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Paternal Inheritance of Mitochondrial DNA of Cucumis hystrix in an Interspecific Cross between C. sativus and C. hystrix

Md. Mizanur Rahim Khan¹, Masaki Iwayoshi², Takashi Arita² and Shiro Isshiki²

¹ Faculty of Agriculture, University of the Ryukyus, Okinawa 903-0213, Japan
² Vegetable and Flower Horticulture Laboratory, Faculty of Agriculture, Saga University, 1 Honjo, Saga, Saga 840-8502, Japan

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Paternal inheritance of mitochondrial DNA (mtDNA) of Cucumis hystrix Chakr, was revealed in the interspecific crosses of cucumber (C. sativus L.) x C. hystrix. The mtDNA was analyzed by RFLP analysis of a PCR amplified nad4 exon1 and nad4 exon2 region of the female parent C. sativus, male parent C. hystrix and the hybrids. All the hybrids showed the identical restriction pattern of C. hystrix. This indicated the paternal inheritance of mtDNA. A similar experiment with chloroplast DNA (cpDNA) was performed by RFLP analysis of a PCR amplified rbcL-ORF106 region. The results showed the identical restriction pattern of the female parent C. sativus in the same hybrids. This indicated the maternal inheritance of cpDNA. Paternal transmission of the mitochondrial genome of C. hystrix was newly discovered in the Cucumis hybrids.

Keywords : cucumber, Cucumis hystrix, mitochondrial DNA, paternal inheritance

INTRODUCTION

Cytoplasmic DNA, including chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) is very interesting to study for phylogenetic reconstitution at various taxonomic levels and its research has become widespread as DNA analysis techniques become generally available (Clegg and Zurawski, 1992). In general, cytoplasmic DNA inheritance is purely maternal in most plants (Reboud and Zeyl, 1994). On the other hand, the paternal inheritance of mtDNA has been observed in Cucumis species (Havey, 1997; Havey et al., 1998).

Cucumis species, i.e., cucumber (C. sativus L., 2n = 14), melon (C. melo L., 2n = 24) et al., have different chromosome numbers and strong cross incompatibility. Therefore, it is extremely difficult to study the inheritance of mtDNA among Cucumis species (Deakin et al., 1971; Singh and Yadava, 1984). However, one interspecific hybrid, the synthesized allotetraploid (C.×hytivus) derived from the female parent (C. hystrix Chakr., 2n=24) and the male parent (C. sativus) was developed by Chen et al. (1997) and paternal transmission of its mtDNA was revealed by Shen et al. (2013). This is the first report of the paternal inheritance of mtDNA in an interspecific hybrid of Cucumis. However, the inheritance of mtDNA of the reciprocal hybrids has not yet been clarified.

In the present study, we analyzed organelle inheritance (cpDNA and mtDNA) of the hybrids between C. sativus (female parent) and C. hystrix (male parent).

MATERIALS AND METHODS

Plant materials

Cucumis hystrix and commercial cucumber (C. sativus) cultivar ‘Jibai kyuri’ and ‘Suyo’ from Japan were used for hybridization. Seeds of Cucumis hystrix which were collected from the Xishuangbanna autonomous region, Yunnan province of China by Chen et al. in September, 1990 (Chen et al., 1994) were grown in this study. The method of hybridization between cucumber cultivars and C. hystrix and the method of embryo culture, were performed following previous report (Chen et al., 1997). In the present study, two individuals of hybrid between ‘Jibai kyuri’ and C. hystrix and three individuals of hybrid between ‘Suyo’ and C. hystrix, were obtained and grown.

Chloroplast DNA and Mitochondrial DNA analysis

Chloroplast DNA and mtDNA were analyzed in cultivars of C. sativus, ‘Jibai kyuri’ and ‘Suyo’, C. hystrix and their hybrids to identify their organelle inheritance. Total DNA was isolated from fresh leaves of the plant materials using the CTAB method described by Murray and Thompson (1980). The cpDNA was analyzed by RFLP analysis of a PCR amplified region between rbcL and ORF106 following the method described by Isshiki et al. (1998) (Table 1). The PCR products of cpDNA were digested with a restriction enzyme XbaI. The mtDNA was analyzed by RFLP analysis of a PCR amplified nad4 exon1 and nad4 exon2 region (Demesture et al., 1995) (Table 1). The PCR products of mtDNA were digested with a restriction enzyme MspI. The DNA restriction fragments were separated on a 1.5% agarose gel containing ethidium bromide and were
photographed under UV illumination.

RESULTS AND DISCUSSION

PCR-RFLP analysis of cytoplasmic DNA is widely used in phylogeny of species and identification of cytoplasm in plants (Demesure et al., 1995). This analysis was also available for identification of cytoplasmic DNA in the present study. Patterns of cpDNA in the two individuals of hybrid between *C. sativus* ‘Jibai kyuri’ and *C. hystrix* were identical as of ‘Jibai kyuri’ which indicates the maternal inheritance of cpDNA (Fig. 1). The identical cpDNA pattern of *C. sativus* ‘Suyo’ was also observed in the three individuals of hybrid between ‘Suyo’ and *C. hystrix*. Since the same pattern appeared even if the cucumber cultivar was changed, it is indicated that there is no doubt that cpDNA is maternally inherited. Maternal inheritance of cpDNA is not surprising in most plants (Reboud and Zeyl, 1994).

On the other hand, patterns of mtDNA in the two individuals of hybrid between *C. sativus* ‘Jibai kyuri’ and *C. hystrix* were identical as of ‘Jibai kyuri’ which indicates the maternal inheritance of cpDNA (Fig. 1). The identical cpDNA pattern of *C. sativus* ‘Suyo’ was also observed in the three individuals of hybrid between ‘Suyo’ and *C. hystrix*. Since the same pattern appeared even if the cucumber cultivar was changed, it is indicated that there is no doubt that cpDNA is maternally inherited.

Table 1  Primers used for amplification.

| Organelle       | Primer name | Sequence                      |
|-----------------|-------------|-------------------------------|
| Chloroplast DNA | *rbcl*      | 5'-ATGTCACCAAAACAGAAACTAAGCAATCAGTCCCACTAAAGCAATG-3' |
|                 | ORF106      | 5'-ACTACAGATCTCATACTACCTACCTACCC-3' |
| Mitochondrial DNA| *nad4 exon1* | 5'-CAGTGGGTGGTCTGTTATG-3' |
|                 | *nad4 exon2* | 5'-TCATATGGCTACTGAGGAG-3' |

From the analysis of their hybrid *C. ×hystrix*, Shen et al. (2013) revealed the paternal inheritance of mtDNA when *C. sativus* used as male parent and *C. hystrix* used as female parent. In the present study, reciprocal hybrids also showed paternal inheritance of mtDNA when *C. hystrix* used as male parent and *C. sativus* used as female parent. *Cucumis hystrix* has chromosome number different from *C. sativus*, but from the viewpoint of mtDNA genetics, it can be said that it is very close to *C. sativus*. A nuclear locus *Psm* that controls the sorting of paternally transmitted mtDNA in cucumbers has been reported (Havey et al., 2004). It is thought that the locus *Psm* also exists in *C. hystrix*, which also shows a close relationship with *C. sativus*.

Paternal transmission of the mitochondrial genome of *C. hystrix* was newly discovered in the *Cucumis* hybrids. Paternal inheritance of mtDNA has been observed in *Cucumis* species (Havey, 1997; Havey et al., 1998). This means that mtDNA is not being suitable for classification because mtDNA would be easily changed in *Cucumis*. Therefore, cpDNA should be available for the classification. This paternal transmission of the mtDNA of *C. hystrix* would be one of the clues of detecting evolution and differentiation of the species in the genus *Cucumis*.

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