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Inheritance of chloroplast DNA in two full-sib *Vitis* populations

by

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Summary: The mode of transmission of chloroplasts in 2 grape populations was determined using restriction fragment length polymorphisms of chloroplast DNA (cpDNA) to trace the origin of plastids in the progeny. The populations examined were formed by crossing 2 complex interspecific hybrids: NY 62.136.2 × Yates and Cayuga White × Aurore. Analysis of the restriction banding patterns of total DNA of the 4 parents probed with cpDNA of grape and petunia revealed a high level of polymorphism (63 %) between parents of the first cross and a low level of polymorphism (15 %) between the parents of the second cross. The restriction banding patterns of the 4 parents were unique, indicating that there were 4 distinct chloroplast genotypes. Analysis of the restriction banding patterns of total DNA of the progeny probed with cpDNA showed that all progeny from both crosses exhibited the banding pattern of the maternal parent. Thus, the mode of plastid transmission in these populations of grape was strictly maternal.

Key words: chloroplast, Vitis, DNA, genetics, RFLP.

Introduction

The inheritance of chloroplast DNA in higher plants has been shown to follow maternal, paternal and biparental modes. Traditionally, chlorophyll deficiency has been the chloroplast encoded trait used in studies of plastid inheritance (KIRK and TILNEY-BASSETT 1978). In recent years, chloroplast-encoded antibiotic resistance has been used to study plastid inheritance, because in conjunction with plant cell culture, it could be used to detect low frequencies of plastid transmission (MEDGYESY *et al.* 1986).

Differences in banding patterns following cleavage of chloroplast DNA (cpDNA) with restriction endonucleases provide yet another means of determining the mode of plastid inheritance. Variations in the restriction patterns of cpDNA among closely related species and genotypes within a species are limited; however, these differences are detectable and useful for studying plastid inheritance (PALMER 1986). Restriction fragment length polymorphism of cpDNA of parents and progeny has been used to demonstrate the mode of plastid transmission in interspecific crosses of wheat (VEDEL *et al.* 1981), corn (CONDE *et al.* 1979), sorghum (PRING *et al.* 1982), soybean (HATFIELD *et al.* 1985) and *Pelargonium* (METZLAFF *et al.* 1981).

The purpose of this study was to determine the mode of inheritance of chloroplast DNA in 2 full-sib grape (*Vitis* sp.) populations using grape cpDNA and *Petunia* cpDNA which would provide almost complete coverage of the grape cpDNA genome.

Materials and methods

Young leaves from the parents and forty progeny of each of 2 crosses, 78.839: 62.136.2 × Yates and 81.316: Cayuga White × Aurore were collected from vines grown in the vineyards of the New York State Agricultural Experiment Station, Geneva, NY. The parents of these crosses were complex interspecific hybrids.

Total cellular DNA was prepared from leaf tissue stored at −70 °C using the method of DOYLE and DOYLE (1987). The DNA was digested using six restriction endo-

nucleases (Eco RI, Eco RV, Bcl I, Bam HI, Hind III and Xba I) according to the manufacturer's instructions and the digest carried out overnight to help insure the enzyme cut the DNA to completion. After separation by electrophoresis on 1 % agarose gels, the DNA fragments were transferred to nylon membranes (GeneScreen Plus-Dupont, Washington, DE) using a modification of the techniques of SOUTHERN (1975) in which the DNA was transferred to the membrane in 0.4 N NaOH (REED and MANN 1985).

A library constructed by Dr. J. D. PALMER consisting of petunia cpDNA fragments (Pst I cut) cloned into pUC 18 served as probes to the filterbound DNA fragments. These 9 probes cover 84 % of the petunia chloroplast genome and another 3 clones from Sal I cut petunia cpDNA cover the remaining 16 % of this genome. The probes derived from Pst I fragments were designated by numbers preceded by the letter 'P' (i.e. P6) and those derived from Sal I cut cpDNA were designated by numbers preceded by the letter 'S' (i.e. S6). Fragments from cpDNA of *Vitis vinifera* cv. Cabernet Sauvignon were isolated from a library generated by cloning Pst I restricted total cellular DNA in pBR322. Plasmids containing a cpDNa insert were identified by dot blotting the fragments in question to a nylon membrane and probing with cpDNA of *Solanum hyporhodium*.

Probe DNA was nick translated following the protocols of MANIATIS *et al.* (1982) except that 4 units of DNase I/Polymerase I (GIBCO BRL, Grand Island, NY) were added to the 30 µl reaction mixture instead of adding each enzyme separately. The nick translation mixture was incubated for 1—2 h at 14 °C, after which the labeled probes were separated from unincorporated nucleotides by spun column chromatography on a Sephadex G-50 1cc column following the method of MANIATIS *et al.* (1982). Prior to hybridization, the labelled probes were denatured in solution with 1N NaOH at room temperature for 10 min.

Table

Summary of cpDNA probes and restriction endonucleases used to detect polymorphisms in the chloroplast genomes of the parents of 2 grape crosses.

cpDNA probe	Cross	
	NY 62.136.2 × Yates	Cayuga White × Aurore
PGG 35	Bcl I	–[a]
PGG 38	Eco RI	×[b]
P 4	×	×
P 6	Xba I, Bam HI	×
P 8	×	×
P 10	Eco RI, Bcl I	Bcl I
P 12	×	×
P 16	×	×
P 18	Hind III	×
P 19	×	×
P 20	×	×
S 6	Bam HI	Eco RI
S 8	Bam HI	×
S 11	Hind III	Hind III

[a] dash (–) indicates parents not screened for polymorphisms using this probe.
[b] "×" indicates no polymorphisms detected between parents. All parents were cut using the following restriction endonucleases: Eco RI, Bam HI, Bcl I, Hind III or Xba I.

The blots were prehybridized for 4—6 h at 65 °C in approximately 10 ml of buffer per 100 cm² of blot. The hybridization buffer consisted of 750 mM NaCl, 125 mM citric acid (trisodium salt, dihydrate), 2.5 mM EDTA, 50 mM $NaPO_4$, 5 × Denhardt's, 0.4 mg/ml calf thymus DNA, 5.0 % dextran sulfate and 0.6 % SDS. Hybridization was carried out in the same buffer for 16—18 h at 65 °C after adding denatured radiolabelled probe. After hybridization the blots were washed 2—3 times in an excess of 2X SSC + 0.1 % SDS for 20 min at 65 °C. The blots were then wrapped in plastic wrap and tape to a Kodak X-ray exposure cassette. Autoradiography was carried out at −70 °C for the appropriate exposure time.

Results and discussion

Autoradiographs of cpDNA fragments of parents demonstrated that four distinct plastid genotypes existed among the parents and that the parents of both crosses had different plastid genotypes. Analysis of the cpDNA restriction patterns of the parental DNA using the cpDNA probes of grape and petunia revealed a high level of polymorphism (63 % of probes revealed polymorphic bands) between the parents of the cross, 62.136.2 × Yates, and a low level of polymorphism (15 %) between the parents of the cross, Cayuga White × Aurore (Table). All polymorphic bands observed for a single probe appeared to be part of a single locus.

The differences observed in the banding patterns of the parental genotypes when probed with the cpDNA probes, P10 (Fig. 1) and P6, showed that there were four distinct plastid genotypes. When Eco RI-digested DNA was probed with P10, there was a unique banding pattern for NY 62.136.2 (Fig. 2). Bcl I cut DNA probed with P10 showed Cayuga White's banding pattern differed from Yates and Aurore. Thus, the banding patterns of Yates, Aurore and Cayuga White were the same for Eco RI digested DNA

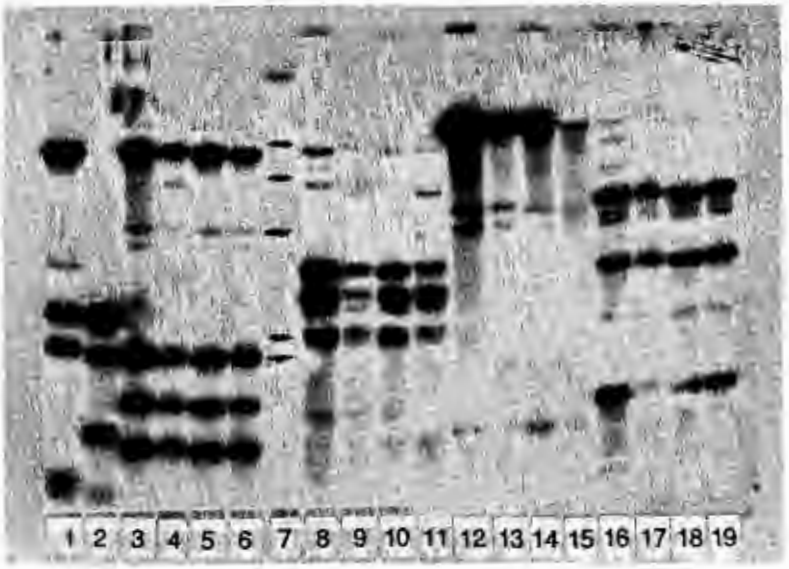

Fig. 1: Restriction patterns of total DNA of 4 grape genotypes cut with four restriction enzymes and probed with cpDNA (P10) from petunia. Lane 1: Eco RI digested total DNA of apple, White Angel; Lane 2: of pea, Sparkle; Lanes 3—6: of Yates, NY 62.136.2, Aurore and Cayuga White (parents); Lane 7: Hind III cut λ DNA; Lanes 8—11: Bcl I digested total DNA of the parents; Lanes 12—15: Hind III cut DNA of the parents; and Lanes 16—19: Bam HI cut DNA of parents.

but differed for Yates and Cayuga White when total DNA was digested with Bcl I. This indicated that these genotypes did not share the same plastid genome sequences. The restriction patterns for Yates and Aurore differed for Xba I cut DNA probed with P6, indicating that the plastid genotypes of these cultivars were different (Fig. 1). From these observations it could be inferred that all four parents carried distinct plastid genomes. The observed differences also indicate that heteroplasmy among the four parental genotypes exists.

Probes polymorphic for the parents were used to screen 40 progeny from each cross. For the cross NY 62.136.2 × Yates the following probes were used to screen the progeny for cpDNA polymorphisms: PGG 35; PGG 38; P6; P10; P18; S6; S8 and S11. No polymorphisms were observed in this population. No segregation occurred in the F1 progeny, as all had the genotype of the female parent, NY 62.136.2. Only three probes (P10, S6 and S11) were polymorphic among the parents of the Cayuga White × Aurore cross. These were used to screen the progeny of this cross and again, uncovered no polymorphisms among progeny; only the banding pattern of the female parent was observed.

Analysis of the cpDNA restriction patterns in the two populations using the cpDNA probes which could distinguish between the parents showed that all progeny had the banding pattern of the female parent. This indicated that chloroplast inheritance in these grape populations was maternal.

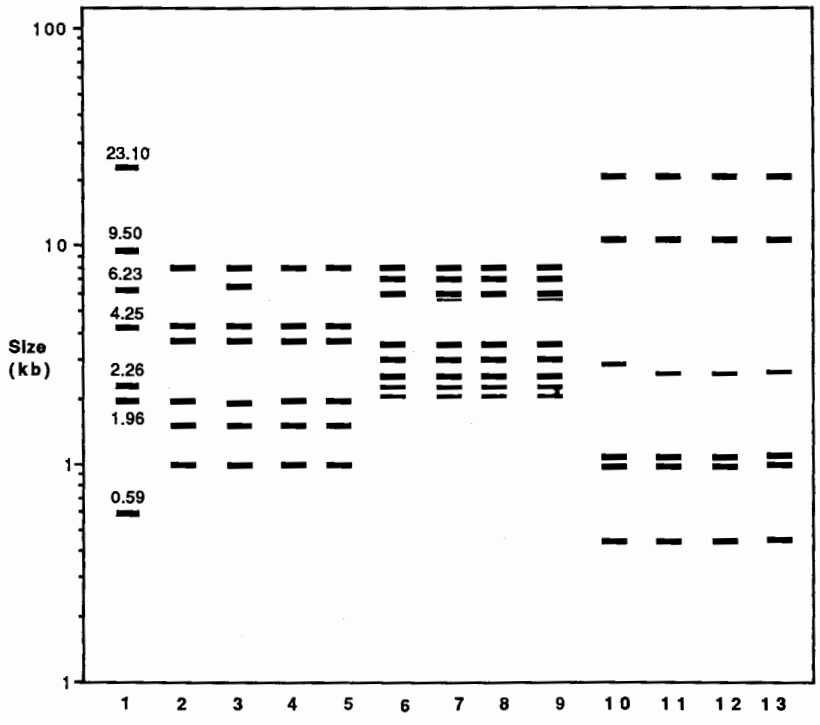

Fig. 2: Schematic of the restriction patterns of total DNA of 4 grape genotypes cut with 3 restriction enzymes and probed with 1 of 2 cpDNA probes (P6 or P10) from petunia. Lane 1: MW-marker λDNA digested with Hind III; Lanes 2—5: Yates, NY 62.136.2, Aurore and Cayuga White, respectively, digested with Eco RI, and probed with P10; Lanes 6—9: same as lanes 2—5 except digested with Bcl I; Lanes 10—13: same as lanes 6—9 but digested with Xba I and probed with P6.

The results of this study demonstrated that there was sufficient variability in grape chloroplast DNA to study the evolutionary relationships among grape species by analysis of the differences in cpDNA among grape species. The small number of differences in restriction patterns of Cayuga White and Aurore may be indicative of their common ancestry in which the original female ancestors, Aramon and Marocain noir, respectively, are both cultivars of *V. vinifera*. Both ancestral cultivars originated in Europe. The ancestral maternal parent of NY 62.136.2 is a selection of *V. labrusca*, the maternal parent of Emily, while the ancestral female of Yates is Trollinger (*V. vinifera*) which originated in Germany. The unrelated origins of the cytoplasms of these two parents accounts well for the high degree of divergence in restriction patterns of the cpDNA.

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