Theor Appl Genet (1995) 90: 607–610

O. P. Rajora · J. D. Mahon

Paternal plastid DNA can be inherited in lentil

Received: 30 April 1994 / Accepted: 10 June 1994

Abstract Restriction fragment analysis was used to study the inheritance of chloroplast DNA (cpDNA) in F_1 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 F_1 progeny from *L. c. orientalis* × *L. c. culinaris* crosses, nine had only maternal cpDNA restriction fragments but one F_1 plant inherited cpDNA fragments from both parents. Nuclear DNA inheritance was biparental in all ten F_1 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 F_1 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

Communicated by R. Hagemann

O .P. Rajora[1](✉) · J. D. Mahon
Plant Biotechnology Institute,
National Research Council of Canada, 110 Gymnasium Place,
Saskatoon, Saskatchewan S7N 0W9, Canada

Present address:
[1] Department of Renewable Resources, University of Alberta,
Edmonton, Alberta T6G 2H1, Canada

NRCC no. 38464

'Eston', and the Syrian accessions 'ILL-5588' and 'ILL-5684' (originally from the International Center for Agricultural Research in the Dry Areas, Aleppo, Syria) of *L. c. culinaris* were obtained as three single-seed descent lines from Dr. A.E. Slinkard of the University of Saskatchewan, and the *L. c. culinaris* varieties 'Brewer' and 'Redchief' and the *L. c. orientalis* accession 'LO4' were obtained from Dr. F.J. Muehlbauer of the USDA/ARS Grain Legume Program, Washington State University. Plants were grown in pots in a controlled growth chamber with a 16-h photoperiod and day/night temperatures of 20°/15 °C.

Intersubspecific crosses of *L.c. orientalis* × *L. c. culinaris* were made by emasculation and hand pollination, and F_1 plants were grown as described above. A total of seven plants from an 'LO4' × 'Laird' parent 1 cross, one plant from an 'LO4' × 'Laird' parent 2 cross and two plants from an 'LO4' × 'Eston' cross were obtained and analyzed. No reciprocal crosses were obtained because of insufficient pollen from 'LO4'.

DNA extraction, digestion, electrophoresis, and Southern blotting

Total cellular DNA was isolated from 0.5 to 2.0 g of fresh leaf tissue from individual parental and F_1 plants by a modification of a CTAB method of Doyle and Doyle (1987). For comparison, purified cpDNA was extracted from a single sample of 'Eston' leaf tissue by the method of Bookjans et al. (1984). Total cellular DNA samples (5–10 µg) were digested with 15–20 units of the restriction enzymes *Ava*I, *Bam*HI, *Bcl*I, *Bgl*II, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I, or *Xho*I 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 *Dra*I.

Total DNA restriction fragments were separated on 20 × 20 cm 0.8% (in TBE buffer) 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 Qasba (1984).

cpDNA and nuclear DNA probes

Three *Pst*I 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 part of the *rbcL* gene, P6, a 15.3-kb *Pst*I fragment from LSC region; and P10, a 9.0-kb *Pst*I 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 all 11 restriction endonucleases and the P3 probe with *Bgl*II, *Eco*RI and *Eco*RV. Two informative RFLPs, revealed by the *Dra*I-P6 and *Hin*dIII-P10 enzyme-probe combinations, were used to examine the inheritance of cpDNA in the intersubpecific crosses of *L. culinaris*. The *Dra*I 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 (Havey and Muehlbauer 1989) was used to probe the same blot of *Dra*I 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 radiolabelling the fragments with α-[^{32}P]-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 *Dra*I digests revealed a 1.3-kb fragment in all *L. c. 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 *Dra*I-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. *Dra*I 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 *Hin*dIII-digested DNA revealed another intersubspecific 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 *Dra*I digests of ten F_1 plants showed the maternal ('LO4') restriction fragments in all of the F_1 plants from three *L. c. orientalis* × *L. c. culinaris* crosses (Fig. 1A: lanes 5–14). However, one F_1 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 *Dra*I 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 *Hin*dIII-digested DNA (Fig. 3) supported the results in Fig. 1. Nine of the ten F_1 plants from the *L. c. orientalis* × *L. c. culinaris* crosses showed only

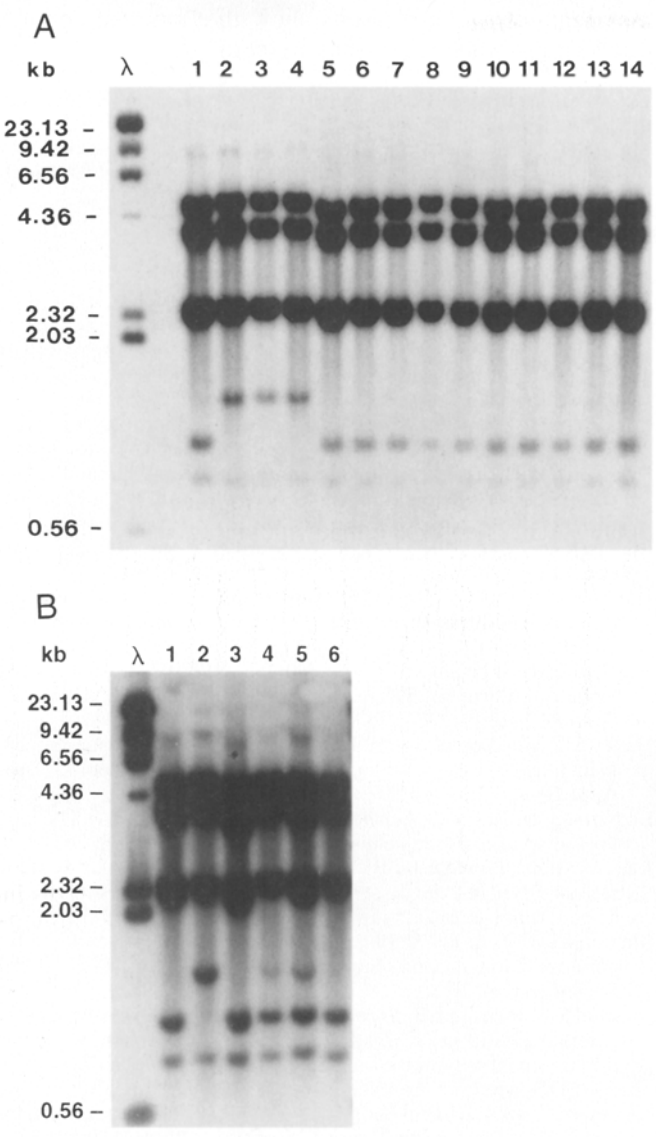
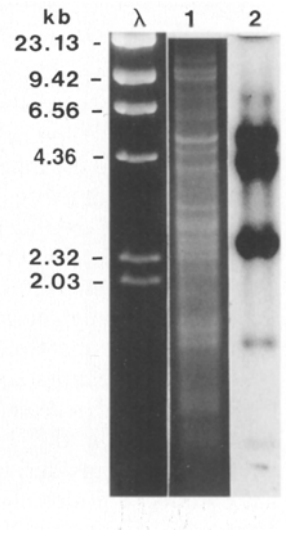


Fig. 1A, B Autoradiograph of cpDNA restriction fragment patterns of parents and F_1 hybrid progeny of *L. c. orientalis* × *L. c. culinaris* crosses obtained by hybridization of the 15.3-kb *Pst*I *Petunia* cpDNA fragment P6 to the *Dra*I-restricted total DNA. **A** *Lane 1* 'LO4', *2* 'Laird' parent 1, *3* 'Laird' parent 2, *4* 'Eston', *5–11* F_1 hybrids 1–7 of 'LO4' × 'Laird' parent 1, *12* F_1 progeny of 'LO4' × 'Laird' parent 2, *13, 14* F_1 hybrids 1, 2 of 'LO4' × 'Eston'. **B** RFLP analysis, repeated with increased lentil DNA for *Dra*I-P6 enzyme-probe combination, for the parents 'LO4' and 'Laird' parent 1 and 3 F_1 hybrids: *Lane 1* 'LO4', *2* 'Laird' parent 1, *3* F_1 hybrid 3, *4* F_1 hybrid 4 of 'LO4' × 'Laird' parent 1-DNA extracted on December 12, 1990, *5* F_1 hybrid 4 of 'LO4' × 'Laird' parent 1-DNA extracted on January 14, 1991, *6* F_1 hybrid 5

Fig. 2 Restriction fragment patterns of purified cpDNA of 'Eston' restricted with *Dra*I. *Lane 1* ethidium bromide-stained gel under the UV light, *2* autoradiograph produced by hybridization of the *Petunia* cpDNA fragment P6 to *Dra*I-restricted DNA fragments from lane 1



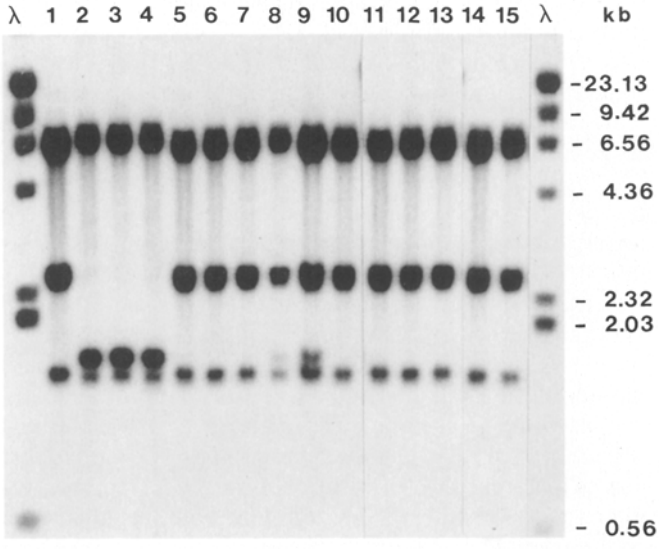


Fig. 3 Autoradiograph of cpDNA restriction fragment patterns of parents and F_1 hybrid progeny of *L. c. orientalis* × *L. c. culinaris* crosses obtained by hybridization of the 9.0-kb *Pst*I *Petunia* cpDNA fragment P10 to *Hin*dIII-restricted total DNA. *Lane 1* 'LO4' , *2* 'Laird' parent 1, *3* 'Laird' parent 2, *4* 'Eston', *5–7* F_1 hybrids 1–3 of 'LO4' × 'Laird' parent 1, *8* F_1 hybrid 4 of 'LO4' × 'Laird' parent 1-DNA extracted on December 12, 1990, *9* F_1 hybrid 4 of 'LO4' × 'Laird' parent 1-DNA extracted on January 14, 1991, *10–12* F_1 hybrids 5–7 of 'LO4' × 'Laird' parent 1, *13* F_1 hybrid of 'LO4' × 'Laird' parent 2, *14, 15* F_1 hybrids 1, 2 of 'LO4' × 'Eston'

the 2.6-kb fragment from the maternal 'LO4' plant, but both the 2.6-kb maternal and the 1.6-kb paternal fragments were visible in the two independent DNA samples from the F_1 hybrid 4 of the 'LO4' × 'Laird' parent 1 cross (Fig. 3: lanes 8, 9). The autoradiographic intensity of the paternal fragments was much weaker in this F_1 hybrid plant than in the 'Laird' parent (Figs. 1B and 3).

Nuclear DNA inheritance

Nuclear DNA inheritance was biparental in all F_1 hybrid progeny from the *L. c. orientalis* × *L. c. culinaris* crosses, as hybridization of the nuclear cDNA probe CMH52 to the same blot shown in Fig. 1A revealed fragments characteristic of both parents for all F_1 plants (data not shown).

Discussion

The RFLPs distinguishing between the chloroplast genomes of *L. c. orientalis* and *L. c. culinaris* made it possible to examine the inheritance of cpDNA in this species. The predominant mode of cpDNA inheritance was uniparental-maternal, as nine of the ten F_1 progeny from the *L. c. orientalis* × *L. c. culinaris* crosses showed only restriction fragments characteristic of the maternal parent. That this resulted from maternal inheritance rather than self-fertilization of the maternal parent was shown by heterozygosity of a nuclear marker in all F_1 progeny. No plants were found with cpDNA from only the paternal parent, but a single F_1 plant from the 'LO4' × 'Laird' parent 1 cross had cpDNA fragments derived from both maternal and paternal parents. The detection of cpDNA from both parents with two different cpDNA probes in DNA samples prepared from plant tissue harvested at different stages of growth provides strong support for the biparental origin of cpDNA in this F_1 plant.

Uniparental-maternal inheritance is the most common mode of cpDNA inheritance in angiosperms, including grain legumes (Hatfield et al. 1985; Corriveau and Coleman 1988; Smith 1989; Polans et al. 1990; Harris and Ingram 1991), and our investigation provides the first evidence of occasional biparental inheritance of cpDNA in the genus *Lens*, or to our knowledge, in any grain legume. Cytological evidence of plastid DNA presence in pollen has been reported for several legumes, including the closely related *Pisum* and *Lathyrus* (Corriveau and Coleman 1988), but results for lentil were negative. The results presented here show the inheritance of both maternal and paternal RFLP markers in one F_1 plant from a cross between two subspecies of *L. culinaris*. Because of the small number of the F_1 plants studied, it was not possible to determine with any accuracy the frequency of paternal transmission of cpDNA. Occasional low frequency transmission of paternal DNA (up to 5%) has been reported for other species (Smith 1989), and with such frequencies, a single example of paternal transmission is not unlikely in ten F_1 plants. It should be noted, however, that the autoradiographic intensity of the paternal cpDNA fragments in the plant exhibiting biparental cpDNA was considerably less than that seen in the paternal parent (Figs. 1b and 3), suggesting that both the number of paternal plastids and their frequency of appearance in this progeny are low. Thus, the total amount of paternal cpDNA inherited may not be great and should perhaps be described as paternal leakage (Wagner et al. 1991). Nevertheless, although the mechanisms responsible and the genetic significance remain unknown, these results show that paternal cpDNA can be transmitted to progeny in lentil.

Acknowledgements The authors wish to thank Colin Kindrachuk for technical assistance, Dr. J. D. Palmer for providing *Petunia* chloroplast DNA fragments and Dr. F. J. Muehlbauer for lentil cDNA clones. This research was supported by the Saskatchewan Agricultural Development Fund (Project No. R-89-05-04580).

References

Bookjans G, Stummann BM, Henningsen KW (1984) Preparation of chloroplast DNA from pea plastids isolated in a medium of high ionic strength. Anal Biochem 141:244–247

Chomczynski P, Qasba PK (1984) Alkaline transfer of DNA to plastic membrane. Biochem Biophys Res Commun 122:340–344

Corriveau JL, Coleman AW (1988) Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Am J Bot 75:1443–1458

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Harris SA, Ingram R (1991) Chloroplast DNA and biosystematics: the effects of intraspecific diversity and plastid transmission. Taxon 40:393–412

Hatfield PM, Shoemaker RC, Palmer RG (1985) Maternal inheritance of chloroplast DNA within the genus *Glycine,* subgenus *Soja.* J Hered 76:373–374

Havey MJ, Muehlbauer FJ (1989) Linkages between restriction fragment length, isozyme and morphological markers in lentil. Theor Appl Genet 77:395–401

Ladizinsky G, Braun D, Goshen D, Muehlbauer FJ (1984) The biological species of the genus *Lens* L. Bot Gaz 145:253–261

Lee DJ, Blake TK, Smith SE (1988) Biparental inheritance of chloroplast DNA and the existence of heteroplasmic cells in alfalfa. Theor Appl Genet 76:545–549

Maniatis T, Fritsch EF, Sambroke J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Masoud SA, Johnson LB, Sorenson EL (1990) High transmission of paternal plastid DNA in alfalfa plants demonstrated by restriction fragment polymorphic analysis. Theor Appl Genet 79: 49–55

Muench DG, Slinkard AE, Scoles GJ (1991) Determination of genetic variation and taxonomy in lentil (*Lens* Miller) species by chloroplast DNA polymorphism. Euphytica 56:213–218

Palmer JD, Shields CR, Cohen DB, Orton TJ (1983) Chloroplast DNA evolution and origin of amphidiploid *Brassica* species. Theor Appl Genet 65:181–189

Polans NO, Corriveau JL, Coleman AW (1990) Plastid inheritance in *Pisum sativum* L. Curr Genet 18:477–480

Rajora OP, Dancik BP (1992) Chloroplast DNA inheritance in *Populus*. Theor Appl Genet 84:280–285

Schumann CM, Hancock JF (1989) Paternal inheritance of plastids in *Medicago sativa*. Theor Appl Genet 78:863–866

Simpson BB, Conner-Ogorzaly M (1986) Economic botany plants in our world. McGraw-Hill, New York

Smith SE (1989) Biparental inheritance of organelles and its implications in crop improvement. Plant Breed Rev 6:361–393

Wagner DB, Dong J, Carlson MR, Yanchuk AD (1991) Paternal leakage of mitochondrial DNA in *Pinus*. Theor Appl Genet 82: 510–514