The Cucumis plastome: physical map, intrageneric variation and phylogenetic relationships

R. Perl-Treves and E. Galun
Department of Plant Genetics, Weizmann Institute of Science, Rehovot 76100, Israel

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Summary. A restriction map of the Cucumis melo L. (melon) plastome was constructed by using several mapping approaches: single and double digestions of the chloroplast DNA (chlDNA) with endonucleases (XhoI, SmaI, SacI and PvuII) and hybridization to heterologous chlDNA probes and to isolated melon chlDNA fragments. Four plastome-coded genes were located using heterologous probes. The overall organization and gene position of the melon plastome was found to be similar to that of tobacco and other angiosperm species. Restriction patterns based on digestion of the chlDNA with nine endonucleases were obtained in over 20 wild species and cultivated varieties of Cucumis. These led to mutational analysis of the restriction sites yielding the most parsimonious phylogenetic tree of the Cucumis plastome. Most African species from a compact group (“Anguria group”) which is distant from the melon, the cucumber and a few other species (C. sagittatus, C. metuliferus and C. humifructus). All of these are also far apart from each other. The distribution of polymorphic restriction sites along the Cucumis plastome is described and conservative regions as well as “hot spots” are suggested.

Key words: Chloroplast DNA – Cucumis – Restriction-patterns – Phylogeny – Plastome – Parsimonious tree

Introduction

The genus Cucumis includes two distinct sets of species, differing in their origin and basic chromosome number (see Frankel and Galun 1977). The African group has 2n=24 (or its polyploid versions), and includes the melon (C. melo) and most other species in this study. Cucumber (C. sativus) and C. hardwickii represent in our study the South-Asian group, and have 2n=14 chromosomes.

Wild C. melo varieties are found in Africa and South Asia, and belong to 2 races: C. melo agrestis and C. melo melo. The evolution within the genus, and in particular the relationship between species having different chromosome numbers are not clear. Except for cucumber and C. hardwickii, barriers between the cultivated species and their wild relatives in the genus are high and crosses between them commonly failed to produce fertile F1.

Physical maps of the plastome, based on chlDNA restriction patterns, were constructed in recent years for numerous species of monocot and dicot plant families (see Vedel and Mathieu 1983). Palmer (1982) derived a cucumber chlDNA map using PvuII and SalI as restriction endonucleases. ChlDNA restriction patterns were also used to investigate taxonomic relations and the evolution of angiosperm species. These phylogenetic studies, based on chlDNA variation, were published for Nicotiana (Rhodes et al. 1981), Brassica ( Erickson et al. 1983; Palmer et al. 1983 b), Lycopersicon (Palmer and Zamir 1982), Triticum and Aegilops (Bowman et al. 1983; Terachi et al. 1984), Coffea (Bethou et al. 1983), Pennisetum (Clegg et al. 1984) and Solanum (Hosaka et al. 1984). These studies varied in the number of restriction endonucleases employed and the method of analyzing the patterns.

The physical plastome map and the chloroplast phylogenetic data obtained in the present study should serve as tools in the genetic investigation of Cucumis at the molecular level. A practical aim would be to utilize the knowledge on plastome relatedness between cultivated and wild species, in order to enable the increase the genetic resources of cultivated melon and cucumber by introgression of wild genes, possibly by a somatic fusion approach (Galun 1984). The restriction patterns could also serve as plastome markers in the hybrids, and for evaluating genetic distance between fusion partners.

In a subsequent article we shall present a Cucumis phylogeny based on isozyme analysis. From the evolutionary and systematic point of view, it is interesting to compare the phylogenies, based on chlDNA and on nuclear-coded isozymes, for a given taxon. The implications of such a bifurcate study on plastome and nuclear evolution shall be discussed in the second article.
Fig. 1. Fruits of wild Cucumis species used in this study. a C. metuliferus (code number 3); b C. longipes (33); c C. humifructus (32); d C. dipsaceus (27); e C. figari (9); f C. meesus (7); g C. hookeri (43); h C. anguria (4); i C. prophetarum (11); j C. ficifolius (6); k C. pustulatus (24); l C. africanus (14); m C. melo var 'agrestis' (8); n C. anguria (5); o C. leptodermis (41); p C. myriocarpus (10); q C. sagittatus (35); r C. dinteri (28); s C. zeyheri (40); t C. zeyheri (12); u C. heptadactylus (34). Fruit size in the photograph is about 1:1 in respect to natural size, except for the fruits in s, t, u which were reduced in the photograph to about half-natural size (1:2 size reduction).

Materials and methods

Plant material

Table 1 lists the cultivated varieties and wild Cucumis species used in this study, and Fig. 1 illustrates the fruits of the wild species. Plants for propagation and for chloroplast DNA extraction were grown in the greenhouse throughout the year (24 ± 4°C). To obtain seed setting and to avoid cross fertilization, hand self-pollinations were performed as required.

Chloroplast DNA extraction

ChlDNA was extracted from fresh, fully-expanded leaves from plants of various ages. In the case of cultivated melon,