The MADS-box gene family, which encodes a type of transcription factor, is a regulatory gene family found widely in eukaryotic genomes. Researchers successively found MADS-box genes in fungi (Passmore et al., 1988), animals (Norman et al., 1988), and plants (Sommer et al., 1990; Yanofsky et al., 1990). The MADS-box genes received their name from the first initials of four transcription factors, MCM1 (Mini Chromosome Maintenance 1) from Saccharomyces cerevisiae (Passmore et al., 1988), AG (AGAMOUS) from arabidopsis [Arabidopsis thaliana (Yanofsky et al., 1990)], DEF (Deficiens) from Antirrhinum majus (Sommer et al., 1990), and Serum Response Factor (SRF) from Homo sapiens (Norman et al., 1988). The proteins contain a conserved region of ≈58–60 amino acids in their N-termini called the MADS-box (Becker et al., 2000; Theissen et al., 2000), which is a domain-binding DNA sequence that recognizes CArG boxes and regulates the expression of target genes (De Bodt et al., 2003). Studies have shown that plant MADS-box transcription factors play an important role in the control of floral organ and fruit development, flowering time, and gametophyte cell division (Ditta et al., 2004; Fang and Fernandez, 2002; Favaro et al., 2003; Liljegren et al., 2000; Ma, 2005; Michaels et al., 2003; Pinyopich et al., 2003).

Plant MADS-box genes can be divided into two categories, types I and II (Alvarez-Buylla et al., 2000). In comparison with type II, type I genes have a simpler gene structure and lack the K domain. In arabidopsis, type I MADS-box genes contain Mo, Mβ, My, and Mδ subfamilies (Pařenícová et al., 2003). Studies on type II genes have been relatively extensive, but type I genes have been less researched and are less well understood. Type II MADS-box genes can also be further subdivided into MIKC-type and 13 MIKC subfamilies (Diaz-Riquelme et al., 2009; Henschel et al., 2002); AG, AGL6, AGL12, AGL15, AGL17, AP1/FUL, AP3/PI, FLC, SCO1, SEP, SVP, BS, and TM8. In addition to the M domain, MADS-box genes have three other conserved domains: the K domain, which is relatively conserved and characterized by a coiled-coil structure, only exists in type II genes and is the basis of the distinction between type I and II genes (Davies et al., 1996; Ma et al., 1991; Pnueli et al., 1991); the I domain, which is located between the M and K domains, may contribute to specificity in the formation of DNA-binding dimers (Riechmann et al., 1996); and the C domain, which has multiple functions, can activate transcription in some MADS-box genes of yeast cells (Kramer et al., 1998). Compared with MIKC-type genes, MIKC-type genes contain a longer I domain and a shorter K domain.

MADS-box genes play a significant role in plant development processes, especially in floral organ development (Causier et al., 2002). The ABC model of floral development was established via studies on model plants, such as arabidopsis and A. majus (Coen and Meyerowitz, 1991). Subsequently, many MADS-box genes and proteins were fully researched and explored, and the widely accepted ABCDE model was proposed (Honma and Goto, 2001). In this model, A-function genes determine sepal development, A- and B-function genes codetermine petal development, B- and C-function genes codetermine stamen development, C-function genes control carpel development, D-function genes determine ovule development, and E-function genes regulate the development of various organs (Kaufmann et al., 2005; Ma, 2000; Pelaz et al., 2000).

Melon, a diploid eudicot plant, is an annual herbaceous vine. Widely cultivated, melon is an important horticultural crop and one of the most popular fruits. Melon has considerable economic value, especially in the Mediterranean and central Asian countries. Through plant genome projects, genome-wide sequencing has been completed for many model plants, such as corn [Zea mays (Schnable et al., 2009)], sorghum [Sorghum bicolor (Paterson et al., 2009)], soybean [Glycine max (Schmutz et al., 2010)], and grape [Vitis vinifera (Jaillon et al., 2007)]. The genome sequence and gene annotations for melon have also been published (Garcia-Mas et al., 2012), which has established a foundation for studying the melon MADS-box gene family. There has been relatively extensive...
research on MADS-box genes in arabidopsis (Pařenicková et al., 2003), poplar [Populus trichocarpa (Leseberg et al., 2006)], rice [Oryza sativa (Arora et al., 2007)], grape (Díaz-Riquelme et al., 2009), soybean (Shu et al., 2013), and cucumber [Cucumis sativus (Hu et al., 2012)], but, so far, in-depth research on melon MADS-box genes has not been performed. Melon, as a model plant for fresh fruit, has important scientific value and significance warranting further discussion and research on the melon MADS-box gene family.

**Materials and Methods**

**Identification of MADS-box genes in melon.** The melon genome was downloaded from the MELONOMICS database (Pere and Jordi, 2012). First, a protein database was established with the genome-wide amino acid sequence using the BioEdit 7.2.0 software (Hall, 1999), and then a Hidden Markov Model (HMM) for the SRF-TF (PF00319) and K-box (PF01486) was found from the Pfam database (Finn, 2014) and used as a probe to perform local protein alignment with the local Basic Local Alignment Search Tool (BLASTp) program. When conducting the alignment, the program was set to the default parameters and the cutoff E-value was 0.001. The alignment results were used to identify candidate genes, and then conserved domain analysis was conducted with the SMART program (Letunic et al., 2015; Schultz et al., 1998) to remove repetitive and redundant sequences. Finally, the complete set of melon MADS-box family genes was obtained.

**Intron–exon structure analysis and chromosomal locations.** In the melon database, the CDS and DNA sequences of 62 melon MADS-box genes were numbered using the FASTA format. The distribution of exons and introns in the melon MADS-box genes was analyzed using the online software GSDS (Hu et al., 2015). According to the positional information for the melon MADS-box genes, we used the MapDraw software (Liu and Meng, 2003) to map the distribution of the melon MADS-box genes on the chromosomes.

**Sequence alignment and phylogenetic analysis.** The arabidopsis MADS-box genome was downloaded from the Arabidopsis Information Resource (TAIR) website (Rhee et al., 2003). The cucumber MADS-box genome was downloaded from the cucumber database (BGI, 2009), which was released by the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (IVF-CAAS). Multiple sequence alignment was performed using CLC Sequence Version 7.6.1 (Qiagen, Duesseldorf, Germany) with default parameters. A phylogenetic tree was built with the MADS-box genes of arabidopsis, cucumber, and melon using the neighbor-joining method in the MEGA 4.1 software (Tamura et al., 2007), with the following parameters: Poisson correction, pairwise deletion, and bootstrapping (1000 replicates). All of the melon MADS-box genes were classified into subfamilies based on the phylogenetic tree.

**Analysis of conserved motifs.** MEME version 4.10.2 (Bailey and Elkan, 1994), a software tool for predicting conserved sequences of genes, was used to analyze the conserved motifs of the melon MADS-box genes (maximum number of motifs: 10, motif width >6 and <200).

**Expression analysis with reverse-transcription polymerase chain reaction and quantitative RT-PCR.** Plants of melon cultivar Hetao were grown under experimental field conditions from May to July 2014 at Inner Mongolia University, Hohhot, China. At the flowering stage, we collected hermaphrodite flowers and fruit at 0 d after pollination (DAP), as well as young roots, leaf blades, and tender stems. Meanwhile, self-pollination was performed by hand and the pollination time was accurately controlled. Only one fruit was kept for each plant. The mesocarp of fruit (15 and 35 DAP) was collected. All harvested samples were immediately washed with sterile water, frozen in liquid nitrogen, and stored at −80 ºC for RNA extraction. TRIzol Reagent (Invitrogen, Carlsbad, CA) was used to extract RNA from all melon samples, and electrophoresis test results showed clear RNA bands and good integrity. Reverse transcription was performed with PrimeScript™ RT Master Mix (TaKaRa, Dalian, China) in a 10-µL reaction system containing 2 µL 5x buffer, 500 ng RNA, and RNase-free dH2O up to 10 µL. The reaction conditions were 37 ºC for 15 min and 85 ºC for 5 s. Aliquots of 4 and 2 µL were taken as templates for reverse-transcription polymerase chain reaction (RT-PCR) and quantitative TR-PCR (qRT-PCR), respectively, after the reverse transcription product was diluted 10 times. We designed primers for the experiment with the Primer 5.0 software (Premier Biosoft, Palo Alto, CA); during the design process, we tried to avoid the conserved regions of MADS-box genes and design primers across long introns as far as possible to remove potential DNA contamination. After primer design was completed, the primers were aligned to the melon database to confirm their specificity. All PCR products were run on 2% agarose gels. For qRT-PCR, a 25-µL reaction system was used containing 12.5 µL 2 × SYBR Premix Ex Taq II (TaKaRa), forward and reverse primers diluted to 0.4 µM, and the templates mentioned above. The reaction conditions were 95 ºC for 30 s, followed by 40 cycles of 95 ºC for 5 s and 60 ºC for 30 s. The reactions were performed with a Chromo 4 Real-Time PCR Detector (Bio-Rad, Hercules, CA). The relative expression of genes was calculated by the 2^(-ΔΔCt) method. At least three technical replicates were performed for each reaction and three biological samples were used for each gene.

**Results**

**Identification, genomic distribution, and annotation of MADS-box genes in melon.** To identify melon MADS-box family genes, we used the HMM model for the SRF-TF (PF00319) and K-box (PF01486) to conduct local protein alignment and collected the results as candidate proteins. After we removed duplicated and functionally redundant genes, 62 MADS-box genes remained. Analysis with the SMART software shows that all 62 genes had a typical MADS-box domain. We named the genes CmMADS01–CmMADS62 according to their distribution of subfamily and collected relative statistics about them (Table 1). Table 1 shows the corresponding statistics and analysis, including amino acid length, number of exons and introns, chromosomal assignment, and subfamily classification.

We mapped the chromosomal locations of the genes based on their location information using the MapDraw software (Fig. 1). This showed that the gene family was differentially distributed across the chromosomes. Chr8 and Chr11 both had eight genes, whereas Chr5 had no genes. The remaining chromosomes contained one to seven genes. Chr1 had two type II genes but no type I genes, whereas Chr2 and Chr10 only had one type I gene each but no type II genes. The gene distribution on the other chromosomes was about equal.
Table 1. Identity of 62 MADS-box genes from a local blast against melon genome and removed repetitive and redundant sequences with the SMART program (Letunic et al., 2015). Obtained the information including amino acid length, number of exons and introns, and chromosomal assignment. The subfamilies were classified based on the phylogenetic tree.

| Gene    | Gene identification | Location                          | Chromosome no. | Protein length (no. of amino acids) | Exons (no.) | Introns (no.) | Subfamily |
|---------|---------------------|-----------------------------------|----------------|-------------------------------------|-------------|---------------|-----------|
| CmMADS01| MELO3C002691T1      | Scaffold00001 5563523–5568056    | 12             | 256                                 | 8           | 7             | AG        |
| CmMADS02| MELO3C007181T1      | Scaffold00007 1322082–1329014    | 8              | 237                                 | 9           | 8             | AG        |
| CmMADS03| MELO3C007181T2      | Scaffold00007 1322082–1329014    | 8              | 242                                 | 9           | 8             | AG        |
| CmMADS04| MELO3C007181T3      | Scaffold00007 1322082–1329266    | 8              | 262                                 | 9           | 8             | AG        |
| CmMADS05| MELO3C022209T1      | Scaffold00051 2262147–2270592    | 9              | 230                                 | 9           | 8             | AG        |
| CmMADS06| MELO3C022516T1      | Scaffold00052 2287507–2292344    | 11             | 187                                 | 7           | 6             | AGL6      |
| CmMADS07| MELO3C022516T2      | Scaffold00052 2287507–2292344    | 11             | 249                                 | 8           | 7             | AGL6      |
| CmMADS08| MELO3C024001T1      | Scaffold00064 990832–994894      | 4              | 251                                 | 8           | 7             | AGL15     |
| CmMADS09| MELO3C019192T1      | Scaffold00036 4133578–4136082    | N/A*           | 135                                 | 4           | 3             | AGL15     |
| CmMADS10| MELO3C003502T1      | Scaffold01596 1775790–1799198    | 4              | 238                                 | 9           | 8             | AGL17     |
| CmMADS11| MELO3C003502T2      | Scaffold01596 1775876–1799198    | 4              | 239                                 | 9           | 8             | AGL17     |
| CmMADS12| MELO3C007700T1      | Scaffold00005 4762147–4767238    | 8              | 150                                 | 4           | 3             | AGL17     |
| CmMADS13| MELO3C018049T1      | Scaffold00005 3970196–3980905    | 7              | 190                                 | 6           | 5             | AGL17     |
| CmMADS14| MELO3C002050T1      | Scaffold00005 900368–905507      | 12             | 248                                 | 8           | 7             | API/FUL   |
| CmMADS15| MELO3C011409T1      | Scaffold00005 4117765–4122123    | 4              | 276                                 | 8           | 7             | API/FUL   |
| CmMADS16| MELO3C005617T1      | Scaffold00005 6499878–6505422    | 9              | 151                                 | 6           | 5             | SCO1      |
| CmMADS17| MELO3C005617T2      | Scaffold00005 6499878–6505422    | 9              | 223                                 | 7           | 6             | SCO1      |
| CmMADS18| MELO3C005393T1      | Scaffold00005 4706570–4715038    | 9              | 160                                 | 7           | 6             | SCO1      |
| CmMADS19| MELO3C006159T2      | Scaffold00006 1394377–1397731    | 6              | 160                                 | 4           | 3             | SCO1      |
| CmMADS20| MELO3C006159T4      | Scaffold00006 1394377–1400874    | 6              | 221                                 | 7           | 6             | SCO1      |
| CmMADS21| MELO3C022049T1      | Scaffold00007 789483–794983     | 12             | 258                                 | 8           | 7             | SEP       |
| CmMADS22| MELO3C022316T1      | Scaffold00007 78877–794983      | 11             | 242                                 | 8           | 7             | SEP       |
| CmMADS23| MELO3C026300T1      | Scaffold00090 352986–362937     | N/A            | 246                                 | 8           | 7             | SEP       |
| CmMADS24| MELO3C026300T2      | Scaffold00090 352986–362937     | N/A            | 216                                 | 7           | 6             | SEP       |
| CmMADS25| MELO3C026300T3      | Scaffold00090 354020–362937     | N/A            | 181                                 | 6           | 5             | SEP       |
| CmMADS26| MELO3C002723T1      | Scaffold00001 5929497–5930018   | 12             | 173                                 | 1           | 0             | Mα        |
| CmMADS27| MELO3C003801T1      | Scaffold00001 531621–534860     | 4              | 188                                 | 1           | 0             | Mα        |
| CmMADS28| MELO3C007148T1      | Scaffold00001 1127180–1128076   | 12             | 298                                 | 1           | 0             | Mα        |
| CmMADS29| MELO3C008942T1      | Scaffold00010 37266274–3726758  | 8              | 102                                 | 2           | 1             | Mβ        |

Continued next page
Furthermore, CmMADS47 and CmMADS48 were located on Contig32319 and Contig38521 on Scaffold00090, the chromosomal location of which remains unknown. The genomic distribution result indicated that 61% of melon MADS-box genes were clustered, and most of them presented in groups of two or more genes separated by 10 to 600 kb. It has been reported that a similar situation exists in arabidopsis, cucumber, and peach (Prunus persica) MADS-box genes. But the distribution of subfamily had no specificity.

We created a schematic of exon and intron structures (Fig. 2) based on the DNA and coding sequences of the genes, which showed that most type II genes had complex exon and intron structures and that there were 14 to 21 kb long introns in CmMADS10, CmMADS11, CmMADS18, CmMADS35, and CmMADS36. Type I genes had simpler structures, with only one exon and no intron for CmMADS37, CmMADS38, CmMADS39, CmMADS41, CmMADS42, CmMADS44, CmMADS46, CmMADS53, CmMADS55, and CmMADS60. A relatively complex exon–intron structure was only observed in CmMADS49, CmMADS51, and CmMADS56 among type I genes; the others only had a few introns.

**Phylogenetic analysis of melon MADS-box genes.** To study the family relationships among the melon MADS-box genes and classify them into subfamilies, a phylogenetic tree was built after multiple sequence alignment of 62 CmMADS-box genes, 58 AtMADS-box genes, and 26 CsMADS-box genes using MEGA 5.1. The results corresponded with previous results for MADS-box genes. The 62 MADS-box genes of melon were divided into types I and II. There were 26 genes (CmMADS37–CmMADS62) in type I, which was further subdivided into the Mα (6 members), Mγ (4 members), Mδ (16 members), and Mβ (0 members) subfamilies (Fig. 3). Type II included 36 genes, which belonged to the MIKC C subfamily and were distributed in 10 subclasses: SEP (5 members), AGL6 (2), AP1/FUL (2), SCO1 (5), AG (5), AGL17 (4), AGL15 (2), AP3/PI (2), SVP (8), and FLC (1); there were no genes in the AGL12, BS, TM8, and MIKC* groups (Fig. 4). Type II included 36 genes, which belonged to the MIKC C subfamily and were distributed in 10 subclasses: SEP (5 members), AGL6 (2), AP1/FUL (2), SCO1 (5), AG (5), AGL17 (4), AGL15 (2), AP3/PI (2), SVP (8), and FLC (1); there were no genes in the AGL12, BS, TM8, and MIKC* groups (Fig. 4).

Meanwhile, we collected and organized the number of MADS-box subfamily of arabidopsis, rice, peach, Chinese cabbage (Brassica rapa), soybean, and cucumber (Table 2), and compared them with melon. According to the statistics, the melon MADS-box gene family has roughly the same

| Gene   | Gene identification | Location   | Chromosome no. | Protein length (no. of amino acids) | Exons (no.) | Introns (no.) | Subfamily |
|--------|---------------------|------------|----------------|-------------------------------------|-------------|--------------|-----------|
| CmMADS55 | MELO3C011410T1 | Scaffold00014 5456438–5456656 | 3 | 72 | 1 | 0 | Mδ |
| CmMADS56 | MELO3C012107T1 | Scaffold00016 3060965–3064708 | 10 | 339 | 11 | 10 | Mδ |
| CmMADS57 | MELO3C022205T1 | Scaffold00051 2234321–2236745 | 9 | 112 | 3 | 2 | Mδ |
| CmMADS58 | MELO3C022315T3 | Scaffold00052 771547–771881 | 11 | 68 | 2 | 1 | Mδ |
| CmMADS59 | MELO3C022515T1 | Scaffold00052 2277559–2277829 | 11 | 72 | 2 | 1 | Mδ |
| CmMADS60 | MELO3C026299T1 | Scaffold00090 201955–202456 | N/A | 75 | 1 | 0 | Mδ |
| CmMADS61 | MELO3C005617T3 | Scaffold00005 6501777–6505422 | 9 | 145 | 3 | 2 | Mδ |
| CmMADS62 | MELO3C005617T4 | Scaffold00005 6504671–6505422 | 9 | 74 | 2 | 1 | Mδ |

*Chromosomal locations of these genes are not available.
characteristics as in the abovementioned species, but also shows obvious specificity. There are no genes distributing on Mβ and MIKC* subfamily in melon, whereas the gene number of Mδ subfamily is more than other pieces.

**Conserved motif analysis.** To examine the characteristics of the melon MADS-box gene family and the conserved motifs of different subfamilies, we searched for motifs via the MEME software and found 10, which we named motifs 1–10 (Fig. 5).
All 62 melon MADS-box proteins had motif 1, which was found in the N-terminus and is an essential conserved motif for the MADS-box gene family as it encodes the MADS-box domain. Motif 2 was recognized as the highly conserved K domain, which is found in all type II genes and is an important criterion to distinguish type I and II genes. Although the K domain was found in the CmMADS09 and CmMADS18 genes by SMART, it was not observed in these genes in the motif distribution diagram. Motif 4 was only found in MIKC-type genes, not in type I MADS-box genes. Motifs 7 and 8 were only found in the SVP subfamily. Motifs 6, 9, and 10 were present in the AG, Mγ, and AGL17 subfamilies, respectively. Motifs 3 and 5 were found in both type I and II genes. The degree of motif conservation was relatively low except for motifs 1 and 2. 

**Expression analysis in different tissues.** To explore the expression patterns of the melon MADS-box genes in different tissues, we conducted RT-PCR analysis on the 62 MADS-box genes in roots, stems, leaves, flowers, and fruit (0 DAP), and qRT-PCR using 20 genes with tissue-specific expression (underlined in Figs. 6 and 7) in roots, stems, leaves, flowers, and fruit (0, 15, and 35 DAP). The qRT-PCR assays of expression levels in fruit at 15 and 35 DAP were performed because 15 DAP represents the melon development period and 35 DAP represents the mature period. Because CmMADS06 shared high sequence similarity with CmMADS07, CmMADS10 with CmMADS11, CmMADS19 with CmMADS20, CmMADS26 with CmMADS27, and CmMADS50 with CmMADS51, the use of gene-specific primer pairs (Supplement 1) was impossible. Therefore, the transcript levels measured for these genes

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**Fig. 3.** Phylogenetic relationships of 26 type I melon MADS-box genes to their homologs in arabidopsis and cucumber. The tree was constructed using the MEGA software [version 4.1 (Tamura et al., 2007)]. The bootstrap value is 1000. Triangles indicate arabidopsis MADS-box genes, squares indicate cucumber, and dots indicate melon.
were only indicative of the mixed transcript level. The gene expression patterns within each group were often conserved to some extent, although the expression levels of specific members varied in different organs. The RT-PCR and qRT-PCR analyses showed that the expression levels of AG subclass genes, which were specifically expressed in fruit, gradually declined during fruit development and ripening, although the expression level of CmMADS04 was too low to show this. Only one gene (CmMADS06) of the AGL6 subfamily was specifically expressed in flowers and fruit (0 DAP) (Fig. 6). This gene had extremely low or no expression in different developmental stages of other tissues or fruit. In the AP1/FUL subfamily, CmMADS14 and CmMADS15 were specifically expressed in flowers and fruit (Fig. 6), and the transcript level of CmMADS14 during the fruit development stage was relatively high (Fig. 8).

Fig. 4. Phylogenetic relationships of 36 type II melon MADS-box genes to their homologs in arabidopsis and cucumber. The tree was constructed using the MEGA software [version 4.1 (Tamura et al., 2007)]. The bootstrap value is 1000. Triangles indicate arabidopsis MADS-box genes, squares indicate cucumber, and dots indicate melon.

Table 2. Numbers of MADS-box subfamily in seven species. The MADS-box genes were divided into type I and II genes. Type I genes contained Ma, Mb, Mg, and Mδ subfamilies. Type II genes contained MIKC* and MIKC* subfamilies (Henschel et al., 2002; Pařenicová et al., 2003).

| Species                     | Type I | Total (no.) |
|-----------------------------|--------|-------------|
|                             | χ       | χ           |
| Arabidopsis thaliana        | 20      | 17 17 21 4   | 43 2 107 |
| Oryza sativa (Arora et al., 2007) | 13      | 9 10 0 38 5   | 75      |
| Prunus persica (Wells et al., 2015) | 21      | 7 12 0 35 4   | 79      |
| Brassica rapa (Duan et al., 2014) | 27      | 16 22 0 84 11 | 160     |
| Glycine max (Shu et al., 2013) | 18      | 5 11 0 67 5   | 106     |
| Cucumis sativus (Hu et al., 2012) | 5       | 2 3 3 29 1   | 43      |
| Cucumis melo                | 6       | 0 4 16 36 0   | 62      |
Although *CmMADS16* had low expression in leaves and fruit, the AP3/PI subfamily showed specific expression in flowers. *CmMADS17* showed an extremely high expression level in flowers (Fig. 8). Most SEP subfamily genes were specifically expressed in flowers and fruit. During fruit development, *CmMADS25* showed rapid downregulation of expression and *CmMADS26* was upregulated and maintained a high transcript level, even entering into the mature period. The expression of AGL17 subclass genes was specific to and high in roots. The expression levels of SVP subclass genes were different in
different tissues, being highest in leaves and hardly detectable in fruit (Figs. 6 and 8). Among type I genes, the expression of Mα and Mγ subfamily members was low (Fig. 7), but relative quantitative results showed that Mα MADS37 and MADS40 had relative high expression in fruit of 35 DAP and flowers (Fig. 8). The Mδ subfamily genes showed diverse expression in different tissues, such as a high expression levels for CmMADS47, CmMADS58, and CmMADS59 in fruit (35 DAP), fruit (0 DAP), and roots, respectively (Fig. 8). CmMADS47 was specifically expressed in flower and the different stage of fruit (Fig. 7), and the expression level was highest at the mature fruit (Fig. 8).

**Discussion**

In recent years, with the rise and development of sequencing techniques, bioinformatics has rapidly expanded. Bioinformatics-based prediction and detailed studies of the MADS-box gene family have been performed in many species. In this study, we identified and analyzed the MADS-box gene family in melon, finding 62 MADS-box genes. There are more MIKCC-type genes than MIKC*-type genes in some plant species, such as arabidopsis, cucumber, and apple, whereas MIKC*-type genes are not found in melon. Previous research has shown that MIKC*-type MADS-box genes have a large influence on pollen viability. MIKC*-type gene mutations lead to extremely reduced pollen fecundity, because of reduced pollen viability, delayed germination, and aberrant pollen tube growth (Adamczyk and Fernandez, 2009). So other genes in melon could regulate the pollen viability and fecundity. Type I MADS-box genes are divided into the Mα, Mβ, Mγ, and Mδ subfamilies, and have a diverse distribution in the representative species mentioned above.
Generally, few or no genes are distributed in Mδ. We found no Mβ subfamily genes in melon, which means that Mβ subfamily genes are not essential for melon or that their functions have been replaced by other genes. Furthermore, we found 16 members in the Mδ subfamily, whose ratio (61.5%) in type I was higher than in the above mentioned species. We know little about the functions of type I MADS-box genes, so the significance of the distribution of these two subfamilies remains unclear. In arabidopsis, the AGL12 gene is closely related with the development of roots (Tapia-López et al., 2008), and overexpression of this gene in rice not only influences root development, but also induces chlorosis, cell death, and pigment accumulation (Lee et al., 2008). However, we did not find any AGL12 subclass genes in melon, suggesting root development and pigment accumulation are not regulated by AGL12 subclass genes in melon. The TT16 gene in arabidopsis, belonging to the BS group, has a confirmed correlation with seed coloring (Nesi et al., 2002). No BS subfamily genes were found in melon, which may be related to the tendency of melon seeds to be colorless. Similarly, the AGL12 and BS subgroups are not found in cucumber (Hu et al., 2012), which has a close genetic relationship with melon, highlighting the affinity between these two species.

Our exon–intron structure analysis showed that melon type I MADS-box genes were relatively short and had simple structures, but MIKC-type genes had a complex structure with short or long introns. The Mα and Mγ genes usually had no intron or a single intron, whereas MIKC genes and several...
M6 genes contained multiple introns. The similar situation existed in arabidopsis, rice, cucumber, and apple (Malus ×domestica) (Arora et al., 2007; Hu et al., 2012; Pašenčícová et al., 2003; Tian et al., 2015). These results suggested that the structure of MADS-box genes was conservative relatively in these species.

Previous studies have shown that MADS-box genes of diverse subfamilies have different expression patterns in different tissues of arabidopsis, grape, apple, and cucumber. For example, AP3/PI subclass genes are specifically expressed in grape flowers, cucumber fruit, and apple stems, leaves, and flowers, whereas their expression was specific to and high in melon flowers. It is reported that the AP3/PI genes controlled the formation of petals and stamens during arabidopsis flower development, and might act as a switch between the activation of male and the repression of female development (Wuest et al., 2012). Thus, their expression suggested a role in melon flower development. AGL6 subclass genes have been found to be specifically expressed in cucumber fruit and grape flowers, whereas their expression was high in melon flowers and fruit. AGL17 genes are expressed at different levels in diverse tissues and are specifically expressed in grape fruit and cucumber roots; our results in melon were consistent with those in cucumber, with specific expression in roots. In melon, the expression levels of Mα and My subfamily genes were low, and some was even undetectable in roots, stems, flowers, and fruit at 0, 15, and 35 DAP. But a low transcript level of MADS37 and MADS38 was detected in fruit at 0 DAP, and MADS40 in flowers. Quantitative results showed that MADS40 was expressed specifically in flowers, and the relative expression levels are about 170 times than other tissues. We infer that genes of both of these subfamilies are pseudo genes, or are expressed only in specific tissues or at a specific stage. SEP-like genes are expressed in a range of species with fleshy fruits, including tomato (Solanum lycopersicum), peach, strawberry (Fragaria ×ananassa), and so on. It was highly expressed during fruit ripening in tomato and strawberry, and suppression of the gene delayed normal fruit ripening (Seymour et al., 2011; Vrebalov et al., 2002). In this study, the expression of SEP-like gene CmMADS26 increased gradually with the mature of melon fruit. Hence, it is tempting to speculate that the gene CmMADS26 may play similar roles in melon. These data generated here will be useful for analysis of the biological functions of MADS-box family genes in melon growth and development.

In brief, 62 MADS-box genes in melon distributed across its 12 chromosomes, often located within the same small chromosomal region in clusters of two or more genes. Phylogenetic comparisons and expression analysis in this study will provide information for understanding the classification, cloning, and predicting biological functions of the family in melon.

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