Chapter 1

Differential Expression of Transcription Factors in Developing Melon Fruits

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Melon (*Cucumis melo*) is an important diploid crop grown worldwide. To understand the development of Melon fruits, time-course gene expression profiling was conducted for two field-grown melon varieties (Western shipper; F39 and Tuscan da Vinci; TTDV) using the Next Generation Sequencing (NGS) approach. A total of 449014207 reads were mapped to the melon genome and a total of 4469 differentially expressed genes in fruits were identified and used to visualize the transition of transcriptomic profiles during the fruit development. Of these, 120 transcriptional regulators were identified and grouped into 23 different classes, that are implied in fruit development, ripening, and metabolic regulations.

Introduction

Muskmelon (*Cucumis melo L.*) is an important horticultural crop with global production of approximately 27 million tons (*1*) and an economic value in US melon production is US$ 345

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million (*2*). Melon is diploid (2n = 24) and has an approximate genome size of 450 Mbp (*3*). The latest version of the high-quality reference genome of melon (DHL92 v4.0) covers 358Mbp pseudomolecules (*4*). The life cycle of cantaloupe melon is 80 to 120 days, and the fruit ripening takes 35 to 45 days after flowering. Melon varieties are classified into two groups, climactic and non-climactic. Ripening of climactic melon is controlled by an increase of respiration and a plant hormone ethylene, and non-climatic melon has no such peak.

Physiology and transcriptional landscape of developing fruits undergo dynamic transitions during the course of fruit maturation. Such a transition is positively and negatively regulated by an array of transcription regulators. We previously conducted time course analysis of transcriptome of developing fruits of two melon varieties and detected several key regulators of fruit ripening (*5*). Here we further mined the dataset and identified groups of transcriptional regulators whose expression dynamics is specific to fruit development and ripening.

Materials and Methods

RNA-seq Data Processing

As detailed in (*5*), RNA-sequencing (RNA-seq) reads for melon cultivar western shipper (F39) and Tuscan da vinci (TTDV) were obtained by Illumina NovaSeq 6000 with 150 bp paired-end mode (Gene Expression Omnibus accession number GSE178084). These reads were aligned to the Melonomics genome sequence: CM3.6.1_pseudomol.fa (https://www.melonomics.net/melonomics.html#/download) along with the annotation reference: CM4.0.gtf, using STAR (v2.5.4) with the setting of -outFilterScoreMinOverLread and –outFilterMatchNminOverLread parameters to be 0.1 for the alignment. The uniquely mapped reads were used to count the number of reads per gene while mapping.

RNA-seq datasets were obtained for differential expression (DE) analysis to identify 4469 differentially expressed genes (DEGs) in fruits as desribed previously in (*5*). To identify transcriptional regulators in DEGs, gene ontology analysis was performed for DEGs in each variety and genes with DNA binding activity (GO:0003677) were extracted using GO slim gene classification at http://cucurbitgenomics.org/funcat. DEGs in GO:0003677 commonly found in F39 and TTDV were identified, and manually curated to generate fruit-specific transcriptional regulator geneset.

Co-Expression Network Visualization and Clustering of DE Transcription Regulators

Based on the generated RNA-seq count matrix by the aforementioned quantification process, we performed normalization on counts using DESeq2 package (v1.30.0). Then, the normalized expression values of the 120 transcriptional regulators across samples were used to calculate the Pearson correlation p-values between each pair of these 120 transcriptional regulators (*6*, *7*). Based on these p-values, the interaction network were constructed and visualized by Igraph (v1.2.6) package (*8*) by varying the threshold to prune the edges among all the pairs of these 120 transcription regulators. We set the Pearson correlation p-value threshold at 1e-6 to be considered as significantly correlated pairs such that the constructed network has a node degree distribution following the power law, which is often manifested in many real-world networks (*9*). The resulting co-expression network identified the edges having the positive Pearson correlation with p-values lower than 1e-6., which naturally leads to the clustering of transcription regulators based on the co-expressoin patterns.

Results and Discussion

To understand the landscape of transcriptional regulators in developing and ripening melon fruits, we performed data mining of our RNA-seq dataset prepared with two field-grown melon varieties (5). The 4469 DEGs were fitered by Gene Ontology analysis and genes commonly detected in two varieties were identified. After removing genes unrelated to transcription factors, expression pattern of 375 DEGs were inspected using heatmap analysis. The expression patterns of total 120 genes were classified as fruit-specific and these genes were classified into 23 groups based on their functional annotations (Table 1). Expression patterns and expected functions of representative groups are discussed below (illustrated in Figure 1).

Table 1. Fruits-Specific Transcription Factors Identified in Melon

ID	*Genes*	*Cluster*	*Degree* [a]	*Class*	*Annotation*
1	MELO3C007572.2[b]	NA	0	AP2	AP2-like ethylene-responsive transcription factor TOE3 (AP2a)
2	MELO3C008318.2	NA	0	AP2	Dehydration-responsive element-binding protein 2C
3	MELO3C024315.2	A	1	AP2	Ethylene-responsive transcription factor
4	MELO3C017940.2	NA	0	AP2	Ethylene-responsive transcription factor
5	MELO3C021306.2	A	13	AP2	Ethylene-responsive transcription factor RAP2-3-like
6	MELO3C011372.2	B	2	ARF	Auxin response factor
7	MELO3C025070.2[b]	NA	0	ARF	Auxin response factor
8	MELO3C007104.2[b]	A	2	ARF	Auxin response factor
9	MELO3C005590.2	NA	0	AT	AT-hook motif nuclear-localized protein 1
10	MELO3C021218.2	A	14	AT	AT-hook motif nuclear-localized protein 9-like
11	MELO3C010905.2	NA	0	B3	B3 domain-containing protein REM16-like
12	MELO3C011167.2	B	1	bZip	Basic leucine zipper 9
13	MELO3C015754.2	A	4	bZip	Basic-leucine zipper (BZIP) transcription factor family
14	MELO3C016835.2	NA	0	bZip	bZIP transcription factor 53-like
15	MELO3C017064.2	A	4	bZip	bZIP transcription factor 60
16	MELO3C009753.2	A	3	bZip	Transcription factor PERIANTHIA
17	MELO3C006784.2	NA	0	bZip	Transcription factor RF2a, putative
18	MELO3C012485.2	A	5	bZip	Transcription factor TGA2-like
19	MELO3C023710.2	A	1	bZip	Transcription factor VIP1
20	MELO3C002099.2	NA	0	CaM	Calmodulin binding protein-like, putative
21	MELO3C021280.2	B	3	CaM	Calmodulin-binding transcription activator
22	MELO3C018642.2	B	2	CaM	Calmodulin-binding transcription activator 2

Table 1. (Continued). Fruits-Specific Transcription Factors Identified in Melon

ID	*Genes*	*Cluster*	*Degree* [a]	*Class*	*Annotation*
23	MELO3C009859.2	B	3	DOF	Cyclic dof factor 2
24	MELO3C007274.2	D	3	DOF	Dof zinc finger protein DOF1.7-like
25	MELO3C013904.2	C	2	GATA	GATA transcription factor 26-like
26	MELO3C023350.2	A	6	GATA	GATA transcription factor
27	MELO3C023076.2	A	17	GATA	GATA transcription factor 1
28	MELO3C023333.2	A	2	HD	BEL1-like homeodomain protein 7
29	MELO3C014565.2	A	4	HD	Homeobox domain-containing protein
30	MELO3C020577.2	NA	0	HD	Homeobox prospero
31	MELO3C011979.2	D	3	HD	Homeobox protein knotted-1-like 6
32	MELO3C010678.2	A	3	HD	Homeobox protein knotted-1-like 6
33	MELO3C011281.2	A	3	HD	Homeobox protein knotted-1, putative
34	MELO3C007260.2	B	4	HD	Homeobox-DDT domain protein RLT1 isoform X1
35	MELO3C012079.2	NA	0	HD	Homeobox-leucine zipper family protein
36	MELO3C007666.2[b]	NA	0	HD	Homeobox-leucine zipper protein
37	MELO3C021978.2	D	3	HD	Homeobox-leucine zipper protein ATHB-20-like
38	MELO3C023514.2	NA	0	HD	Homeobox-leucine zipper protein HOX11-like
39	MELO3C013937.2	B	1	HD	Homeodomain-like superfamily protein
40	MELO3C002209.2	A	18	HD	Homeobox leucine zipper family protein
41	MELO3C003468.2	A	3	HD	Homeobox leucine-zipper protein
42	MELO3C005992.2	A	7	HD	Homeobox-leucine zipper protein ATHB-7
43	MELO3C013727.2	A	8	HD	WUSCHEL-related homeobox 13
44	MELO3C010841.2	NA	0	HD	WUSCHEL-related homeobox 4
45	MELO3C017032.2	NA	0	HD	WUSCHEL-related homeobox 8-like
46	MELO3C007560.2	NA	0	HSF	Heat stress transcription factor
47	MELO3C006891.2	A	4	HSF	Heat stress transcription factor B-2b-like
48	MELO3C018723.2	A	3	LSH	Protein LIGHT-DEPENDENT SHORT HYPOCOTYLS 10
49	MELO3C020521.2	NA	0	LSH	Protein LIGHT-DEPENDENT SHORT HYPOCOTYLS 6
50	MELO3C019694.2	C	2	MADS	AGAMOUS MADS box factor transcription factor

Table 1. (Continued). Fruits-Specific Transcription Factors Identified in Melon

ID	*Genes*	*Cluster*	*Degree* [a]	*Class*	*Annotation*
51	MELO3C002691.2[b]	NA	0	MADS	MADS box transcription factor AGAMOUS (TAGL1)
52	MELO3C022316.2[b]	C	1	MADS	MADS-box transcription factor (RIN)
53	MELO3C026300.2[b]	NA	0	MADS	MADS-box transcription factor (RIN)
54	MELO3C024001.2	B	1	MADS	MADS-box transcription factor (AGL15)
55	MELO3C013580.2	NA	0	MBF1	Multiprotein-bridging factor 1b
56	MELO3C004553.2	NA	0	MBF1	Multiprotein-bridging factor 1c
57	MELO3C024440.2	F	1	Myb	Myb family transcription factor family protein
58	MELO3C024799.2	NA	0	Myb	Transcription factor MYB44-like
59	MELO3C022302.2	B	1	Myb	Protein LHY-like isoform X1
60	MELO3C005840.2	C	4	Myb	Protein REVEILLE 6-like
61	MELO3C025151.2	A	1	Myb	Transcription factor DIVARICATA
62	MELO3C019904.2	NA	0	Myb	Transcription factor DIVARICATA
63	MELO3C009188.2	NA	0	Myb	Transcription factor DIVARICATA-like
64	MELO3C017315.2	A	2	Myb	Transcription factor MYB24-like
65	MELO3C007586.2	A	1	Myb	Transcription factor MYB44
66	MELO3C025894.2	A	7	Myb	Two-component response regulator
67	MELO3C017185.2	NA	0	NAC	NAC domain protein
68	MELO3C016540.2[b]	B	3	NAC	NAC domain protein (NOR)
69	MELO3C010632.2	NA	0	NAC	NAC domain-containing protein (ATAF1)
70	MELO3C001996.2	NA	0	NAC	NAC domain-containing protein 100-like
71	MELO3C010923.2	NA	0	NAC	NAC domain-containing protein 13-like
72	MELO3C012391.2	A	13	NAC	NAC domain-containing protein 16
73	MELO3C019954.2	A	10	NAC	NAC domain-containing protein 17-like
74	MELO3C013173.2	G	1	NAC	NAC domain-containing protein 17-like
75	MELO3C014510.2	A	3	NAC	NAC domain-containing protein 40
76	MELO3C002628.2	NA	0	NAC	NAC domain-containing protein 73-like
77	MELO3C019845.2	B	1	NAC	NAC domain-containing protein 78-like isoform X2
78	MELO3C008056.2	A	1	NAC	NAC domain-containing protein 82
79	MELO3C014505.2	NA	0	NAC	NAC domain-containing protein 90
80	MELO3C000247.2	NA	0	NAC	"NAC domain-containing protein, putative"
81	MELO3C017754.2	A	15	NAC	NAC domain-containing protein
82	MELO3C022342.2	A	3	NAC	NAC domain-containing protein 83

Table 1. (Continued). Fruits-Specific Transcription Factors Identified in Melon

ID	*Genes*	*Cluster*	*Degree* [a]	*Class*	*Annotation*
83	MELO3C015196.2	A	4	NFY	Nuclear transcription factor Y subunit A-1
84	MELO3C014590.2	A	9	NFY	Nuclear transcription factor Y subunit A-1-like protein
85	MELO3C023554.2	NA	0	NFY	Nuclear transcription factor Y subunit A-9-like
86	MELO3C009309.2	A	4	NFY	Nuclear transcription factor Y subunit B
87	MELO3C011726.2	C	2	NFY	Nuclear transcription factor Y subunit B
88	MELO3C003375.2	NA	0	RR	Two-component response regulator-like protein APRR2
89	MELO3C013874.2	A	4	RR	Two-component response regulator-APRR2-like protein
90	MELO3C005370.2	H	1	SEU	Transcriptional corepressor SEUSS
91	MELO3C009639.2[b]	A	8	SPL	Squamosa promoter binding protein (CNR)
92	MELO3C002048.2	A	4	SPL	Squamosa promoter-binding-like protein 12 isoform X1
93	MELO3C014895.2	A	13	SPL	Squamosa promoter-binding-like protein 13A
94	MELO3C021144.2[b]	A	10	SPL (CNR)	Squamosa promoter-binding-like protein 8
95	MELO3C016092.2	C	2	TCP	Transcription factor TCP4
96	MELO3C019745.2	E	1	TCP	Transcription factor TCP4-like
97	MELO3C024459.2	F	1	TCP	Transcription factor, TCP
98	MELO3C012086.2	A	5	TCP	Transcription factor TCP15
99	MELO3C022331.2	A	12	TCP	Transcription factor TCP20-like
100	MELO3C024673.2	A	1	TTF	Trihelix transcription factor
101	MELO3C022356.2	H	1	TTF	Trihelix transcription factor ASR3
102	MELO3C000615.2	NA	0	TTF	Trihelix transcription factor GT-2
103	MELO3C008175.2	E	1	WRKY	WRKY transcription factor
104	MELO3C010223.2	A	2	WRKY	WRKY transcription factor
105	MELO3C024209.2	G	1	WRKY	WRKY transcription factor 1 isoform X1
106	MELO3C022014.2	C	1	WRKY	WRKY transcription factor, putative
107	MELO3C000030.2	A	5	WRKY	LOW QUALITY PROTEIN: probable WRKY transcription factor 48
108	MELO3C009097.2	NA	0	WRKY	WRKY transcription factor
109	MELO3C014066.2	A	7	WRKY	WRKY transcription factor

Table 1. (Continued). Fruits-Specific Transcription Factors Identified in Melon

ID	*Genes*	*Cluster*	*Degree* [a]	*Class*	*Annotation*
110	MELO3C015611.2	NA	0	ZF	ABSCISIC ACID-INSENSITIVE 5-like protein 2
111	MELO3C019925.2	NA	0	ZF	Abscisic acid-insensitive 5-like protein 2
112	MELO3C005595.2	A	9	ZF	Zinc finger A20 and AN1 domain-containing stress-associated protein 1-like
113	MELO3C014608.2	A	13	ZF	Zinc finger A20 and AN1 domain-containing stress-associated protein 8
114	MELO3C023537.2	A	15	ZF	Zinc finger A20 and AN1 domain-containing stress-associated protein 8-like
115	MELO3C021987.2	D	3	ZF	Zinc finger homeodomain protein 1
116	MELO3C006469.2	A	12	ZF	Zinc knuckle (CCHC-type) family protein, putative
117	MELO3C000099.2	A	4	ZF	Zinc-finger homeodomain protein 10-like
118	MELO3C007832.2	A	12	ZF	Zinc-finger homeodomain protein 2
119	MELO3C012094.2	A	5	ZF	Zinc-finger homeodomain protein 9
120	MELO3C002157.2	A	1	ZF	Zinc-finger homeodomain protein 9-like

[a] Degree of coexpression. [b] The information of tomato homologous genes is described in the main text.

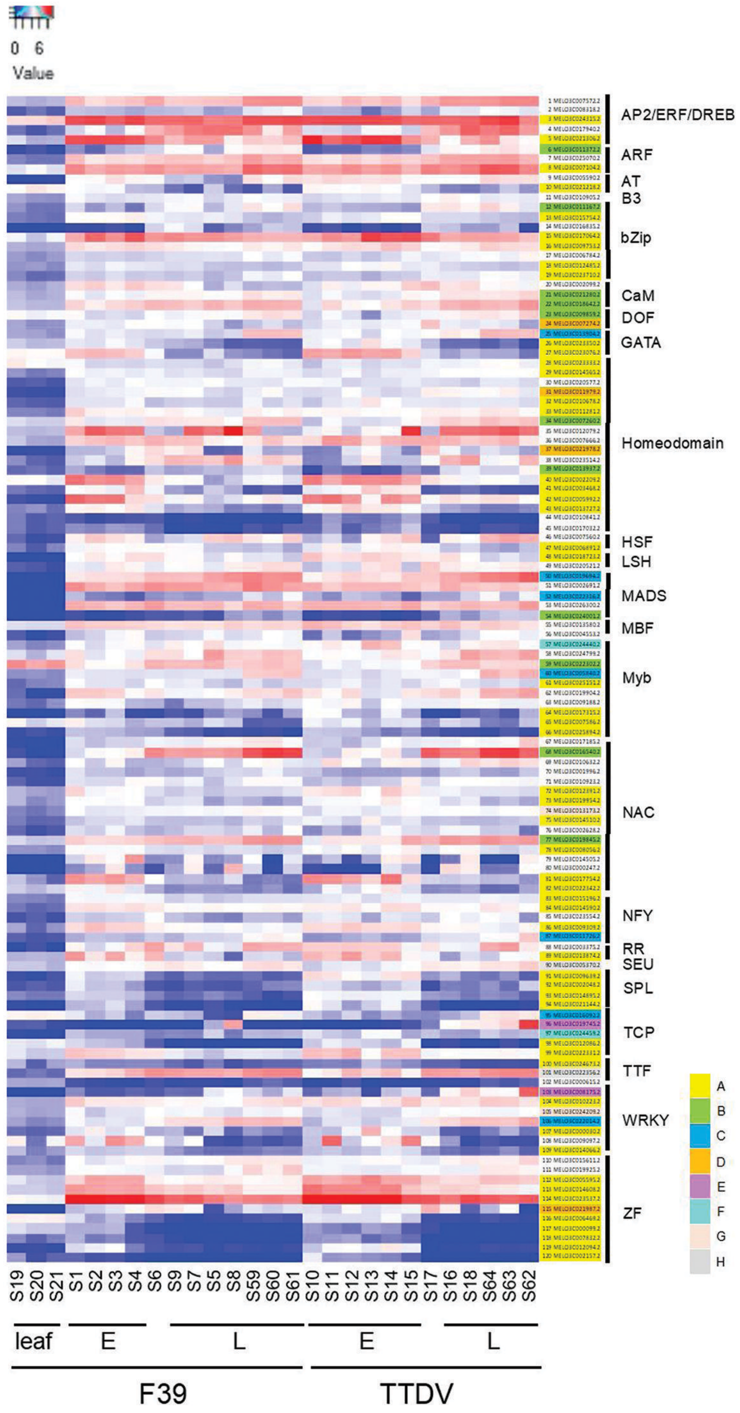

Figure 1. Heat map of TF expression during the melon fruit development. E and L denote early (E) and late (L) maturity stages. The samples are 14-day-old leaves (S19, S20, S21) and fruit flesh harvested at 20-25 days (S1, S2, S3, S10, S11, S12), 30-35 days (S4, S5, S6, S13, S14, S15), 40 days (S7, S8, S9, S16, S17, S18), and 45 days (S59, S60, S61, S62, S63, S64) after flowering.

AP2/ERF/DREB

Five TF DEG belonged to this family. MELO3C007572 that express throughout the time course is a likely ortholog of tomato APETALA2a, which function as a negative regulator of ethylene production, but a positive regulator of other ripening aspects, such as chlorophyll degradation and carotenoid biosynthesis (*10*, *11*). MELO3C024315.2, MELO3C017940.2, and MELO3C021306.2 are homologous to Arabidopsis RAP2.2, HRE2, and RAP2.3, respectively, important for hypoxia response.

ARF Family

MELO3C011372.2, MELO3C025070.2, and MELO3C007104.2 represent homolog of Arabidopsis ARF17, ARF8, and ARF6, respectively. ARF6 and ARF8 are known to promote jasmonic acid production and flower maturation. Downregulation of tomato ARF6/ARF8 leads to floral development defects and female sterility.

AT-hook Family

MELO3C005590.2 and MELO3C021218.2 are homolog of AHL (AT-hook nuclear localized protein family) that can bind to AT-rich DNA sequences. AT-hook family protein is conserved in both prokaryotes and eukaryotes, and Arabidopsis AHL proteins were able to regulate various growth and developmental processes, including hypocotyl elongation, enlargement of leaves and flowers, and fruits (*12*).

B3 Domain Family

MELO3C010905.2 is homologous to REM (Reproductive Meristem) family of AP2/B3-like transcriptional factors, which are targets of flower development regulator genes (*13*). MELO3C010905.2 shows high homology to VRN1 and RVN1, which are required for flowering induction by vernalization (*14*).

bZip Family

MELO3C011167.2 is homologous to AtbZip9 involved in vascular development (*15*). MELO3C017064.2 is a homolog of Arabidopsis bZip60, a regulator of ER-stress signals (*16*). MELO3C009753.2 is a homolog of PERIANTHIA important for gynoecium and fruit development in Arabidopsis. MELO3C009753.2 and MELO3C012485.2 also show high homology to AHBP-1B/AtTGA2, which interacts with a key regulator in SA signaling pathway NPR1 to activate PR1 transcription (*17*). MELO3C006784.2 and MELO3C023710.2 are homolog of Arabidopsis group I bZIP proteins bZIP69 and VIP1, respectively (*18*), and respond to hypoosmotic stress (*19*).

CaM Family

MELO3C002099.2 is a homolog of SAR DEFICIENT 1(SARD1), which is required for SA production and defense responses (*20*). MELO3C021280.2 is a homolog of CAMTA5, a regulator for cold-inducible DREB1B and DREB1C expressions (*21*). MELO3C018642.2 is a homolog of Arabidopsis CAMTA2, which is involved in plant immunity (*22*) and aluminum transport (*23*).

DOF Family

MELO3C009859.2 is a homolog of CDF2, which controls the miRNA expression CDF2 and flowering time in Arabidopsis (*24*).

GATA

MELO3C013904.2 is a homolog of GATA26 in Arabidopsis. MELO3C023350.2 is a homolog of GATA5. MELO3C023076.2 is a homolog of GATA1, a regulator of chloroplast development, growth, and division with a nucleus-localized transcription factor GNC in Arabidopsis. These transcription factors have high expression in green tissues, and cytokinin affects their expression level (*25*).

Homeodomain (HD)

MELO3C023333.2 is a homolog of a transcriptional repressor BEL1-like Homeodomain 6 (BLH6), which forms a heterodimer with KNOTTED-like from Arabidopsis 7 (KNAT7) and involves in the formation of the secondary cell wall (*26*). MELO3C011979.2 and MELO3C010678.2 show high homology to Arabidopsis KNAT6, which functions in shoot apical meristem maintenance (*27*). MELO3C011281.2 is a homolog of KNAT3, a member of the class II KNAT family. KAT3 works with KANT7 to synthesize secondary cell walls (*28*). MELO3C007260.2 is a homolog of HOMEOBOX-1(HB-1)/RINGLET 1 (RLT1). RLT1 is a subunit of the Imitation of Switch (ISWI) complex, ATP-dependent chromatin remodeling factors. In Arabidopsis, the ISWI is required to control fertility (*29*). MELO3C012079.2 and MELO3C002209.2 are homologous genes of ATHB13 in a homeodomain leucine zipper class I (HD-ZipI). ATHB13 is induced by low temperature, drought, and salinity (*30*). MELO3C007666.2 is a homolog of ABA INSENSITIVE GROWTH 1(ABIG1)/HAT22. ABIG1 is a part of absisic acid (ABA) signaling to conrol plant growth, especially growth inhition and senecense induced by drought (*31*). Tomato HAT22 is implied in the fruit metabolic shift from early development and subsequent ripening (*32*). MELO3C021978.2 is a homolog of ATHB20. Lack of *ATHB20* increased seed dormancy and ABA sensitivity in Arabidopsis (*33*). MELO3C023514.2 is a homolog of HOMEOBOX FROM ARABIDOPSIS THALIANA 14 (HAT14). MELO3C013937.2 is a homolog of DUO POLLEN 3 (DUO3). DUO3 is known as an important regulator of male germline development and embryogenesis (*34*). MELO3C003468.2 has homology to ATHB8, which works to stabilize preprocambial cell specification to form leaf vein (*35*). MELO3C005992.2 is a homolog of ATHB7, which of transcripts are induced by ABA. ATHB7 involves in the development and abiotic stress responses like drought stress (*36*, *37*). MELO3C013727.2 and MELO3C017032.2 are homologs of WUSCHEL RELATED HOMEOBOX 13 (WOX13)/HB-4. Arabidopsis WOX13 is a positive regulator of replum and important in the mediolateral patterning of the fruit (*38*). MELO3C010841.2 is a homolog of WOX4, a regulator of cambium activity (*39*).

Heat Shock Factor (HSF)

MELO3C007560.2 is a homolog of HSF4, also known as HSFB1 in Arabidopsis. HSF1 belongs to class B in the HSF transcription factor family, and the members work as transcriptional repressors. Lack of HSF4 and another The class B HSF, HSFB2b show high transcriptional level of the defensin gene PDF1.2A/B (*40*). The HSB2b homolog in melon is MELO3C006891.2 in the Table1.

LSH

MELO3C018723.2 and MELO3C020521.2 belongs to LIGHT-DEPENDENT SHORT HYPOCOTYLS family involved in light signaling (*41*).

MADS

MELO3C026300.2 and MELO3C022316.2 are homolog of tomato RIN (ripening inhibitor) (*42*). MELO3C002691.2 is homolog of tomato TAGL1 involved in fruit metabolite biosynthesis (*43*). MELO3C024001.2 is a homolog of Arabidopsis AGAMOUS-LIKE 15(AGL15). In Arabidopsis, AGL15 works with AGL18 to repress the floral transition in Arabidopsis (*44*).

Multiprotein Bridging Factor 1 (MBF1)

MBP1 is a conserved transcription co-activators to regulate various physiological processes. Three MBP1 proteins were found in the Arabidopsis genome. AtMBP1a and AtMBP1b belong to Class I, and AtMBP1c belongs to Class II (*45*). MELO3C013580.2. is a homolog of AtMBF1B and MELO3C004553.2 is a homolog of AtMBF1C. Heat stress and H_2O_2 induced *AtMBP1C* expression drastically, although *AtMBF1B* expression did not change (*45*).

Myb Family

MELO3C024440.2 is homologous to NID1 involved in nitrogen response. MELO3C024799.2 and MELO3C007586.2 are homologous to Myb73 involved in auxin signaling (*46*). MELO3C025894.2 is homologous to BROTHER OF LUX ARRHYTHMO involved in circadian clock (*47*). MELO3C022302.2 is homologous to circadian clock gene LATE ELONGATION OF HYPOCOTYL 1 (*48*). MELO3C005840.2 is homolog of circadian clock gene REVEILLE 6 (*49*). MELO3C025151.2, MELO3C019904.2 and MELO3C009188.2 encodes DIVARICATA homologs involved in ventral identity during floral development in Antirrhinum (*50*).

NAC Family

MELO3C016540 is a homolog of tomato NOR (NO RIPENING), and its expression gradually increased during the fruit development. MELO3C010632.2 is homologous to ATAF1 involved in ABA biosynthesis (*51*). MELO3C019845.2 expresses higher in the mature stage, and is homologous to NAC78, regulating flavonoid biosynthesis. MELO3C012391.2 is homologous to NAC52/53, functioning in histone demethylation (*52*). MELO3C017754.2 is homologous to VNI2 involved in xylem formation (*53*).

NF-Y Family

MELO3C015196.2, MELO3C014590.2, and MELO3C023554.2 are homologous to NF-YA1 and A9, involved in fruit development. MELO3C009309.2 and MELO3C011726.2 are homologous to NF-YB8 and B10, implicated in fruit ripening (*54*).

APRR Family

MELO3C003375.2, and MELO3C013874.2 are homologous to APRR2, that interact with TCP family protein, and affect fruit pigments (*55*, *56*).

SEUSS

MELO3C005370.2 is homologous to corepressor SEUSS involved in diverse developmental events (*57*).

SPL Family

MELO3C009639.2 and MELO3C021144.2 are squamosa promoter binding protein-like family and homologous to tomato COLORLESS NO RIPE protein (*58*).

TCP Family

MELO3C016092.2 and MELO3C019745.2 are homologous to Arabidopsis TCP4. TCP family is associated with various aspect of cell growth and development in plants (*59*). MELO3C012086.2 and MELO3C022331.2 are homologous to TCP15 and TCP20, which interact with ARR2 (*60*).

Trihelix Family

MELO3C000615.2 is homologous to Arabidopsis GTL1 cpntrolling proidy levels (*61*). MELO3C022356.2 is homologous to ASR4 involved in immunity (*62*). MELO3C024673.2 is homologous to GT2L controlling cold and salt responses (*63*).

WRKY Family

MELO3C010223.2, MELO3C024209.2, and MELO3C022014.2 are homologous to Arabidopsis WRKY3 and WRKY4 involved in pathogen responses (*64*). MELO3C008175.2 and MELO3C009097.2 are homologous to WRKY75 and WRKY70, respectively, also involved in pathogen responses (*65*). MELO3C014066.2 is homologous to WRKY2/WRKY34 involved in pollen development (*66*).

Zinc Finger Family

MELO3C015611.2 and MELO3C019925.2 are homologous to ABA-RESPONSIVE ELEMENT BINDING PROTEIN 3 involved in ABA-inducible gene expression (*67*). MELO3C005595.2, MELO3C014608.2, and MELO3C023537.2 belongs to stress associated proteins (SAPs) containing A20/AN1 zinc finger domains, novel regulators of stress responses (*68*). MELO3C021987.2, MELO3C000099.2, MELO3C007832.2, MELO3C012094.2, and MELO3C002157.2 encode Zinc-Finger homeodomain proteins whose functions are implied in frolar development (*69*). MELO3C006469.2 is homologous to zinc knuckle protein important for phytochrome A signaling (*70*).

Clusters of Transcription Factors

To identify the regulatory network of fruit gene expression, we set to identify the co-expression network of transcription factors identified above. Based on Pearson correlation analysis, eight clusters (A-H) were identified (Figure 2). A major cluster (cluster A) contained 53 genes and represented genes with decreasing trends over the time course of the fruit development. TFs in SPL and ZF gene families are predominant in Cluster A. These genes correspond to COLORLESS NO

RIPE homolog and 4 ZF-HD proteins. However, ABI5-like proteins in ZF family showed increasing trends and did not cluster with other ZF TFs. Also many HD (8/18), including WUSCHEL-like HD, and MYB (4/10) and NAC (6/16) TFs were associated with cluster A. These TFs may function in early fruit development. Clusters B and C represented TFs with increasing trends over the course of the fruit development. Master regulators of fruit ripening, such as NOR homolog (B) and RIN homolog (C), were included in these clusters. Together, clusters B and C contain 18 TFs. Cluster D (4 genes) shows relatively stable expression but had expression peaks around the transition stage S6 (F39) and S17 (TTDV). DOF1.7-like protein, HB protein ATHB20-like, and Zinc-Finger homeodomain 1 protein belong to this cluster. Minor clusters E-H contain only 2 genes/cluster. We were not able to identify co-expression patterns in 47 genes with any clusters or among each other. Several unclustered TFs are known ripening regulators, such as AP2a homolog (MELO3C007572.2), RIN homolog (MELO3C026300.2) and TAGL1 homolog (MELO3C002691.2). In our dataset, these TFs are expressed throughout the fruit development, and may regulate gene expression in cell metabolism directly (*71*), rather than through cascades of secondary transcription factors.

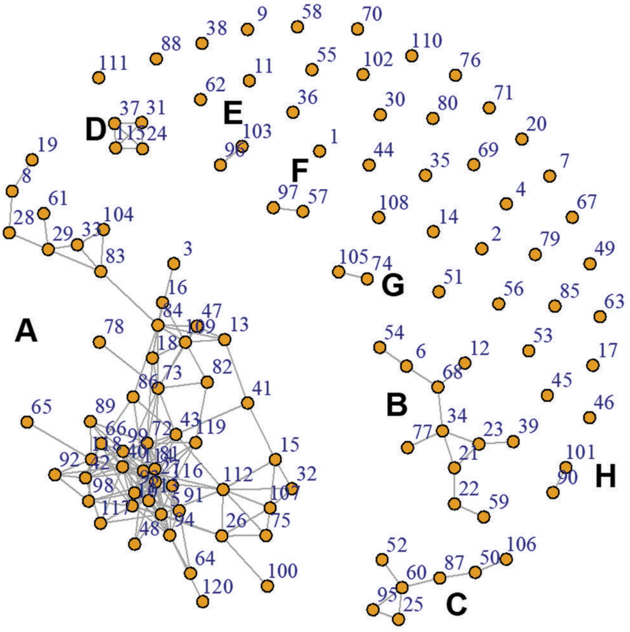

Figure 2. Association of transcription factors in Table 1 during melon fruit ripening. Edges indicate coexpression with $P<10^{-6}$ in Pierson correlation analysis.

Conclusion

The transcriptome analysis based on the time-course RNA-seq data from F39 and TTDV identified 120 melon TFs that are specifically expressed in ripening fruits. Inspection of functional annotations and clustering analysis of expression patterns showed that a large number of TFs manifested declining expression levels over the course of the fruit development, followed by a cluster with increasing trends. Several known master regulator TFs produced distinct expression patterns, such as early expression of CNR, late expression of NOR and AGL15. Other master TFs, AP2a, RIN, TAGL1, and ATAF1, showed rather steady expression and failed to connect with other TFs. Furthermore, some TFs with increasing trends failed to be associated with the existing clusters. This may indicate direct regulation of expression of ripening-related enzymes by master regulators, yet, it is possible that the dataset used in this study was too small to detect weak to moderate co-expressions. The larger dataset with diverse growth conditions is currently under analysis and will likely strengthen our analysis to establish the fine regulatory network of TFs in the melon fruit development.

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