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Mitochondrial genome is paternally inherited in *Cucumis* allotetraploid (*C.* × *hytivus*) derived by interspecific hybridization

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ABSTRACT

Paternal transmission of the mitochondrial (mt) genome in cucumber (*Cucumis sativus* L.) was revealed previously using intraspecific crosses. The present study reports the first paternal transmission of mtDNA observed between *Cucumis* species. Mitochondrial primers specific to *nad1*, *cob*, and *nad7* were used to amplify the DNAs from the female parent (*C. hystrix*), male parent (*C. sativus*) and the allotetraploid (*C.* × *hytivus*) derived the interspecific hybridization. By sequence alignment, we found that the explicit nucleotide polymorphisms of the allotetraploid were all inherited from male parent, *C. sativus*. Similar experiment with chloroplast (cp) genome was conducted and the results showed that while mtDNA was paternally inherited, cpDNA was still maternally inherited between *Cucumis* species. This work provides novel evidence of paternal transmission of the mitochondrial genome between plant species and lays the foundation for further research on the genetic mechanism of mitochondrial transmission in *Cucumis*.

1. Introduction

Maternal inheritance of mitochondrial genome is reported in the most angiosperms (Boynton et al., 1987; Neale and Sederoff, 1989). However, occasional biparental or paternal mitochondrial transmissions have been observed in some species (Erickson et al., 1989), contrary to the strict maternal transmission in animals. Previous studies have found that the mitochondrial genome of cucumber (*Cucumis sativus* L., $2n = 14$) and melon (*C. melo* L., $2n = 24$) were paternal transmission (Havey et al., 1998). In addition, the *Cucumis* mitochondrial genomes are several-fold larger than other plants. These features make the genus *Cucumis* an excellent system for study of mechanisms of transfer, evolution, and function of the mitochondrial genome (Bartoszewski et al., 2004; Wang et al., 2010). However, all these researches were limited to intraspecific crosses.

Cucumis species with different numbers of chromosome are separated by strong cross incompatibilities, which make it almost impossible to study interspecific transmission of mtDNA (Deakin et al., 1971; Singh and Yadava, 1984). Chen and Kirkbride (2000) successfully developed new synthetic allotetraploid, *Cucumis* × *hytivus* Chen and Kirkbride (HHCC), from a wide cross between the cultivated cucumber (CC) and a wild *Cucumis* species, *C. hystrix* Chakr. ($2n = 2x = 24$, HH). Our previous studies found that some DNA fragments in the allotetraploid were paternally inherited in reciprocal crosses (Chen et al., 2007). Given to the paternal transmission of the mitochondrial genome of the genus *Cucumis*, these paternal inherited genes might come from mitochondria. If confirmed true, then *Cucumis* would be distinguished as a distinct genus with respect to mtDNA inheritance and would enhance our knowledge on mtDNA transmission between species.

In this study, we cloned partial sequences of *nad1*, *cob*, and *nad7* in the mitochondria of the female parent (*C. hystrix*), male parent (*C. sativus*) and synthesized allotetraploid (*C.* × *hytivus*), respectively. Meanwhile, in order to detect chloroplast genome effect, *trnL* and *trnS-trnG* intergenic spacers (*trnS-G*), and *rpl20-rps12* (*rpl-s*) in chloroplast were also cloned. By sequence alignment, the nucleotide polymorphisms of the three materials were identified and used to evaluate the transmission of mitochondria and chloroplast genomes. The results provide useful insights regarding the transmission and evolution of the mitochondrial sequences in *Cucumis*.

2. Materials and methods

2.1. Plant materials and isolation of nucleic acids

The plant material consisted of the two diploid inbred parents, a common cucumber (*C. sativus* cv. 'Beijingjietou', paternal parent) and a wild *Cucumis* species *C. hystrix* (maternal parent), and the S_5 generation of the synthetic allotetraploid *C.* × *hytivus* (genome HHCC, $2n = 4x = 38$). The primary allotetraploid (S_0) was previously obtained from interspecific hybridization followed by embryo

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Table 1
Primers used for amplification.

Primer name	Sequence
*nad*1	5′-TTCTTATTGTGCGCCTTGTGA-3′ 5′-GGGGCTATCCTTGTACTAAGT-3′
cob	5′-GATTCTCTCTTCTTAAACAA-3′ 5′-CCAAGAGACATATAAAAACTG-3′
*nad*7	5′-ATGAATGATCGAGGATTCAAG-3′ 5′-GCAGATCTTGTGCCACTCCA-3′
trnL	5′-GATTCCAAGACCTCCAAGT-3′ 5′-CCTCTGCTCTACCAACTG-3′
trnS-G	5′-AAGCCCTTCTTTATCTCCC-3′ 5′-AGGAATCGAACCTTGATTCT-3′
rpl-s	5′-CTTCCCGACCACGATTTC-3′ 5′-CCAGTGACTAGGATAGAATGAA-3′

rescue and chromosome doubling. The S_5 generation was produced after four self-pollinations of S_0. The allotetraploid plants had the expected karyotype with 38 chromosomes at mitotic metaphase (Chen and Kirkbride, 2000). All plants were grown in a plastic house in consecutive spring seasons of 2008, 2009 and 2010. Young expanded leaves from eight individual plants were collected for DNA isolation.

Genomic DNA of *C.* × *hytivus* (S_5) and the two diploid parents was isolated by a modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980), and adjusted to a concentration of 50 ng μL^{-1}.

2.2. Polymerase chain reaction (PCR)

Using Primer premier 5.0 software, specific primers were designed for the cucumber *nad1*, *cob*, *nad7* and *trnL*, *trnS-G*, *rpl-s* according to the published mitochondria and chloroplast sequences, respectively (GenBank accessions AF288044, FJ007641, FJ007643, DQ536765, HM597105, and HM596984) (Table 1). Using total genomic DNA as a template, six genes were amplified with the specific primers. PCR amplifications were performed using 20 μL reaction mixtures with 50 ng DNA, 50 pmol of each primer, 0.2 mmol/L of dNTP, 2.5 mmol/L of $MgCl_2$ and 1 U of Taq polymerase (Takara, Japan). Reactions for *cob* were denatured at 94 °C for 3 min, followed by 35 cycles of 1 min at 94 °C, 30 s at 51 °C and 1.5 min at 72 °C, with a final elongation step of 10 min at 72 °C, while the annealing temperature for *nad1*, *nad7*, *trnL*, *trnS-G*, *rpl-s* was 55.4 °C, 52 °C, 56 °C, 54.5 °C and 56.5 °C, respectively.

The PCR products were separated by electrophoresis on 1% agarose gel in 1× TAE buffer and visualized under UV light after staining with ethidium bromide.

2.3. Cloning and sequences analysis

The PCR products of the expected size were recovered and purified using a DNA Gel Extraction Kit (Karroten, China). The purified fragments were cloned into the vector pMD^{TM} 19-T (Takara, Japan) and then transformed into *Escherichia coli* strain DH5α. Recombinant clones were immediately used as templates for PCR. The correct insert size was identified and then sequenced. Clones of the expected size were sequenced by Invitrogen Bio-Technology Co., Ltd., Shanghai, China.

The target sequences obtained were analyzed using multiple sequence alignments and homology analysis using DNAMan 5.2 software (Zhang et al., 2006). To ensure reliability, two clones from all fragments were randomly picked to sequence, and only consistent results were utilized.

3. Results

3.1. Paternal inheritance of mtDNA in interspecific crosses of Cucumis

Specific primers for *nad1* yielded an expected 951 bp fragment from *C. hystrix, C. sativus* cv. 'Beijingjietou' and *C.* × *hytivus*. Multiple sequence alignment showed 7 nucleotide polymorphisms among the three fragments, accounting for 0.7% of the total sequence. The nucleotide polymorphisms were all located in transition and intronic regions. In addition, 6 of the 7 bases (85.7%) in *C.* × *hytivus* were the same as those in the male parent, *C. sativus* cv. 'Beijingjietou'. The remaining base did not match either parent.

The *cob* sequence of *C.* × *hytivus* was 909 bp in length. It almost completely matched the sequences of the two parents but one polymorphism at 556 bp with base T was observed only in the female parent, *C. sativus* cv. 'Beijingjietou'. This indicated that the *cob* gene in *C.* × *hytivus* originated from the male parent, *C. hystrix*.

The length of the *nad7* PCR products for all the three cases was 880 bp. Multiple sequence alignments revealed that the three sequences had a 290 bp exon and a 590 bp intron with 15 nucleotide polymorphisms in the intron. Whereas polymorphisms of these 15 nucleotides were similar in *C.* × *hytivus* and the male parent, they were different from the female parent.

Regardless of the sequence, the comparison of the three genes showed that 22 of 23 polymorphic bases of the allotetraploid and male parent, *C. sativus* cv. 'Beijingjietou' were the same. At 150 bp of *nad1*, both parents had same base (A), whereas *C. hytivus* showed bas G. The details of all the informative nucleotide polymorphisms are summarized in Table 2.

3.2. Maternal inheritance of cpDNA in interspecific crosses of Cucumis

Three specific primers for partial chloroplast sequences yielded expected 623, 511, and 580 bp fragments for *trnL*, *trnS-G*, *rpl*, respectively. Multiple sequence alignments revealed that a total of 26 nucleotide polymorphisms were detected in these three sequences (Table 3). This accounted for 1.52% of these three sequences. The polymorphic loci percentage in chloroplast was higher than mitochondria. As expected, all the polymorphisms completely matched the sequences of the female parent, *C. hystrix*. This fully demonstrates the transmission of chloroplast DNA in this *Cucumis*-interspecific cross is maternal.

4. Discussion

We studied the inheritance of mitochondrial and chloroplast genomes in the interspecific cross of the genus *Cucumis* through gene cloning and sequence alignment. This approach is faster and more convenient than restriction fragment length polymorphisms and other methods. Using this approach, we established that the polymorphic loci of the mitochondrial genome were 95.7% similar to the paternal material, *C. sativus* cv. 'Beijingjietou', whereas they were different from *C. hystrix*, the maternal parent. The remaining one locus (accounting for 4.3%) differed from both parents, which might be due to mitochondrial nucleotide mutation within the inbred lines. Although the numbers of nucleotide polymorphisms were different among these three genes, nearly all of them were located within the introns rather than exons. In *nad7*, all 15 nucleotide polymorphisms were intronic. On the other hand, 26 nucleotide polymorphisms from the partial chloroplast genome matched the sequences of *C. hystrix*. Our findings from this interspecific cross are consistent with the results of previous studies that demonstrated differential transmission of the three genomes

Table 2
The comparison of nucleotide polymorphisms and base type of mitochondria *Nad1*, *cob* and *Nad7* from three species.

nad1	Base site and base type of *nad1* intron (951 bp)						
Sequence	34	35	36	150	191	210	379
C. hystrix	T	T	A	A	T	G	G
C. sativus	G	C	C	A	C	A	T
C. × hytivus	G	C	C	G	C	A	T

cob	Base site and base type of *cob* (909 bp)
Sequence	556
C. hystrix	G
C. sativus	T
C. × hytivus	T

nad7	Base site and base type of *nad7* intron (880 bp)														
Sequence	66	67	126	275	277	279	281	283	285	287	288	289	290	292	348
C. hystrix	T	C	G	T	A	T	G	G	G	A	G	T	C	G	A
C. sativus	G	A	A	C	T	A	T	T	T	G	A	G	A	A	C
C. × hytivus	G	A	A	C	T	A	T	T	T	G	A	G	A	A	C

Table 3
The comparison of nucleotide polymorphisms and base type of chloroplast *trnL*, *trnS-G* and *rpl-s* from three species.

trnL	Base site and base type of *trnL* (623 bp)							
Sequence	83	103	193	233	371	407	446	526
C. hystrix	T	A	A	T	G	T	G	C
C. sativus	C	C	T	G	A	G	A	T
C. × hytivus	T	A	A	T	G	T	G	C

trnS-G	Base site and base type of *trnS-G* (511 bp)								
Sequence	22	391	396	397	421	422	423	424	425
C. hystrix		T	T	C					
C. sativus	C	G	G	A	A	A	A	A	A
C. × hytivus		T	T	C					

rpl-s	Base site and base type of *rpl-s* (580 bp)								
Sequence	62	63	65	66	67	69	70	71	531
C. hystrix	A	A	A	A	C				
C. sativus	C	T	G	G	T	T	A	A	A
C. × hytivus	A	A	A	A	C				

in *Cucumis* (Havey et al., 1998). Based on the current findings, we can conclude that several DNA fragments that were predominantly paternal in our previous study using reciprocal crosses emanated from mitochondrial genome (Chen et al., 2007). So we could infer that the mitochondrial genome is paternal inherited in reciprocal crosses between *Cucumis* species.

Mitochondrial genes are crucial components in plant breeding programs. Breeders must consider potential nuclear-mitochondrial compatibilities with regards to performance of interspecific hybrids. The desire to improve the narrow genetic base of *Cucumis* by importing alien genes from other plants or wild relatives is usually accompanied transmission of nonhereditary undesired traits emanating from the mitochondrial genome. This might partially explain why the interspecific F_1 from *C. sativus* cv. 'Beijingjietou' × *C. hystrix*, allotetraploid (doubled F_1 chromosome) produced almost no fertile pollen, whereas the allotetraploid from *C. hystrix* × *C. sativus* cv. 'Beijingjietou' formed significant amounts of viable pollen and produced seeds after pollination. We suspected that the mtDNA from *C. hystrix* might be responsible for incompatibility in hybrids. It is necessary to investigate this phenomenon further in order to gain more insights on the nucleo-cytoplasmic interaction of interspecific *Cucumis* crosses.

DNA transfers from organelle genomes to nuclear DNA, and vice versa, appears to be a common phenomenon associated with the redistribution of genetic material between nuclear and organelle genomes (Kubo and Mikami, 2007). Currently, all the three cucumber genomes have been fully sequenced (Alverson et al., 2011; Huang et al., 2009; Kim et al., 2006). Thus, it is feasible to confirm the acquisition of cucumber mitochondrial sequences from these diverse sources including the nuclear and chloroplast genomes. This interspecific cross provides an excellent system for the study of the evolution of extremely large mitochondrial genomes, which has allowed us to study intercompartmental sequence exchange, especially by the interspecific cross, *Cucumis*, *C. × hytivus*, whose genomes were recently assembled. This allotetraploid may undergo "genomic shock" in interspecific hybrids and allopolyploids (Ha et al., 2009). The newly formed nuclear, mitochondrial, and chloroplast genomes interactions were unstable. A nuclear locus, *Psm*, controlling sorting of paternally transmitted mtDNA in cucumber has been reported (Havey et al., 2004) and it is interesting to find out the fate of *Psm* in *C. × hytivus*. Whereas previous studies focused on nuclear genome-wide relaxation of gene expression, the question of whether interspecific crosses and chromosome doubling could have triggered the evolution of the mitochondrial or chloroplast genome remains to be answered.

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