horticulturae

MDPI

Article

Comprehensive Analysis of N6-Methyladenosine Regulatory Genes from *Citrus grandis* and Expression Profilings in the Fruits of "Huajuhong" (*C. grandis* "Tomentosa") during Various Development Stages

Yuzhen Tian [1,2,3], Jiwu Zeng [1,2,3,*] and Ruiyi Fan [1,2,3,*]

[1] Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China; tianyzh@126.com
[2] Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture and Rural Affairs, Guangzhou 510640, China
[3] Guangdong Provincial Key Laboratory of Tropical and Subtropical Fruit Tree Research, Guangzhou 510640, China
* Correspondence: zengjiwu@gdaas.cn (J.Z.); fanruiyi@gdaas.cn (R.F.)

Abstract: *Citrus grandis* "Tomentosa" ("Huajuhong") is a famous traditional Chinese medicine. The aim of the present study is to provide a comprehensive characterization of the m^{6}A regulatory genes from *C. grandis*, and examine their expression patterns in fruits of *C. grandis* "Tomentosa" during various developmental stages. A total of 26 N6-methyladenosine (m^{6}A) regulatory proteins were identified from the genome of *C. grandis*, which were distributed across nine chromosomes in *C. grandis*. Phylogenetic relationships revealed that all m^{6}A regulatory genes were divided into groups of m^{6}A writers, erasers, and readers. The m^{6}A writer groups included CgMTA, CgMTB, and CgMTC three MTs (methyltransferases), one CgVIR (virilizer), one CgHAKAI (E3 ubiquitin ligase HAKAI), and one CgFIP37 (FKBP interacting protein 37). Moreover, 10 CgALKBH (α-ketoglutarate-dependent dioxygenase homolog) members (numbered from CgALKBH1 to CgALKBH10) and 10 CgECT (C-terminal region) members (numbered from CgECT1 to CgECT10) in *C. grandis* were identified as m^{6}A erasers and readers, respectively. The domain structures and motif architectures among the groups of m^{6}A writers, erasers, and readers were diverse. *Cis*-acting elements in the promoters of the 26 m^{6}A regulatory genes predicted that the abscisic acid-responsive (ABA) element (ABRE) was present on the promoters of 19 genes. In addition, the expression profiles of all m^{6}A regulatory genes were examined in the fruits of two varieties of *C. grandis* "Tomentosa" during different growth stages to give basic hints for further investigation of the function of the N6-methyladenosine regulatory genes in *C. grandis* "Tomentosa".

Keywords: *Citrus grandis*; "Huajuhong"; N6-methyladenosine; RNA methylation

Citation: Tian, Y.; Zeng, J.; Fan, R. Comprehensive Analysis of N6-Methyladenosine Regulatory Genes from *Citrus grandis* and Expression Profilings in the Fruits of "Huajuhong" (*C. grandis* "Tomentosa") during Various Development Stages. *Horticulturae* **2022**, *8*, 462. https://doi.org/10.3390/horticulturae8050462

Academic Editors: Jinzhi Zhang, Pingxian Zhang and Changfei Guan

Received: 7 April 2022
Accepted: 15 May 2022
Published: 20 May 2022

1. Introduction

Methylation is an epigenetic modification which can regulate the heritage information to affect its function without altering the DNA sequence [1–3]. In general, methylation is divided into DNA methylation, histone modification, and RNA methylation according to molecular biological processes [3]. The methylation of RNA usually occurs in the internal modification of RNA. N6-methyladenosine (m^{6}A) plays an important role in eukaryotic RNA methylation in animals and plants [4,5]. According to the three pivotal proteins, a sophisticated regulatory network of m^{6}A is composed of m^{6}A writers (methyltransferases), m^{6}A erasers (demethylases), and m^{6}A reader proteins [5–7]. The m^{6}A writers add methyl groups to the conserved sequence. Similarly, m^{6}A erasers can remove methylation. In addition, m^{6}A readers have a specific recognition function that decodes the specific proteins of m^{6}A modifications in RNA levels [5,6].

Previous research has been conducted on the m^{6}A modification in animals. However, to the best of our knowledge, m^{6}A modification in plants has rarely been reported. Recently, regulatory complexes of m^{6}A writers have been identified from *Camellia sinensis* and *Arabidopsis thaliana* [8]. The m^{6}A writers are not only regulated by methyltransferases (MTs), but are also controlled by a series of m^{6}A writer components, such as FKBP interacting protein 37 (FIP37), virilizer (VIR), and E3 ubiquitin ligase HAKAI [9]. The MT family is the first m^{6}A writer component which contains three subfamilies of methyltransferase A (MTA), methyltransferase B (MTB), and methyltransferase C (MTC) [10]. MTA subfamilies contain the MT-A70 domain which can activate RNA modification. Subsequently, MTB is regarded as one of the second most-active proteins in regulating m^{6}A level [10]. The MTC subfamily, which also belongs to the MT family, was recently discovered. Therefore, little information has been reported about the function of MTC in m^{6}A modification [11]. Apart from the aforementioned, m^{6}A writers contain other components, including FIP37, VIR, and HAKAI. Among them, FIP37 contains the Wilms' tumor 1-associating protein (WTAP) domain [12]. Previous research has shown that the suppression of FIP37 could lead to a decrease of the m^{6}A modification level [12]. It has shown that FIP37 also plays an indispensable role in regulating m^{6}A modification. As the third component of the m^{6}A writer, VIR could regulate the MTA–MTB heterodimer to affect the level of RNA modification [13]. Similarly, inhibition of HAKAI expression could reduce m^{6}A methylation [13]. Additionally, previous studies have found that HAKAI can interact with other components and together can regulate RNA levels [13].

Unlike m^{6}A writers, m^{6}A erasers can also regulate RNA methylation in plants. Previous studies have confirmed that the α-ketoglutarate-dependent dioxygenase homolog (ALKBH) could effectively remove the specific region of m^{6}A [14]. A conserved clavaminate synthase-like domain was included in the ALKBH family which plays an important role in the demethylation of m^{6}A-modified RNAs. Different from m^{6}A methylation, the demethylations of m^{6}A only include an ALKBH family [14]. The abundance of ALKBH in *A. thaliana* and *Solanum lycopersicum* is related to the decrement of m^{6}A level [15,16]. Nevertheless, the m^{6}A erasers in other plants need to be further confirmed. As the third component of regulatory m^{6}A genes, the m^{6}A readers have been divided into two types of CPSF30 (cleavage and polyadenylation specificity factor 30) and ECT (C-terminal region) [17,18]. A highly conserved YTH domain is contained in ECT, which could specifically regulate m^{6}A methylation. Moreover, CPSF30 contains a conserved C3H1-type zinc finger domain at the N-terminal region, which mainly contributes to sweeping away deleterious mRNA transcripts [19].

Citrus grandis (also annotated as *Citrus maxima*) is one of the three ancestors of the Citrus family [20]. In addition to the freshly consumed pummelo, the immature fruits of *C. grandis* "Tomentosa", named as "Huajuhong", are processed to be a famous traditional Chinese medicine for effectively curing chronic cough [21]. Nowadays, "ZhengMao" (ZM) is the main cultivated variety of "Huajuhong" whose fruits are covered with a thick layer of trichomes. There is also a local pummelo variety called "GuangQing" (GQ) whose fruits are smooth without trichome. Our previous study indicated that the terpenoid compositions and contents in the fruits of ZM and GQ were somehow different [22]. Whether the trichomes are related to the internal fruit quality is not yet known. It has been reported that m^{6}A regulatory genes played important roles in the trichome branching [8]. Hence, it is necessary to study RNA methylation in *C. grandis*, which has not yet been reported.

In this research, the m^{6}A regulatory genes family was comprehensively investigated in the genome from *C. grandis*. Firstly, all m^{6}A regulatory genes were identified using HMMER and their chromosomal localization was investigated. The phylogenetic relationships of the m^{6}A regulatory genes from *C. grandis*, *S. lycopersicum*, and *A. thaliana* combined with the conserved domains were analyzed. Gene structure and cis-element analysis of all the identified m^{6}A regulatory genes were performed. Expression levels of m^{6}A regulatory genes in the fruits of the two varieties of *C. grandis* "Tomentosa" were examined by RNA-seq and q-RT-PCR. The objective of this investigation is to give general information about

the m^{6}A regulatory genes from *C. grandis*, and to lay a basis for the future research on the function of RNA methylation during the development of *C. grandis* "Tomentosa" fruits.

2. Materials and Methods

2.1. Identification and Chromosomal Localization of the m^{6}A Regulatory Genes from C. grandis

To identify all the m^{6}A regulatory genes from *C. grandis*, the sequences of the m^{6}A regulatory genes in *A. thaliana* and *S. lycopersicum* were downloaded from the Arabidopsis Information Resource (TAIR) (https://www.arabidopsis.org/, (accessed on 10 January 2022)) [23] and the Sol Genomics Network (SGN) database (https://solgenomics.net/, (accessed on 10 January 2022)) [24], respectively. These sequences were queried against the genome sequences of *C. grandis* using BLAST with the parameter E-value < 1.0×10^{-5}. Then, the hidden Markov model (HMM) profiles of the MT-A70 family (PF05063), VIR-N domain (PF15912) and WTAP family (PF17098), 2OG-FeII-Oxy-2 superfamily (PF13532), and YTH domain (PF04146) downloaded from the Pfam 35.0 database (http://pfam.xfam.org/, (accessed on 10 January 2022)) were used to identify the m^{6}A regulatory genes of the *C. grandis* genome (https://www.citrusgenomedb.org/, (accessed on 10 January 2022)). Moreover, the obtained m^{6}A regulatory genes of *C. grandis* were further confirmed through the online SMART database (http://smart.embl.de/smart/set_mode.cgi?GENOMIC=1#, (accessed on 10 January 2022)) [25], NCBI Conserved Domain Search databases (NCBI CDD) (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi, (accessed on 10 January 2022)) [26] and Pfam 35.0 (http://pfam.xfam.org/, (accessed on 10 January 2022)) databases. The amino acids number (AAs), molecular weights (Mw), and isoelectric points (pI) of the identified m^{6}A regulatory genes from *C. grandis* were examined using the ExPasy tool (http://web.expasy.org/protparam/, (accessed on 10 January 2022)) [27]. The subcellular location of the m^{6}A regulatory genes was predicted using the online software WoLF PSORT II (https://www.genscript.com/wolf-psort.html, (accessed on 10 January 2022)) [28].

All m^{6}A regulatory genes from *C. grandis* were mapped on the chromosomes according to their positions in the database. The database was obtained from annotated gff3 files. The chromosomal distribution was graphically visualized using Mapchart 2.32 software created by Roeland E. Voorrips which could be download and freely licensed in Guangzhou, China [29].

2.2. Phylogenetic Analysis of the m^{6}A Regulatory Genes from C. grandis, S. lycopersicum, and A. thaliana

The phylogenetic tree of the m^{6}A regulatory genes from *C. grandis*, *S. lycopersicum*, and *A. thaliana* was generated by MEGA X64 with 1000 bootstrap replicates. The phylogenetic tree was employed by using the neighbor-joining (NJ) algorithm and the pairwise gap deletion mode [30]. The conserved domain information of m^{6}A writers, erasers, and readers was obtained from the SMART database (http://smart.embl.de/smart/set_mode.cgi?GENOMIC=1#, (accessed on 11 January 2022)) and NCBI CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi, (accessed on 11 January 2022)), and was graphically visualized using DOG 2.0 software [31].

2.3. Gene Structure Analysis, Cis-Element Analysis, and Heat Map Construction of the m^{6}A Regulatory Genes from C. grandis

The phylogenetic tree of the m^{6}A regulatory genes from *C. grandis* was generated by MEGA X64. The gene structures of the m^{6}A regulatory genes in *C. grandis* were graphically visualized using the TBtools software [32]. Furthermore, for cis-acting element analysis, the promoter sequences of the m^{6}A regulatory genes from *C. grandis* which were in the 1500 bp upstream region were collected. Moreover, the promoter sequences were obtained using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, (accessed on 11 January 2022)) [33]. The expression profiles of the m^{6}A regulatory genes from *C. grandis* were visualized with heat maps using TBtools [32]. FPKM-normalized counts were regarded as thresholds.

2.4. Plant Materials and Treatments

"Huajuhong" (*C. grandis* "Tomentosa") fruits, which were regarded as samples, were collected from an orchard of Huazhou Traditional Chinese Medicine Company located in Hexi District, Huazhou, Guangdong province. The fruits of 2, 4, 6, and 8 cm in diameters were harvested in the year 2019. The fruits were cut and immediately frozen in liquid nitrogen, and stored at −80 °C before usage.

2.5. RNA Isolation and RT-PCR Analysis

The total RNA from each abovementioned sample was isolated and treated to eliminate any potential contamination with DNA using the PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Dalian, China), following the manufacturer's instructions. Then, the real-time quantitative polymerase chain reaction (qRT-PCR) of the m^{6}A regulatory genes from *C. grandis* was conducted as described previously (2019) [34]. Primer pairs of each m^{6}A regulatory gene from *C. grandis* were designed using the online software program Primer 3 (http://primer3.ut.ee/, (accessed on 12 January 2022)), and they are listed in Table 1. *Actin* was used as a reference gene which normalized the gene expression. The relative gene expression of the m^{6}A regulatory genes from *C. grandis* was calculated by the comparative $2^{-\Delta\Delta Ct}$ method [35], and the values are shown as the mean ± standard deviation of three independent biological replicates.

Table 1. The primers used for qRT-PCR.

Genes Names	Forward Primers	Reverse Primers
Actin	ATCTGCTGGAAGGTGCTGAG	CCAAGCAGCATGAAGATCAA
CgMTA	GCGTCCTGAATATTGTTCTGAAGTA	AACTCCAAATTGTCCTAAAATGTCC
CgMTB	CAACTCGAAAGCCTGAAGATATGTA	GCAAAGTTTTTAATGTATGCCTCCT
CgMTC	AACTTACTCATTCAGAGGGAGCACT	CATCTAGCTTCACCTTCAACCAGTA
CgVIR	TGATTTTCTTGCTAGTTTGTTGGAG	CACCAGCTAGTCAAAGTAGAACCAA
CgHAKAI	TAGCTAGGGAAGAAGGTATGGTGTC	AGTGTCTTCACAGGCTCATACAATC
CgFIP37	ATGAAATGGTGCTTATGTTACGAGA	AACTGAATCATTCTTGTCCTCTTCC
CgALKBH1	TTGAAAGAGAAAACGTATTCAGCAC	GATCACTTTAAACAGATGAGGCAGA
CgALKBH2	ATTGGAATTCAGATTGGAGAAACTC	TCTTTAGTATCAGCTATGGCAGGTG
CgALKBH3	TGGAAATTCTTTGATTACCTCAACA	ATTGTGTCACTCCTTCACTAGCAAC
CgALKBH4	TCATTCTAAATGGTAATGGAGCTGA	ACTTGTTTGAGGGAGAATTAACCAC
CgALKBH5	TCATTTCCAAACCTTGATGATTCTA	AGGCTGAAAATTAATCCTTCAAATG
CgALKBH6	CATCAATGAATACCAACCTAACCAA	AAATGCATCACCATTAGAACTTTCA
CgALKBH7	CTAGAACCAATTCCAGCTCTTCTTC	ATCAAAGAAGTTAATGATGCAACCA
CgALKBH8	AGGGAAGAATAGATAATCCACATGC	ATTTATCTCGTGCTTCCAAAGGTAG
CgALKBH9	CGAAAGCAGATACAGTCTCAAGAAG	CCCCACCAAATATTAGTACATCTCC
CgALKBH10	CCCATAGATGACTTATTCAATGCTG	AAAATTTCCACCTATGAGCATCTTC
CgECT1	GTTAATACAAGTGGGCAGTTTGTTG	AAGTCATTCCAGTTTGTCCTAGCTG
CgECT2	TAACATGTACAAGCTGGGTTTTTGT	AAATGTTTATTAGGGTTGCCATGAT
CgECT3	ACAGTCTATGAAACCAGTGAGCAAG	AAGCCTTGTTTTTGGTTAGAAAATG
CgECT4	GCTAAACAACAAGTTAGCCTGACAA	TTGAGAGAGCACCAATAATACTTGC
CgECT5	GGAAGCCTTTCATAACTCTGGTAAA	AATCGAAGCCATTTGACTTTAAAAC
CgECT6	GAAGATTTTCCCGAGAGTTACTCAG	ATATGCTGCATCAAGCTTCTTATTG
CgECT7	TTTCTGTTGTTTTCGGTAAATGCTA	CATCCTTAATTATGTGCCACTTGAC
CgECT8	TTCTTTATGTGGTTGCAATGGTACT	TCAAGAATCTTAACCACAGACTTGC
CgECT9	GGAAATGTATAATGCGTCTGACAAC	AATAAACTCAGAGGCTCCTTCGTTA
CgECT10	ACCTTTAGAGGAAATGATGACGAAC	ATCCATAATGTGCTCCAGTCTGATA

3. Results

3.1. Identification of the m^{6}A Regulatory Genes from C. grandis

In this paper, a total of 6 m^{6}A writers, 10 m^{6}A erasers, and 10 m^{6}A readers of m^{6}A regulatory genes were identified from the genome of *C. grandis* according to the characteristics of m^{6}A regulatory gene domain-containing proteins. The m^{6}A regulatory genes family were divided into the MT-A70 family (PF05063), VIR-N domain (PF15912), WTAP

family (PF17098), 2OG-FeII-Oxy-2 superfamily (PF13532), and YTH domain (PF04146)). All 26 m^{6}A regulatory genes were identified based on the genome of *C. grandis*. The 26 m^{6}A regulatory genes from *C. grandis* were named according to the homologs of m^{6}A regulatory genes identified from *S. lycopersicum* [24] and *A. thaliana* [23]. As illustrated in Table 2, the number of amino acids (AAs) in the m^{6}A regulatory proteins ranged from 195 (CgALKBH6) to 2199 (CgVIR) amino acids. The molecular weight (MW) of the m^{6}A regulatory proteins varied from 22.14 (CgALKBH6) to 241.16 (CgVIR) kDa. Similarly, the isoelectric point (pI) of the 26 identified m^{6}A regulatory proteins were quite different. For instance, the highest pI was observed in CgALKBH3 (9.50), while CgALKBH8 had the lowest pI (4.50). As shown in Table 2, the subcellular localization of 19 m^{6}A regulatory proteins were in the nucleus (nucl), while a total of 7 m^{6}A regulatory proteins targeted to the cytoplasm (cyto).

Table 2. The m^{6}A regulatory genes identified in *C. grandis*.

Gene Names	Annotation Number	AAs	Mw (kDa)	pI	Subcellular Localization
CgALKBH1	Cg1g002100.1	515	57.94	6.59	cyto
CgALKBH2	Cg1g012910.1	624	68.53	7.58	nucl
CgECT1	Cg2g000250.1	711	77.99	7.57	nucl
CgECT2	Cg2g039370.1	701	76.31	6.26	nucl
CgALKBH3	Cg2g046540.1	245	28.73	9.50	nucl
CgECT3	Cg3g001240.1	572	63.06	6.34	nucl
CgALKBH4	Cg3g004030.1	464	51.92	8.52	nucl
CgALKBH5	Cg3g010130.1	874	97.61	6.09	nucl
CgMTC	Cg3g022350.1	408	46.90	6.47	cyto
CgECT4	Cg4g020150.1	616	68.11	4.94	nucl
CgMTA	Cg5g000680.1	710	78.81	5.94	cyto
CgFIP37	Cg5g005360.1	342	38.76	5.21	nucl
CgECT5	Cg5g009390.1	398	45.00	6.32	nucl
CgALKBH6	Cg5g012920.1	195	22.14	6.05	cyto
CgECT6	Cg5g031130.1	611	66.88	6.51	nucl
CgALKBH7	Cg5g036060.1	547	59.98	5.56	cyto
CgALKBH8	Cg5g037150.1	260	29.84	4.50	nucl
CgHAKAI	Cg6g006090.1	779	83.60	8.23	cyto
CgALKBH9	Cg6g010210.1	458	51.51	9.06	cyto
CgVIR	Cg6g014480.1	2199	241.16	5.34	nucl
CgECT7	Cg7g016930.1	631	69.52	6.45	nucl
CgALKBH10	Cg7g016980.1	361	40.75	6.04	nucl
CgECT8	Cg8g024900.1	656	71.85	5.41	nucl
CgECT9	Cg9g000560.1	696	76.43	5.88	nucl
CgMTB	Cg9g027190.1	1189	133.49	8.19	nucl
CgECT10	Cg9g029620.1	552	61.01	6.08	nucl

3.2. Chromosomal Localization of the m^{6}A Regulatory Genes from C. grandis

The genome location information shown in Figure 1 demonstrated that a total of 26 m^{6}A regulatory genes were unevenly anchored to the chromosomes of *C. grandis*. The results revealed that the 26 m^{6}A regulatory genes in *C. grandis* were distributed across nine chromosomes, including in chr1, chr2, chr3, chr4, chr5, chr6, chr7, chr8, and chr9. Seven of the m^{6}A regulatory genes were distributed on chromosome 5 (chr5). In contrast, only one gene was distributed on chr4 and chr8.

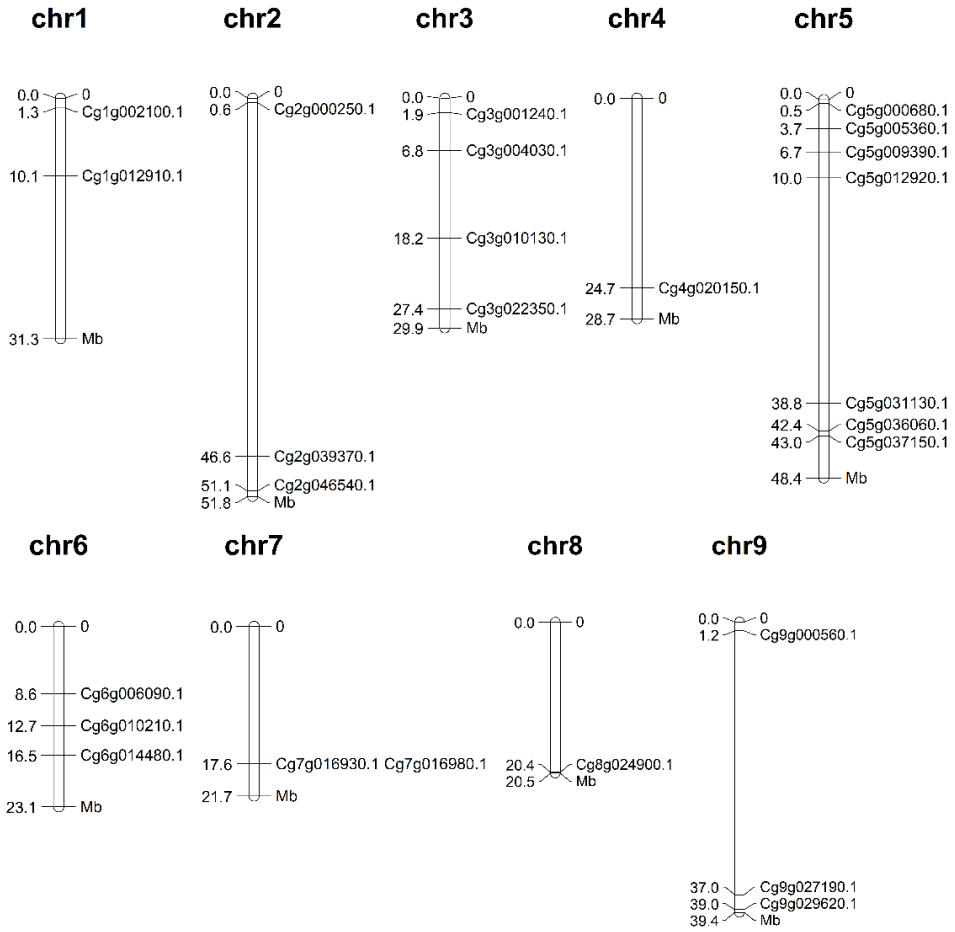

Figure 1. Chromosomal locations of m^{6}A regulatory genes in *C. grandis*. The chromosomal position of each m^{6}A regulatory gene was mapped according to the genome of *C. grandis*. The chromosome number is marked at the top of each chromosome and the unit for the scale is megabases (Mb).

3.3. Phylogenetic Analysis of the m^{6}A Regulatory Genes from C. grandis, S. lycopersicum, and A. thaliana

To study the phylogenetic relationships of the m^{6}A regulatory genes from *C. grandis*, *S. lycopersicum*, and *A. thaliana*, a phylogenetic tree was constructed using MEGA X64 with the m^{6}A regulatory protein sequences (Figure 2A). According to previously reported conserved domains from *S. lycopersicum* and *A. thaliana*, all m^{6}A regulatory members were divided into three groups classified as m^{6}A writers, erasers, and readers [5,6]. As illustrated in Figure 2A, three groups of m^{6}A writers, erasers, and readers included three MT genes (*CgMTA*, *CgMTB*, and *CgMTC*), one *CgVIR*, one *CgHAKAI*, one *CgFIP37*, 10 *CgALKBH* genes (*CgALKBH1* to *CgALKBH10*), and 10 *CgECT* members (*CgECT1* to *CgECT10*) in *C. grandis*, respectively. It is obvious that *CgALKBH* and *CgECT* were the two largest groups, including 10 family members, respectively. Different categories contained different domain structures. In the m^{6}A writer groups, the domain architectures were diverse. In the m^{6}A writer groups, MT, VIR, HAKAI, and FIP37 subfamilies were identified. According to the phylogenetic analysis, the MT gene family can be classified into three categories, namely, MTA, MTB, and MTC (Figure 2A). One *CgMTA*, one *CgMTB*, and one *CgMTC* were identified as illustrated in Figure 2A. The MTA, MTB, and MTC were present in *C. grandis*, *S. lycopersicum*, and *A. thaliana*. Similarly, only one gene was identified in the components of VIR, HAKAI, and FIP37 from *C. grandis*, respectively (Figure 2A). Additionally, in the m^{6}A eraser groups, all of the CgALKBH proteins contained only one highly conserved 2OG-FeII-Oxy-2 domain. Similarly, all 10 m^{6}A eraser proteins from *C. grandis* were closely clustered with their homologs in *S. lycopersicum* and *A. thaliana*. Nevertheless, m^{6}A reader

components of ECT and CPSF30 were identified through phylogenetic relationship analysis. A total of 10 *CgECT* family genes which had one conserved YTH domain were found in *C. grandis*. However, another key component of the m^{6}A readers, CPSF30, with a ZnF-C3H1 domain previously identified from *A. thaliana*, was not identified from *C. grandis*.

As illustrated in Figure 2B–D, the structure characteristics of the m^{6}A regulatory genes from *C. grandis* were examined. In Figure 2B, *CgMTA*, *CgMTB*, and *CgMTC* possessed a typical MT-A70 conserved domain. Among other m^{6}A writer components, the structures of CgVIR, CgHAKAI, and CgFIP37 proteins are conserved with a VIR-N, a ZnF-C2H2, and one WTAP domain, respectively. As shown in Figure 2C, compared with the m^{6}A writer components, all members of the *CgALKBH* family contain a highly conserved 2OG-FeII-Oxy-2 domain in the m^{6}A eraser groups. Similarly, in the m^{6}A readers, all ECT family members with a conserved YTH domain were identified (Figure 2D).

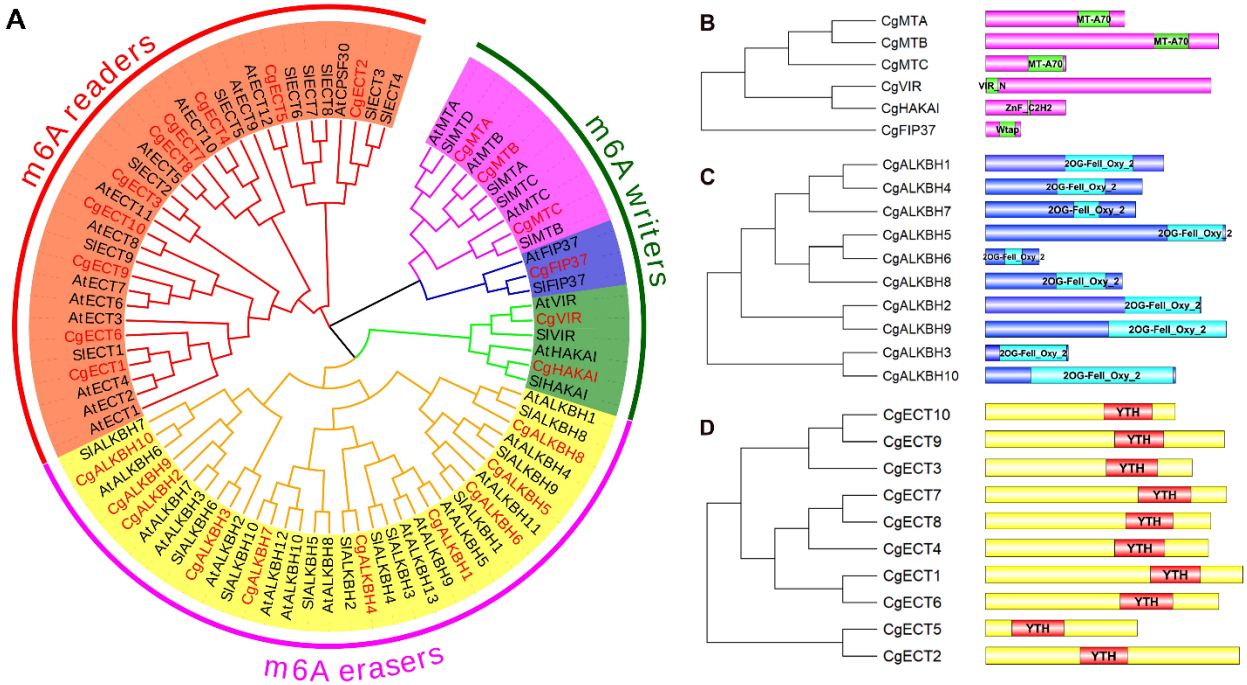

Figure 2. Phylogenetic relationship of the m^{6}A regulatory genes from *C. grandis*, *S. lycopersicum*, and *A. thaliana*. (**A**) Phylogenetic relationship of m^{6}A regulatory genes from *C. grandis*, *S. lycopersicum*, and *A. thaliana*. (**B**) MT-A70, VIR-N, ZnF-C2H2, and WTAP conserved domains of m^{6}A writer groups (Pink represents' MTs; Blue represents' FIP37s; Green represents' VIRs and HAKAIs). (**C**) One highly conserved 2OG-FeII-Oxy-2 domain of m^{6}A eraser groups (Yellow represents' ALKBHs). (**D**) YTH conserved domain of m^{6}A reader groups (Orange represents' ECTs).

3.4. Gene Structure Analysis of the m^{6}A Regulatory Genes from C. grandis

The gene structures and conserved motif characters of the m^{6}A regulatory genes from *C. grandis* were analyzed and are illustrated in Figure 3. As shown in Figure 3B, motif 1 and motif 2 were observed in almost all 10 CgETC subgroup members of the m^{6}A eraser groups. Intriguingly, in the m^{6}A reader groups, motif 4 and motif 7 were observed in *CgALKBH1*, *CgALKBH4*, and *CgALKBH7*. Nevertheless, any conserved motif characters were not detected from three *CgMT* members, one *CgVIR*, one *CgHAKAI*, and one *CgFIP37* member in the m^{6}A writer groups. The structural diversity of the m^{6}A regulatory genes from *C. grandis* was investigated by the analysis of the exon/intron structures' architectures. The exon number of the m^{6}A regulatory genes caused a huge change, as shown in Figure 3C. Moreover, no introns were contained in *CgMTB* and *CgHAKAI* (Figure 3C).

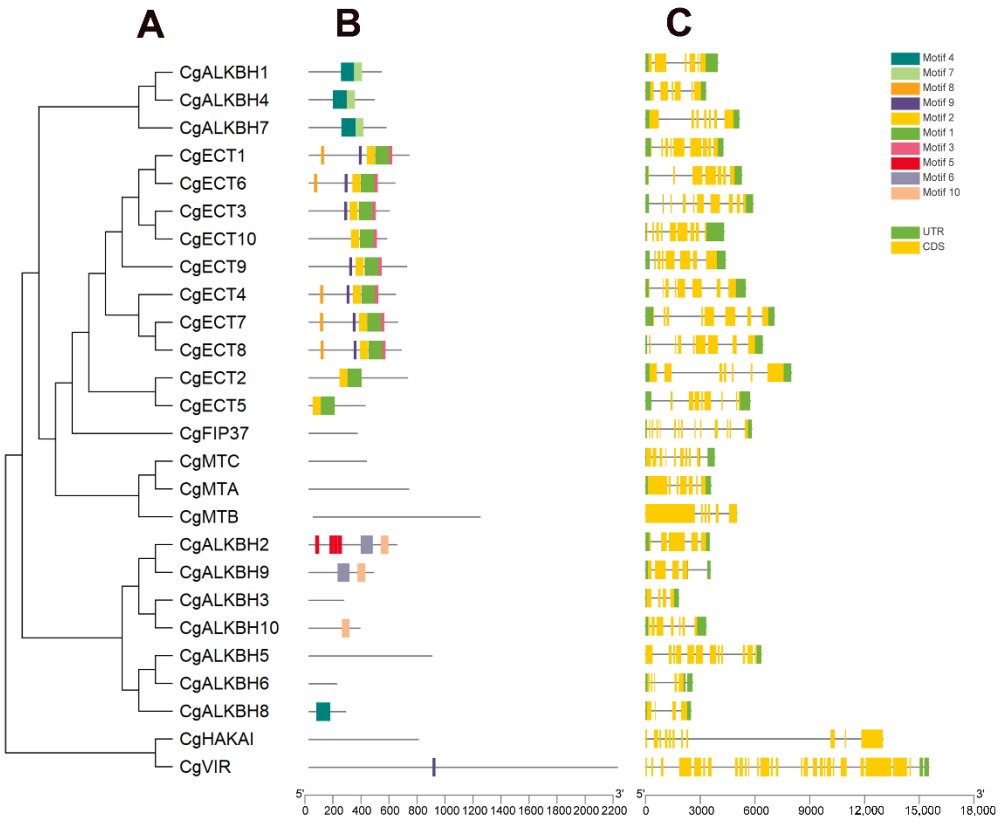

Figure 3. The gene structure analysis of m^{6}A regulatory genes from *C. grandis*. (**A**) Phylogenetic relationship of m^{6}A regulatory genes. (**B**) Conserved motifs analysis of m^{6}A regulatory genes from *C. grandis*. All motifs were identified by the MEME database with the complete amino acid sequences of m^{6}A regulatory genes from *C. grandis*. The detailed information for each motif is provided in Figure S1. (**C**) The exon and intron structures of m^{6}A regulatory genes. Yellow represents exons.

3.5. Cis-Element Analysis of the m^{6}A Regulatory Genes from C. grandis

To further elucidate the potential biological functions and expression mechanisms of the m^{6}A regulatory genes from *C. grandis*, the 1500 bp upstream sequences were firstly obtained. Then, PlantCARE was used to predict the *cis*-elements in the 1500 bp upstream sequences which are usually regarded as the promoters. The frequently appearing *cis*-elements are illustrated in Figure 4, and detailed information is also provided in Table S1. Defense and stress responsiveness, light responsiveness, and hormones responsiveness related elements were predicted from the promoters of the m^{6}A regulatory genes from *C. grandis*. In Figure 4, 12 *cis*-elements were presented on the promoters of 26 m^{6}A regulatory genes from *C. grandis*. *Cis*-elements TGA-element (auxin), ABRE (ABA), CGTCA-motif (MeJA), TCA-element (SA), and GARE-motif (GA) are involved in hormones in Figure 4. Among these elements, most of the m^{6}A regulatory genes from *C. grandis* promoters include ABRE (ABA). According to the *cis*-elements analysis, TATC-box, P-box, and GARE-motif were involved in response to gibberellic acid (GA). In the defense and stress responses category, several types of stress-responsive elements were identified in the promoter regions of the m^{6}A regulatory genes from *C. grandis*. TC-rich, LTR, and MBS were discovered, related with the defense and stress response, low-temperature response, and drought-inducibility, respectively. Moreover, light response elements were present in the promoter regions of 19 m^{6}A regulatory genes, which indicated that the expression levels of the m^{6}A regulatory genes from *C. grandis* might relate to light signaling.

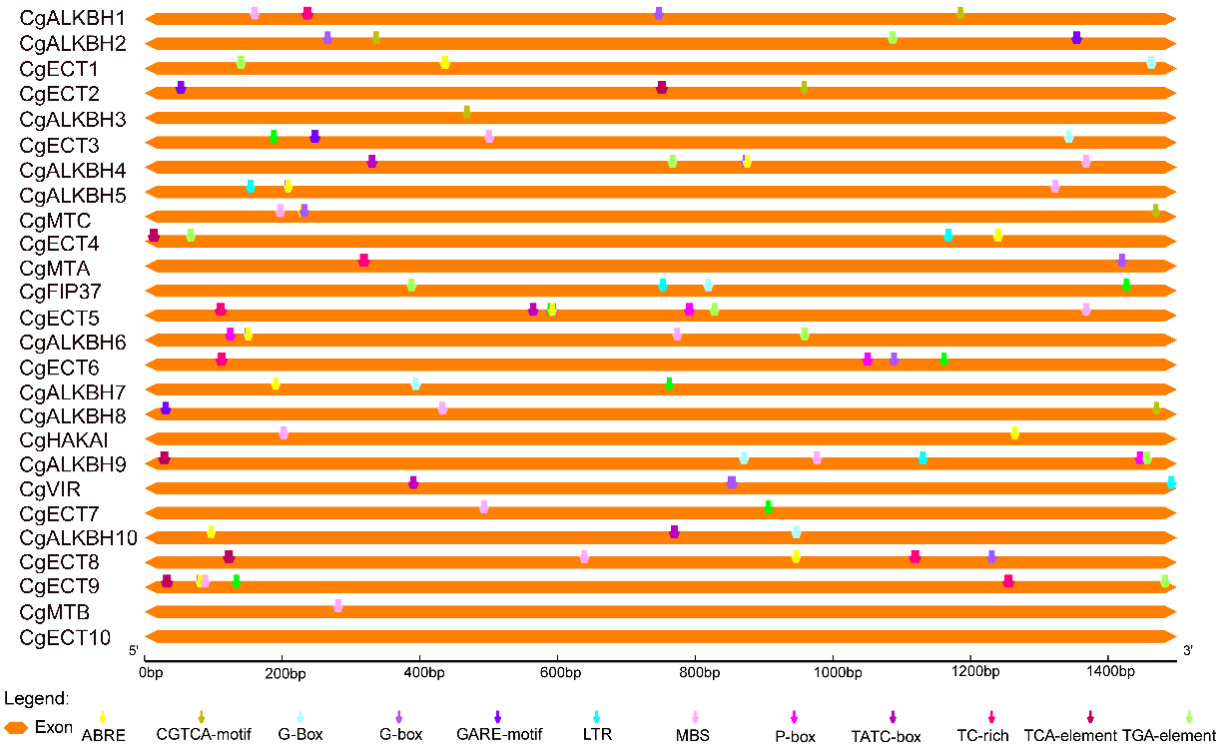

Figure 4. *Cis*-element analysis of 26 identified m^{6}A regulatory genes in *C. grandis* from the upstream 1500 bp sequences to the transcription start site.

3.6. Expression Patterns in the Fruits of C. grandis "Tomentosa" at Four Growth Points

To illustrate the expression patterns of the m^{6}A regulatory genes in *C. grandis*, the transcriptional profiles of the ZM and GQ fruits of various developmental points were investigated by RNA-seq and qRT-PCR, respectively. The heatmap showing the expression profiles of the genes was plotted based on the FPKM-normalized log2 transformed values. As illustrated in Figure 5 and Table S2, in the m^{6}A reader groups, the expression levels of *CgECT1* and *CgECT2* in ZM and GQ were higher compared with that of other m^{6}A regulatory genes. However, in the m^{6}A eraser groups, the FPKM values of *CgALKBH7*, *CgALKBH9*, and *CgALKBH10* were close to 0, indicating that almost no expression of the three genes was observed in these samples. Furthermore, the qRT-PCR analysis shown in Figure 6 and Figure S2 indicated that the gene expression patterns of most of the m^{6}A regulatory genes were consistent with the RNA-Seq data. Nevertheless, the expression levels of *CgMTA* and *CgMTC* were not consistent with the RNA-Seq values (Figures 6 and S2).

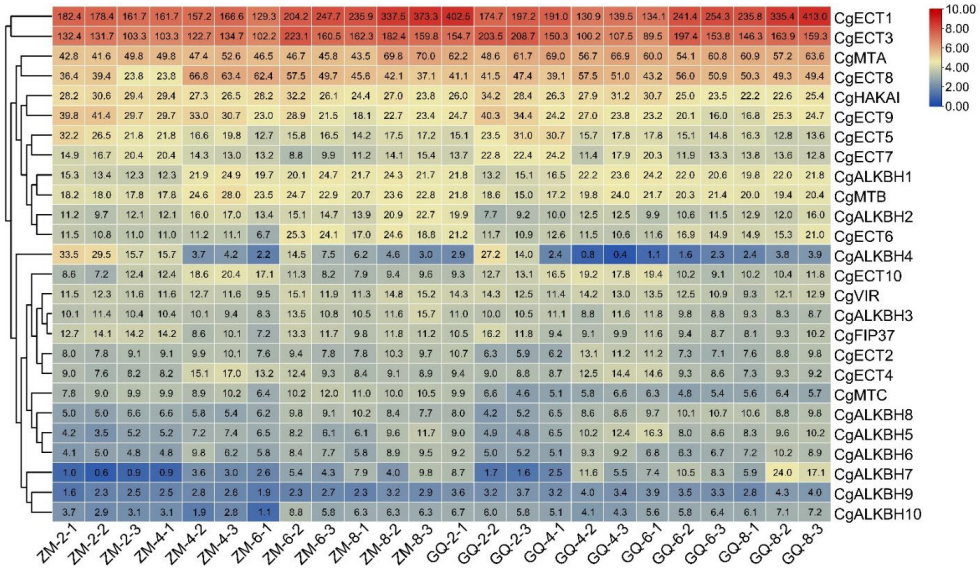

Figure 5. Expression patterns of m^{6}A regulatory genes from *C. grandis* in the fruits of 2, 4, 6, and 8 cm diameters of two varieties. The bar at the right of the heat map represents relative expression values. FPKM-normalized log2 transformed counts. (ZM and GQ are the names of the two varieties, the first number followed with ZM or GQ stands for the diameter of the fruits and the last number represents the biological replicate).

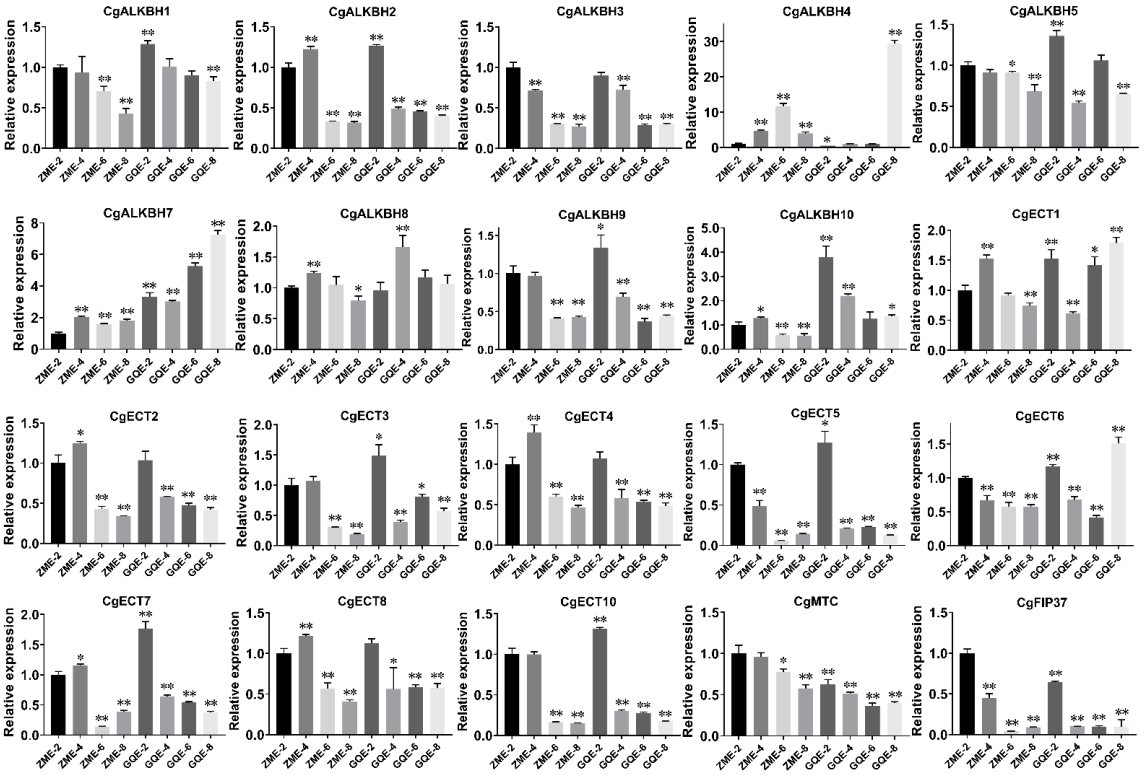

Figure 6. Relative expression of 20 m^{6}A regulatory genes in the fruits of ZM and GQ during various growth points. Actin was used for the qRT-PCR data normalization. The name of the genes is indicated above each bar diagram. Error bars indicate the standard deviation, Student's *t*-test was used for statistical analysis; * $p < 0.05$, ** $p < 0.01$. (ZM and GQ are the names of the two varieties, the first number followed with ZM or GQ stands for the diameter of the fruits and the last number represents the biological replicate).

4. Discussion

In general, according to molecular biological processes, methylation is divided into DNA methylation [36], histone modifications [37], and RNA methylation [38]. Previous studies demonstrated that N6-methyladenosine (m^6A) plays an important role in the formation of eukaryotic RNA methylation in animals and plants [5,6]. To date, the research has shown that RNA methylation plays a prominent role in the defense and stress response, including for drought [39,40] and cold [41]. Meanwhile, m^6A can reduce the damage of abiotic stress [42–45]. Furthermore, the regulatory mechanism of m^6A is related to growth and development in *A. thaliana* [13]. Nevertheless, there is limited knowledge about the m^6A regulatory genes from *C. grandis*. In the current study, a total of 26 m^6A regulatory genes from *C. grandis* were ultimately identified, while 32, 26, and 34 of them were reported in *A. thaliana*, *S. lycopersicum*, and *C. sinensis*, respectively [5,46]. Compared with other species, the number of m^6A regulatory genes in *C. grandis* is less than that in *A. thaliana* (32) and *C. sinensis* (34). Moreover, gene duplication and expansion events of m^6A regulatory genes were not observed in *C. grandis*. Hence, during the evolutionary process of *C. grandis*, the m^6A regulatory genes underwent a shrink and some redundant genes might have been deleted from the genome with certain unknown events.

Previous studies have shown that m^6A is composed of m^6A readers, writers, and erasers [5,6]. The effects of m^6A on mRNA are mediated by an expanding list of m^6A readers and m^6A writer-complex components, as well as potential erasers that currently have an unclear relevance to m^6A prevalence in the transcriptome [47]. The m^6A writers include the components of MTs, FIP37, VIR, and HAKAI [9]. The conserved domain in m^6A genes include MT-A70, WTAP, VIR-N, and ZnF-C2H2, respectively [5,6]. The MT family was further classified as MTA, MTB, and MTC subfamilies [5]. The ALKBH family has one highly conserved 2OG-FeII-Oxy-2 domain in m^6A erasers and the ECT family and CPSF30 possess the conserved YTH domain and ZnF-C3H1 domain in m^6A reader groups, respectively [5,6,8]. In this study, MTs, FIP37, VIR, and HAKAI components were detected in the m^6A writers from *C. grandis*. However, only one highly conserved 2OG-FeII-Oxy-2 domain and another conserved YTH domain were discovered in the m^6A erasers and readers of *C. grandis*, respectively. Additionally, the phylogenetic relationship and gene structure investigations identified three MT genes (*CgMTA*, *CgMTB*, and *CgMTC*), 10 *CgALKBH* genes, and 10 *CgECT* genes for reading, writing, and erasing mRNA methylation, respectively. However, compared with *A. thaliana*, *S. lycopersicum*, and *C. sinensis*, members from the CPSF30 family were not identified from *C. grandis*. The results further support the point of view of the shrinking of m^6A regulatory genes from *C. grandis* during the long-term evolutionary course. Furthermore, it was reported that 30 m^6A regulatory genes were randomly distributed on 13 chromosomes in *C. sinensis*, which is a descendant of *C. grandis* [5]. In the present study, m^6A regulatory genes were distributed at nine chromosomes, including in chr1, chr2, chr3, chr4, chr5, chr6, chr7, chr8, and chr9. In addition, the evolution of gene functional differentiation was associated with the variation of structure and the expression profile [48]. The exon/intron structures of m^6A regulatory genes obviously change, which may be related to the mutation during the process of post-transcriptional regulation [49]. *Cis*-acting elements are important for the down-stream gene transcriptional expression (activated or repressed) due to its potential binding ability with transcription factors (TFs) [50]. In the paper, the abscisic acid-responsive (ABA) element (ABRE) was presented on 19 m^6A regulatory genes from *C. grandis*. Moreover, elements related with defense and stress responsiveness, light responsiveness, and hormones responsiveness were discovered from the promoters of the m^6A regulatory genes of *C. grandis*. These results suggest that the transcriptional expressions of m^6A regulatory genes might be affected by hormones (could be internal or external) and stress (could be abiotic or biotic).

The expression levels of all m^6A regulatory genes in the fruits of ZM and GQ, which are two local varieties of *C. grandis*, during four growth stage points were examined by both RNA-seq and qRT-PCR. The varieties of ZM and GQ are different in both the internal quality and morphology of fruits; the immature fruits of ZM are usually used for the production

of a famous traditional Chinese medicine named "Huajuhong" with the medical effect of curing chronic cough [51,52]. The variety of GQ is a local pumelo (*C. grandis*). The results demonstrated that the expression patterns of *CgECT1* and *CgECT3* increased, while the expression amount of *CgALKBH4*, *CgALKBH7*, *CgALKBH9*, and *CgALKBH10* decreased during the fruit development of the two varieties. Moreover, among all the identified m^6A regulatory genes, the expression levels of *CgECT1* and *CgECT3* were notably high in the fruits of two varieties of *C. grandis* during four development points (Figure 5). It was reported that ECT2/3/4 proteins played important roles in the timing and regulation of leaf formation and normal leaf morphology in *Arabidopsis* [8]. *CgECT3* identified from this study might play a vital role in the development of fruits of *C. grandis*, which could be investigated in the future.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae8050462/s1, Figure S1. Sequence logos for the conserved motifs of m6A regulatory genes domain proteins from Citrus grandis. Conserved motifs and the sequence logos were generated using the MEME search tool. Numbers on the horizontal axis represent the sequence positions in the motifs and the vertical axis represents the information content measured in bits, Figure S2: Relative expression of 6 m6A regulatory genes in the fruits of ZM and GQ during various growth points, Table S1: The detailed information of *cis*-elements, Table S2: The FPKM values of 26 m6A regulatory genes in *C. grandis*.

Author Contributions: Conceptualization, R.F. and J.Z.; methodology, R.F.; software, Y.T.; validation, Y.T. and R.F.; investigation, R.F. and J.Z.; resources, J.Z.; data curation, Y.T.; writing—original draft preparation, Y.T.; writing—review and editing, R.F. and J.Z.; visualization, Y.T.; supervision, R.F.; project administration, J.Z.; funding acquisition, R.F. and J.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Guangdong Basic and Applied Basic Research Foundation (2019A1818011378), the National Natural Science Foundation of China (32001999), the Fundamental and Applied Fundamental Research Fund of Guangzhou City (2021020493), the Special fund for scientific innovation strategy-construction of high level Academy of Agriculture Science (No: R2021PY-QF004; R2020PY-JG001), the Guangdong Province Science and Technology Plan Project (2019B090905002) and the China Scholarship Council (202108440301).

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Matouk, C.C.; Marsden, P.A. Epigenetic regulation of vascular endothelial gene expression. *Circ. Res.* **2008**, *102*, 873–887. [CrossRef] [PubMed]
2. Luger, K.; Richmond, T.J. The histone tails of the nucleosome. *Curr. Opin. Genet. Dev.* **1998**, *8*, 140–146. [CrossRef]
3. Zhang, X. The epigenetic landscape of plants. *Science* **2008**, *320*, 489–492. [CrossRef] [PubMed]
4. Fu, Y.; Dominissini, D.; Rechavi, G.; He, C. Gene expression regulation mediated through reversible m(6)A RNA methylation. *Nat. Rev. Genet.* **2014**, *15*, 293–306. [CrossRef] [PubMed]
5. Zhu, C.; Zhang, S.; Zhou, C.; Xie, S.; Chen, G.; Tian, C.; Xu, K.; Lin, Y.; Lai, Z.; Guo, Y. Genome-Wide Investigation of N6-Methyladenosine Regulatory Genes and Their Roles in Tea (*Camellia sinensis*) Leaves during Withering Process. *Front. Plant Sci.* **2021**, *12*, 702303. [CrossRef]
6. Yang, Y.; Hsu, P.J.; Chen, Y.S.; Yang, Y.G. Dynamic transcriptomic m(6)A decoration: Writers, erasers, readers and functions in RNA metabolism. *Cell Res.* **2018**, *28*, 616–624. [CrossRef]
7. Saito, K. Phytochemical genomics—A new trend. *Curr. Opin. Plant Biol.* **2013**, *16*, 373–380. [CrossRef]
8. Yue, H.; Nie, X.; Yan, Z.; Weining, S. N6-methyladenosine regulatory machinery in plants: Composition, function and evolution. *Plant Biotechnol. J.* **2019**, *17*, 1194–1208. [CrossRef]
9. Sledz, P.; Jinek, M. Structural insights into the molecular mechanism of the m(6)A writer complex. *eLife* **2016**, *5*, e18434. [CrossRef]
10. Wang, P.; Doxtader, K.A.; Nam, Y. Structural Basis for Cooperative Function of Mettl3 and Mettl14 Methyltransferases. *Mol. Cell* **2016**, *63*, 306–317. [CrossRef]

11. Liang, Z.; Riaz, A.; Chachar, S.; Ding, Y.; Du, H.; Gu, X. Epigenetic Modifications of mRNA and DNA in Plants. *Mol. Plant* **2020**, *13*, 14–30. [CrossRef] [PubMed]
12. Shen, L.; Liang, Z.; Gu, X.; Chen, Y.; Teo, Z.W.; Hou, X.; Cai, W.M.; Dedon, P.C.; Liu, L.; Yu, H. N(6)-Methyladenosine RNA Modification Regulates Shoot Stem Cell Fate in Arabidopsis. *Dev. Cell* **2016**, *38*, 186–200. [CrossRef] [PubMed]
13. Ruzicka, K.; Zhang, M.; Campilho, A.; Bodi, Z.; Kashif, M.; Saleh, M.; Eeckhout, D.; El-Showk, S.; Li, H.; Zhong, S.; et al. Identification of factors required for m(6) A mRNA methylation in Arabidopsis reveals a role for the conserved E3 ubiquitin ligase HAKAI. *New Phytol.* **2017**, *215*, 157–172. [CrossRef]
14. Scarrow, M.; Chen, N.; Sun, G. Insights into the N(6)-methyladenosine mechanism and its functionality: Progress and questions. *Crit. Rev. Biotechnol.* **2020**, *40*, 639–652. [CrossRef]
15. Martinez-Perez, M.; Aparicio, F.; Lopez-Gresa, M.P.; Belles, J.M.; Sanchez-Navarro, J.A.; Pallas, V. Arabidopsis m(6)A demethylase activity modulates viral infection of a plant virus and the m(6)A abundance in its genomic RNAs. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 10755–10760. [CrossRef]
16. Zhou, L.; Tian, S.; Qin, G. RNA methylomes reveal the m(6)A-mediated regulation of DNA demethylase gene SlDML2 in tomato fruit ripening. *Genome Biol.* **2019**, *20*, 156. [CrossRef] [PubMed]
17. Addepalli, B.; Hunt, A.G. A novel endonuclease activity associated with the Arabidopsis ortholog of the 30-kDa subunit of cleavage and polyadenylation specificity factor. *Nucleic Acids Res.* **2007**, *35*, 4453–4463. [CrossRef]
18. Arribas-Hernandez, L.; Bressendorff, S.; Hansen, M.H.; Poulsen, C.; Erdmann, S.; Brodersen, P. An m(6)A-YTH Module Controls Developmental Timing and Morphogenesis in Arabidopsis. *Plant Cell* **2018**, *30*, 952–967. [CrossRef]
19. Pontier, D.; Picart, C.; El Baidouri, M.; Roudier, F.; Xu, T.; Lahmy, S.; Llauro, C.; Azevedo, J.; Laudie, M.; Attina, A.; et al. The m(6)A pathway protects the transcriptome integrity by restricting RNA chimera formation in plants. *Life Sci. Alliance* **2019**, *2*, e201900393. [CrossRef]
20. Velasco, R.; Licciardello, C. A genealogy of the citrus family. *Nat. Biotechnol.* **2014**, *32*, 640–642. [CrossRef]
21. Tian, Y.; Fan, R.; Zeng, J. 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. *Horticulturae* **2021**, *7*, 592. [CrossRef]
22. Fan, R.; Zhu, C.; Qiu, D.; Zeng, J. Comparison of the bioactive chemical components and antioxidant activities in three tissues of six varieties of Citrus grandis 'Tomentosa' fruits. *Int. J. Food Prop.* **2019**, *22*, 1848–1862. [CrossRef]
23. Berardini, T.Z.; Reiser, L.; Li, D.; Mezheritsky, Y.; Muller, R.; Strait, E.; Huala, E. The Arabidopsis information resource: Making and mining the "gold standard" annotated reference plant genome. *Genesis* **2015**, *53*, 474–485. [CrossRef] [PubMed]
24. Bombarely, A.; Menda, N.; Tecle, I.Y.; Buels, R.M.; Strickler, S.; Fischer-York, T.; Pujar, A.; Leto, J.; Gosselin, J.; Mueller, L.A. The Sol Genomics Network (solgenomics.net): Growing tomatoes using Perl. *Nucleic Acids Res.* **2011**, *39*, D1149–D1155. [CrossRef] [PubMed]
25. Letunic, I.; Bork, P. 20 years of the SMART protein domain annotation resource. *Nucleic Acids Res.* **2018**, *46*, D493–D496. [CrossRef] [PubMed]
26. Lu, S.; Wang, J.; Chitsaz, F.; Derbyshire, M.K.; Geer, R.C.; Gonzales, N.R.; Gwadz, M.; Hurwitz, D.I.; Marchler, G.H.; Song, J.S.; et al. CDD/SPARCLE: The conserved domain database in 2020. *Nucleic Acids Res.* **2020**, *48*, D265–D268. [CrossRef]
27. Artimo, P.; Jonnalagedda, M.; Arnold, K.; Baratin, D.; Csardi, G.; de Castro, E.; Duvaud, S.; Flegel, V.; Fortier, A.; Gasteiger, E.; et al. ExPASy: SIB bioinformatics resource portal. *Nucleic Acids Res.* **2012**, *40*, W597–W603. [CrossRef]
28. Zuo, C.; Liu, H.; Lv, Q.; Chen, Z.; Tian, Y.; Mao, J.; Chu, M.; Ma, Z.; An, Z.; Chen, B. Genome-Wide Analysis of the Apple (*Malus domestica*) Cysteine-Rich Receptor-Like Kinase (CRK) Family: Annotation, Genomic Organization, and Expression Profiles in Response to Fungal Infection. *Plant Mol. Biol. Report.* **2019**, *38*, 14–24. [CrossRef]
29. Xie, T.; Chen, C.; Li, C.; Liu, J.; Liu, C.; He, Y. Genome-wide investigation of WRKY gene family in pineapple: Evolution and expression profiles during development and stress. *BMC Genom.* **2018**, *19*, 490. [CrossRef]
30. Hu, R.; Qi, G.; Kong, Y.; Kong, D.; Gao, Q.; Zhou, G. Comprehensive analysis of NAC domain transcription factor gene family in Populus trichocarpa. *BMC Plant Biol.* **2010**, *10*, 145. [CrossRef]
31. Ren, J.; Wen, L.; Gao, X.; Jin, C.; Xue, Y.; Yao, X. DOG 1.0: Illustrator of protein domain structures. *Cell Res.* **2009**, *19*, 271–273. [CrossRef] [PubMed]
32. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* **2020**, *13*, 1194–1202. [CrossRef] [PubMed]
33. Lescot, M.; Dehais, P.; Thijs, G.; Marchal, K.; Moreau, Y.; Van de Peer, Y.; Rouze, P.; Rombauts, S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* **2002**, *30*, 325–327. [CrossRef] [PubMed]
34. Li, B.; Fan, R.; Yang, Q.; Hu, C.; Sheng, O.; Deng, G.; Dong, T.; Li, C.; Peng, X.; Bi, F.; et al. Genome-Wide Identification and Characterization of the NAC Transcription Factor Family in Musa Acuminata and Expression Analysis during Fruit Ripening. *Int. J. Mol. Sci.* **2020**, *21*, 634. [CrossRef] [PubMed]
35. Takeuchi, T.; Yamazaki, Y.; Katoh-Fukui, Y.; Tsuchiya, R.; Kondo, S.; Motoyama, J.; Higashinakagawa, T. Gene trap capture of a novel mouse gene, jumonji, required for neural tube formation. *Genes Dev.* **1995**, *9*, 1211–1222. [CrossRef]
36. Akimoto, K.; Katakami, H.; Kim, H.J.; Ogawa, E.; Sano, C.M.; Wada, Y.; Sano, H. Epigenetic inheritance in rice plants. *Ann. Bot.* **2007**, *100*, 205–217. [CrossRef]

37. Shen, Y.; Conde, E.S.N.; Audonnet, L.; Servet, C.; Wei, W.; Zhou, D.X. Over-expression of histone H3K4 demethylase gene JMJ15 enhances salt tolerance in Arabidopsis. *Front. Plant Sci.* **2014**, *5*, 290. [CrossRef]
38. Sharma, A.; Singh, K.; Almasan, A. Histone H2AX phosphorylation: A marker for DNA damage. *Methods Mol. Biol.* **2012**, *920*, 613–626. [CrossRef]
39. Scutenaire, J.; Deragon, J.M.; Jean, V.; Benhamed, M.; Raynaud, C.; Favory, J.J.; Merret, R.; Bousquet-Antonelli, C. The YTH Domain Protein ECT2 Is an m(6)A Reader Required for Normal Trichome Branching in Arabidopsis. *Plant Cell* **2018**, *30*, 986–1005. [CrossRef]
40. Miao, Z.; Zhang, T.; Qi, Y.; Song, J.; Han, Z.; Ma, C. Evolution of the RNA N (6)-Methyladenosine Methylome Mediated by Genomic Duplication. *Plant Physiol.* **2020**, *182*, 345–360. [CrossRef]
41. Hu, J.; Manduzio, S.; Kang, H. Epitranscriptomic RNA Methylation in Plant Development and Abiotic Stress Responses. *Front. Plant Sci.* **2019**, *10*, 500. [CrossRef] [PubMed]
42. Zhou, J.; Wan, J.; Gao, X.; Zhang, X.; Jaffrey, S.R.; Qian, S.B. Dynamic m(6)A mRNA methylation directs translational control of heat shock response. *Nature* **2015**, *526*, 591–594. [CrossRef] [PubMed]
43. Zhou, J.; Wan, J.; Shu, X.E.; Mao, Y.; Liu, X.M.; Yuan, X.; Zhang, X.; Hess, M.E.; Bruning, J.C.; Qian, S.B. N(6)-Methyladenosine Guides mRNA Alternative Translation during Integrated Stress Response. *Mol. Cell* **2018**, *69*, 636–647.e637. [CrossRef]
44. Duan, H.C.; Wei, L.H.; Zhang, C.; Wang, Y.; Chen, L.; Lu, Z.; Chen, P.R.; He, C.; Jia, G. ALKBH10B Is an RNA N(6)-Methyladenosine Demethylase Affecting Arabidopsis Floral Transition. *Plant Cell* **2017**, *29*, 2995–3011. [CrossRef] [PubMed]
45. Kramer, M.C.; Janssen, K.A.; Palos, K.; Nelson, A.D.L.; Vandivier, L.E.; Garcia, B.A.; Lyons, E.; Beilstein, M.A.; Gregory, B.D. N(6)-methyladenosine and RNA secondary structure affect transcript stability and protein abundance during systemic salt stress in Arabidopsis. *Plant Direct.* **2020**, *4*, e00239. [CrossRef] [PubMed]
46. Xia, E.; Tong, W.; Hou, Y.; An, Y.; Chen, L.; Wu, Q.; Liu, Y.; Yu, J.; Li, F.; Li, R.; et al. The Reference Genome of Tea Plant and Resequencing of 81 Diverse Accessions Provide Insights into Its Genome Evolution and Adaptation. *Mol. Plant* **2020**, *13*, 1013–1026. [CrossRef]
47. Zaccara, S.; Ries, R.J.; Jaffrey, S.R. Reading, writing and erasing mRNA methylation. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 608–624. [CrossRef]
48. Tian, R.; Yang, Y.; Chen, M. Genome-wide survey of the amino acid transporter gene family in wheat (*Triticum aestivum* L.): Identification, expression analysis and response to abiotic stress. *Int. J. Biol. Macromol.* **2020**, *162*, 1372–1387. [CrossRef]
49. Pellagatti, A.; Boultwood, J. Splicing factor gene mutations in the myelodysplastic syndromes: Impact on disease phenotype and therapeutic applications. *Adv. Biol. Regul.* **2017**, *63*, 59–70. [CrossRef]
50. Sun, Z.; Wang, X.; Qiao, K.; Fan, S.; Ma, Q. Genome-wide analysis of JMJ-C histone demethylase family involved in salt-tolerance in *Gossypium hirsutum* L. *Plant Physiol. Biochem.* **2021**, *158*, 420–433. [CrossRef]
51. Li, P.L.; Liu, M.H.; Hu, J.H.; Su, W.W. Systematic chemical profiling of Citrus grandis 'Tomentosa' by ultra-fast liquid chromatography/diode-array detector/quadrupole time-of-flight tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2014**, *90*, 167–179. [CrossRef] [PubMed]
52. Yu, X.; Liu, Q.; Xie, Z.; Lam, S.; Xu, X. Chromatographic Fingerprint Analysis of Exocarpium Citri Grandis by High-Performance Liquid Chromatography Coupled with Diode-Array Detector. *Food Anal. Methods* **2014**, *8*, 1868–1875. [CrossRef]