Identification of MYC genes in four *Cucurbitaceae* species and their roles in the response to temperature stress

Tao Liu[1], Yani Zheng[1], Jingyu Yang[1], Rourou Li[1], Huan Chang[1], Nanyang Li[1,2], Wang Suna[1,2], Liping Wang[1,2] and Xing Wang[1,2*]

Abstract

Background Myelocytomatosis (*MYC*) transcription factors are crucial mediators of the response of plants to environmental stresses through via binding to DNA regulatory regions. However, few systematic characterizations of *MYC* genes are available in *Cucurbitaceae* species.

Results In this study, we identified 10, 8, 12, and 10 MYC genes in *Cucumis sativus*, *Cucumis melo*, *Citrullus lanatus*, and *Benincasa hispida*, respectively. Characterization revealed that all of the MYC proteins contain a highly conserved H4-V5-E6-E8-R9-R11-R12 sequence, which is essential for the binding of DNA regulatory regions. Evolutionary analysis enabled us to categorize 40 predicted MYC proteins from seven species into five distinct groups and revealed that the expansion of the MYC genes occurred before the divergence of monocots and dicots. The upstream promoter regions of the MYC genes contain a variety of developmental, stress, and hormone-responsive regulatory elements. The expression of cucumber MYC genes varies significantly across organs, with particularly high expression of *CsaV3_3G001710* observed across all organs. Transcriptomic analysis revealed that certain cucumber *MYC* genes undergo specific upregulation or downregulation in response to both biotic and abiotic stressors. In particular, under temperature stress, the cucumber genes *CsaV3_3G007980* and *CsaV3_3G001710* were significantly upregulated. Interestingly, the homologs of these two genes in *C. lanatus* presented a similar expression pattern to that in *C. sativus*, whereas in *B. hispida*, they presented the opposite pattern, i.e., significant downregulation. These findings indicated that these two genes indeed respond to temperature stress but with different expression patterns, highlighting the divergent functions of homologous genes across different species.

Conclusions This study analyzed the size and composition of the MYC gene family in four *Cucurbitaceae* species and investigated stress-responsive expression profiles, especially under temperature stress. All the results showed that MYC genes play important roles in development and stress responses, laying a theoretical foundation for further investigations of these response mechanisms.

Keywords Myelocytomatosis, *Cucurbitaceae*, stress responses

*Correspondence:
Xing Wang
wangxing@hebeu.edu.cn
[1]School of Landscape and Ecological Engineering, Hebei University of Engineering, Handan 056038, China
[2]Hebei Engineering Research Center for Seedling Breeding of Solanaceae Vegetables, Handan 056038, China

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Background

Myelocytomatosis oncogene (*MYC*) transcription factors (TFs) are an important class of TFs belonging to the basic helix-loop-helix (bHLH) TF family and contain two conserved functional domains, namely, the bHLH_MYC_N domain in the N-terminal region and the bHLH region in the C-terminal region [1–3]. As a subfamily of the bHLH family, *MYC* plays important roles in plant growth and development, secondary metabolism and signal transduction [4].

The first *MYC* gene, *AtMYC1*, was cloned from *Arabidopsis thaliana*, and functional studies revealed that it plays a certain role in plant seed development [5]. Successively, more *MYC* genes have been found in *Arabidopsis*. *MYC2* can regulate leaf aging by antagonizing bHLH IIId subfamily transcription factors [6] and can also interact with jasmonate ZIM-domain 7 (*JAZ7*) to inhibit leaf aging under dark conditions [7]. In *Arabidopsis thaliana*, *AtMYC2* can cooperate with *AtMYC3* and *AtMYC4* to regulate leaf development [8], chlorophyll degradation [9], seed production and seed storage protein accumulation [10, 11]. Previous studies also revealed that *AtMYC2* can inhibit the growth of leaf veins by inhibiting the synthesis of auxin in plant leaves [12]. In addition, the *Arabidopsis* Aborted Microspores (*AMS*) gene, which encodes a *MYC* transcription factor, plays a crucial role in tapetum cell development and pollen wall formation [13]. In addition, MYC genes in other plants are involved in plant growth and development. In apple (*Malus pumila* Mill.), at the fruit ripening stage, *MdMYC2* can affect ethylene biosynthesis and promote fruit ripening by promoting the expression of *MdACS1* and *MdACO1* [14]. In rice (*Oryza sativa*), overexpressed *OsMYC2* can interact with *OsJAZ1* and activate the downstream gene *OsMADS1*, which then regulates the development of spikelets [15]. The *MYC* genes also have important effects on the accumulation of plant secondary metabolites. For example, overexpression of *AtMYC3* and *AtMYC4* resulted in excessive accumulation of anthocyanins in *Arabidopsis*. Similarly, wheat (*Triticum aestivum*) *MYC1* can regulate anthocyanin synthesis in the pericarp [16]. *CrMYC2* can control jasmonate-responsive expression of the *ORCA* genes, which regulate alkaloid biosynthesis in *Catharanthus roseus*. In a few cases, *TcJAMYC* could negatively regulate the jasmonic acid-responsive expression of taxol biosynthesis genes in cultured cells of *Taxus cuspidata* [17]. In *Artemisia annua*, *AaMYC2* can bind to *AaJAZ1-4* and activate the expression of the artemisinin biosynthetic enzymes *CYP71AV1* and *DBR2*, which positively regulate artemisinin biosynthesis [18].

Although many studies on MYC genes in various species have been conducted, studies on MYC genes in *Cucurbitaceae* crops are still lacking. *Cucurbitaceae* is one of the most important edible plant families in the world, among which cucumbers, melons, watermelons and wax gourds are widely grown worldwide and have great economic benefits. In this study, we aimed to identify the MYC genes in four *Cucurbitaceae* crops and compare MYC gene evolution and variation among species through bioinformatics analysis. We also analyzed the expression patterns of MYC genes under biotic and abiotic stresses, with a focus on identifying MYC genes involved in the temperature stress response, to provide theoretical support for stress-resistant breeding in *Cucurbitaceae* crops.

Methods

Identification and bioinformatics analysis of the MYC gene family in *Cucurbitaceae* crops

The HMM model files (PF14215.7 and PF00010) for the MYC gene family were downloaded from the Pfam database (http://pfam.xfam.org/), and the protein sequence files of *Cucumis sativus* L., *Cucumis melo* L., *Citrullus lanatus*, and *Benincasa hispida* were downloaded from the Cucurbitaceae Genomic Database (http://cucurbitgenomics.org; [19–22]). The hidden Markov model (HMM) plugin in the HMMER v3 software package was used to predict candidate MYC gene family members in the *Cucumis sativus* L., *Cucumis melo* L., *Citrullus lanatus*, and *Benincasa hispida* genomes [23], and the sequence information of high-quality candidate proteins was extracted via Perl scripts ($E<1\times10^{-5}$). The candidate *MYC* gene family genes were subsequently identified via the BLASTP program and NCBI-Conserved Domain Data (CDD) (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi; [24]).

MYC gene family characterization and phylogenetic tree construction in *Cucurbitaceae* crops

Using the online tool ExPASy (https://web.expasy.org/protparam/) [25], we analyzed the amino acid length, molecular weight, isoelectric point, instability coefficient, aliphatic index, and average hydrophobicity of the members of the MYC gene family. We used the online website CELLO (http://cello.life.nctu.edu.tw/) for subcellular localization prediction. We employed the online software MEME (http://meme-suite.org/) to analyze the conserved motifs of MYC family proteins, with the parameters set as follows: 10 motifs and optimal motif width ranging from 6 to 200. Multiple sequence alignment was performed via ClustalX 2.0 and visualized via Jalview [26]. Phylogenetic analysis was performed via MEGA 7 [27] via the neighbor-joining (NJ) method, and the parameters used were the Poisson model, pairwise deletion, and 1000 bootstrap replications [28].

Chromosomal distribution and collinearity analysis of MYC genes

Based on the physical location information in the genome database, we used a mapchart to map the MYC gene family members onto *Cucurbitaceae* crop chromosomes [29]. The chromosomal distribution of the *Cucurbitaceae* crop SRS gene family was visualized via TBtools software [30]. Gene duplication events were analyzed via the multiple collinearity scan tool (MCScanX) [31]. A collinearity analysis plot was generated via Dual Synteny Plotter software (https://github.com/CJ-Chen/TBtools) [32].

Gene expression analysis

Transcriptome sequencing data related to cucumbers were downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/). The SRA-to-Fastq plugin in TBtools was used to convert the downloaded SRA data into Fastq format. Data quality was assessed via the FastQC plugin [33], and adapter sequences and low-quality sequences were removed via the Trimmomatic plugin [34], resulting in clean data. The filtered transcriptome data were aligned to the cucumber ChineseLong_V3 genome via the STAR plugin, generating SAM files [35]. Gene expression levels were analyzed via the StringTie Quantify plugin [36]. Finally, differential gene expression analysis was conducted via the DESeq2 plugin [37].

Tissue-specific expression analysis of cucumber MYC genes

Using transcriptome sequencing data from the NCBI database (PRJNA80169) [38], we analyzed the tissue-specific expression of the cucumber MYC gene family in various tissues and organs, including cucumber leaves, stems, female flowers, male flowers, unfertilized ovaries, fertilized ovaries, ovaries, roots, tendrils, and tendril bases. We utilized TBtools software to create an expression heatmap depicting the specific expression patterns of the cucumber MYC gene family in different cucumber tissues and organs.

Stress-responsive expression analysis of cucumber MYC genes

Using transcriptome sequencing data from the NCBI database, including PRJNA634519 [39], PRJNA438923 [40], PRJNA477930 [41], PRJNA321023 [42], and PRJNA419665 [43], we analyzed the specific expression patterns of the cucumber MYC gene family in response to various stress conditions, such as high temperature, low temperature, high salinity, silicon, powdery mildew, downy mildew, and southern root-knot nematodes. We used TBtools software to create expression heatmaps illustrating the gene expression responses of the cucumber MYC gene family under abiotic and biotic stress conditions.

Temperature stress treatment, RNA extraction and qRT–PCR

The cultivars Jinyan-4 (*Cucumis sativus*), Harukei-3 (*Cucumis melo*), B227 (*Benincasa hispida*), and 8424 (*Citrullus lanatus*) provided by the Hebei Engineering Research Center for Seedling Breeding of Solanaceae and Fruit Vegetables of Hebei University Engineering were used to explore gene expression under temperature stress. The seedlings (two-leaf and one-heart stage) of the four cultivars were treated at 42 °C, and the leaves of the seedlings were removed at 0, 3, 6 and 12 h after treatment for qRT–PCR. At the same time, the seedlings of the four cultivars were treated at 10 °C, and the leaves of the seedlings were removed at 0, 3, 6 and 12 h after treatment for qRT–PCR. Three biological replicates were prepared for all samples, which were frozen in liquid nitrogen and then immediately stored at -80 °C.

Total RNA was extracted via the RNAprep Pure Plant Kit (DP432; Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. cDNA was synthesized from total RNA via the PrimeScript RT Kit (Takara). Specific primers for each gene were designed through Primer 6 (Table 1). The synthesized cDNA was subsequently subjected to qRT–PCR on an Opticon

Table 1 List of primers used in quantitative RT–PCR. All primers used were designed with primer 6

Gene	Sense Primer	Anti-sense Primer
CsaV3_3G007980	TAGTCAGTGGAGTCAGAGAT	CTACACGGTTAATCACAGAAG
CsaV3_3G001710	GGATGCGATGATAAGGATTC	TCTTCACTGTTGCTTGTTG
Bhi01M000362	GCGGTTCTATGCTCTACG	TTGAGGTGAGGCTGGATT
Cla97C05G080890	CTAGTTAATGGAGTCAGAGATG	CACGGTTAATCACAGAAGG
MELO3C006016	CGGTGAGGAATGATGAGAA	AATGAGACAGTTGCCAGAA
MELO3C013851	CGAGACGAGTTCTTGGATT	ACGGTGGTGGTAATTGAAT
Bhi11M000137	CGACTCAGACCACTCAGA	GATTCAATGGCTCTTCTCTTC
Cla97C10G186220	GGACTCAGACCACTCAGA	GATTCAATGGCTCTTCTCTTC
Bhi10G001911(Actin)	ATGTTCACAACCACTGCCGA	GTCGAGCGCAACATAAGCAA
MELO3C008032(Actin)	CATGTTCACCACCACTGCCGA	TGGCTGGAATAGAACTTCTGGGC
Cla97C02G026960.1(ClActin)	CCATGTATGTTGCCATCCAG	GGATAGCATGGGGTAGAGCA
Csa6M484600(CuActin)	CTGGTGATGGTGTGAGTC	AGAGATGGCTGGAATAGAAC

thermocycler (CFX96 Connect Real-Time System; Bio-Rad, Hercules, CA) with SYBR Green PCR master mix (Vazyme, Nanjing, China) according to the manufacturer's instructions. The 2-ΔΔCT method was used to calculate the relative expression of the MYC genes [44]. The value of relative expression showed the log2 FC (Fold change) of each gene compared with that of the control. The data analysis was conducted by Excel.

Results

Identification and physicochemical property analysis of MYC genes

A total of 40 MYC genes were identified in the genomes of four *Cucurbitaceae* crops: 10 in *C. sativus*, 8 in *C. melo*, 12 in *C. lanatus*, and 10 in *B. hispida*. The MYC genes were unequally distributed on each chromosome. For example, *C. sativus* L. contains 6 MYC genes on chromosome 3, whereas there are no MYC genes on chromosomes 1, 2, 4, or 5. In *C. melo* L., chromosomes 2, 3, 5, 7, 8, 9, 10, and 12 contain no MYC genes, but the other chromosomes each have up to 3 MYC genes (Fig. 1). Sequence analysis revealed that the amino acid lengths encoded by the *CsMYC* genes varied from 431 aa (*CsMYC8*) to 694 aa (*CsMYC5*), those encoded by the *CmMYC* genes varied from 433 aa (*CmMYC8*) to 745 aa (*CmMYC1*), those encoded by the *ClMYC* genes varied from 423 aa (*ClMYC3*) to 969 aa (*ClMYC1*), and those encoded by the *BhMYC* genes varied from 501 aa (*BhMYC4*) to 968 aa (*BhMYC3*). Except for the acidic proteins encoded by *CsMYC1*, *CmMYC5*, *ClMYC10* and *BhMYC1*, the MYC proteins in these *Cucurbitaceae* crops are alkaline proteins. Most proteins are unstable (with an instability index greater than 40), with the exceptions of *CsMYC5*, *CmMYC1*, *ClMYC12*, *BhMYC7*, and *BhMYC9.* The average hydropathicity values of all the proteins are less than 0, indicating that all the proteins are hydrophobic. Subcellular localization prediction revealed that most MYC proteins are localized in the cell nucleus, followed by chloroplasts. (Table 2).

Structure and phylogenetic analysis of the *Cucurbitaceae* crop MYC genes

Motif prediction analysis of the protein sequences of the MYC family members revealed that among 10 categorized motifs, no MYC family member contained all of them (Fig. 2). Motifs 1, 2, 5, 7, and 8 were relatively conserved and were common to all MYC genes. Gene structure analysis revealed that the number of exons in the MYC genes ranged from 1 to 15 (Fig. 2). The gene structures of most MYC genes within the same lineage are similar, further indicating the conservation of protein motifs and gene structures within each *MYC* evolutionary branch.

According to an alignment of the bHLH domains in the MYC proteins, there is a basic amino acid region (Basic) composed of approximately 12 amino acids. This region contained a highly conserved H4-V5-E6-E8-R9-R11-R12 sequence, which was essential for the binding of bHLHs to target genes. This region also included two helical structures, consisting of approximately 37 amino acids. Notably, the 22nd and 38th amino acids in the HLH domain, both leucine (Leu), were highly conserved, indicating their necessity for dimer formation (Fig. 3).

To clarify the evolutionary relationships among the MYC gene family members in the *Cucurbitaceae* crops and in *Zea mays*, *Brachypodium distachyon*, and *Oryza sativa*, we constructed a phylogenetic tree. All the MYC proteins can be divided into five subgroups, labeled I to V. Each subgroup included both monocotyledonous and dicotyledonous plants, indicating that the MYC genes were relatively conserved during the evolution of both monocots and dicots. Group IV is the largest subgroup, consisting of 4, 4, 6, 3, 2, 1, and 1 MYC proteins from *C. sativus* L., *C. melo* L., *C. lanatus*, *B. hispida*, *Z. mays*, *B.*
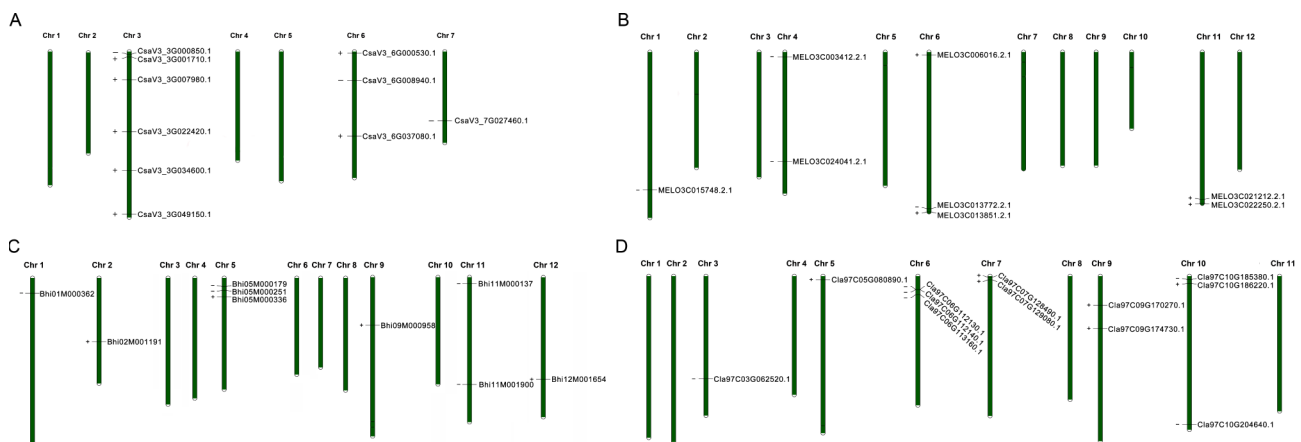
Fig. 1 Distribution of MYC family genes on the chromosomes of four Cucurbitaceae crop species. (**A**), *C. sativus* (**B**), *C. melo* (**C**), *B. hispida* (**D**), *C. lanatus*

**Table 2** Protein information for MYC gene family members in Cucurbitaceae crops

Species	Gene ID	Name	Number of amino acids (aa)	Molecular weight (D)	pI	Instability index	Aliphatic index	Average of hydropathicity	Prediction of sub-cellular location
Cucumis sativus L.	CsaV3_3G000850.1	CsMYC1	447	49398.41	8.65	43.61	82.37	-0.416	Nucleus
	CsaV3_3G001710.1	CsMYC2	642	69911.19	6.21	50.44	64.14	-0.578	Nucleus
	CsaV3_3G007980.1	CsMYC3	649	71943.89	5.83	49.00	77.66	-0.490	Nucleus
	CsaV3_3G022420.1	CsMYC4	501	55301.14	5.70	44.81	78.60	-0.445	Nucleus
	CsaV3_3G034600.1	CsMYC5	694	78486.44	5.24	38.28	84.84	-0.370	Chloroplast/Nucleus
	CsaV3_3G049150.1	CsMYC6	688	75666.20	5.11	58.15	69.71	-0.617	Nucleus
	CsaV3_6G000530.1	CsMYC7	644	72769.50	5.51	43.25	78.57	-0.500	Nucleus
	CsaV3_6G008940.1	CsMYC8	431	48394.52	5.42	47.63	76.43	-0.429	Nucleus
	CsaV3_6G037080.1	CsMYC9	650	71869.01	5.91	48.30	83.31	-0.347	Nucleus
	CsaV3_7G027460.1	CsMYC10	691	76190.07	5.66	41.31	76.58	-0.372	Nucleus
Cucumis melo L.	MELO3C015748.2.1	CmMYC1	745	82003.24	5.70	37.16	81.25	-0.269	Nucleus
	MELO3C003412.2.1	CmMYC2	723	79597.55	5.32	56.07	68.62	-0.628	Nucleus
	MELO3C024041.2.1	CmMYC3	501	55262.12	6.16	48.09	78.06	-0.486	Nucleus
	MELO3C006016.2.1	CmMYC4	586	65067.91	5.55	47.76	80.00	-0.535	Nucleus
	MELO3C013772.2.1	CmMYC5	442	48984.93	7.68	48.03	81.52	-0.439	Nucleus
	MELO3C013851.2.1	CmMYC6	662	72281.88	6.03	49.58	64.24	-0.570	Nucleus
	MELO3C021212.2.1	CmMYC7	656	74227.22	5.61	42.86	79.07	-0.473	Nucleus
	MELO3C022250.2.1	CmMYC8	433	48684.93	5.59	47.11	76.07	-0.442	Nucleus
Citrullus lanatus	Cla97C03G062520.1	ClMYC1	969	105839.97	6.15	47.45	78.86	-0.415	Nucleus
	Cla97C05G080890.1	ClMYC2	618	68589.08	5.85	46.81	76.99	-0.547	Nucleus
	Cla97C06G112130.1	ClMYC3	423	47569.45	5.14	52.02	71.70	-0.513	Nucleus
	Cla97C06G112140.1	ClMYC4	427	47692.74	5.09	52.44	52.44	-0.424	Nucleus
	Cla97C06G113160.1	ClMYC5	645	72922.74	5.78	46.22	79.64	-0.469	Nucleus
	Cla97C07G128490.1	ClMYC6	637	70287.21	6.36	45.59	81.93	-0.368	Nucleus
	Cla97C07G129080.1	ClMYC7	501	55179.91	5.96	49.98	76.47	-0.504	Nucleus
	Cla97C09G170270.1	ClMYC8	690	76046.16	5.87	40.82	80.36	-0.345	Nucleus
	Cla97C09G174730.1	ClMYC9	680	74878.38	5.24	53.48	70.68	-0.610	Nucleus
	Cla97C10G185380.1	ClMYC10	463	51021.92	8.77	53.53	79.74	-0.471	Nucleus
	Cla97C10G186220.1	ClMYC11	694	76681.99	6.35	49.20	64.81	-0.573	Nucleus
	Cla97C10G204640.1	ClMYC12	695	78085.01	4.97	38.55	83.87	-0.326	Chloroplast/Nucleus
Benincasa hispida	BhiUN179M27	BhMYC1	453	49798.89	8.19	47.72	83.43	-0.371	Nucleus
	Bhi01M000362	BhMYC2	617	68250.67	5.77	44.99	78.36	-0.520	Nucleus
	Bhi02M001191	BhMYC3	968	105559.53	6.12	44.22	79.96	-0.421	Nucleus
	Bhi05M000179	BhMYC4	501	55182.03	6.02	45.65	79.20	-0.464	Nucleus
	Bhi05M000251	BhMYC5	647	71471.31	5.91	43.64	80.06	-0.370	Nucleus
	Bhi05M000336	BhMYC6	682	75237.76	5.22	48.52	71.17	-0.632	Nucleus
	Bhi09M000958	BhMYC7	604	66789.67	6.01	39.23	80.83	-0.350	Nucleus
	Bhi11M000137	BhMYC8	660	72104.63	5.99	47.48	65.35	-0.570	Nucleus
	Bhi11M001900	BhMYC9	697	78638.85	5.07	39.16	84.61	-0.314	Chloroplast/Nucleus
	Bhi12M001654	BhMYC10	643	72375.09	5.60	47.35	80.34	-0.451	Nucleus

distachyon, and *O. sativa*, respectively. Group I was the smallest, with only 4 MYC genes (Fig. 4). In group II, there were 7 MYC genes, only 1 of which was from a monocotyledonous plant.

Collinearity analysis of the MYC genes among Cucurbitaceae crops
A total of 36 MYC genes (9 in *C. sativus* L., 7 in *C. melo* L., 11 in *C. lanatus*, and 9 in *B. hispida*) were located within synteny blocks in the four *Cucurbitaceae* genomes

(Fig. 5; Table 3). We identified five orthologous gene pairs that exist among all four species. These MYC genes were conserved during the evolution of all four *Cucurbitaceae* species, suggesting conserved roles. Furthermore, some MYC genes have been lost in some species. For example, certain MYC genes, such as the *CsaV3_3G000850/ MELO3C013772.2/Cla97C10G185380* collinear gene pair, were found in *C. sativus* L., *C. melo* L., and *B. hispida* but were absent in *B. hispida*. These results revealed the specific traits of different *Cucurbitaceae* species
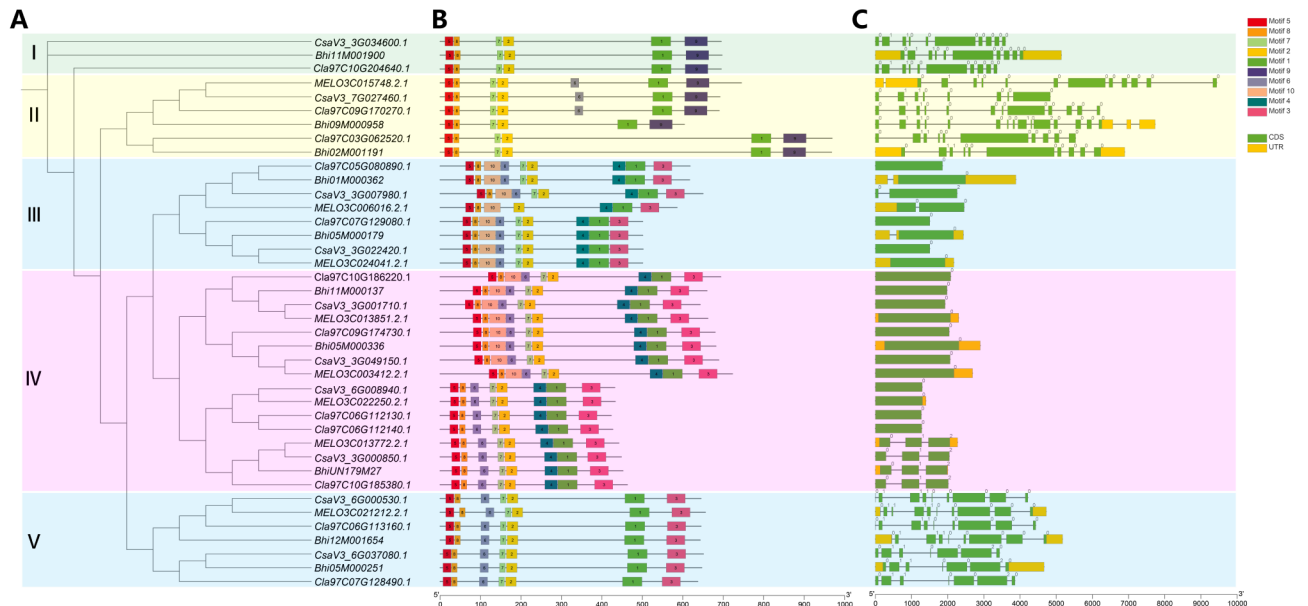
Fig. 2 Phylogenetic tree, gene structure and conserved domains of the MYC genes of the four Cucurbitaceae crop species. (**A**), The MYC phylogenetic tree is divided into five groups, and different colors indicate different branches. (**B**), Conserved sequence of the MYC genes. (**C**), MYC gene structure; green indicates the CDS, yellow indicates the untranslated region, and the black line indicates introns
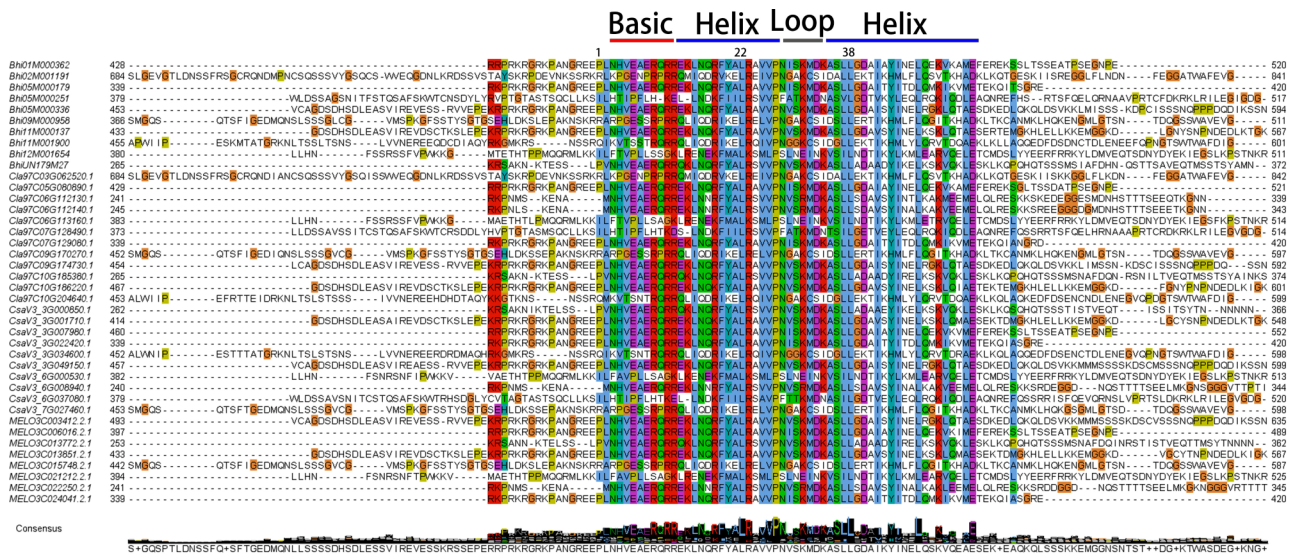

Fig. 3 Sequence alignment of the MYC proteins of the four *Cucurbitaceae* species

during evolution and amplification of the genome. In addition, two collinear gene pairs were detected in only *C. lanatus* and *B. hispida*.

Regulatory TFs of the MYC genes

The 1.5-kb upstream sequences of the MYC genes were selected for prediction of the TFs that regulate them. Three types of *cis*-elements related to development, hormone stress, and abiotic stress were identified (Fig. 6). Among the *cis*-elements related to development, the number of G-box (CACGTC) elements, which are

light-responsive elements, is the greatest. For example, the genes *MELO3C021212.2.1*, *MELO3C003412.2.1*, and *Cla97C10G186220.1* contain 9 G-box elements, indicating that they might be regulated by the light environment. Among the *cis*-elements related to hormone stress, the abscisic acid (ABA)-responsive element (ABRE) (ACGTG) is present in relatively large proportions, with 8 in *MELO3C021212.2.1*, *MELO3C003412.2.1*, and *Cla97C10G186220.1* and 6 in *Bhi02M001191*, *CsaV3_3G001710.1*, and *Bhi11M000137*. Among the *cis*-elements related to abiotic stress, anaerobic induction
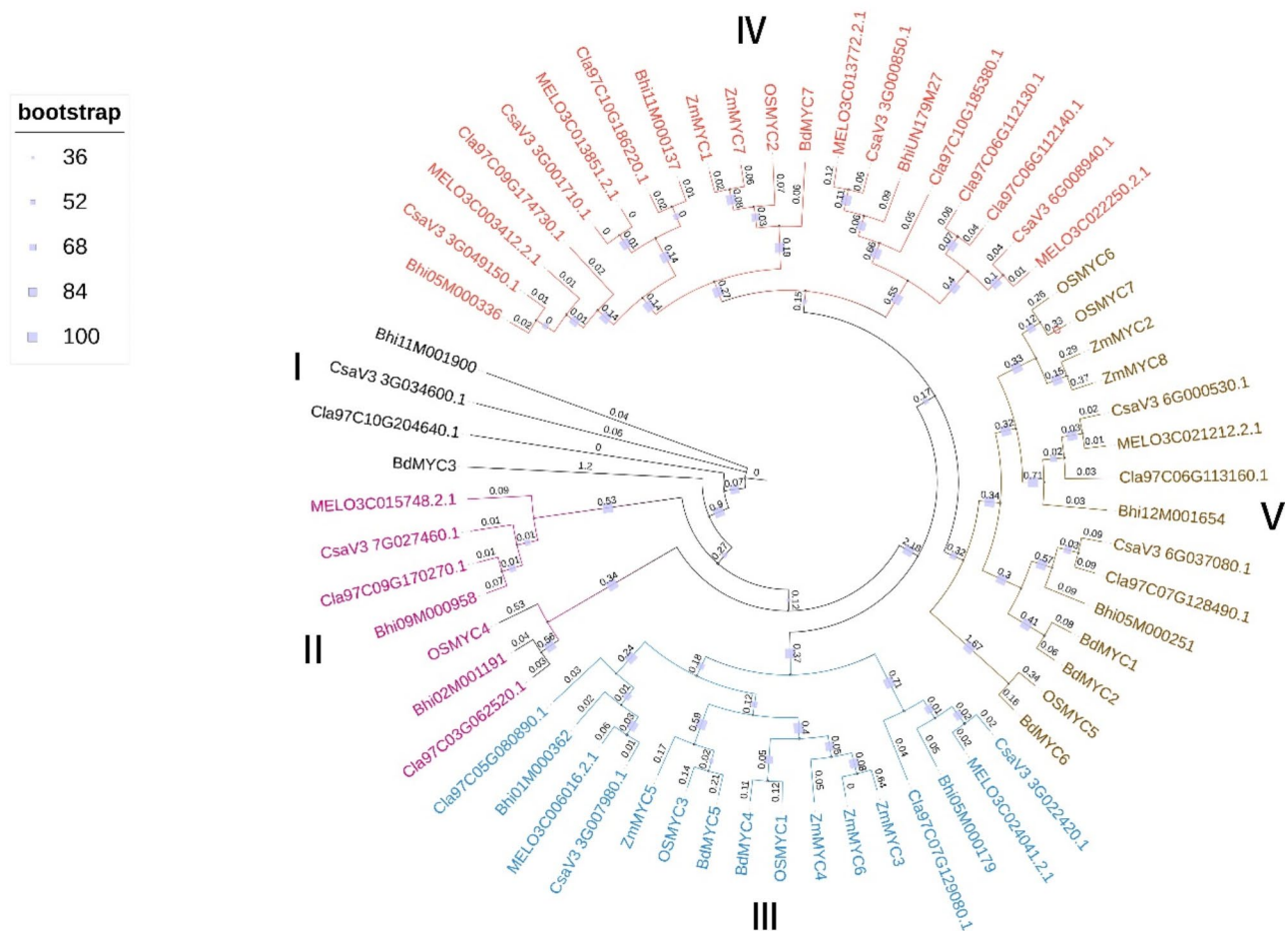
Fig. 4 Phylogenetic tree of MYC family members in *C. sativus*, *C. melo*, *C. lanatus*, *B. hispida*, *Z. mays*, *B. distachyon*, and *O. sativa*

AREs (AAACCA) were detected in a series of members, with 6 in *Cla97C07G128490.1* and *CsaV3_3G007980.1* and 5 in *Cla97C05G080890.1*.

### Tissue-specific expression analysis of the MYC genes in *C. Sativus*

To investigate the expression profiles of the MYC gene family members in different tissues, using cucumber as a representative, we conducted transcriptome analysis on various tissues based on publicly available cucumber transcriptome sequencing data (PRJNA80169). The results revealed significant variation in the expression of MYC gene family members across different tissues (Fig. 7). For example, while the *CsaV3_3G001710* gene presented a relatively high level of expression across all tissues or organs, three genes (*CsaV3_3G000850*, *CsaV3_6G008940*, and *CsaV3_6G037080*) presented relatively low expression levels across all tissues or organs. Some genes presented significant tissue-specific expression patterns. For example, the *CsaV3_3G049150* gene presented relatively high expression in roots and

fertilized ovaries but low expression in other tissues or organs. Similarly, compared with other tissues or organs, the *CsaV3_7G027460* gene presented greater expression in roots. These results indicate that cucumber MYC family genes play distinct roles in the development of tissues or organs, contributing to various functions in plant growth and development.

Expression analysis of cucumber MYC genes under different stress conditions

Using publicly available transcriptome data from the NCBI SRA database, we analyzed the expression levels of the cucumber MYC genes under both biotic and abiotic (high temperature, low temperature, salt and silicon stress, powdery mildew, and southern root-knot nematode) stress conditions.

Under high-temperature stress, most MYC genes were not significantly differentially expressed (Fig. 8). For example, the expression levels of the genes *CsaV3_6G037080*, *CsaV3_3G000850*, and *CsaV3_6G008940* did not change under
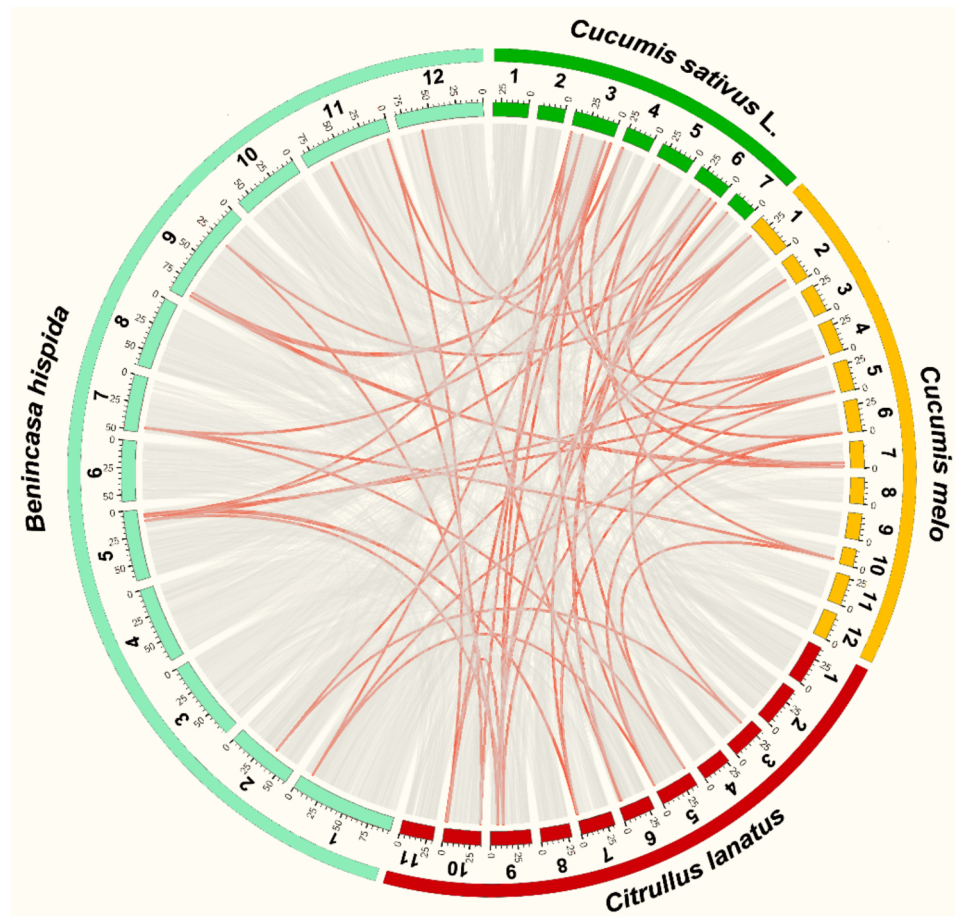
Fig. 5 Syntenic analysis of the MYC genes among *C. sativus*, *C. melo*, *C. lanatus*, and *B. hispida*. The gray lines in the background indicate the collinear blocks within the *C. sativus*, *C. melo*, *C. lanatus*, and *B. hispida* genomes, whereas the red lines highlight the homologous gene pairs
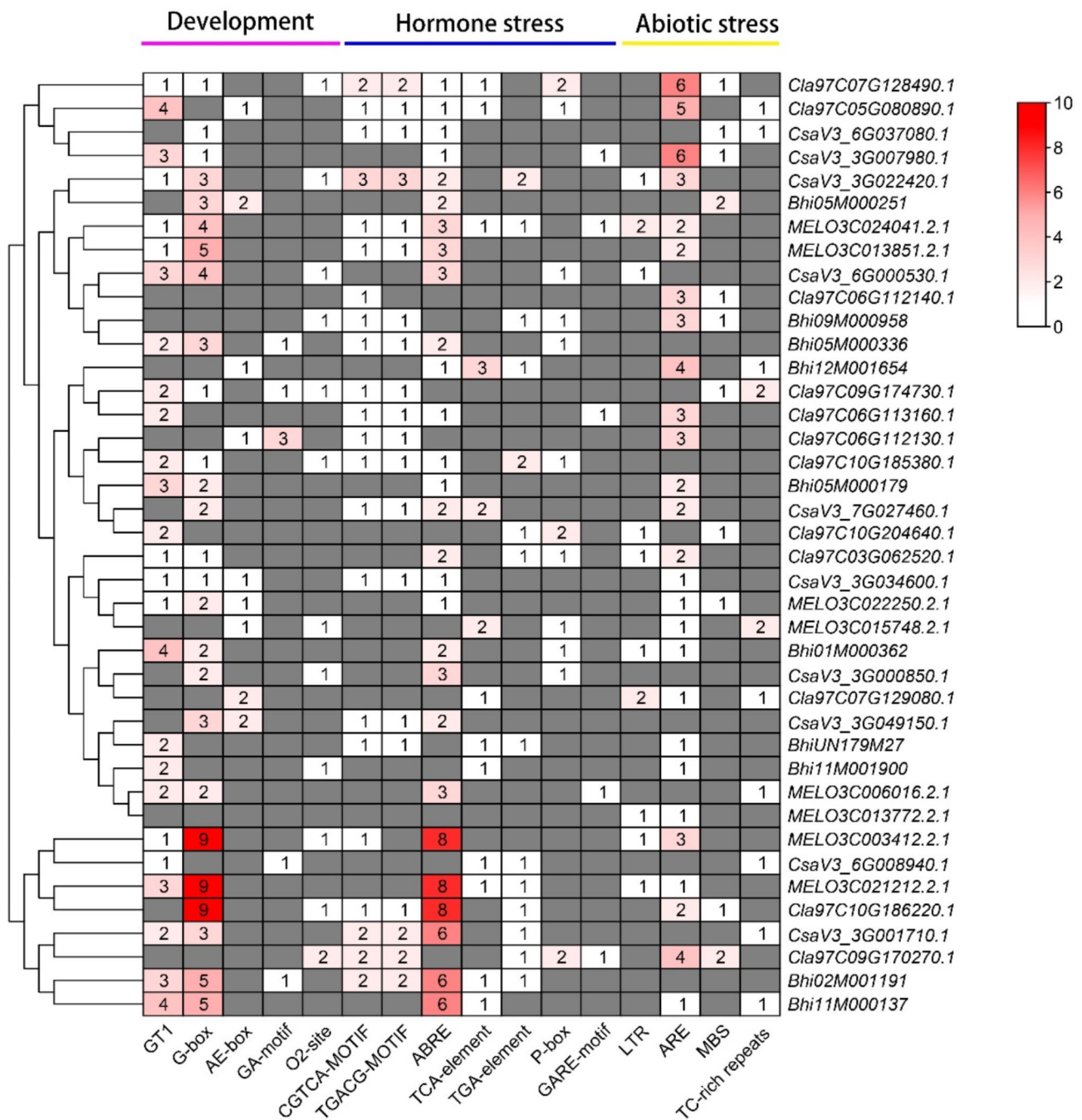
Table 3 Collinear gene pairs present in the four *Cucurbitaceae* genomes

Number	Cucumber	Melon	Watermelon	Wax gourd
1	CsaV3_3G000850	MELO3C013772.2	Cla97C10G185380	-
2	CsaV3_7G027460	MELO3C015748.2	Cla97C09G170270	Bhi09M000958
3	CsaV3_6G037080	-	Cla97C07G128490	Bhi05M000251
4	CsaV3_6G008940	MELO3C022250.2	Cla97C06G112130	-
5	CsaV3_6G000530	MELO3C021212.2	Cla97C06G113160	Bhi12M001654
6	CsaV3_3G049150	MELO3C003412.2	Cla97C09G174730	Bhi05M000336
7	CsaV3_3G001710	MELO3C013851.2	Cla97C10G186220	Bhi11M000137
8	CsaV3_3G007980	MELO3C006016.2	Cla97C05G080890	Bhi01M000362
9	CsaV3_3G034600	-	Cla97C10G204640	Bhi11M001900
10	-	-	Cla97C03G062520	Bhi02M001191
11	-	-	Cla97C07G129080	Bhi05M000179

high-temperature stress, and their expression levels were relatively low. However, the *CsaV3_3G001710* gene was significantly upregulated at 6 h after high-temperature treatment (6 hph), whereas the *CsaV3_3G007980* gene presented high expression levels at both 3 hph and 6 hph. These results suggest that the *CsaV3_3G007980* gene is likely involved in the response of cucumber to high-temperature stress.

Under low-temperature stress, the expression levels of four genes (*CsaV3_6G037080, CsaV3_3G000850, CsaV3_6G008940,* and *CsaV3_6G000530*) did not significantly change and remained relatively low. Two genes presented relatively high expression levels during the low-temperature treatment, with the *CsaV3_3G007980* gene being significantly upregulated at 6 hph. The expression levels of the other genes remained unchanged before

| Gene	GT1	G-box	AE-box	GA-motif	O2-site	CGTCA-MOTIF	TGACG-MOTIF	ABRE	TCA-element	TGA-element	P-box	GARE-motif	LTR	ARE	MBS	TC-rich repeats
Cla97C07G128490.1	1	1			1	2	2	1	1		2			6	1	
Cla97C05G080890.1	4		1			1	1	1	1		1			5		1
CsaV3_6G037080.1		1				1	1	1							1	1
CsaV3_3G007980.1	3	1						1				1		6	1	
CsaV3_3G022420.1	1	3			1	3	3	2		2			1	3		
Bhi05M000251		3	2					2							2	
MELO3C024041.2.1	1	4				1	1	3	1	1		1	2	2		
MELO3C013851.2.1	1	5				1	1	3						2		
CsaV3_6G000530.1	3	4			1			3			1		1			
Cla97C06G112140.1						1								3	1	
Bhi09M000958				1	1	1				1	1			3	1	
Bhi05M000336	2	3		1		1	1	2			1					
Bhi12M001654			1			1	3	1						4		1
Cla97C09G174730.1	2	1		1	1	1	1							1	2	
Cla97C06G113160.1	2					1	1	1				1		3		
Cla97C06G112130.1			1	3		1	1							3		
Cla97C10G185380.1	2	1			1	1	1	1		2	1					
Bhi05M000179	3	2						1						2		
CsaV3_7G027460.1		2				1	1	2	2					2		
Cla97C10G204640.1	2								1	2		1			1	
Cla97C03G062520.1	1	1						2	1	1		1		2		
CsaV3_3G034600.1	1	1	1			1	1	1						1		
MELO3C022250.2.1	1	2	1					1						1	1	
MELO3C015748.2.1			1		1				2		1			1		2
Bhi01M000362	4	2						2			1		1	1		
CsaV3_3G000850.1		2			1			3			1					
Cla97C07G129080.1			2						1			2	1			1
CsaV3_3G049150.1		3	2			1	1	2								
BhiUN179M27	2					1	1		1	1				1		
Bhi11M001900	2			1					1					1		
MELO3C006016.2.1	2	2						3				1				1
MELO3C013772.2.1													1	1		
MELO3C003412.2.1	1	9			1	1		8					1	3		
CsaV3_6G008940.1	1			1					1	1						1
MELO3C021212.2.1	3	9						8	1	1			1	1		
Cla97C10G186220.1		9			1	1	1	8			1			2	1	
CsaV3_3G001710.1	2	3				2	2	6			1					1
Cla97C09G170270.1					2	2	2				1	2	1	4	2	
Bhi02M001191	3	5			1	2	2	6	1	1						
Bhi11M000137	4	5						6	1					1		1

Fig. 6 Heatmap of various *cis*-elements in the promoters of each *Cucurbitaceae* MYC gene. The colors represent the quantity of the *cis*-elements, with deeper red indicating greater quantities. The numbers in the image represent the counts of the *cis*-elements

and after low-temperature treatment. Additionally, the expression of *CsaV3_3G049150* was significantly downregulated at both 6 hph and 12 hph. Notably, the expression levels of all the genes did not significantly change at 3 hph. These results suggest that the *CsaV3_3G007980* and *CsaV3_3G049150* genes play key roles in the response of cucumber to prolonged low-temperature stress and that *CsaV3_3G007980* is positively regulated, whereas *CsaV3_3G049150* is negatively regulated (Fig. 9).

Under salt and silicon treatment, most genes did not show differential expression after NaCl and silicon treatments. One gene (*CsaV3_3G000850*) was significantly downregulated after NaCl treatment and significantly upregulated after silicon treatment. However, when the plants were treated simultaneously with NaCl and silicon, a greater degree of downregulation was found (Fig. 10). Despite the significant differential expression of the *CsaV3_3G00850* gene after treatment, its expression
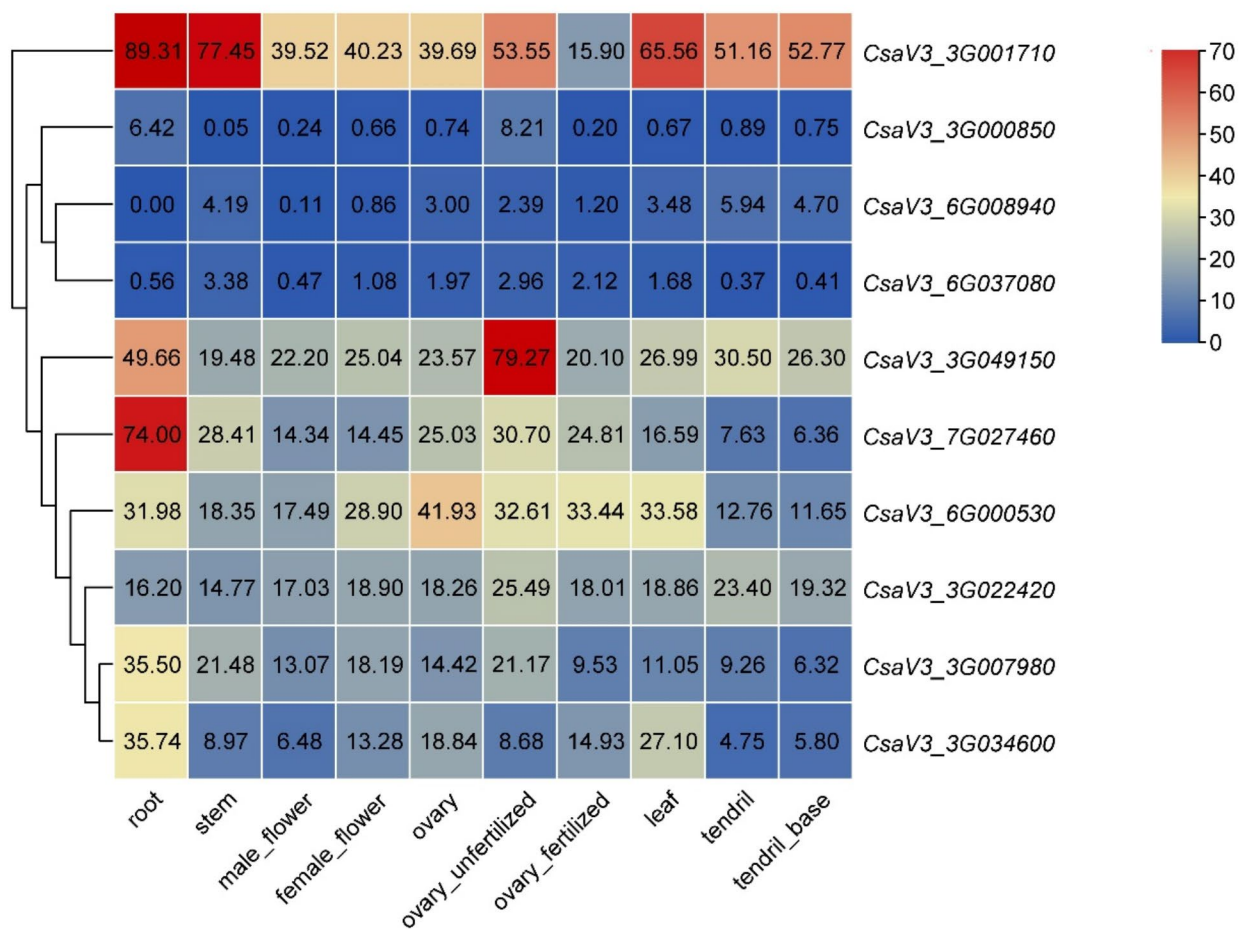
Fig. 7 Expression heatmaps of MYC family genes in different tissues of *C. sativus*

level remained relatively low under both the control and stress conditions.

Similarly, we analyzed the response of the MYC genes to biotic stress. Forty-eight hours after inoculation with powdery mildew, the expression of most genes did not significantly differ between the resistant (SSL508-28) and susceptible (D8) materials (Fig. 11). However, certain MYC genes presented differential expression patterns between SSL508-28 and D8. For example, *CsaV3_3G000850* was upregulated to a significantly greater degree in D8 than in SSL508-28 after powdery mildew treatment. Interestingly, post-inoculation, the absolute expression level of *CsaV3_3G000850* in D8 was much lower than its expression in SSL508-28. In addition, after inoculation with powdery mildew, *CsaV3_3G049150* presented significantly downregulated expression in D8 and some degree of upregulation in SSL508-28.

After inoculation with root-knot nematodes (*Meloidogyne incognita*), the expression levels of most genes, such as *CsaV3_3G000850* and *CsaV3_3G034600*, in both resistant (IL10-1) and susceptible (CC3) materials

generally exhibited similar trends (Fig. 12). However, two genes, *CsaV3_6G000530* and *CsaV3_6G037080*, were upregulated in resistant materials but downregulated in susceptible materials. Nevertheless, the degree of differential expression of these genes is relatively low between the resistant and susceptible materials.

The response of eight genes in the four *Cucurbitaceae* crops under temperature stress

Eight genes in the four *Cucurbitaceae* crops (*CsaV3_3G007980, CsaV3_3G001710, MELO3C006016, MELO3C013851, Bhi01M000362, Bhi11M000137, Cla97C10G186220,* and *Cla97C05G080890*) were selected for analysis of the response to temperature stress. As shown in Fig. 13, in *Cucumis sativus*, the genes *CsaV3_3G007980* and *CsaV3_3G001710* were upregulated under both low- and high-temperature stress. Especially under high temperature treatment, its expression level increased significantly as the treatment time increased. Which is relatively consistent with the transcriptome results, indicating their involvement in the cucumber response to temperature stress (Fig. 13A
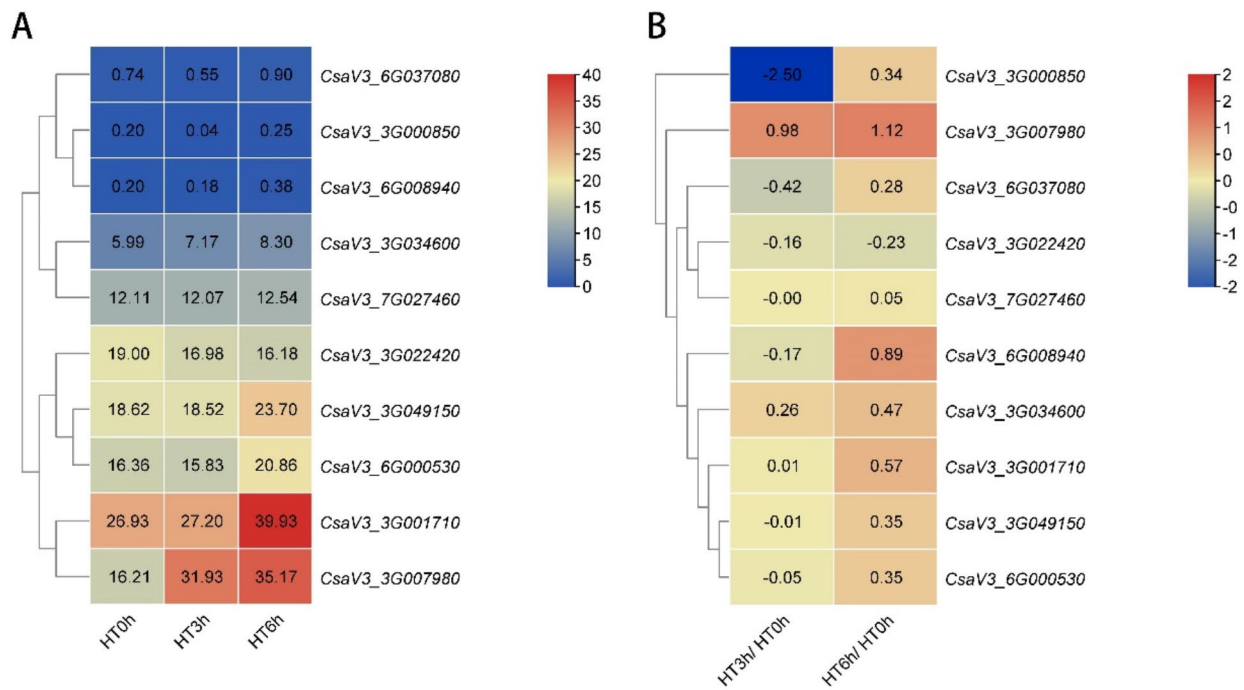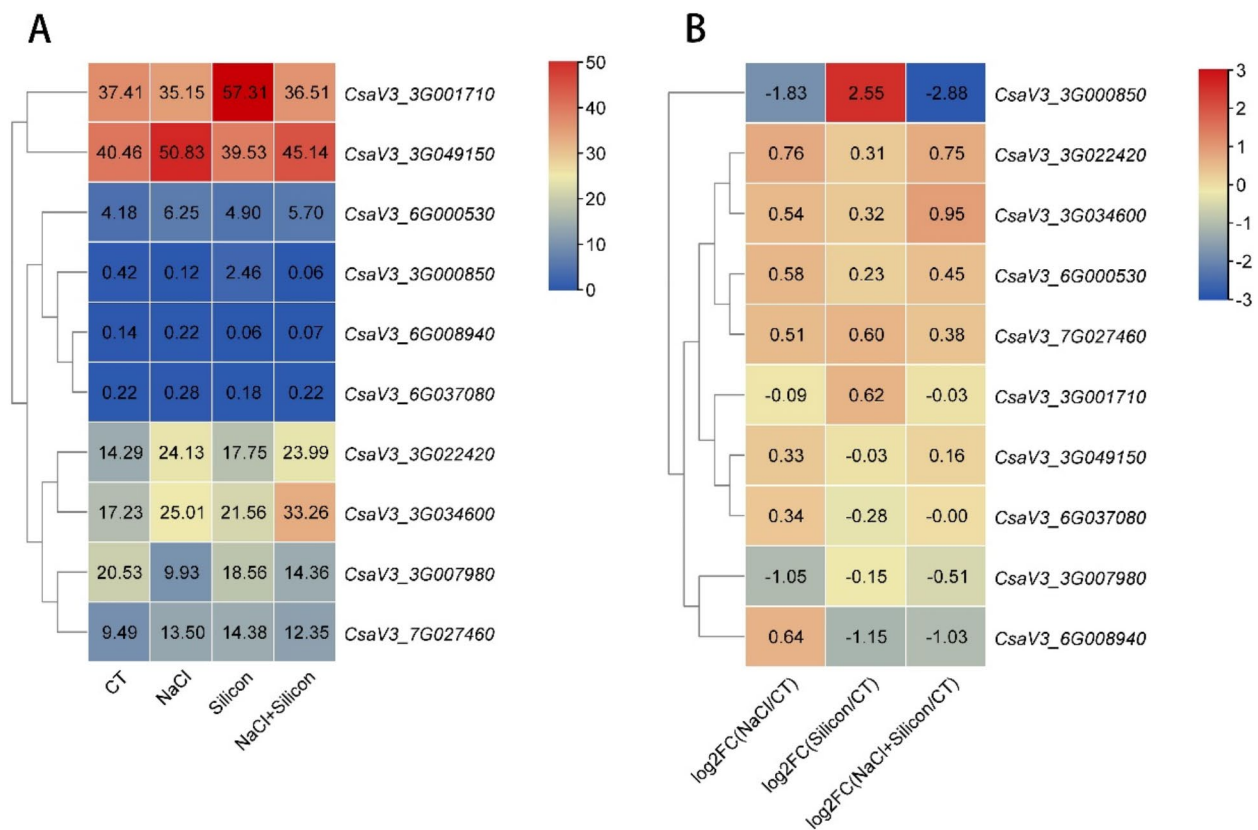
Fig. 8 Expression heatmap of the cucumber MYC genes under high-temperature stress. HT0h represents the control treatment, HT3h represents high-temperature treatment for 3 h, and HT6h represents high-temperature treatment for 6 h. (**A**), The data in the table represent the raw FPKM values. (**B**), The data in the table represent the log2 FC values of the raw FPKM values
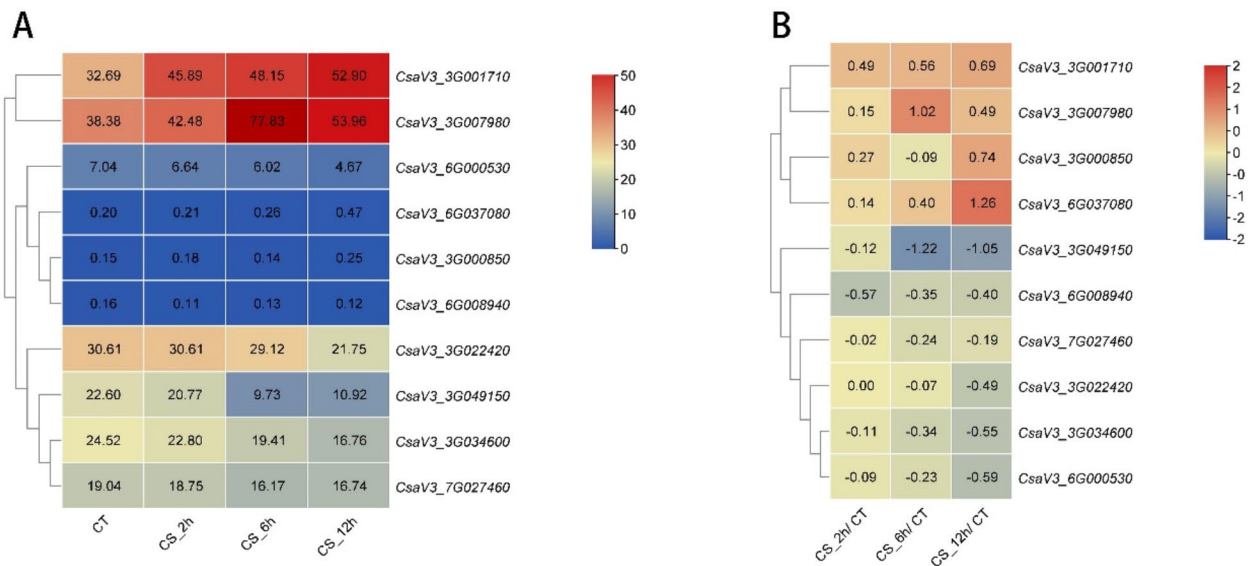
Fig. 9 Expression heatmap of cucumber MYC family genes under low-temperature stress. CT represents the control treatment, CS_2h represents low-temperature treatment for 2 h, CS_6h represents low-temperature treatment for 6 h, and CS_12h represents low-temperature treatment for 12 h. (**A**), The data in the table represent the raw FPKM values. (**B**), The data in the table represent the log2 FC of the raw FPKM values

and B). This expression pattern also appeared in *Cucumis melo* and *Citrullus lanatus*. The *CsaV3_3G007980* homologs *MELO3C013851* and *Cla97C05G080890* were upregulated under high-temperature stress (Fig. 13C and G). However, the homologous gene *MELO3C006016* was

significantly downregulated at 3 and 6 h of high-temperature treatment and then upregulated at 12 h of high-temperature treatment (Fig. 13D). However, the expression pattern of this gene was opposite under low-temperature treatment compared to high-temperature treatment.

Fig. 10 Expression heatmap of the cucumber MYC family genes under salt and silicon treatment treatments. CT represents the control treatment, NaCl represents salt treatment, Silicon represents silicon treatment, and NaCl + Silicon represents combined salt and silicon treatment. (**A**), The data in the table represent the raw FPKM values. (**B**), The data in the table represent the log2 FC of the raw FPKM values

Under low-temperature treatment, gene *MELO3C006016* was upregulated at 3 h of high-temperature treatment and then significantly downregulated at 6 and12 hours of high-temperature treatment. In *Benincasa hispida*, the expression of the two homologous genes *Bhi01M000362* and *Bhi11M000137* was downregulated at all time points under both the low- and high-temperature treatment groups (Fig. 13E and F).

Discussion

Characteristics of the MYC genes in the Cucurbitaceae crops

The MYC transcription factors have been reported to participate in various life activities in plants, playing crucial roles in regulating the growth and development of plant organs and in modulating tolerance to abiotic stress responses. The MYC protein has a bHLH_MYC_N domain in the N-terminal region, which consists of two subdomains: JID and TAD. The former is essential for interacting with JAZ proteins, whereas the latter is a putative transcriptional activation domain [45]. In the C-terminal region, the conserved bHLH domain determines its specificity and affinity for DNA sequence binding, and

it can facilitate the formation of various homodimers and heterodimers [46]. The bHLH domain comprises a basic region and an HLH region. The basic region is located at the N-terminus of the domain and contains sites for DNA recognition and binding. In this study, a highly conserved H4-V5-E6-E8-R9-R11-R12 sequence was found in the basic region, in which the highly conserved Leu at residues 22 and 38 were necessary for dimer formation (Fig. 3); it has been shown [47] that mutations at these two Leu sites significantly affect bHLH dimerization in *Arabidopsis*. bHLH-type transcription factors can be classified into six main groups (designated A to F) according to the differences in the recognition mode between the basic region and the *cis*-acting elements [1]. Most of the MYC genes in *Cucurbitaceae* crops can specifically bind to the G-box (5'-CACNTG-3'), which could be bounded by the GBF family of bZIP proteins [48], and belong to Group B. Consistent with previous findings, the C-terminus of the domain includes a conserved helix−loop−helix (HLH) structure that can form homodimers or heterodimers with other proteins.

Gene structure analysis revealed that the MYC genes in groups III and IV had fewer introns (less than or equal
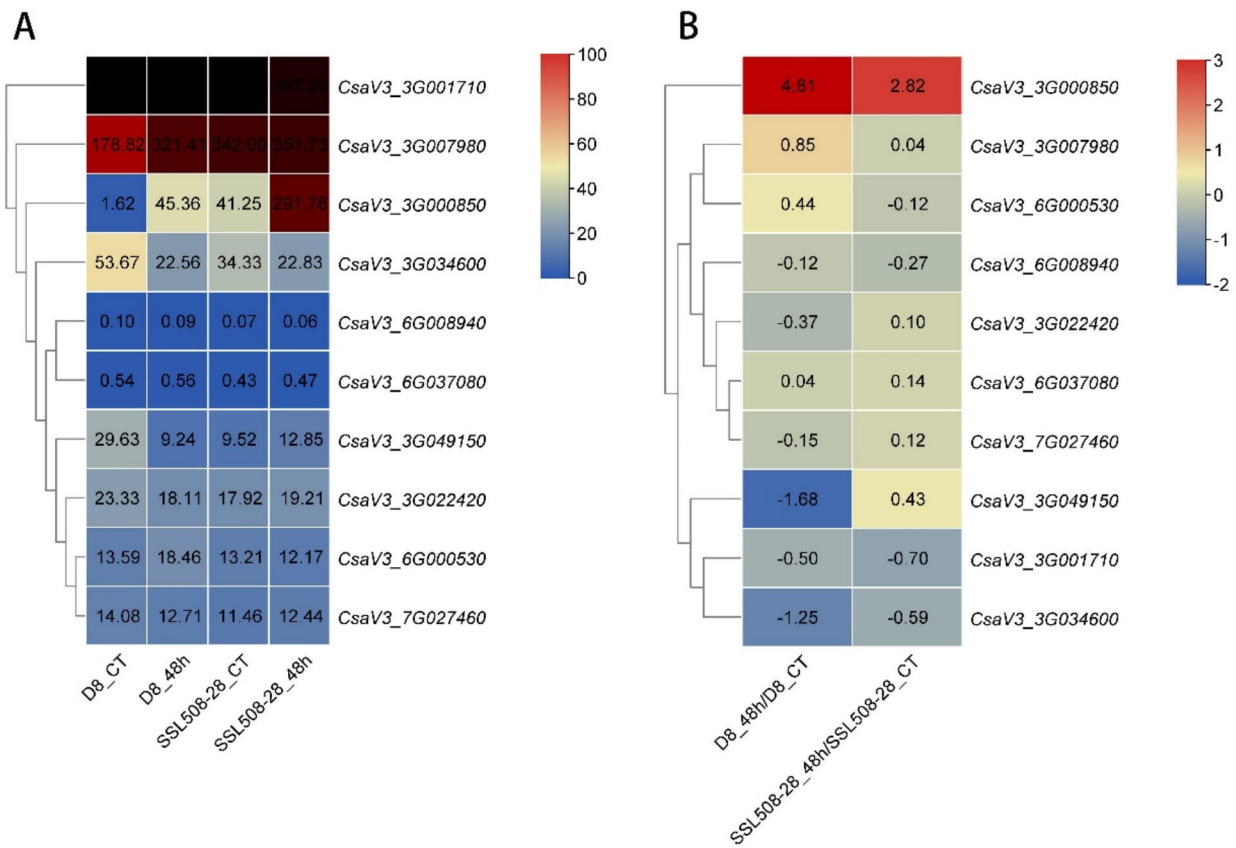
Fig. 11 Expression patterns of the cucumber MYC family genes under powdery mildew stress treatment. SSL508-28: resistant material; D8: susceptible material; CT: uninfected; 48 h, 48 h after inoculation. (**A**), The data in the table represent the raw FPKM values. (**B**), The data in the table represent the log2 FC of the raw FPKM values
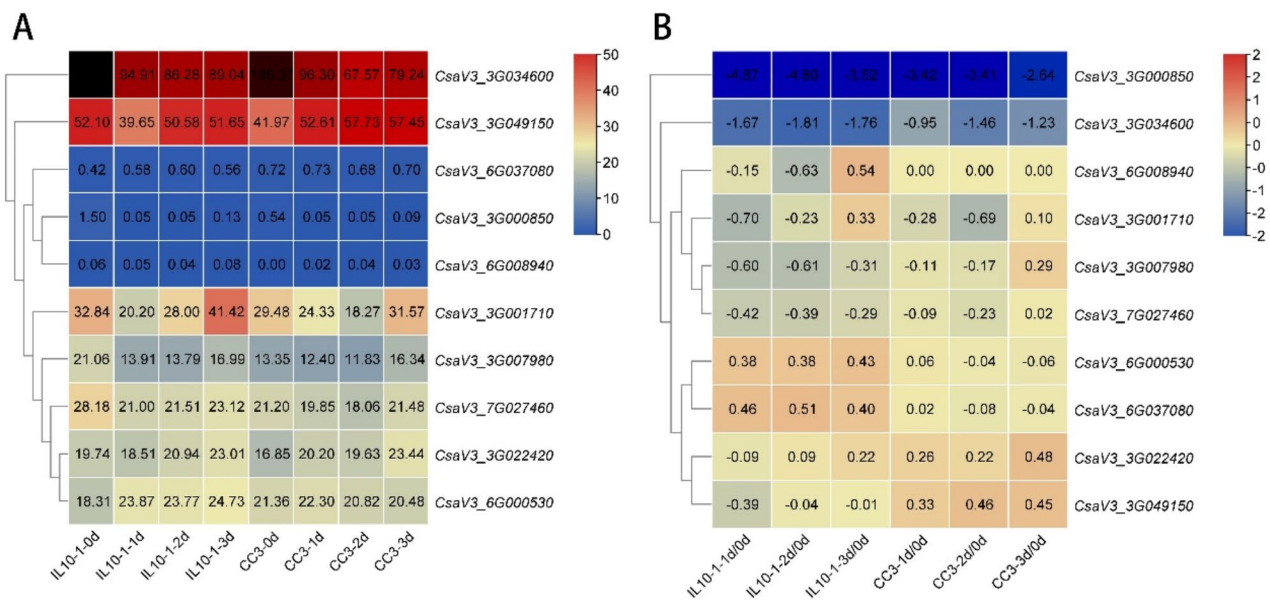
Fig. 12 Expression patterns of the cucumber MYC family genes under southern root–knot nematode stress treatment. IL10-1: resistant material; CC3: susceptible material; 0d, 1d, 2d, and 3d represent 0 days, 1 day, 2 days, and 3 days after inoculation, respectively. (**A**), The data in the table represent the raw FPKM values. (**B**), The data in the table represent the log2 FC of the raw FPKM values
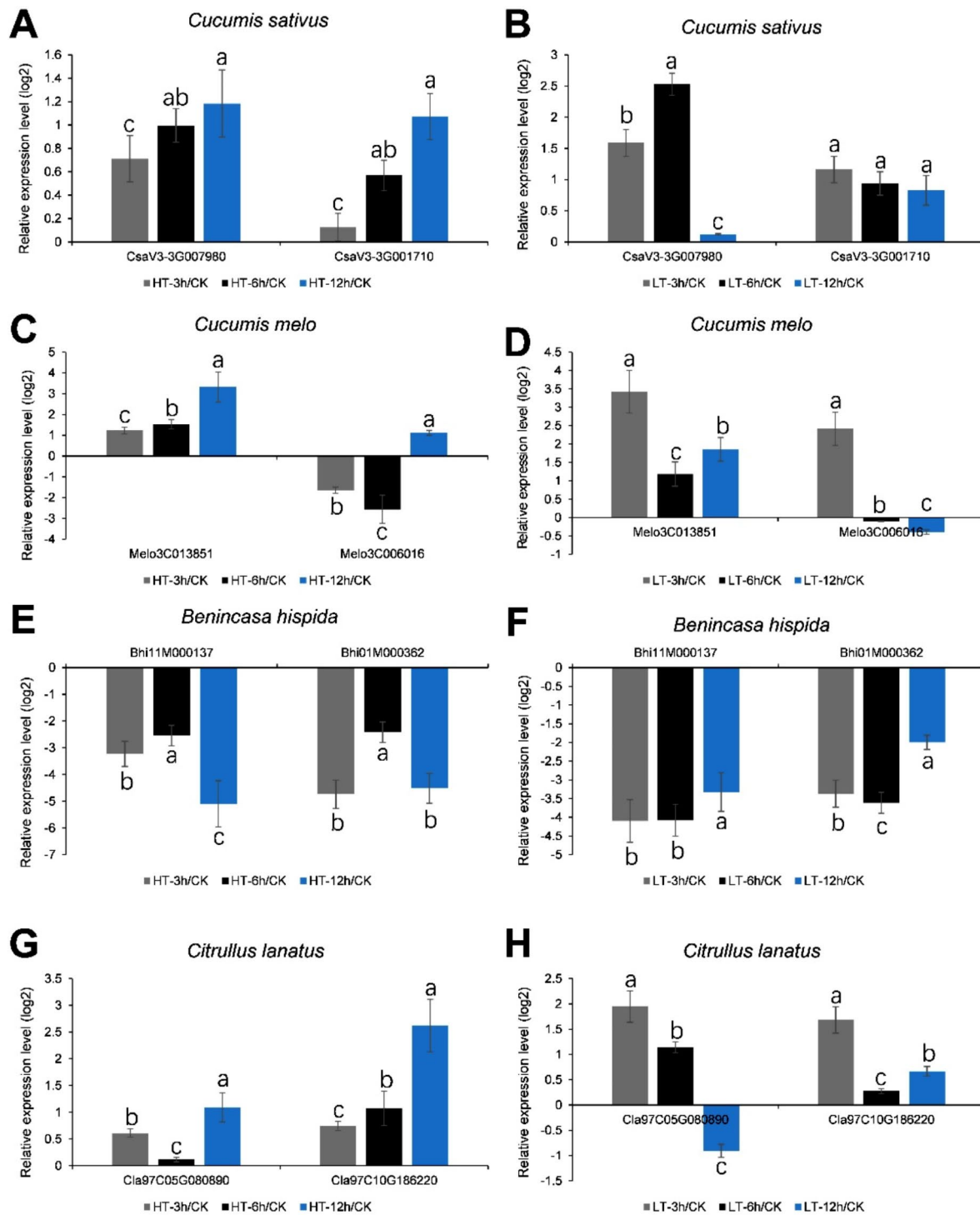
Fig. 13 Expression of MYC genes in the four *Cucurbitaceae* crops under high-temperature and low-temperature stress. HT: high-temperature; LT: low-temperature; CK: control. The error bars in the graphs indicate SD. The value of relative expression showed the log2 FC of each gene compared with that of the control. Letters (**a**, **b**, **c**) indicates a significant difference at $p < 0.05$

to 2), with approximately 50% of all MYC genes lacking introns. In contrast, the MYC genes in the other three groups contained many introns. Previous studies have indicated that introns and exons play important roles

in the diversity and evolution of gene families through gain/loss and insertion/deletion events [49, 50]. The significant difference in the number of introns among the MYC genes suggests that the *Cucurbitaceae* crops have

undergone intron loss events during their evolution to adapt to environmental changes. Jeffares et sl. reported that having fewer introns in genes enables plants to respond more rapidly to environmental changes [51]. In addition, in an evolutionary analysis between monocots and dicots, all the groups included both monocot and dicot species, indicating that the MYC genes have been relatively conserved during the evolution of both monocots and dicots.

Functions of the MYC genes and their role in the response to temperature stress

Many studies have shown that MYC transcription factors play significant roles in the growth and development of plants. For example, MYC TFs are involved in regulating processes such as plant seed production [52], stamen development [53], hormone regulation [54], and secondary metabolism [55]. In this study, the majority of the MYC genes were expressed in roots, leaves, and unfertilized ovaries (Fig. 7). Coupled with the identification of numerous *cis*-elements related to development and hormone stress, these findings further underscore their functions in growth and development.

Moreover, MYC genes play important roles in the response to abiotic and biotic stresses. Previous research found that silicon application promotes the growth of plants under salt stress, significantly reduces the Na^+ content, especially in the leaves, and counteracts the effects of NaCl on gas exchange [56]. Zhu et al. found that silicon confers resistance to salt stress in cucumber by regulating proline and cytokinins [57]. In this study, The *CsaV3_3G000850* gene was significantly downregulated after NaCl treatment and significantly upregulated after silicon treatment. These results indicate that silicon treatment induced high expression of *CsaV3_3G000850*, thereby increasing salt tolerance in cucumber. Similar results have been reported in *Arabidopsis*, *MYB2* and *MYC2* function in ABA-inducible gene expression of the *RD22* gene, where overexpression of the *AtMYC2* gene significantly increased osmotic stress tolerance [58]. In recent years, there has been a growing focus on the responses of MYC genes to temperature stress. Overexpressing *SlICE1*, which encodes a MYC-type transcription factor, enhances cold tolerance in tomato [59]. In *Arabidopsis*, *MYC67* and *MYC70* interact with *ICE1*, leading to negative regulation of cold tolerance [60]. The overexpression of *PtrbHLH*, a basic helix-loop-helix transcription factor from *Poncirus trifoliata*, confers enhanced cold tolerance in pummelo (*Citrus grandis*) by regulating Catalase (CAT) to modulate the level of H_2O_2 [61]. Under cold conditions, *StICE1* in potato enhances the stability of cell membranes by increasing the expression of the StLTI6A gene, thereby increasing its tolerance [62]. The MYC-type TF *MdbHLH4*

negatively regulates apple cold tolerance by inhibiting the expression of *MdCBF1/3* and the promoter-binding activity of *MdICE1L*, as well as by promoting the expression of *MdCAX3L-2* and the cold-induced degradation of *MdICE1L* [63]. In this study, we identified two MYC genes (*CsaV3_3G007980* and *CsaV3_3G001710*) in cucumber that are significantly differentially expressed under temperature stress (Figs. 8 and 9). To validate the involvement of these two genes in the temperature stress response in the other cucurbit species, we analyzed their homologous genes for their reactions under temperature stress (Fig. 13). Gene expression analysis revealed differential expression of these two genes across all four species, albeit with varying patterns. Comparative functional genomics research has indicated that if regulatory elements in evolutionarily related species are conserved, then gene expression characteristics within species are correspondingly conserved [64]. In this study, *cis*-regulatory element analysis revealed certain differences in both the type and quantity of these elements among the eight homologous genes, which may account for the differential expression of homologous genes across species. These findings suggest that *CsaV3_3G007980* and *CsaV3_3G001710*, along with their homologs in the other *Cucurbitaceae* crops, are highly responsive to temperature stress. However, the differential expression patterns between species remain unresolved. Further exploration of the response mechanisms of these genes to temperature stress will be the focus of future research.

Conclusions

In summary, we identified 10, 8, 12, and 10 MYC genes in *C. sativus*, *C. melo*, *C. lanatus*, and *B. hispida*, respectively, each of which play distinct roles in plant development. In particular, under environmental stress, some genes respond actively to external pressures through the upregulation or downregulation of expression. Additionally, we identified two genes that are relatively more sensitive to temperature stress, namely, *CsaV3_3G007980* and *CsaV3_3G001710*. However, these two genes exhibit contrasting expression patterns across the different species. This finding implied that some degree of alterations in gene function occurred following species divergence. These results provide valuable insights for future functional studies of MYC genes and present potential candidate genes for enhancing the environmental adaptability of *Cucurbitaceae* species.

Abbreviations
MYC   Myelocytomatosis
TF   Transcription factor
JAZ   Jasmonate ZIM-domain
AMS   Arabidopsis Aborted Microspores
CDD   Conserved domain data
ABA   Abscisic acid
Hph   Post high-temperature treatment

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HLH   Helix-loop-helix

Data availability
The authors confirm that the data supporting the findings of this study from NCBI database with accession numbers of PRJNA80169 [39], PRJNA634519 [39], PRJNA438923 [40], PRJNA477930 [41], PRJNA321023 [42], and PRJNA419665 [43] are available within the manuscript.

Declarations

Ethics approval and consent to participate
This study did not directly involve humans, animals or plants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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