the cucumber GASA gene family. It revealed that the expansion of GASA genes in cucumber mainly resulted from segmental duplications, indicating that each species performed gene expansion in a specific way. This phenomenon also existed in the other plant gene families [81,82]. The synteny analysis of the GASA family genes in *Arabidopsis*, rice and cucumber found that nine cucumber GASA genes were collinear with *Arabidopsis* GASA genes, while only six cucumber GASA genes were collinear with rice GASA genes, indicating that the cucumber GASA family genes have higher homology with *Arabidopsis* GASA genes in evolution.

Due to the rapid development of high-throughput sequencing technology in recent years, many researchers have conducted transcriptome sequencing in cucumber, forming cucumber transcriptome sequencing big data. Therefore, using these transcriptome sequencing data effectively could not only reduce research costs but also enable in-depth data mining. Moreover, the molecular biological functions of different gene families in

cucumber could be studied by using cucumber transcriptome sequencing big data under different treatments. In this study, with the published cucumber transcriptome sequencing big data, the tissue-specific expression patterns and the stress-responsive gene expression patterns of nine cucumber GASA family genes were analyzed. The results showed that only *CsaV3_2G029490* was lower expressed or not expressed in all tissues. *CsaV3_3G041480* was highly expressed in all tissues, while the other cucumber GASA family genes showed a tissue-specific expression pattern. The tissue-specific expression pattern of these GASA family genes in different tissues cooperatively regulated the plant growth and development of cucumber. By analyzing the expression patterns of cucumber GASA genes in response to GA stress, the results showed that *CsaV3_2G026300* and *CsaV3_3G042060* were induced by GA stress. It was also found that some GASA genes, such as *GASA14,* were regulated by GA in *Arabidopsis* and rice [4,5,22]. It was found that *CsaV3_3G042060* and *GASA14* were orthologous genes. By analyzing the expression profiles of cucum- ber GASA genes in response to biotic and abiotic stress, the results showed that eight GASA genes, except for *CsaV3_2G026300*, were involved in stress responses. Among them, *CsaV3_3G042060, CsaV3_3G041480, CsaV3_1G008270 and CsaV3_3G042990* were all involved in response to biotic and abiotic stress, *CsaV3_3G045860* was only up-regulated in response to silicon stress, and *CsaV3_4G000520, CsaV3_6G043760* and *CsaV3_3G045860* were only involved in response to biological stress. Previous research has found that the *CsGASA4* gene (*Csa3G826660* in the cucumber V2 genome and *CsaV3_3G041480* in the cucumber V3 genome) is involved in the response to downy mildew and corynespora leaf spot, and is up-regulated in the resistant varieties [83], which is consistent with the expression pattern of *CsGASA4* gene in resistant varieties in this study.

5. Conclusions

In this study, a total of 114 GASA family genes were identified in 10 Cucurbitaceae species including cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), pumpkin (*Cucurbita moschata*), wax gourd (*Benincasa hispida*), sponge gourd (*Luffa cylindrica*), bottle gourd (*Lagenaria siceraria*), bitter gourd (*Momordica charantia*), chayote (*Sechium edule*), and snake gourd (*Trichosanthes anguina*), which were divided into three subgroups. The gene structure and protein conserved motifs in the same subgroup were highly conservative, and the distributions of exon/intron and motifs were different among different subgroups. The tissue-specific expression patterns and the stress-responsive gene expression patterns of cucumber GASA family genes were analyzed by reanalyzing the cucumber transcriptome sequencing data with cucumber ChineseLong_V3 genome. The results showed that the expression patterns of cucumber GASA family genes in different tissues and stress responses were different, which synergistically regulated the plant growth and development of cucumber. Among them, *CsaV3_3G042060* and *CsaV3_3G041480* were differentially expressed under multiple biotic and abiotic stresses, indicating that these two GASA genes were actively involved in stress responses. This study will provide a theoretical reference for the further study of molecular biological functions of cucumber GASA genes, and provide favorable genes for cucumber resistance molecular breeding.

Author Contributions: X.W. and L.J. conceived the research and designed the experiments. K.Z. performed research, analyzed the data and wrote the manuscript. Y.H. and D.Y. participated in downloading transcriptome sequencing data and helped with the bioinformatics analysis. C.Y., N.L. and Z.L. analyzed and interpreted the data. M.K.N. wrote and modified the manuscript. All authors have read and agreed to the published version of the manuscript.

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