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Structural characteristics of the melon mitochondria genome

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Abstract

The mitochondrial genome can provide important genetic information of melon. We reported the mitochondrial genome sequence of melon in a previous study, the structural characteristics of the melon mitochondrial genome were further analyzed in the present study. The mitochondrial genome of melon is comprised of three circular DNA molecules, with a total length of about 2.9 Mb, contains 4,861 pairs of homologous repeats, 439 pairs of inverted repeats, 653 tandem repeats and 218 SSR sequences. The coding genes accounted for 1.54% and non-coding gene sequences accounted for 98.46% of the melon mitochondrial genome. The total repetitive sequence of mitochondrial genome of melon was the highest among *Cucumis melo*, *Cucumis sativus*, *Cucurbita pepo* and *Citrullus lanatus*. The large number of repeated sequences and nuclear genome sequences were the main reason for the increasing size and variation of melon mitochondrial genome. Melon mitochondrial genome has the highest GC content and tRNA quantity. These regions were the main source of mitochondrial genome differences among all species here analyzed.

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INTRODUCTION

Liverwort (*Marchantia polymorpha*) was the first complete mitochondrial genome sequenced[1]. In Cucurbitaceae, sequencing of the mitochondrial genome of watermelon, zucchini[2] and cucumber[3] have been completed. Ward et al.[4] suggested that the mitochondrial genome of melon was about 2.9 Mb. In 2011, the mitochondrial genome sequence of melon was reported, mainly composed of five scaffolds and four contigs[5]. In 2020, we reported the completed mitochondrial genome of melon including a main loop and two small loops[6]. Melon mitochondrial genome can provide additional genetic information, such as cytoplasmic male sterility and mitochondrial RNA editing. Therefore, further research focusing on the mitochondrial genome of melon is expected.

Plant mitochondrial genomes have more complex structural characteristics. The size of plant mitochondrial genomes varies greatly between plants, ranging from 187 kb in liverwort[1] to 11.3 Mb in flycatcher[7], but there is no linear relationship between the size of the mitochondrial genome and the number of genes it encodes, flycatcher has 32 coding genes, but ground money has 74 coding genes. The GC content of the mitochondrial genome is about 43%–45%. The number of protein-coding genes in plant mitochondrial genomes is typically 30–50, mainly including *complex I-V* genes, cytochrome C biosynthetic genes, ribosomal protein genes, *matR* genes, *mttB* genes and ORFs of unknown function. The non-protein-coding genome includes rRNA and tRNA genes. rRNA includes 5s rRNA, 18s rRNA and 28s rRNA; tRNA includes three different types of tRNAs, including its own intrinsic tRNA and tRNAs transferred from the chloroplast and nuclear genomes, but with the exception of liverwort, the mitochondrial genomes of all other plants do not cover tRNA genes encoding all 21 amino acids.

RNA editing refers to a predetermined codon modification caused by nucleotide changes at the RNA level[8]. This molecular process mainly exists in chloroplasts and mitochondria of plants, where it maintains the normal biological functions of these organelles. Mitochondrial RNA editing mostly occurs along protein coding regions. In this case, RNA editing can increase the conservation of the encoded protein product, with regards to its primary structure, between different species[9]. The number of RNA editing sites can vary greatly among different species. There are only 11 RNA editing sites in the mitochondrial genome of the moss *Physcomitrella patens*[10], in contrast to 456 and 692 RNA editing sites identified, respectively, in the mitochondria of *Arabidopsis thaliana*[11] and *Gossypium spp*[12]. Lu et al.[13] studied the process of RNA editing in eight species from four families of gymnosperms and found a substantial difference in the number of RNA editing sites as well as their positions along the DNA of distinct families and genera of gymnosperms. Mitochondrial RNA editing can affect many important traits in plants. RNA editing of the cotton mitochondrial *Ghatp1* gene, at the C1292 and C1415 loci, affects ATPase production and promotes epidermal hair and fiber elongation[12]. In tomato, a decreased RNA editing of *nad3* and *sdh4* genes can disrupt the biological function of mitochondria, therefore, reduce the respiratory efficiency of the fruit, which can ultimately inhibit its ripening[14]. Inadequate and deviated mitochondrial RNA editing may be associated

with certain biological conditions, such as cytoplasmic male sterility[15]. The structural analysis of the mitochondrial genome will provide a theoretical basis for an in-depth study of the genetic characteristics of melon mitochondria.

MATERIALS AND METHODS

Materials

Mitochondrial genomes were sequenced and assembled using dark-treated MR-1 yellowing seedlings as plant material[6].

Genome annotation

The annotation of protein-coding genes in the mitochondrial genome was carried out using BLASTN and BLASTX to search the nucleotide and protein libraries of the NCBI/GenBank database. rRNA and tRNA annotations were carried out using RNAmmer (rnammer -S euk-m lsu,ssu,tsu -xml melon.xml -gff melon.gff -hmelon.hmmreport < melon.fsa) and tRNAScan-SE (tRNAscan-SE -o tRNA.out -f rRNA.ss -m tRNA.stats /home/gjs /fasta/h.fa).

Forward repeats in the mitochondrial genome were analyzed using Reputer software[16] with minimum repeat size set to 20 bp, hamming distance of 0, and similarity greater than 90%. Inverted repeats were analyzed using IRF (Inverted Repeats Finder) software[17] with parameters set to: match 2, mismatch 3, delta 5, match probability 80, indel probability 10, min-score 40, max-length to report 500,000, max-loop 500,000. Tandem repeat was analyzed using TRF (Tandem Repeat Finder) software with parameters set to: min. align. score 50, max. period size 500. Simple sequence repeat was analyzed using MISA software[18] with parameters set to 1 base. The parameters were set to 10 and more repetitions of 1 base, five and more repetitions of 2 bases, four and more repetitions of 3 bases, four and more repetitions of 4 bases, four and more repetitions of 5 bases, four and more repetitions of 6 bases, and only those base repetitions meeting the criteria were considered as microsatellite sequences.

Comparative analysis of mitochondrial genomes

Analyses were delineated using Easyfig[19], and then illustrated with MapChart 2.2[20]. Comparative analysis of sequences between organelle and nuclear genomes was performed by BLASTN and Tbtools.

Prediction of mitochondrial RNA editing sites

The RNA editing sites of all protein-coding gene sequences, located in the mitochondrial genomes of melon (*Cucumis melo*), cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*) and zucchini (*Cucurbita pepo*), were predicted by PREP-Mt[21].

Phylogenetic analysis

Mitochondrial genomes were aligned using ClustalX (www.clustal.org/clustal2). A phylogenetic tree was constructed *via* Neighbor Joining (NJ) using Mega7 software.

RESULTS

Structural characteristics of the melon mitochondrial genome

The melon mitochondrial genome contains 4,861 pairs of forward repeats, 439 pairs of inverted repeats, 653 tandem repeats and 218 SSR sequences. The total length of these repeats is about 44.2% of those detected in the whole genome. The coding genes accounted for 1.54% of *C. melo* mitochondrial genome (Table 1). In fact, non-coding gene sequences accounted for 98.46% of the mitochondrial genome, but their function still require more detailed characterization.

Collinearity analysis of mitochondrial genomes

By comparing the mitochondrial genomes of four cucurbit plants, we discovered that sequences, with a consistency of no less than 80% in *C. sativus*, *C. pepo* and *C. lanatus*, accounted for 33%, 40% and 65% of the whole mitochondrial genome length in melon, respectively. The sequence shared by the four species accounts for about 6% of the full length of the melon mitochondrial genome. In addition, both melon and the other three crops have similar gene coding regions, but the non-coding regions are quite distinct (Fig. 1).

The linear relationships of mitochondrial coding genes among *C. melo*, *C. sativus*, *C. pepo* and *C. lanatus* were compared. Two or more collinear gene groups are called gene clusters, and we get 7 to 13 gene clusters with different gene numbers (Table 2). A higher number of collinear gene clusters was identified in *C. melo* and *C. sativus*, contrarily, a fewer number of collinear gene clusters was found in *C. pepo* and *C. lanatus*.

Comparison of four mitochondrial genomes

There were no significant differences in mitochondrial genomic GC content among *C. melo*, *C. sativus*, *C. pepo* and *C. lanatus* (between 42.8 and 45.1%, Table 3). The total number of repeat sequences in the melon mitochondrial genome was the highest among all four species, accounting for 44.2% of the total genome sequence. The number of repeat sequences in the mitochondrial genome of *C. sativus*, *C. pepo* and *C. lanatus* was 44.1%, 24.4% and 9.6% of the total genome, respectively.

A total of 40 protein-encoding genes were annotated in the mitochondrial genome of melon. The number of protein-encoding genes in the mitochondrial genome of *C. sativus*, *C. pepo* and *C. lanatus* was 37, 38 and 39, respectively. Melon mitochondrial genome lost *rps19* gene, two copies of *atp1* gene, and two more ORF genes (*orf1* and *orf2*). The

Table 1. The basic features of the *C. melo* mitochondrial genome.

Feature	Value
Total length (bp)	2,906,673
Chromosome number	3
GC content	44.77%
Gene number	88
Protein genes	40
rRNA genes	8
tRNA genes	40
Genes with introns	10
Trans-spliced genes	3
Coding sequence	1.54%
Protein coding	1.23%
tRNAs and rRNAs	0.31%
Non-coding sequence	98.46%
Repetitive content	44.2%
SSRs	0.1% (218)
Tandem repeats (TRs)	2.1% (653)
Inverted repeats (IRs)	2.4% (439)
Forward repeats (FRs)	39.4 (4,861)
Chloroplast-like	2.73%
Nuclear-like	48.62%

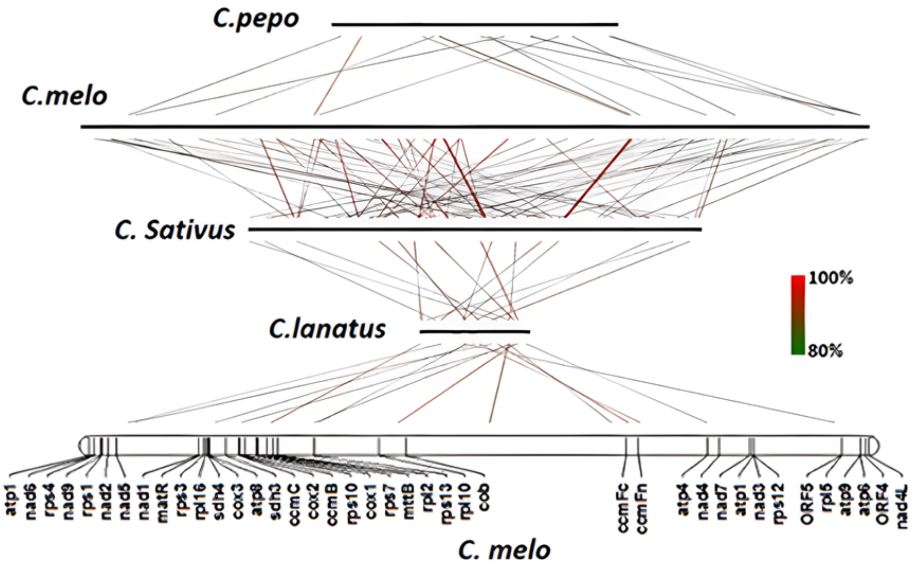

Fig. 1 Collinearity analysis of *C. melo* mitochondrial genome with other three Cucurbitaceae plants.

Table 2. The gene clusters of melon collinearity with three other mitochondrial genomes.

Species	Amount	*C. melo*
C. lanatus	7	***nad6-rps4***; *trnY-nad2*; *trnF-trnS*; ***rps3-rpl16***; ***sdh4-cox3-atp8***; ***rrn5s-rrn18s***; ***nad3-rps12***
C. sativus	13	***nad6-rps4***; *nad9-rps1*; *nad2-sdh3*; *trnF-trnS*; *matR-trnH*; ***rps3-rpl16***; ***sdh4-cox3-atp8***; ***rrn5S-rrn18S***; *rpl10-trnD*; *ccmFc-trnW-atp4*; ***nad3-rps12***; *atp9-atp6*
C. pepo	8	***nad6-rps4***; *nad9-rps1*; ***rps3-rpl16***; ***sdh4-cox3-atp8***; ***rrn5S-rrn18S***; ***nad3-rps12***; *atp9-atp6*; *trnM-trnG*

Gene clusters in **bold** are the gene clusters common to the four mitochondrial genomes.

Table 3. Mitochondrial genome summary of *C. melo*, *C. sativus*, *C. pepo* and *C. lanatus*

Feature	*C. melo*	*C. lanatus*	*C. sativus*	*C. pepo*
Genome				
Accession	MG947207 MG947208 MG947209	NC_014043	NC_016004 NC_016005 NC_016006	NC_014050
Size in bp	2,906,673	379,236	1,644,236	982,833
Chromosome number	3	1	3	1
Topology Structure	Circle	Circle	Circle	Circle
GC content (%)	44.8%	45.1%	44.3%	42.8%
Gene				
Protein-coding genes	40	39	37	38
Protein-coding genes in bp (%)	35,613 (1.23%)	32,370 (8.5%)	32,550 (3.31%)	32,032 (3.26%)
Single-copy protein genes	37	37	37	37
Single-copy protein genes in bp (%)	34,080 (1.17%)	31,986 (8.4%)	32,550 (3.31%)	31,806 (3.23%)
Intron				
Trans-spliced	5	4	5	5
Cis-spliced	17	20	18	19
Cis-spliced introns in bp (%)	46,000 (1.6%)	32,476 (8.6%)	47,996 (2.9%)	30,557 (3.1%)
tRNA genes	40	18	20	13
Native	17	3	7	3
Chloroplast-derived	23	15	13	10
Total tRNAs in bp (%)	2,999 (0.1%)	1,358 (0.4%)	1,486 (0.09%)	966 (0.1%)
rRNA genes	8	3	6	3
Total rRNAs in bp (%)	5,815 (0.2%)	5,148 (1.4%)	11044 (0.67%)	5,109 (0.5%)
Noncoding regions in bp (%)	2,862,246 (98.5%)	340,360 (89.7%)	1,599,156 (97.3%)	944,726 (96.1%)
Repetitive content				
SSR (num.)	0.1% (218)	0.2% (54)	0.1% (144)	0.2% (144)
TR (num.)	2.1% (653)	0.3% (14)	0.4% (120)	1.9 (287)
IR (pairs)	2.4% (439)	0.4% (14)	6.3% (539)	0.2% (17)
FR (pairs)	39.6% (4861)	8.7% (209)	37.3% (4974)	22.1% (1608)
Maximum large repeat length (bp)	5,532	7,286	17,159	621
Number of repeats (>1 kb)	87	1	10	0
Total repeats (%)	44.2%	9.6%	44.1%	24.4%
Chloroplast-like in bp (%)	79,463 (2.73%)	28,703 (7.6%)	70,702 (4.3%)	113,347(11.5%)
Mitochondrial-like in bp (%)	967,450 (33.3%)	159,032 (41.9%)	907,251 (55.2%)	180,008 (18.3%)
Nuclear-like in bp (%)	1,413,224 (48.62%)	24,352 (6.4%)	501,491 (30.5%)	20,638 (2.1%)

mitochondrial genome of these four plants contained three different types of ribosomal genes (*rrn5S*, *rrn18S* and *rrn26S*). The highest number of rRNAs were detected in melon, including six copies of *rrn5S*, and two single copies of *rrn18S* and *rrn26*. Six rRNA genes were present in the cucumber mitochondrial genome, and each ribosomal gene presented two repeats. Only three rRNAs were observed in watermelon and zucchini, all of which were single-copy genes (Table 4).

We compared the tRNA use of *C. melo*, *C. sativus*, *C. pepo* and *C. lanatus* (Table 5). The results showed that the mitochondrial genome sequences of all four species could not encode a complete set of tRNA that could recognize all codons or transport a complete set of 20 amino acids.

Prediction of mitochondrial RNA editing sites

Our results show that *C. lanatus* had the largest number of RNA editing sites along its mitochondrial genome (509 sites). *C. melo* and *C. sativus* contained 507 and 498 RNA editing sites, respectively. *C. pepo* presented the lowest number of RNA editing sites (total of 486). *Nad4*, *ccmB* and *ccmFn* were the three genes with the highest RNA editing sites (Table 6).

Phylogenetic analysis

In this study, the phylogenetic relationship of *C. melo*, *C. sativus*, *C. pepo* and *C. lanatus* were analyzed based on 10 conserved coding genes (*atp6*, *nad6*, *cox3*, *rps12*, *atp1*, *nad4*, *nad9*, *nad7*, *nad4L*) that are present in all 14 species. Evolutionary relationships were analyzed (Fig. 2) and it was found that grapes and Cucurbitaceae are closely related, and Cucurbitaceae can cluster well in a clade, but the genetic distance of *C. pepo* in the phylogenetic tree is closer to *C. melo* and *C. sativus* compared to *C. lanatus*.

Table 4. Comparison of the gene content among *C. melo*, *C. sativus*, *C. pepo* and *C. lanatus* mitochondrial genome.

Gene	*C. melo*	*C. lanatus*	*C. sativus*	*C. pepo*
Complex I				
nad1,2,3,4,4L,5,6,7,9	+	+	+	+
Complex II				
sdh3	+	2	+	+
sdh4	+	+	+	+
Complex III				
cob	+	+	+	+
Complex IV				
cox1,2,3	+	+	+	+
Complex V				
atp1	2	+	+	+
atp4,6,8,9	+	+	+	+
Cytochrome c biogenesis				
ccmB, C, Fc, Fn	+	+	+	+
Ribosomal RNAs				
rrn5S	6	+	2	+
rrn18S	+	+	2	+
rrn26S	+	+	2	+
Ribosomal proteins				
rpl2,5,16	+	+	+	+
rpl10	+	–	+	–
rps1,3,4,7,10,12,13	+	+	+	+
rps19	–	2	–	2
Other ORFs				
matR, mttB	+	+	+	+
orf1,2	+	–	–	–
Total number	48	42	43	41

+: indicates the presence and uniqueness of this gene; –: represents the absence of this gene, and the number represents the copy number of this gene.

DISCUSSION

Our study corroborates with previous reports that utilized renaturation kinetics and restriction endonuclease technology to analyze the mitochondrial genome analysis of Cucurbitaceae[4]. The structure of both *C. melo* and *C. sativus* mitochondrial genomes is polycyclic. Likewise, this genome structure has been observed in other plants, including wheat[22] and rape[23].

The protein-coding genes in melon mitochondrial genome are like those in the three mitochondrial genomes. Similar conclusions have been reached in studies of other higher plants, where the coding regions of plant mitochondrial genomes are more conserved than the non-coding regions[24]. Melon mitochondria contain two more ORF genes than the other three reference genomes, and ORFs may encode proteins with important functions. In fact, some mitochondrial ORFs have been associated with cytoplasmic male sterility in many plants.

With the expectation of *T-urf13*, *atp6* of radish and *orf256* of wheat, it has been verified that most of the transcripts of protein-coding genes are edited in the mitochondria of higher plants, but the editing degree of transcripts from different genes is variable[25,26]. The male sterility of plants may be caused by some genes in the mitochondrial genome recombining with ORF to form chimeric genes or inadequate RNA editing[27]. In the present study, a slightly distinct number of RNA editing sites was detected in the mitochondrial genomes of four Cucurbitaceae crops. The elucidation of these RNA editing sites may provide data support for the research of RNA editing in cucurbits.

The mitochondrial genomes of both melon and three other kinds of Cucurbitaceae plants were linearly analyzed. *C. melo*

Table 5. Comparison of the tRNA genes.

tRNA	*C. melo*	*C. lanatus*	*C. sativus*	*C. pepo*
trnC-GCA	M	MM	M	M
trnD-GUC	CCMM	–	C	–
trnE-UUC	M	M	MM	M
trnF-GAA	CM	M	CCC	M
trnfM-CAU	–	M	M	M
trnG-GCC	CM	MM	M	M
trnH-GUG	CCM	C	CC	C
trnI-CAU	–	M	MM	M
trnK-UUU	–	M	–	M
trnL-CAA	CM	–	–	–
trnM-CAU	CCCMMM	C	M	C
trnN-GUU	CCMM	C	–	C
trnP-UGG	M	M	M	M
trnQ-UUG	MM	MM	M	M
trnR-ACG	M	–	M	–
trnS-GCU	M	M	M	–
trnS-UGA	C	M	–	–
trnV-GAC	C	–	–	–
trnW-CCA	CCCMMMM	–	C	–
trnY-GUA	M	M	M	M
Choloroplast-derived	17	3	7	3
Mitochondrial-derived	23	15	13	10
Total (type)	40 (18)	18 (15)	20 (15)	13 (13)

Table 6. Number of RNA editing sites in the four mitochondrial genomes.

Order	Genes	*C. melo*	*C. lanatus*	*C. sativus*	*C. pepo*
1	*Atp1*	4	5	5	5
2	*Atp4*	11	13	12	13
3	*Atp6*	23	22	22	22
4	*Atp8*	2	4	2	2
5	*Atp9*	5	6	5	6
6	*ccmB*	34	34	33	30
7	*ccmC*	28	27	27	26
8	*ccmFc*	17	17	18	17
9	*ccmFn*	36	36	36	35
10	*Cob*	16	14	16	14
11	*Cox1*	17	17	18	19
12	*Cox2*	13	13	13	13
13	*Cox3*	8	9	8	7
14	*matR*	12	12	12	12
15	*mttB*	27	24	23	24
16	*Nad1*	21	21	21	20
17	*Nad2*	25	25	25	24
18	*Nad3*	12	12	12	10
19	*Nad4*	37	38	36	33
20	*Nad4L*	13	13	13	13
21	*Nad5*	28	27	28	23
22	*Nad6*	15	10	10	10
23	*Nad7*	25	27	27	26
24	*Nad9*	7	7	7	7
25	*Rpl2*	5	3	3	2
26	*Rpl5*	10	10	9	8
27	*Rpl16*	5	5	5	5
28	*Rps1*	3	4	3	4
29	*Rps3*	7	9	7	8
30	*Rps4*	17	17	17	18
31	*Rps7*	2	2	2	2
32	*Rps10*	6	5	6	5
33	*Rps12*	5	7	7	7
34	*Rps13*	4	3	3	4
35	*Rps19*	pseudo	3	pseudo	4
36	*Sdh3*	3	4	3	5
37	*Sdh4*	4	4	4	3
Total	–	507	509	498	486

Note: pseudo indicates that the gene is a pseudogene.

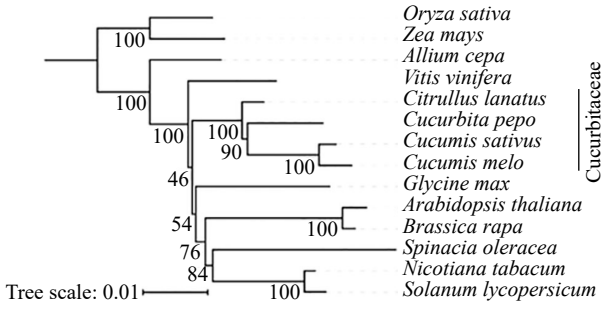

Fig. 2 The construction of phylogenetic tree among 14 species based on mitochondrial conserved genes.

and *C. sativus* showed more collinearity between mitochondrial gene clusters, which further shows that *C. melo* has a closer relationship with *C. sativus*. In the four kinds of Cucurbitaceae plants some gene cluster, *rps3-rpl16* is widespread in the plant mitochondrial genome typical gene cluster[28].

CONCLUSIONS

This study upon in-depth comparative analysis of the mitochondrial gene structure of *C. melo*, *C. sativus*, *C. pepo* and *C. lanatus*, revealed that the large number of repetitive and nuclear genome sequences were the potential reasons for the increasing scale and variation of the melon mitochondrial genome. These results provide the basis for the genetic variation of the mitochondrial genome in Cucurbitaceae plants.

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Conflict of interest

The authors declare that they have no conflict of interest.

Dates

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