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Paternal plastid DNA can be inherited in lentil

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Abstract Restriction fragment analysis was used to study the inheritance of chloroplast DNA (cpDNA) in F1 progeny from crosses between Lens culinaris ssp. orientalis and L. culinaris ssp. culinaris. Twenty-five combinations of 11 restriction enzymes and three heterologous probes from Petunia hybrida cpDNA were used to screen six accessions of L. c. culinaris and one accession of L. c. orientalis for restriction fragment length polymorphisms (RFLPs). No variation in cpDNA was observed within the subspecies L. c. culinaris, but the L. c. orientalis accession was unambiguously distinguished from all six L. c. culinaris accessions by two RFLPs. Of ten F1 progeny from L. c. orientalis × L. c. culinaris crosses, nine had only maternal cpDNA restriction fragments but one F1 plant inherited cpDNA fragments from both parents. Nuclear DNA inheritance was biparental in all ten F1 progeny.

Key words Lens culinaris · Chloroplast DNA Maternal plastid inheritance · Biparental plastid inheritance · Restriction fragment length polymorphism

Introduction

The most common mode of plastid inheritance in angiosperms is uniparental-maternal, although there is evidence for the inheritance of paternal plastids in many species, including several of the Leguminosae (Smith 1989; Harris and Ingram 1991). Analysis of restriction fragment length polymorphisms (RFLPs) now provides a powerful tool to examine the inheritance of chloroplast (cp) DNA. In the Fabaceae (formerly Leguminosae: Papilionaceae), both maternal and paternal modes of chloroplast inheritance have been reported (Corriveau and Coleman 1988; Smith 1989; Harris and Ingram 1991). RFLP analysis has shown only maternal cpDNA inheritance in Glycine (Hatfield et al. 1985) and Pisum sativum (Polans et al. 1990), but biparental or even predominantly paternal cpDNA inheritance in Medicago sativa (Lee et al. 1988; Schumann and Hancock 1989; Masoud et al. 1990).

Lentil (Lens culinaris Medik.), a member of Fabaceae, is an important food legume in India, the Middle East and North Africa because of its high nutritional quality and drought resistance (Simpson and Conner-Ogorzaly 1986). The species is subdivided into three cross-compatible subspecies (Ladizinsky et al. 1984): ssp. culinaris (cultivated), ssp. orientalis (wild) and ssp. odemensis (wild). Restriction fragment length variation has been reported for cpDNA in Lens (Muench et al. 1991), but as yet this variability has not been used to examine the inheritance of cpDNA. On the basis of cytological evidence from fluorochrome epifluorescence microscopy, Corriveau and Coleman (1988) suggested that plastid inheritance is maternal in L. culinaris.

In the study presented here, RFLP analysis was used to examine the cpDNA inheritance in F1 progeny from crosses between the Lens culinaris subspecies L. c. orientalis and L. c. culinaris. Cloned fragments from Petunia hybrida cpDNA were used as probes for detecting lentil cpDNA fragments. The results demonstrate the possibility of cpDNA inheritance from both parents in addition to the predominant uniparental-maternal inheritance pattern.

Materials and methods

Lentil cultivars and controlled crosses

Six accessions of Lens culinaris ssp. culinaris and one accession of L. culinaris ssp. orientalis were studied. The varieties 'Laird' and
AvaI, BamHI, BclI, BgIII, ClaI, DraI, EcoRI, EcoRV, HindlII, XbaI, HindIII-P

Three cpDNA and nuclear DNA probes

The method of Bookjans et al. (1984). Total cellular DNA samples (5–10 μg) were digested with 15–20 units of each of the restriction enzymes Aval, BamHI, BclI, BglII, EcoRI, EcoRV, HindIII, XbaI, or XhoI for 5 h. RNA was removed by treating with 1 μg RNAase at 37°C for 1 h. Approximately 1.0 g of cpDNA was digested with 50 units of DraI.

Total DNA restriction fragments were separated on 20 x 20 cm agarose gels containing ethidium bromide by electrophoresis at 1.5–1.75 V/cm for about 18 h in TBE buffer (Maniatis et al. 1982), whereas purified cpDNA fragments were separated on a 1% agarose gel. DNA fragments were transferred to nylon membranes (Gene Screen Plus, DuPont Canada, Mississauga, Ontario) or Hybond-N+ (Amersham Canada, Oakville, Ontario) using the alkaline transfer method of Chomczynski and Sashe (1984).

cpDNA and nuclear DNA probes

Three PstI fragments of Petunia hybrida cpDNA (Palmer et al. 1983) were used as hybridization probes for lentil cpDNA fragments: P3, a 21-kb fragment from the large single copy (LSC) region including the rbcL gene, P6, a 15.3-kb PstI fragment from LSC region; and P10, a 9.0-kb PstI fragment from the LSC region including part of the psbA gene. Southern blots of DNA fragments from the seven accessions of lentil were screened using the P6 and P10 probes with 11 restriction endonucleases and the P3 probe with BglII, EcoRI and EcoRV. Two informative RFLPs, revealed by the DraI-P6 and HindIII-P10 enzyme-probe combinations, were used to examine the inheritance of the cpDNA in the interspecific crosses of L. culinaris. The DraI restriction digest of purified cpDNA from ‘Eston’ was also hybridized to Petunia cpDNA probe P6. To examine nuclear gene inheritance, a 0.64-kb lentil cDNA clone CMH52 (Hayve and Muehlbauer 1989) was used to probe the same blot of DraI digests of the parents and progeny DNA as was used for cpDNA analysis with the P6 probe.

Probe preparation, hybridization, washing, and autoradiography

Petunia cpDNA or lentil cDNA probes were prepared by radiolabeling the fragments with α-32P-dCTP by random priming. Prehybridizations for 6–8 h and hybridizations for a minimum of 16 h were conducted at 60°C (Rajora and Dancik 1992) with or without 10 mg denatured salmon/herring sperm DNA. Hybridized blots were washed (Rajora and Dancik 1992) and then exposed to X-ray films with intensifying screens for 3–48 h (cpDNA) or 8 days (nuclear DNA) at ~70°C.

Results

cpDNA restriction fragment variation

A total of 103 restriction fragments was revealed by 25 combinations of 11 restriction enzymes and three cpDNA probes. All accessions of the same L. culinaris subspecies, including the different plants of one accession, shared the same cpDNA fragments. Two RFLPs unambiguously distinguished L. culinaris ssp. culinaris from L. culinaris ssp. orientalis. Hybridization of the P6 cpDNA probe to blots of DraI digests revealed a 1.3-kb fragment in all L. culinaris accessions examined, including the ‘Laird’ and ‘Eston’ varieties (Fig. 1A: lanes 2–4), but a 1.0-kb fragment in the L. c. orientalis accession ‘LO4’ (Fig. 1A: lane 1). Hybridization of this probe to a blot of a DraI-digested purified cpDNA of ‘Eston’ showed that the hybridizing fragments (Fig. 2: lane 2) corresponded to the cpDNA fragments visible on the ethidium bromide-stained gel (Fig. 2: lane 1) and were the same as those seen in the equivalent blot of total DNA samples (Fig. 1A: lane 4). This supports the chloroplastic origin of the hybridizing bands. DraI digestion of cpDNA from ‘Eston’ produced at least 26 visible restriction fragments, ranging in size from 0.7 to 15.8 kb (Fig. 2: lane 1). A summation of the fragment sizes indicated a chloroplast genome size of approximately 125 kb, which is in agreement with a size reported previously (Muench et al. 1991). Hybridization of the P10 cpDNA probe to a blot of HindIII-digested DNA revealed another intersubspecies RFLP. All L. c. culinaris accessions, including ‘Eston’ and ‘Laird’ (Fig. 3: lanes 2–4), had a 1.6-kb fragment, whereas the L. c. orientalis accession ‘LO4’ had a 2.6-kb fragment (Fig. 3: lane 1).

cpDNA inheritance

Hybridization of the P6 cpDNA probe to DraI digests of ten F1 plants showed the maternal (‘LO4’) restriction fragments in all of the F1 plants from three L. c. orientalis × L. c. culinaris crosses (Fig. 1A: lanes 5–14). However, one F1 plant (hybrid 4) from a ‘LO4’ × ‘Laird’ parent 1 cross also showed the 1.3-kb fragment characteristic of the paternal ‘Laird’ parent, although it is not apparent in Fig. 1A (lane 8). To confirm this RFLP pattern and minimize the possibility that it was the result of sample contamination, a second DNA sample was extracted from this plant at a later stage of growth. With larger samples of DNA, the 1.3-kb DraI fragment was clearly identified by the P6 probe in the two independent samples from this plant (Fig. 1B: lanes 4,5) and not in the other two hybrids. Hybridization of the cpDNA probe P10 to HindIII-digested DNA (Fig. 3) supported the results in Fig. 1. Nine of the ten F1 plants from the L. c. orientalis × L. c. culinaris crosses showed only...