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Comprehensive Analysis of Jumonji Domain C Family from *Citrus grandis* and Expression Profilings in the Exocarps of "Huajuhong" (*Citrus grandis* "Tomentosa") during Various Development Stages

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Abstract: *Citrus grandis* "Tomentosa" ("Huajuhong") is a famous Traditional Chinese Medicine. In this study, a total of 18 jumonji C (JMJC) domain-containing proteins were identified from *C. grandis*. The 18 CgJMJCs were unevenly located on six chromosomes of *C. grandis*. Phylogenetic analysis revealed that they could be classified into five groups, namely KDM3, KDM4, KDM5, JMJC, and JMJD6. The domain structures and motif architectures in the five groups were diversified. *Cis*-acting elements on the promoters of 18 CgJMJC genes were also investigated, and the abscisic acid-responsive element (ABRE) was distributed on 15 CgJMJC genes. Furthermore, the expression profiles of 18 CgJMJCs members in the exocarps of three varieties of "Huajuhong", for different developmental stages, were examined. The results were validated by quantitative real-time PCR (qRT-PCR). The present study provides a comprehensive characterization of JMJC domain-containing proteins in *C. grandis* and their expression patterns in the exocarps of *C. grandis* "Tomentosa" for three varieties with various development stages.

Keywords: *Citrus grandis*; "Huajuhong"; jumonji C domain (JMJC); histone demethylation; epigenetic regulation

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1. Introduction

In eukaryotes that carry the genetic and regulatory information of organisms, the structure and conformation of chromatin can directly affect the heritage changes in gene expression that occur without a change in the DNA sequence [1]. This is known as "epigenetics". The basic unit of chromatin is the nucleosome, which consists of 146-bp double-stranded DNA wrapped around the core histone [2]. DNA methylation, histone modifications, and RNA interference are distinct but highly interrelated means of regulating the structure of chromatin, and they further affect the expression of genes [1,3]. During the past decades, epigenetics has been developed into a new trend in life science research, especially in human diseases. Nevertheless, with the wide application of methylation-sensitive amplification polymorphism (MASP) in fruit plants [4–6], special epigenetic phenomenon will be revealed.

Recently, histone demethylation has attracted the attention of researchers as it is a reversible process of histone methylation. Previous studies showed that two types of histone lysine demethylases (KDMs) have been identified: KDM1/LSD1-like (lysine-specific demethylase 1) and jumonji C (JMJC) domain-containing demethylases [7,8]. Studies on

plants indicate that JMJCs play vital regulatory roles in growth and developmental processes [7,9]. After the first histone demethylase, LSD1, was discovered in 2004 [10], the study of the demethylation of histone in relation to the determination of the epigenetic characteristics of plants has become a research hotspot. LSD1 belongs to the flavin-dependent amine oxidase family, which is highly specific for the H3 mono/di-methylation of lysine 4 (H3-K4me and H3-K4me2), while the other known major methylation sites of H3 and H4 do not serve as substrates for this protein [10,11]. Subsequently, the jumonji C (JMJC) domain-containing protein family was also characterized as being involved in histone demethylation [12,13]. Increased evidence shows that JMJCs epigenetically regulate various biological processes in plants, with their functions including the regulation of flowering time [14–16], the repression of leaf senescence [17], the control of seed germination [18], and the regulation of shoot regeneration [19]. There are 21 and 20 JMJC members in *Arabidopsis* and Rice, respectively, and these are classified into the subfamilies of KDM5/JARID1, KDM4/JHDM3, KDM3/JHDM2, KDM3/JHDM2, and JMJC domain-only [20]. Until now, the JMJC family has been characterized in some other plants, including *Glycine max* [21], *Zea mays* [22], *Citrus sinensis* [23], and *Gossypium hirsutum* L. [24].

As an ancient variety of *Citrus grandis*, "Huajuhong" (*C. grandis* "Tomentosa"), has been utilized as famous folk medicine against chronic cough for thousands of years. *C. grandis* "Tomentosa" originated from Huazhou town in Guangzhou Province of Southern China and also received China GI (Geographical Indication) protection. At present, there are three major varieties, known as "ZM" (ZhengMao), "FM" (FuMao), and "GQ" (GuangQing). The three varieties are different in terms of both fruit appearance and internal quality. Interestingly, a thick layer of trichomes is covered on the surface of the "ZM" fruits, and the density of trichomes on the "FM" fruits is lighter than that of "ZM", while there is no trichome on the "GQ" fruits. Moreover, we previously reported that the some secondary metabolites in those varieties of "Huajuhong", including flavonoid and volatile compound contents, were different [25]. However, little information has been reported regarding the regulatory mechanism of the outer morphology and inner quality of *C. grandis* "Tomentosa". In addition, as far as we know, there is limited information about the epigenetics of *C. grandis*.

In the present study, the JMJC domain-containing protein family was comprehensively investigated in the genome of *C. grandis*. The total JMJC genes were first identified by blasting using HMMER of JMJC and then chromosomal localization was analyzed. A phylogenetic analysis combined with the conserved domains was conducted and the results were compared with those for *Arabidopsis*. Gene structure, motif composition and cis-element analysis were performed for all the identified JMJC genes. Expression patterns of the JMJC family in the exocarps of "Huajuhong" ("ZM", "FM" and "GQ") during various development stages were finally examined. The aim of this study was to provide important and helpful information for further research on the regulatory mechanisms of the outer morphology and fruit quality of *C. grandis*.

2. Materials and Methods

2.1. Identification of JMJC Genes from Citrus grandis

A Hidden Markov Model (HMM) profile of the JMJC domain (Pfam: PF02373) downloaded from the Pfam database (http://pfam.xfam.org, accessed on 27 November 2021) was used for the identification of JMJC genes from the *Citrus grandis* genome (https://www.citrusgenomedb.org/, accessed on 27 November 2021). The cutoff value was 0.01 and the parameter was set to the default value. To confirm the presence of the JMJC domain, the sequences of all the obtained CgJMJC genes were further identified through the online SMART (http://smart.embl.de/smart/, accessed on 27 November 2021), NCBI Conserved Domain Search databases (NCBI CDD) (https://www.ncbi.nlm.nih.gov/cdd/, accessed on 27 November 2021) and PFAM (http://pfam.xfam.org/, accessed on 27 November 2021) databases. The redundant sequences were eliminated. Protein signatures, such as the amino acids number (AAs), molecular weights (Mw) and isoelectric points (pI) of the

identified CgJMJC genes, were obtained using the ExPasy website (http://web.expasy.org/protparam/, accessed on 27 November 2021).

2.2. Chromosomal Localization of CgJMJC Genes

According to the locations of the identified CgJMJC genes in the database, they were obtained from annotated gff3 files. MapChart software was used to analyze the chromosomal distribution [26].

2.3. Phylogenetic Analysis of JMJC Genes from Citrus grandis and Arabidopsis

The phylogenetic analysis of the CgJMJC gene family in *Citrus grandis* and *Arabidopsis thaliana* was performed by MEGA X with 1000 bootstrap replicates. The phylogenetic tree was plotted by employing the neighbor-joining (NJ) method and the pairwise gap deletion mode [27]. The domain information was obtained from the SMART database (http://smart.emblheidelberg.de/, accessed on 27 November 2021) and the NCBI Conserved Domain Search database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi, accessed on 27 November 2021). The conserved domain information was graphically visualized using DOG 2.0 software [28].

2.4. Gene Structure Analysis

The gene exon/intron structures of the CgJMJC gene family in *Citrus grandis* were graphically visualized using the gene structure display server (GSDS2.0) (http://gsds.cbi.pku.edu.cn/index.php, accessed on 28 November 2021) [29].

2.5. Cis-Element Analysis and Heat Map Construction

For cis-acting element analysis, genomic DNA sequences in the 1500 bp upstream region of all JMJCs were obtained, and the sequences were identified using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 28 November 2021) [30]. To further understand the expression patterns of CgJMJCs, RNA-Seq data were visualized with heat maps using TBtools [31]. FPKM-normalized log2 transformed counts were regarded as thresholds to define significant differences in gene expression. CgJMJCs expression levels were analyzed in relation to the expression patterns in exocarp tissue.

2.6. Plant Material and Treatments

The exocarps of "ZM", "FM" and "GQ" fruits were peeled from fruits of 4, 6, and 8 cm diameter, which were harvested from an orchard with GAP (Good Agricultural Practice) certified by the Chinese government in Hexi District, Huazhou, Guangdong province. After obtaining the exocarps, they were chopped into small pieces and frozen immediately by liquid nitrogen and stored at −80 °C.

2.7. RNA Isolation and RT-PCR Analysis

The total RNA was extracted as described by Asif et al. (2000) [32]. Using gDNA Eraser (TaKaRa, Tokyo, Japan), the total RNA was treated to eliminate any potential contamination with DNA. Then, DNA-free total RNA was reverse-transcribed into cDNA with a reverse transcription kit (TaKaRa, Tokyo, Japan), following the manufacturer's instructions. Real-time Quantitative Polymerase Chain Reaction (qRT-PCR) was then performed as described by Li et al. (2019) [33]. Primer pairs of each JMJC gene were designed using the online software program Primer 3 (http://primer3.ut.ee/, accessed on 28 November 2021). The primers are listed in Table 1. *Actin* was used as a reference gene. The expression levels were normalized to that of the reference gene and calculated by the comparative $2^{-\Delta\Delta Ct}$ method [34], and the data are shown as the mean ± standard deviation of three independent biological replicates.

Table 1. The primer sequences used for RT-PCR.

Genes	Forward Primers	Reverse Primers
Actin	ATCTGCTGGAAGGTGCTGAG	CCAAGCAGCATGAAGATCAA
Cg2g031440	GGGTCTCCTTAATGAAGCTGAAGAG	TTTCATTATCTGTTCTTCCCGGCAA
Cg2g038450	GAAGCTTTGGAGGGAGGATTAGATG	CCATTGCCCTCCACGATATTTTCTA
Cg2g041150	AAAATAAAAGGCCAAAACCCGTCTG	ACAACTTGCACAGCTTCTATGGTAA
Cg3g007850	TGAAGGAAGATGTGGTCAGTTGATG	GCTGTCTACTATTCGCTTCCTCATC
Cg3g014720	TCAGAACTCTAGCCAGGAAGAAGAA	GAAGAAACCTGGTAGATGTCAAGCA
Cg5g003020	TTACCATGTCTCAGCTTGTTGAACA	ATCAATTTGTTCACCATGTCCCTGA
Cg5g005310	ATCTTGCAGCTAACACAGGAATGAA	TACAAGATTCAATGCCAAGTGTCGA
Cg5g006280	GTCTTGAACATGCTGTGGAAGTAGA	TCTTCTCGTCTTCTTTGGTAGCAAC
Cg5g018250	CAAGCATGGCAAAAGGAGAAGAAAA	GTCTCTGATTTTGATGCTTCTTCGC
Cg5g033850	CAAACGGGAATGCAACATATGTCTC	ACAGCTGCTTTACATGATTCAGACA
Cg6g013700	TTCAATCCCTTACATGTGAAGTGCA	TGTTGGTAGATCAAATTGGGCATCA
Cg7g006840	AAGGTTGGAGGATGTTCTGAAGTTC	GCAGTAGTATGATTTTGCAGCTGTG
Cg7g013930	GGTGAAATTGACATGACCAACAGTG	GCCACTGGAAATGCTTTAAATCTCC
Cg8g010020	TCCTGTGTTCTACCCTACTGAAGAG	CACGCGTAACAAATGTAGAACTGTC
Cg2g045510	AACATGACTGATTGCGAAAAGGTTG	TTGCCACAAAGAGTCTCTGATACAC
Cg5g034880	CCTGTTCTCTCCAGTCTTCTTCAAC	TGCCATAACAACAAAGACTTCCTGA
Cg2g002200	GTTTTGGTGAAAGAGAAGCTAAGCG	AAGTCATTCCAGTTTGTCCTAGCTG

3. Results

3.1. Identification and Chromosomal Localization of JMJC Genes from Citrus grandis

In present study, according to the characteristics of the JMJC domain-containing proteins (Pfam: PF02373), all 39 JMJC protein sequences were identified from *Citrus grandis* and *Arabidopsis thaliana* using the PFAM (http://pfam.xfam.org/, accessed on 27 November 2021) and SMART (http://smart.embl.de/smart/set_mode.cgi?GENOMIC=1, accessed on 27 November 2021) online databases. A total of 18 and 21 members were present in *Citrus grandis* and *Arabidopsis thaliana* [20,24], respectively. As illustrated in Table 2, the number of amino acids (AAs), molecular weight (Mw), and theoretical pI was varied from 456 (Cg5g005310) to 1849 (Cg5g003020), 53.43 (Cg5g005310) to 189322.74 (Cg2g045510) kDa, and 5.06 (Cg5g005310) to 9.03 (Cg5g012890), respectively.

Table 2. JMJC identified in *Citrus grandis*.

Annotation Number	AAs	Mw (kDa)	pI
Cg5g033850	789	88.59	6.70
Cg6g013700	1010	118.93	5.38
Cg2g031440	947	107.39	5.46
Cg5g018250	1755	193.70	8.22
Cg3g014720	976	111.60	5.25
Cg8g010020	1259	140.86	7.50
Cg7g013930	1135	128.62	7.30
Cg3g007850	874	98.63	8.18
Cg7g006840	1635	181.41	5.82
Cg5g005310	456	53.43	5.06
Cg5g006280	1666	187.79	8.67
Cg5g012890	926	106.20	9.03
Cg5g003020	1849	210.07	7.64
Cg2g041150	1004	114.88	8.28
Cg2g038450	1048	117.76	5.83
Cg2g045510	1395	189,322.74	8.55
Cg5g034880	518	59,185.24	5.24
Cg2g002200	401	45,456.52	5.23

Genome chromosomal location analyses using the MapChart software showed that all 18 CgJMJCs were unevenly anchored to six chromosomes of *Citrus grandis*. Figure 1

includes the detailed location information. The results revealed that 18 CgJMJCs were distributed across six chromosomes, including in chr2, chr3, chr5, chr6, chr7, and chr8. Chromosome 5 (chr5) included the largest number (seven) of CgJMJC genes, followed by five on chr2. In contrast, only one gene was distributed on chr6 and chr8.

3.2. Phylogenetic Analysis of JMJC Genes in Citrus grandis, Citrus sinensis, and Arabidopsis

To understand the phylogenetic relationships of the JMJC genes from *Citrus grandis*, *Citrus sinensis*, and *Arabidopsis thaliana*, JMJC domain-containing protein sequences were obtained to construct a phylogenetic tree (Figure 2). According to previous reported conserved domains, all JMJC members were divided into five groups of KDM3, KDM4, KDM5, JMJC, and JMJD6 [7]. As illustrated in Figure 2, the groups of KDM3, KDM4, KDM5, JMJC, and JMJD6 included 6, 3, 4, 3, and 2 CgJMJC genes, respectively. There is no doubt that KDM3 and KDM5 were the two largest groups. Different groups contained different domain structures. In the KDM5 group, the domain architecture was diverse. Frequently, five different conserved domains were shown in the KDM5 group, including JMJ-N, JMJ-C, zf-C5HC2, FYRN, and FYRC. In addition, two genes contained additional ARID, PLU-1, and PHD domains. Nevertheless, KDM3, KDM4, and JMJD6 included some simple domains, such as Ring, WRC, zf-C5HC2, JMJ-N, JMJ-C, and FBOX. However, the JMJC group only had a JMJC domain, and thus, could be referred to as the JMJC-domain-only group. Interestingly, all JMJC members contained a common JMJC domain. It was reported that the JMJC domain is a unique feature of the JMJC gene family, and is related to the regulation of histone lysine demethylation [24]. Different domain structures also performed different functions. For the five groups, the Ring, Zf-C5HC2, PLU-1, ARID, FYRN, and FYRC domains had DNA binding functions. The DNA-binding domain may contribute to the functioning of JMJC genes [24].

3.3. Gene Structure Analysis

To investigate and illustrate the gene structure and conserved motif characters of CgJMJCs, a combined figure is presented in Figure 3, which contains a phylogenetic tree and the gene structures for each CgJMJC gene. Since the CgJMJC members were divided into five groups, including KDM3, KDM4, KDM5, JMJC, and JMJD6, the gene structures of 18 CgJMJCs were also annotated within the phylogenetic context and visualized using TBtools [31], as shown in Figure 3A. To gain more insight into the structural diversity of the CgJMJC genes, the exon and intron structures of all CgJMJC genes were examined by employing Gene Structure Display Server 2.0 [29]. As shown in Figure 3B, the exon number caused a huge change, which may be associated with the splicing mutation in the process of post-transcriptional regulation [35].

3.4. Cis-Element Analysis

Cis-acting elements are specific binding targets of transcription factors (TFs), which can bind with TFs to activate or repress gene transcription. The expression ability and level of the downstream genes regulated this process. The 1500 bp upstream sequences from 18 CgJMJC genes were obtained to further elucidate the regulatory mechanisms of JMJC-containing genes' expression. Furthermore, the cis-elements were predicted using PlantCARE (Figure 3, Table S1). In Figure 4, we present 11 cis-elements on the promoters of 18 CgJMJC genes. Several cis-elements are involved in hormones, such as ABRE (ABA), CGTCA-motif (MeJA), TCA-element (salicylic acid), TGA-element (auxin), and GARE-motif (gibberellin). Among these components, all 15 CgJMJC genes included the abscisic acid-responsive element (ABRE), which could be in response to abscisic acid and other stresses [36]. These results suggested that CgJMJCs might be involved in the regulation of metabolism in hormones that may control the fruit quality of "Huajuhong" (*Citrus grandis* "Tomentosa").According to the analysis, TGA-element and GARE-motif were discovered in 10 genes, and these are an auxin-responsive element and gibberellic acid, respectively [37]. Eleven genes contained the CGTCA-motif, which is a MeJA element [37]. In addition,

19 elements involved in light responsiveness were identified as containing GATA-motif and G-box (light). Consequently, the *cis*-elements' predictions suggested that CgJMJCs might be involved in the regulation of light responsiveness and metabolism in hormones.

3.5. Expression Patterns in the Exocarps of "Huajuhong" (Citrus grandis "Tomentosa") during Various Development Stages

Three major varieties, "ZM" (ZhengMao), "FM" (FuMao), and "GQ" (GuangQing), were used in this study. The three varieties are different in terms of both fruit appearance and internal quality. To identify the expression pattern of the CgJMJC genes, the expression profiles of the JMjC family in the exocarps of "Huajuhong" ("ZM", "FM", and "GQ"), with fruits of 4, 6, 8 cm in diameter, were examined. Transcriptome data for in the exocarps of "ZM", "FM", and "GQ" during various development stages were obtained from the Illumina RNA-Seq data generated in this study. The heatmap was created based on the FPKM-normalized log2 transformed values from different samples, which were plotted to a heatmap employing TBtools. As illustrated in Figure 5 and Table S2, the expression levels of Cg2g045510 in three varieties were quite high compared with other CgJMJC genes, while the FPKM value of Cg5g012890 was close to 0, indicating that no gene expression of Cg5g012890 was observed in these samples. Consequently, the relative expression levels of 17 CgJMJC genes were determined by qPCR. As shown in Figure 6, the gene expression patterns measured by qPCR were consistent with the RNA-Seq results. In addition, we used the first sample (ZME-4, FME-4 and GQE-4) as a control to conduct statistical analysis. The expression levels of Cg2g038450 and Cg8g010020 significantly decreased during the fruit development in all three varieties. The expression amounts of Cg2g031440 and Cg3g007850 also decreased significantly during the fruit development of "ZM" and "GQ", while Cg3g007850 first increased then decreased in "FM". Moreover, during the development of "FM" and "GQ", the expression levels of Cg7g006840 and Cg7g013930 decreased. The Cg2g041150 expression level only reduced in the fruits of "GQ". The expression amount of Cg3g014720 first increased and then decreased during the development of the three fruit varieties. However, the general expression levels of the four CgJMJC genes (Cg5g003020, Cg5g006280, Cg5g005310, and Cg6g013700) remained stable for the three varieties.

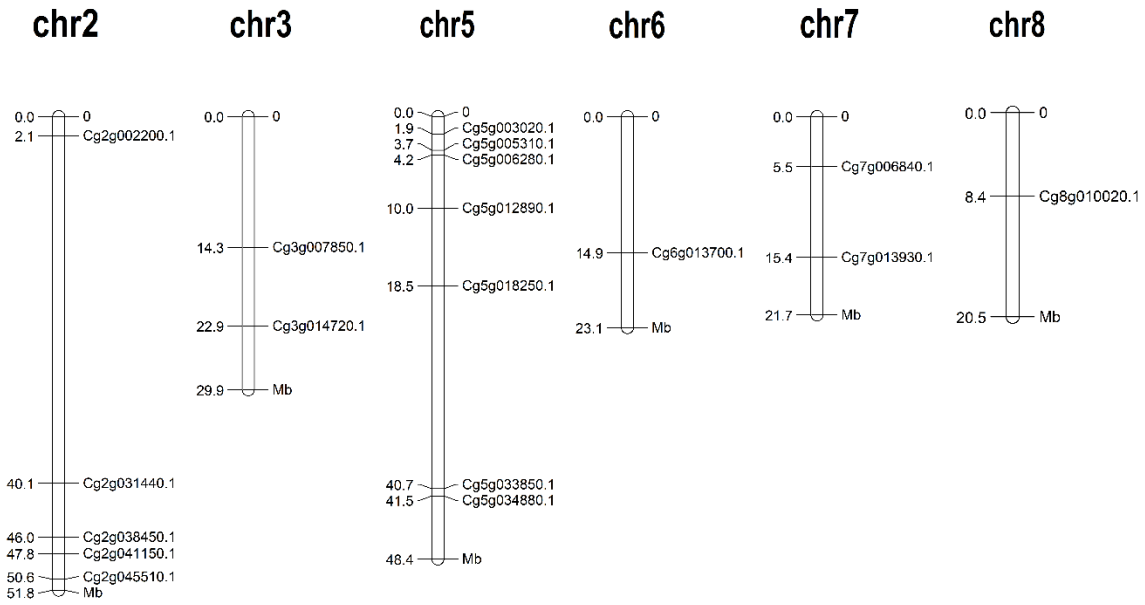

Figure 1. Chromosomal locations of JMJC genes in *Citrus grandis*. The chromosomal position of each CgJMJC gene was mapped according to the genome of *Citrus grandis*. The chromosome number is marked at the top of each chromosome and the unit for the scale is megabases (Mb).

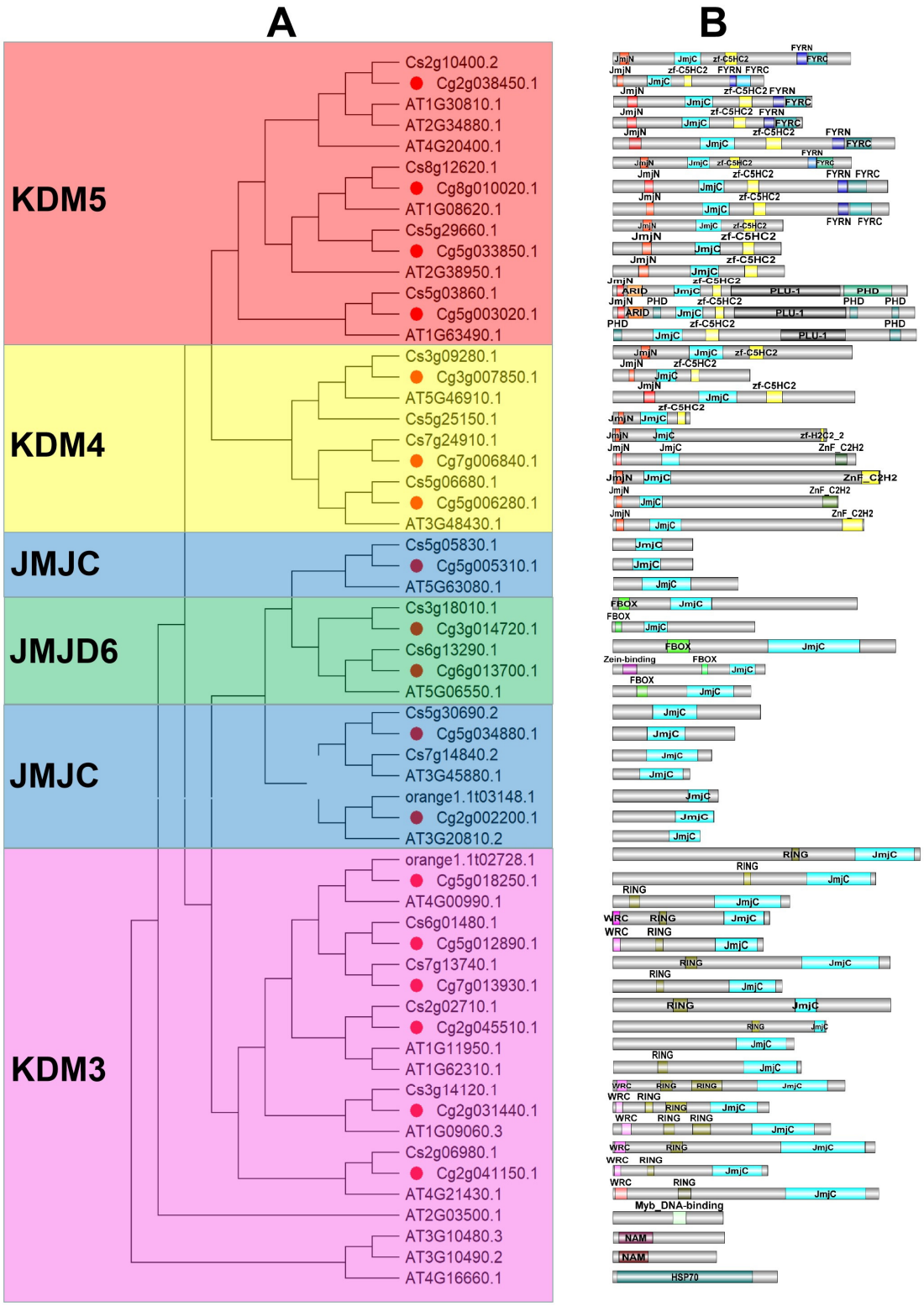

Figure 2. Phylogenetic relationship of JMJC genes in *Citrus grandis*, *Citrus sinensis*, and *Arabidopsis*. Multiple-sequence alignment was performed using Clustalx1.83. MEGAX was used to perform phylogenetic tree analysis. (**A**) Phylogenetic relationship of CgJMJC genes. The group labels are displayed on the left side of the figure. The five subgroups are marked by different colors. (**B**) The conserved domains of CgJMJC genes.

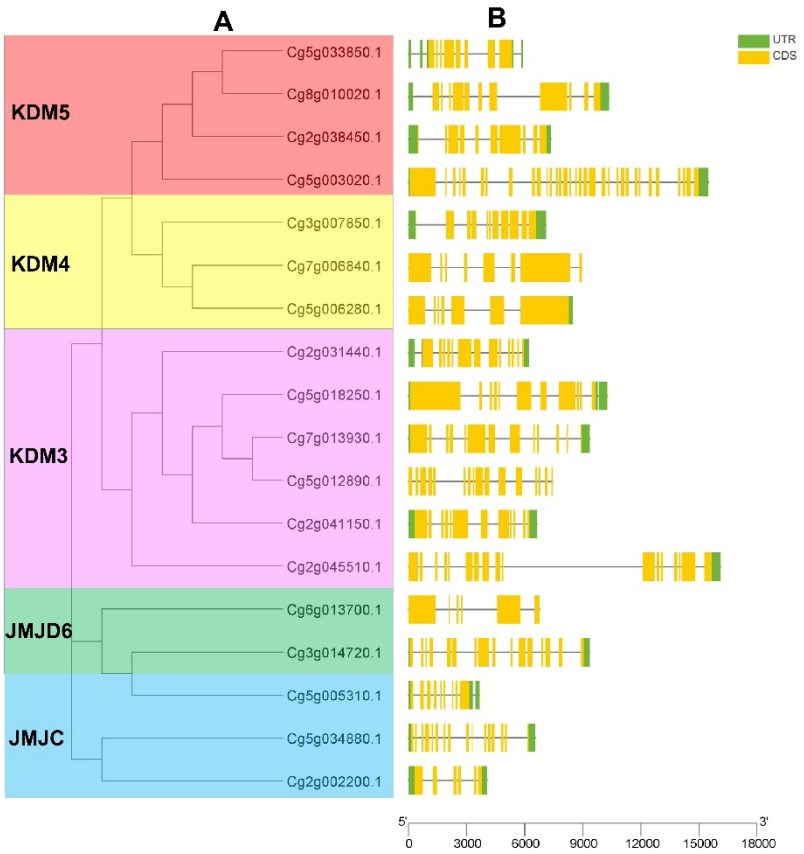

Figure 3. The gene structure analysis of JMJC gene family from *Citrus grandis*. (**A**) Phylogenetic relationship of CgJMJC genes. (**B**) The exon and intron structures of CgJMJC genes. Yellow represents'exons.

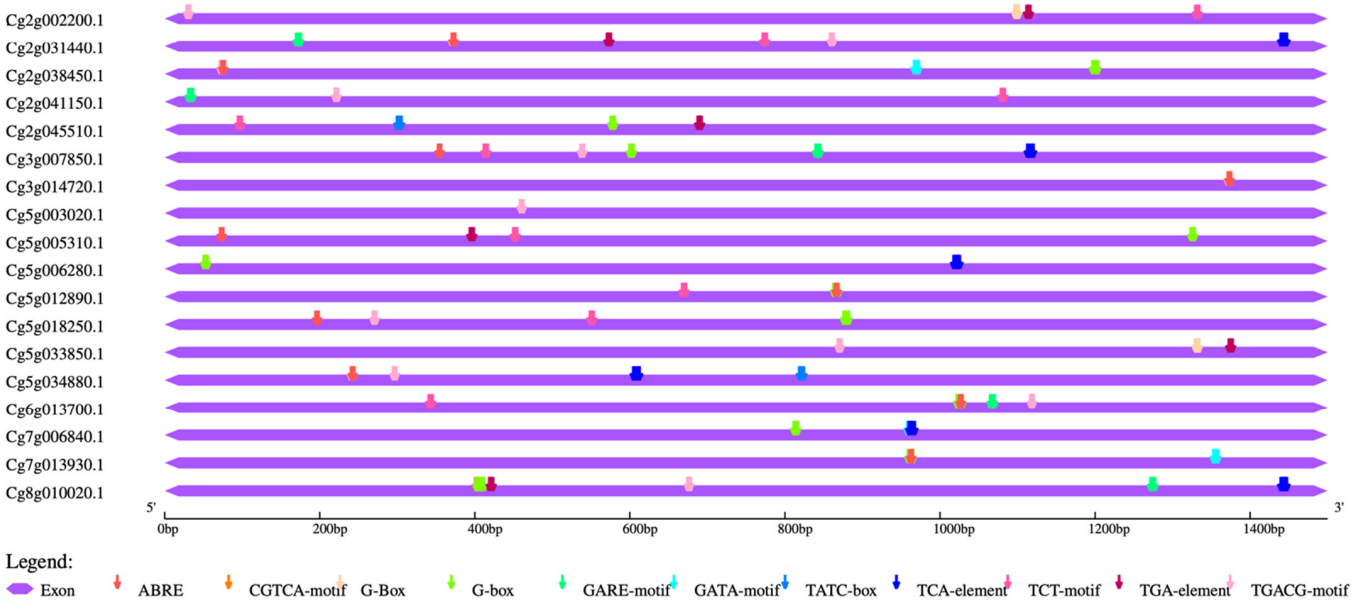

Figure 4. Cis-element analysis of 18 identified CgJMJC genes from the upstream 1500 bp sequence to the transcription start site.

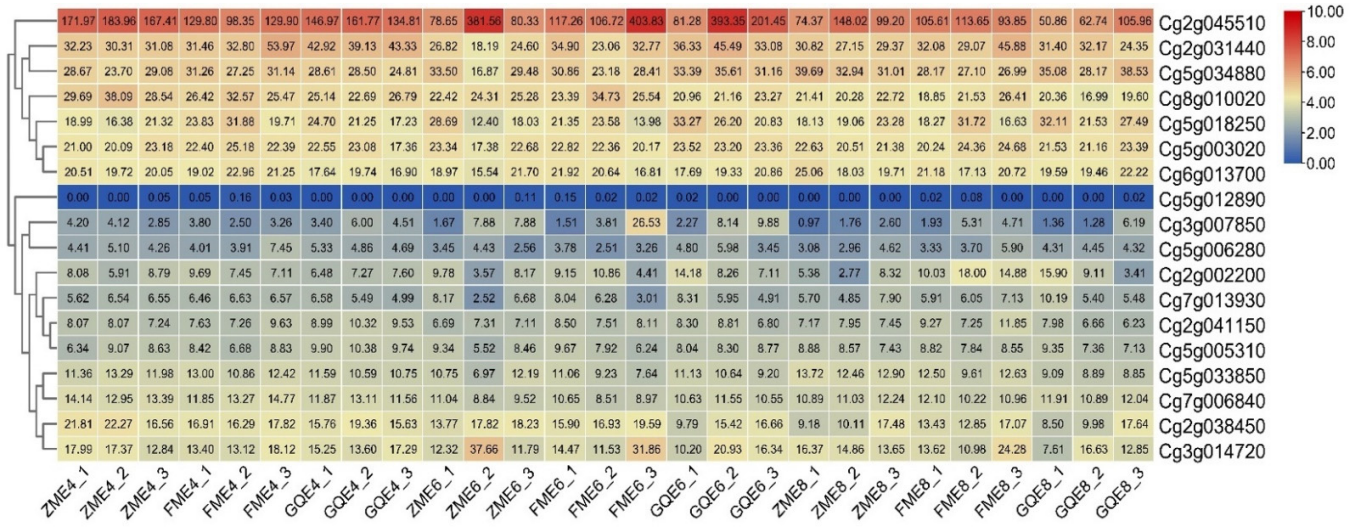

Figure 5. Expression patterns of CgJMJC genes in the exocarps of three varieties from fruits of 4, 6, and 8 cm diameter. TBtools was used to generate the heat map. The bar at the right of the heat map represents relative expression values. FPKM-normalized log2 transformed counts. (ZM (Zheng Mao); FM (Fu Mao); GQ (Guang Qing)). E4, E6, and E8 stand for the exocarps of three varieties from fruits of 4, 6, and 8 cm diameter, respectively, with three biological repeats.

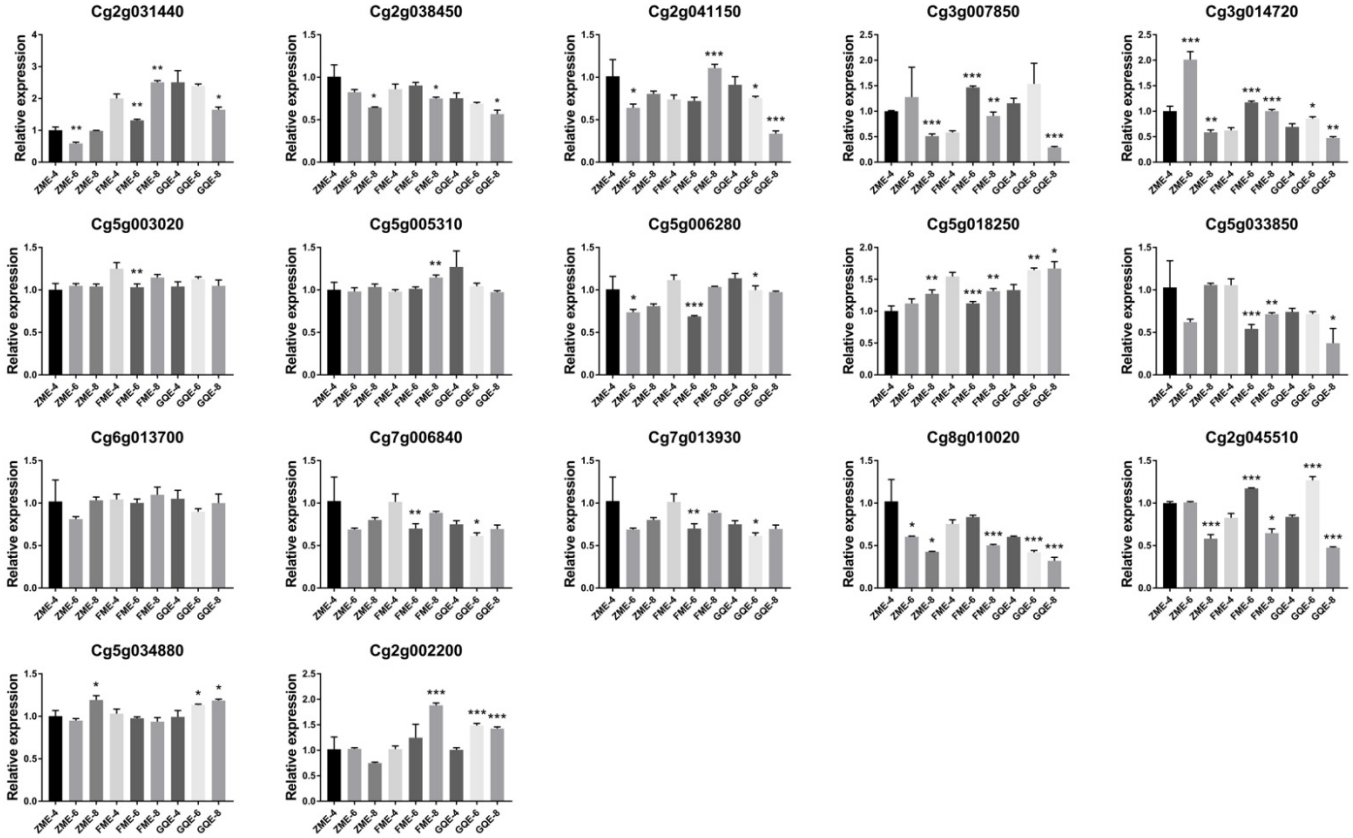

Figure 6. Relative expression of 17 selected CgJMJC genes in "ZM", "FM", and "GQ" during various development stages. qRT-PCR data were normalized using *Actin*. The name of the gene is indicated above each bar diagram. Error bars indicate the standard deviation (ZM (ZhengMao); FM (FuMao); GQ (GuangQing)). E4, E6, and E8 stand for the exocarps of three varieties from fruits of 4, 6, 8 cm diameter, respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

As a famous traditional Chinese medicine, the fruits of *Citrus grandis* "Tomentosa" have been utilized as a cure for chronic cough [38,39]. However, little information has been reported about the epigenetic aspects of *C. grandis*. Previous studies demonstrated that epigenetic modification can regulate the expression of genes, but the genetic characteristics do not change [40], with these including DNA methylation [40], histone methylation [41], and histone phosphorylation [42]. The JMJC-containing gene family was characterized as being involved in histone demethylation [34]. The JMJC genes play an important role in plant growth and development [23]. For instance, they can regulate the flowering period [43]. In present study, 18 JMJC genes from *Citrus grandis* were researched in terms of protein characteristics, chromosome location, phylogenetic relationships, protein domains, and cis-elements.

Further studies showed that JMJC protein family members not only contained the JMJC domain in animals, but also exhibited the histone demethylase function in plants [20]. Previous studies demonstrated that the JMJC members from *Arabidopsis* can be divided into five subgroups, namely JMJC, JMJD6, KDM3, KDM4, and KDM5 [7]. Different subgroups contain different conserved domains. Notwithstanding, in this study, the results of the analysis of the phylogenetic tree and gene structure showed that all 18 JMJC genes identified from *Citrus grandis* were distributed into five subgroups. Therefore, the results demonstrated that the JMJC members contained similarly conserved domains in one subgroup, whereas there appeared to be significant differences among different subgroups. The genes of one subgroup were in the same clade of a phylogenetic tree, which contained a similar conserved domain and gene structure. However, different motif information and gene structures were found among different subgroups. Through genome-wide screening and phylogenetic analysis of the genes encoding JMJC domain proteins in Arabidopsis and rice, the results found 21 and 20 JMJC protein family members, respectively, to be present in Arabidopsis and rice [20]. Compared to *Arabidopsis* and rice, the number of CgJMJC genes decreased (from 21, to 20, to 18), which indicated that the number of CgJMJC genes shrinks in the process of evolution [23]. Furthermore, CsJMJs were distributed at chromosomes 2, 3, 5, 6, 7, and 8. Chromosome 5 included six CsJMJs [23]. In the present study, the results revealed that 18 CgJMJCs were distributed across six chromosomes, including in chr2, chr3, chr5, chr6, chr7, and chr8. Chromosome 5 (chr5) included the largest number (7) of CgJMJC genes. The distribution of CgJMJC genes on the chromosomes was identical to that of CsJMJs.

Previous studies demonstrated that the abscisic acid-responsive element (ABRE) is related to fruit development in strawberry [44]. In this paper, according to the cis-element analysis, the promoters of 18 CgJMJC genes contained the ABRE element, which might be related to the development of *Citrus grandis* "Tomentosa" in an ABA-dependent manner. In addition, the expression patterns of 18 CgJMJC genes were identified via RNA-seq and 17 of them were confirmed using qRT-PCR. The results revealed that, with the exception of four CgJMJC genes (Cg5g003020, Cg5g006280, Cg5g005310, and Cg6g013700), the expression levels of the remaining 13 CgJMJCs significantly changed (increased or decreased) during the fruit development process, indicating that they might be involved in fruit growth. In addition, some CgJMJC genes (e.g., Cg5g018250, Cg2g031440, Cg3g007850, etc.) exhibited different expression patterns in the three varieties of *C. grandis* "Tomentosa", suggesting that they might play a role in the formation of unique phenotypes such as fruit appearance or secondary metabolites synthesis.

The functions of the JMJC family are diverse, and are related to both stress resistance and fruit development [23]. Moreover, JMJC genes also regulate the expression of related genes through epigenetic modification to ensure the integrity of genome structure and function [45]. In summary, the analysis of the characteristics and expression patterns of the JMJC gene family could provide a basis for further research on the mechanism underpinning JMJC gene functioning during the developmental regulation of *C. grandis* "Tomentosa" fruits.

5. Conclusions

In conclusion, the JMJC genes from *Citrus grandis* "Tomentosa" were identified and compared with the JMJC family members from *Arabidopsis*, and *Citrus sinensis*. The phylogenetic tree analysis, conserved motifs, and gene structure characterization of the 18 JMJC genes indicated the conserved nature of these genes when comparing them in terms of subgroups, while there was also significant divergent in these groups. In addition, the chromosomal location analyses showed that all 18 CgJMJCs were unevenly anchored to six chromosomes. Furthermore, RNA sequencing analysis suggested that the JMJC gene family from *Citrus grandis* "Tomentosa" may play a role in the fruit's development, along with the equilibrating of gene expression, by regulating histone methylation. The comprehensive characterization of JMJC family from *C. grandis* may provide a basis for the future research of CgJMJC genes.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7120592/s1, Table S1: Detailed information on the *cis*-elements. Table S2: The FPKM values of all 18 CgJMJC genes.

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