Comparative Analysis of the Complete Mitochondrion of *Chlorophytum Comosum* with 9 Other Species

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Abstract

Chlorophytum comosum belongs to genus *Chlorophytum* of family Asparagaceae that has more than 215 species. It is among 120 plant species assayed for phytoremediation of pollutants from indoor air. It has the ability to remove formaldehyde, nitrogen dioxide, carbon oxide, ozone, benzene, toluene, cigarette smoke and ammonia. It also is an important Chinese traditional medicine material. Aim to get more information about its mitochondrial genome, we sequenced the complete mitochondrial genome of *C. comosum*. The length of mitochondrial genome was 950316 bp with 36 protein coding genes, 5 rRNAs and 21 tRNAs. Although it has the biggest mitochondrial genome, the total PCGs content were the second least. *C. comosum* had the most repeats sequence and it may effect the size of mitochondrial genome, but base the result we found that the quantity of repeats sequence could not have the positive correlation with the mitochondrial size. Compared with other 9 species, we found in the mitochondrial genome of *C. comosum*, there were at least one PCGs suffered from the positive selection after divergence. Finally, the result of phylogenetic showed that *C. comosum* did not have close relationship with other species.

Introduction

Chlorophytum comosum belongs to genus *Chlorophytum* and family Asparagaceae [1]. Genus *Chlorophytum* distributed in Africa (possibly the center of origin), Australia, Asia, Madagascar, and India and *C. comosum* mainly native to the tropics and southern Africa [2,3]. It has long and narrow leaves from the root. Its leaf is green color or with white stripes on the edges and sometimes it also has white flowers [4]. It is known as indoor or outdoor ornamental plant, however, an important ability of *C. comosum* was remove hazardous in air such as formaldehyde, nitrogen dioxide, carbon oxide, ozone, benzene, toluene, cigarette smoke and ammonia [5-9]. Moreover, *C. comosum* have good accumulation on heavy metal in soil and easily removable, so it was widely used in interior design and urban microlandscape to improve the environment [10-13]. *C. comosum* is also well known as a kind of medicinal characteristics, it could be used to make steroidal and triterpenoidal saponin related medicines [14]. At same time, it is used to treat burns, bronchitis, fractures [15], and also has antitumor activity [16].

Mitochondria are complex intracellular organelles that have long been considered the power plants of cells because they play an important role in oxidative energy metabolism. Mitochondria, as metabolic centers, can rapidly adapt to different environmental signals and metabolic changes to meet the bioenergy requirements of cells, which has recently been defined as mitochondrial plasticity [17]. Mitochondria are highly plastic and a dynamic network of signaling organelles that play a multifunctional and critical role in cell metabolism, proliferation and survival [18]. Notably, various preclinical and clinical studies have shown that metabolic and/or inherited mitochondrial changes are involved in the pathogenesis of a large number of diseases, including cancer [19, 20].

Plant mitochondrial (mt) genomes are usually over 1000 times larger than those of animals, by frequent re-combination occurred they are structurally more complex [21]. The notably large size of plant mt genomes is shaped by a combination of several factors, including a rich gene set along with the abundant introns that carries, and the capability of uptaking and integrating intracellular transferred sequences from the chloroplast and nucleus [22, 23], and horizontally transferred genes from foreign donors [24, 25]. The studies about *Chlorophytum comosum* continued for long time, however, base the mt genomes of plants in genus *Chlorophytum* are fewer than other family. The data that we could find in NCBI about complete mitochondrial genome of family Asparagaceae was no more than 10 records. This state the study about this field could be woefully insufficient.

So this study, we will sequence the mitochondrial of *Chlorophytum comosum* and analysis (1) the characters of the mt genome, (2) comparative analysis the structure and of mt genomes with other species like codon usage, repeat sequence, gene cluster, Selective Pressure, (3) phylogenetic analysis.

Methods

2.1 Plant materials

In this study, the sample was collected from the flower market at 22 Yongwu Road, Chaoyang Street, Xixiangtang District, Nanning City, Guangxi province (108°20'43"E, 22°54'7"N), the voucher specimen was stored in the Laboratory of the Ministry of Education, Nanning Normal University (specimen code NN202010). The study complies with relevant institutional, national, and international guidelines and legislation

2.2 Sequencing, genome assembly and annotation

We used the leaves of *Chlorophytum comosum* to extract total genome by using modified CTAB method [26]. Then we used Nanodrop (Nanodrop Technologies, Wilmington, DE, US), agarose gel electrophoresis and Qubit (Thermo Fisher Scientific, Waltham, MA, USA) to get the high quality DNA fragments. In sequencing step, we used both Illumina novaseq sequencing platform (Illumina, San Diego, CA, USA) and Nanopore PromethION sequencing platform (Nanodrop Technologies, Wilmington, DE, US). After using SQK-LSK109 to construct library. Large fragments with an average size of 10.72 kb were purified by magnetic bead enrichment. Then 12.07Gb raw data was sequenced by Nanopore PromethION sequencing platform. Then the raw data was filtered and reedited by NanoFilt and NanoPlot in Nanopack [27], and we got 11.93 Gb clean data with longest reads size 156,720 bp. At the same time the high quality DNA fragments was constructed by using the NexteraXT DNA Library Preparation Kit. Then the libraries were sequenced on Illumina Novaseq 6000 platform and the average length of the generated read was 150bp. The 5.19Gb Illumina raw sequence read were edited using the NGS QC Tool Kit v2.3.3 [28]. After using de novo assembler Canu2.1 [29] and software Blastn [30] to assemble both Illumina and Nanopore clean data with the reference genome of *Asparagus officinalis* (MT483944), we got the mitochondrion contig with 950,316 bp length. Then we ran the correction step by BWA v0.7.12-r103 [31], and yielding a circular molecule of 950,316bp. Following, we used Blastn and tRNAscan-SE 2.0 [32, 33] to annotate the protein coding genes, rRNA

genes and tRNA genes. Finally, OGDRAW 1.2 was used to create gene clusters and the mitochondrial genome map[34]. The mitochondrial genome of *C. comosum* was submitted to NCBI under the accession No. MW411187

2.3 Codon usage analysis, Selection pressure analysis and identification of repeat sequences

In codon usage analysis, we selected all protein coding genes from 10 MT genomes and then Synonymous codon usage and the relative synonymous codon usage (RSCU) was conducted to determine if the plastid genes were under selection by using codonW v1.4.4 [35]. Following step, we eliminated the stop codons and used Mafft v7.429[36] align the selected protein coding genes, and converted aligned files to PAML format. Nonsynonymous (Ka) and synonymous (Ks) substitution ratio (Ka/Ks) of the mitochondrial PCGs of *C. comosum* were predicted using DnaSP v5.10.01 to identify the genes that are under selection pressure [37,38], finally PAML [39] was used to analyze Ka/Ks with default setting. And repeat sequences were identified by software REPuter v2.74 with setting repeat sequence ≥ 30 bp and repeat identity $\geq 90\%$ [40]

2.4 Phylogenetic Analysis

We selected the homologous genes that existed in all selected MT genomes and only had 1 copy in genomes. Then they were aligned by Mafft v7.429 [36] and cleaned by Gblocks v0.19b[41]. Using the model-finders[42] chose the model TIM+G4, then ML tree constructed by IQTree v1.6 [43].

Results

3.1 Mitochondrion structure and gene content.

Based on the result, we found that the complete mitochondrial genome of *Chlorophytum comosum* had 950316 bp long with circle structure (Figure 1). The total GC content covered 46.65%, Adenosine covered 26.62%, Thymine covered 26.73%, Cytosine covered 23.34% and Guanine covered 23.31%. There were 62 genes in this mitochondrial genome with 36 protein coding genes, 5 rRNAs and 21 tRNAs. We also found the protein coding genes covered very small proportion in genome, the size only was 31500 bp in total. In protein coding genes, GC content covered 43.66%, Adenosine covered 26.54%, Thymine covered 29.8%, Cytosine covered 21.82% and Guanine covered 21.84%. This was similar as the total GC content. Furthermore, based on the result all protein coding genes only had one copy and 9 (*nad7*, *nad1*, *nad4*, *ccmFC*, *rps10*, *rps3*, *nad2*, *nad5* and *cox2*) genes were containing one or more introns. For RNA, there were 2 rRNA genes (*rrn5* and *rrn8*) had 2 copies, and 7 tRNA genes (*trnM-CAT*, *trnY-GTA*, *trnN-GTT*, *trnE-TTC*, *trnQ-TTG*, *trnH-GTG* and *trnW-CCA*) had 2 or more copies especially *trnM-CAT* had 3 copies.

3.2 Competitive analysis in Angiospermae

Aim to acquire more information about the mitochondrial genome of *C. comosum*, we selected 9 complete mitochondrial genomes in Angiospermae from NCBI and compared with *C. comosum* by codon usage, repeat sequence, gene cluster, Selective Pressure and phylogenetic analysis.

3.2.1 Codon usage analysis

Based on the result we found that there were 3 start codons and 3 stop codons. In start codons, ATG was the most frequent start codon that had been used in 32 protein coding genes in mitochondrial genome of *C. comosum*, then start codon ACG was used in 2 genes and start codon ATC was used in just 1 protein coding gene. In stop codons, stop codon TAA was the most frequent stop codon that had been used in 19 protein coding genes, then stop codon TGA was used in 10 genes and TAG was used in 7 genes. We calculated the relative synonymous codon usage (RSCU) to determine whether there was codon usage bias in coding sequences of *C. comosum*, and found that Methionine AUG and tryptophan UGG had no usage bias (RSCU = 1), 30 codons were preferred codons (RSCU > 1) (Figure 2).

In this study, we found another interesting thing that the quantity of codons was independent with the size of the mitochondrial genome. The largest mitochondrial genome was 950,316 bp and from *C. comosum*, but it did not contain the most codons, however, it just contained the second least codons (10500 codons) that meant the size of total protein coding genes was 31500 bp. And the most codons were contained in the mitochondrial genome of *Cocos nucifera*, the quantity was 18777 codons, however the size of this mitochondrial genome was 678,653 bp. But we also found that the smallest mitochondrial genome contained the least codons. It was *Allium cepa* and contained 8490 codons. The leaves of codons from the species in study were between 8490 and 18777, these meant that the range of the total size of protein coding genes would be between 25470 and 56331 bp (Supplementary table S1).

Then we analyzed the codon usage in 10 species, and found that the percentage of different codons were similar, the Leucine was the most amino acids around 10% in all of species in this study. Cysteine and Tryptophan were the least two amino acids in the species. In species *Cocos nucifera*, *Lolium perenne* and *Phoenix dactylifera* Tryptophan was the least amino acids and Cysteine was the least amino acids in other species around 1.3-1.67% (Figure 3).

3.2.2 Repeat sequence analysis

Repeat sequence analysis has been widely used for species identification, genetic diversity studies, and evolutionary analysis, due to their high polymorphism level among the related species [44]. We detected repeat sequences of species in study. Based on table 1 and figure 4, we could find that 3833 repeats were detected in *C. comosum* that contained the most quantity of repeat sequence. 133 repeat sequences were detected in *Phoenix dactylifera* that

contained the least quantity of repeats sequence. We divided these repeats sequence by the length, then we found that the sequence with the length 30-49 bp would be the most repeats sequence in all species in this study. But the least repeats sequence was different among the species, such as in *C. comosum* the least repeats should be the length over 1000bp, there were only 7 repeats, however in *Allium cepa*, there was no repeat with the size between 150 and 199 bp, so the least repeat sequence would be the repeat range around 150-199 bp.

Table 1 the repeat sequence in the mitochondrial genome of *Chlorophytum comosum* and other 9 higher plant mitochondrial genomes.

	<i>Allium cepa</i>	<i>Bambusa oldhamii</i>	<i>Chlorophytum comosum</i>	<i>Cocos nucifera</i>	<i>Eleusine indica</i>	<i>Lolium perenne</i>	<i>Oryza rufipogon</i>	<i>Phoenix dactylifera</i>	<i>Tripsacum dactyloides</i>	<i>Zea luxurians</i>
30-49	155	169	3436	91	96	111	188	89	1771	147
50-69	12	40	223	38	45	39	172	21	936	29
70-99	21	33	67	25	18	20	14	11	569	11
100-149	4	8	30	12	7	14	14	5	229	1
150-199	0	8	14	5	2	3	4	4	46	2
200-999	1	21	56	13	6	20	3	2	6	7
≥1000	1	0	7	11	6	16	10	1	6	3

Normally, the quantity of repeats may affect the size of genome, such as *C. comosum* has largest quantity of repeats sequence and the size of mitochondrial genome also was the largest one. However, not all quantity had the positive correlation with the size of mitochondrial genome, such as *Phoenix dactylifera*, the mitochondrial genome of *Phoenix dactylifera* was the second largest mitochondrial genome, however it just had the least number of repeats sequences. *Tripsacum dactyloides* and *Phoenix dactylifera* had similar size around 700 kb, but the number of repeats was huge different. *P. dactylifera* only had 133 repeats but *T. dactyloides* has 3563 repeats. so it can hardly state that the repeats had the positive correlation with the size of mitochondrial genome.

3.2.3 gene cluster analyses

With the advancement of genomic sequencing and molecular biology, some similar clustered gene organization has been described in mitochondria of a number of green plants, from charophytic algae to angiosperms [45-48]. By the reason of the repeat mediated homologous recombination, sequence duplications, genome expansion and shrinkage, and incorporation of foreign DNAs, plant mitogenomes are highly fluid in genome structure [49]. In many instances, the ancestral ribosomal gene clusters are broken into much smaller ones containing no more than four genes [50,51]. However, in large scale phylogenetic, some gene clusters are still conserved [52]. But in most situation the gene organization greatly differs among plant mt genomes. In this study, we compared the gene orders in the 10 mt genomes and counted the number of syntenic gene clusters (genes that remain in the same order). Gene clusters (rps12-nad3) was found to exist in all species mt genomes in this study (Figure 5, Supplementary Table S2). Gene cluster rrn18-rrn5 was absent in mt genome of *Allium cepa*, but existed in others. (nad5)-nad1-matR was lost in *Oryza rufipogon*, and rpl5-rps14 was only exist in species *Chlorophytum comosum*, *Lolium perenne* and *Phoenix dactylifera*, but absented or lost in others. It means these gene clusters would be highly conserved in plant mt genomes. The gene cluster matR-rps1-ccmFN was absented in *Phoenix dactylifera*, *Cocos nucifera*, *Chlorophytum comosum* and *Allium cepa* but existed in other 6 species. However, the gene cluster atp4-nad4L was only exist in *Allium cepa*, *Chlorophytum comosum*, *Cocos nucifera* and *Phoenix dactylifera*.

Generally, the gene order and clusters would be the more similar by closer relationship in different species. it would be large different in gene order and clusters between different families, because genome recombination can disrupt clusters [53,54]. In this study, the selected species came from different family, but base the result we found gene clusters (rps12-nad3) was conserved plants mt genomes, and another two gene clusters (nad5-nad1-matR and rrn18-rrn5) conserved in most mt genomes (Table S2). These may reason that multiple recombination events can generate similar syntenic gene clusters, leading to vast differences in the gene order among plant mt genomes [53-56]. In our study, the characteristics of the gene clusters in the *Chlorophytum comosum* mt genome were consistent with the general conservation of most monocotyledons, indicating that the gene clusters in the *Chlorophytum comosum* mt genome were more conserved during plant mt genome evolution.

3.2.4 Selective Pressure Analysis

One way to evaluate the selective pressures on protein evolution is to compare the rate of synonymous and non-synonymous nucleotide substitutions [57]. Ks is the estimated number of synonymous changes per synonymous site and corresponds to the rate of amino acid- neutral evolution. Ka, on the other hand, is the number of nonsynonymous substitutions per non-synonymous site. Under neutral protein- level evolution Ka should be equal to Ks and hence the ratio Ka/Ks = 1 [58]. We compared 18 protein coding gene between *Chlorophytum comosum* and other species, then calculated the Ka/Ks. we found that most of Ka/Ks value were under than 1, at same time 19 Ka/Ks value were over 1. The highest value of Ka/Ks was 2.115 that happened on gene

rps14 compare with *L. perenne*. In addition, there were 10 genes that the Ka/Ks value >1, they happened on gene *atp4*, *ccmC*, *nad2* compare with *A. cepa*, gene *rpl5* compare with *B. oldhamii*, gene *ccmB*, *mttB* and *nad2* compare with *C. nucifera*, gene *nad2* compare with *E. indica*, gene *mttB* and *rps14* compare with *L. perenne*, gene *mttB*, *nad3* and *rps1* compare with *O. rufipogon*, gene *ccmB* and *nad2* complete with *P. dactylifera*, gene *mttB* and *rps1* compare with *T. dactyloides*, gene *mttB* compare with *Z. luxurians*. From the result, we could know that the most gene would be under stabilizing during evolution, but 10 genes would be suffered from the positive selection after divergence. (Figure 6)

3.2.5 Phylogenetic analysis

To confirm the phylogenetic position and understand the relationship of *Chlorophytum comosum* within Angiospermae. The complete mitochondrial genome of *Allium cepa*, *Bambusa oldhamii*, *Eleusine indica*, *Tripsacum dactyloides*, *Zea luxurians*, *Oryza rufipogon*, *Lolium perenne*, *Cocos nucifera* and *Phoenix dactylifera* were collected and aligned with *Chlorophytum comosum*, then got the result. The figure7 shows that *Chlorophytum comosum* did not have the very close relationship with other, however, we found *Tripsacum dactyloides* and *Zea luxurians* had close relationship, at the same time *Cocos nucifera* and *Phoenix dactylifera* also had close relationship. (Figure 4)

Conclusion

Through the result, we get the complete mitochondrial genome of *Chlorophytum comosum* with the length 950,316bp. It was the largest one in this study. There were 36 protein coding genes, 5 rRNAs and 21 tRNAs. Although it has the biggest mitochondrial genome, the total PCGs content were the second least. *C. comosum* had the most repeats sequence and it may affect the size of mitochondrial genome, but base the result we found that the quantity of repeats sequence could not have the positive correlation with the mitochondrial size. Compared with other 9 species, we found in the mitochondrial genome of *C. comosum*, there were at least one PCGs suffered from the positive selection after divergence. Finally, the result of phylogenetic showed that *C. comosum* did not have closed relationship with other species.

Declarations

Availability of data and materials: the raw sequence data of this study can be found in NCBI under Bio-project: PRJNA785105, and SRA Number: SRR17082800 and SRR17082801. The chloroplast genome sequence was under the accession number: MW411187 on NCBI website. Supplementary materials can be found at website

Consent for publication: the details/images/videos will be freely available on the internet and may be seen by the general public

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Ethics approval and consent to participate: Not applicable

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Figure 2

RSCU of mitochondrial genome of *C. comosum*

Figure 3

codon usage of mitochondrial genome in 10 plants.

Figure 4

the repeat sequence in the mitochondrial genome in 10 plant mitochondrial genomes.

Figure 5

Analysis of conservative gene clusters between the *Chlorophytum comosum* mitochondrial genome and other higher plant mitochondrial genomes.

Figure 6
The value of Ka/Ks of between *Chlorophytum comosum* and other nine species

Figure 7
Maximum-likelihood phylogenetic tree for *Chlorophytum comosum* based on 10 complete mitochondrial genomes.

Supplementary Files

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- [SupplementaryMaterial.zip](#)
- [tableS1thecodonsof10higherplantmitochondrialgenomes.xlsx](#)
- [TableS2AnalysisofconservativegeneclustersbetweentheChlorophytumcomosummitochondrialgenomeandotherhigherplantmitochondrialgenomes.xlsx](#)