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Mitochondrial and nuclear DNA variation, genotype fingerprinting and genetic relationships in lentil (*Lens culinaris* Medik.)

Om P. Rajora[1] and John D. Mahon[2]

[1]*Department of Renewable Resources, 751 General Services Building, University of Alberta, Edmonton, Alberta, Canada T6G 2H1;* [2]*Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada S7N 0W9.* *NRCC No. 40723. Received 6 September 1996, accepted 24 April 1997.*

Rajora, O. P. and Mahon, J. D. 1997. **Mitochondrial and nuclear DNA variation, genotype fingerprinting and genetic relationships in lentil (*Lens culinaris* Medik.)**. Can. J. Plant Sci. **77**: 515–521. Mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) variations were examined in six cultivars of *Lens culinaris* ssp. *culinaris* and two (mtDNA) or one (nuDNA) accession(s) of L. *culinaris* ssp. *orientalis*. Total leaf DNA was digested with up to 15 restriction endonucleases, separated by agarose gel electrophoresis and trasferred to nylon membranes. To examine mtDNA variation, blots were probed with mtDNA coding for cytochrome c oxidase I (*cox*I) and ATPase 6 (*atp*6) of both wheat and maize as well as apocytochrome b (*cob*) and Orf25 (*orf*25) of wheat. Sixteen combinations of mtDNA probes and restriction enzymes revealed 34 fragments that discriminated between at least two lentil accessions. For nuDNA analysis, probes from cDNA and genomic DNA clones of lentil were used to probe the same blots, and identified 46 diagnostic fragments from 19 probe/enzyme combinations. Each lentil accession could be unequivocably distinguished from all others on the basis of both mitochondrial and nuclear DNA fragment patterns. The mitochondrial restriction fragment similarities ranged from 0.944 to 0.989, with a mean of 0.970 but nuclear restriction fragment similarities varied from 0.582 to 0.987, with a mean of 0.743. The apparent genetic relationships among accessions differed according to the source of DNA examined, although the commercial varieties Laird, Brewer and Redchief showed similarly high levels of mean similarity with both nuclear (0.982) and mitochondrial DNA (0.983).

Key words: *Lens culinaris* Medik., genetic variation, mitochondrial, nuclear, DNA, lentil

Rajora, O. P. et Mahon, J. D. 1997. **Variation de l'ADN dans les mitochondries et dans le noyau, empreintes génétiques et rapports génétiques chez la lentille (*Lens culinaris* Medik.)**. Can. J. Plant Sci. **77**: 515–521. Nous avons examiné les variations de l'ADN mitochondrial (ADNmt) et nucléaire (ADNnu) chez six cultivars de *Lens culinaris* spp. *culinaris* et chez 2 (ADNmt) et 1 (ADNnu) obtention de *L. culinaris* spp. *orientalis*. L'ADN foliaire total était digéré en présence d'endonucléases de restriction (jusqu'à 15), séparé par électrophorèse sur gel d'agarose, puis transféré sur membrane de nylon. Pour examiner les variations de ADNmt, les empreintes étaient mises en présence de ADNmt codant pour la cytochrome c oxydase I (*cox*I) et pour l'ATPase 6 (*atp*6) du blé et du maïs, ainsi que pour l'apocytochrome b (*cob*) et Orf25 (*orf*25) du blé. Seize combinaisons de sonde ADNmt et d'enzymes de restriction ont mis au jour 34 fragments différents entre au moins 2 obtentions de lentilles. Pour l'analyse de ADNnu, les sondes provenant de clones d'ADNc et de clones d'ADN génomique de lentilles étaient liées sur les mêmes empreintes. Elles ont permis d'identifier 46 fragments diagnostiques à partir de 19 combinaisons sonde-enzyme. Chaque obtention de lentille pouvait être distinguée sans équivoque de toutes les autres d'après la longueur des fragments d'ADN mitochondrial et nucléaire. Le degré de similarité des fragments de restriction mitochondriaux allait de 0,944 à 0,989, pour une moyenne de 0,970, mais pour les fragments de restriction nucléaire il variait de 0,582 à 0,987, pour une moyenne de 0,743. Les rapports génétiques apparents entre les obtentions différaient selon la provenance de l'ADN examiné, bien que les variétés du commerce Laird, Brewe et Redchief manifestaient les mêmes niveaux élevés de similarité moyenne, tant avec l'ADN nucléaire (0,982) qu'avec l'ADN mitochondrial (0,983).

Mots clés: *Lens culinaris* Medik., variation génétique, mitochondrial, nucléaire, ADN lentille

Lentil (*Lens culinaris* Medik), a legume of the Fabaceae, is subdivided into three cross compatible subspecies: spp. *culinaris* (cultivated), ssp. *orientalis* (wild), and ssp. *odemensis* (wild) (Ladizinsky et al. 1984). The cultivated forms are an important food crop of the Indian subcontinent, the Middle-East and North Africa, because of their high nutritional quality and drought resistance (Simpson and Conner-Ogorzaly 1986). The ability to identify cultivars and accessions and an understanding of their phylogenetic relationships could contribute to the effectiveness of lentil breeding programs. Recent genetic studies have examined Lens for genetic variation in allozymes (Pinkas et al. 1985; Hoffman et al. 1986; Erskine and Muehlbauer 1991) and restriction fragment length polymorphisms (RFLPs) in nuclear (Havey and Muehlbauer 1989a) and chloroplast DNA (Muench et al. 1991; Mayer and Soltis 1994). RFLPs have also been used to examine the inheritance of the chloroplast (Rajora and Mahon 1995 and mitochondrial (Rajora and Mahon 1994) genomes in *Lens culinaris*. Although no other information on variation in *Lens* mitochondrial DNA (mtDNA) has been

Abbreviations: **mtDNA**, mitochondrial DNA; **nuDNA**, nuclear DNA; **RFLP**, restricted fragment length polymorphism

published, mtDNA variation in other Leguminosae has been used to examine the structure of mitochondrial genomes (Negruk and Kaushik 1988) as well as genetic diversity and taxonomic relationships (Khairallah et al. 1990, 1991; Grabau et al. 1992; van de Van et al. 1993; Lee et al. 1994).

In *Lens*, generally high similarities have been observed among cultivars and even between the subspecies *culinaris* and *orientalis* (Pinkas et al. 1985; Hoffman et al. 1986; Havey and Muehlbauer 1989a; Muench et al. 1991; Mayer and Soltis 1994). In the present study, we used RFLP analysis to examine the variation in mtDNA and nuclear DNA (nuDNA) using eight selected accessions of *L.c. culinaris* and *L.c. orientalis*. The results demonstrate unique genomes for each accession and varying levels of genetic similarity among accessions.

MATERIALS AND METHODS

Lentil Cultivars and Accessions

Six accessions of *Lens culinaris* ssp. *culinaris* (Laird, Eston, Brewer, Redchief, ILL-5588 and ILL-5684) and two accessions of *L. culinaris* ssp. *orientalis* (LO4 and LO24) were examined. The varieties Laird and Eston and the Syrian accessions ILL-5588 and ILL-5684 (originally from the International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria) were obtained as three single-seed descent lines each and the *L. culinaris* ssp. *orientalis* accession LO24 as a single seed lot from Dr. A. E. Slinkard of the Crop Development Centre, University of Saskatchewan. The varieties Brewer and Redchief and 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.

DNA Extraction, Restriction, Electrophoresis, and Southern Blotting

Total cellular DNA was isolated from 0.5 to 2.0 g fresh weight of young leaf tissue from individual plants after 2, 4, and 8 wk of planting (seeding) by a modification of the CTAB method of Doyle and Doyle (1987). In three separate DNA extractions, good quality DNA with identical results was obtained. DNA (5–10 μg) from individual plants was digested for 5 h with 15–20 units of restriction enzymes *Ava*I, *Bam*HI, *Bcl*I, *Bgl*II, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Kpn*I, *Pst*I, *Pvu*II, *Sca*I, *Xba*I, and *Xho*I according to the manufacturer's instructions, and restriction fragments were separated on 0.8% agarose gels, and transferred to nylon membranes (Rajora and Mahon 1994).

DNA Probes and Probe-enzyme Combinations

The sources and characteristics of the DNA fragments used as probes to detect polymorphisms in mitochondrial and nuclear DNA are summarized in Table 1. Mitochondrial DNA variation was examined among all eight lentil cultivars using all 84 combinations of six mtDNA probes (maize *Atp*6, maize *Cox*I, wheat *Atp*6, wheat *Cob*, wheat *Cox*I, and wheat *Orf*25) and 14 restriction enzymes (*Ava*I, *Bam*HI, *Bcl*II, *Bgl*I, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, *Xba*I, and *Xho*I). Nuclear DNA variation was examined among seven lentil cultivars (Laird, Eston, Brewer, Redchief, ILL-5588, ILL-5684, and LO4) using the following 29 combinations of four probes and 10 restriction enzymes: CMH-34 with *Bam*HI, *Bcl*I, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Sca*I, and *Xba*I; CMH-52 with *Bam*HI, *Bcl*I, *Bgl*II, *Dra*I, *Hin*dIII, *Kpn*I, and *Xba*I; CMH-69 with *Bam*HI, *Bcl*I, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, and *Xba*I; and EMH-1 with *Bcl*I, *Dra*I, *Hin*dIII, and *Xba*I.

Probe Preparation, Hybridization, Washing, and Autoradiography

The probes were prepared by random primer labeling with α-^{32}P-dCTP. Prehybridizations for 6–8 h and hybridizations for a minimum of 16 h were conducted at 60°C (Rajora and Dancik 1992). Hybridized blots were washed (Rajora and Dancik 1992), and then exposed to X-ray films with intensifying screens at –70°C for 3–48 h for mtDNA and 3–14 d for nuDNA probes. For quantitative estimates of radioactivity, autoradiograms were scanned with a Molecular Dynamics (Sunnyvale, CA, 94086) Computing Densitometer and relative band densities were determined by linear integration of lanes.

Genetic Relationships Among Lentil Accessions

The proportions of nuclear or mitochondrial DNA fragments shared by any two cultivars (F) and nucleotide substitutions per site between any two cultivars were determined according to Nei (1987). The DNA restriction fragment similarities between any two cultivars were calculated as $F = 2n_{AB}/n_A+n_B$ (Nei 1987), where n_{AB} is the number of restriction fragments common to cultivars A and B, n_A is the number of restriction fragments in cultivar A, and n_B is the number of restriction fragments in cultivar B. The restriction fragment similarities calculated from this method are not affected by the duplication or multiplication of observations that may result from same or similar RFLPs revealed by different restriction enzymes with the same probe. Clustering of the lentil varieties on the basis of mtDNA and nuDNA restriction fragment similarities was determined by using the unweighted pair-group with arithmetic mean method (UPGMA; Sneath and Sokal 1973).

RESULTS

Mitochondrial DNA

Eighty-four combinations of six mtDNA probes and 14 restriction enzymes produced a total of 197 restriction fragments of which 163 were common to all accessions. Sixteen of these combinations demonstrated RFLPs in lentil mtDNA and no two accessions had the same patterns in all blots (Table 2). Even probes from the *Atp*6 regions of maize and wheat mitochondrial DNA produced different restriction fragment patterns with the same enzymes (*Bam*HI, *Eco*RV), although in both cases the difference was restricted to a single genotype (Table 2). The maize *Cox*I probe identified a unique fragment pattern in ILL-5588 DNA cut with *Bgl*II (Table 2; Fig. 1a). The fragment sizes of 3.6, 2.5 and 1.1 kb in ILL-5588, and the consistently greater intensity of the

Table 1. Probes for mitochondrial and nuclear DNA

Probe	Plant source	Size (kb)	Gene content	Clone	Reference
Mitochondrial DNA Probes					
Maize Atp6	Maize	2.7	ATPase subunit 6 (*atp*6)		Dewey et al. (1985)
Maize CoxI	Maize	10.0	Cytochrome c oxidase subunit I (*cox*I)		Isaac et al. (1985)
Wheat Atp6	Wheat	3.6	*Atp*6	B302	Bonen and Bird (1988)
Wheat Cob	Wheat	5.0	Apocytochrome b (*cob*)	B376	Boer et al. (1985)
Wheat CoxI	Wheat	4.8	*Cox*I	B342	Bonen et al. (1987)
Wheat Orf25	Wheat	4.8	*Orf*25	B314	Bonen et al. (1990)
Nuclear DNA Probes					
CMH-34	Lentil	1.1	cDNA clone	CMH-34	Havey and Muehlbauer (1989b)
CMH-52	Lentil	0.64	cDNA clone	CMH-52	Havey and Muehlbauer (1989b)
CMH-69	Lentil	1.0	cDNA clone	CMH-69	Havey and Muehlbauer (1989b)
EMH-1	Lentil	0.76	Genomic DNA clone	EMH-1	Havey and Muehlbauer (1989b)

Table 2. Restriction fragment polymorphisms of mitochondrial DNA observed in lentil accessions

				mtDNA fragment variants (size in kb)							
Region	Enzyme	Probe		Laird	Eston	ILL-5588	ILL-5684	Brewer	Redchief	LO4	LO24
*Cox*I	*Bgl*II	Maize	CoxI	3.6, 3.6	3.6, 3.6	3.6, 2.5, 1.1	3.6, 3.6	3.6, 3.6	3.6, 3.6	3.6, 3.6	3.6, 3.6
	*Dra*I	Wheat	CoxI	–	–	–	–	–	1.6	–	–
	*Hin*dIII	Maize	CoxI	0.9	0.9	0.9	0.9	0.9	0.9	2.4	2.4
ATPase 6	*Bam*HI	Maize	Atp6	–	4.0	–	4.0	–	–	–	–
		Wheat	Atp6	–	4.0	3.6, 3.3	4.0	–	–	–	–
	*Eco*RV	Maize	Atp6	–	2.0	–	–	–	–	–	2.9
		Wheat	Atp6	–	2.2	–	–	–	–	–	–
	*Xba*I	Maize	Atp6	2.9	–	2.9	–	2.9	2.9	–	–
	*Xho*I	Maize	Atp6	8.2	8.2	8.2	9.8	9.8	9.8	9.8	9.8
Cob	*Hin*dIII	Wheat	Cob	2.1	2.1	2.1	2.1	2.1	2.1	2.1	–
	*Xba*I	Wheat	Cob	–	19.4	–	–	–	–	–	–
*Orf*25	*Bcl*I	Wheat	Orf25	7.2	8.5	6.1	8.5, 4.1	7.9	7.4	9.5	9.0, 6.5, 5.0, 4.1
	*Eco*RV	Wheat	Orf25	–	–	–	–	–	–	–	4.9
	*Hin*dIII	Wheat	Orf25	–	–	3.3	–	–	–	–	2.4
	*Xba*I	Wheat	Orf25	–	–	–	–	–	18.2	–	–
	*Xho*I	Wheat	Orf25	19.9	19.9	19.9	19.9	19.9	13.4	19.9	19.9

3.6 kb band in the other seven accessions suggested that all had two copies of this fragment. However, an additional *Bgl*II site in one of these fragments in ILL-5588 would have produced 2.5 and 1.1 kb fragments. Densitometry of the autoradiogram indicated that 38% of the total radioactivity of ILL-5588 was in the 3.6 kb as compared with 90% in the other genotypes. As the same band patterns were apparent after digestion of the DNA samples with increased amounts of restriction enzyme, it is unlikely that incomplete digestion was responsible for the *Bgl*II-CoxI polymorphism. The RFLP revealed by the maize CoxI probe with *Hin*dIII unambiguously distinguished between the *L.c. culinaris* and *L.c. orientalis* subspecies (Table 2; Fig. 1b). The greatest genotypic variability was consistently seen with the Orf25/*Bcl*I probe/enzyme combination which produced 10 restriction fragments with unique fragment patterns for each of the accessions, when repeated at least three times with or without increased amount of restriction enzymes (Table 2; Fig. 1c). Wheat CoxI/*Dra*I and wheat Orf25/*Xba*I combinations both produced restriction fragments unique to Redchief (Table 2). DNA cut with *Bam*HI and probed with wheat Atp6 (Table 2) showed a 4.0 kb fragment in both Eston and ILL-5684, but 3.6 and 3.3 kb fragments in ILL-5588. However, with the maize Atp*6* probe, only the 4.0 kb fragments were seen on the same blot (Table 2).

Mitochondrial DNA restriction fragment similarities (*F*) averaged 0.970 (Table 3), with a standard deviation of 0.011 (range 0.944–0.989). Brewer and Laird were the most similar to each other and ILL-5588 and LO24 were the most divergent (Table 3). Brewer was most similar (0.979) to all other accessions and LO24 had the least mean similarity (0.959) to the other accessions. The estimates of nucleotide substitutions per site ranged from 0.00064 between Laird and Brewer to 0.00321 between ILL-5588 and LO24, with an average of 0.00169 among all the accessions.

Nuclear DNA

Nuclear DNA variation was examined in only seven of the lentils, as the accession LO24 was not available for analysis when this work was done. Twenty-nine combinations of four lentil DNA probes (CMH-34, CMH-52, CMH-69 and EMH-1) with 10 restriction enzymes (*Bam*HI, *Bcl*I, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Sca*I, and *Xba*I), revealed a total of 64 restriction fragments. Each accession produced 39 or 40 fragments, of which 18 were common to all seven accessions. Genotype differences in restriction fragment lengths were identified from the 19 probe/restriction enzyme combinations (Table 4). In almost all cases, only a single restriction fragment was apparent in each accession (suggesting single-copy gene patterns), but with

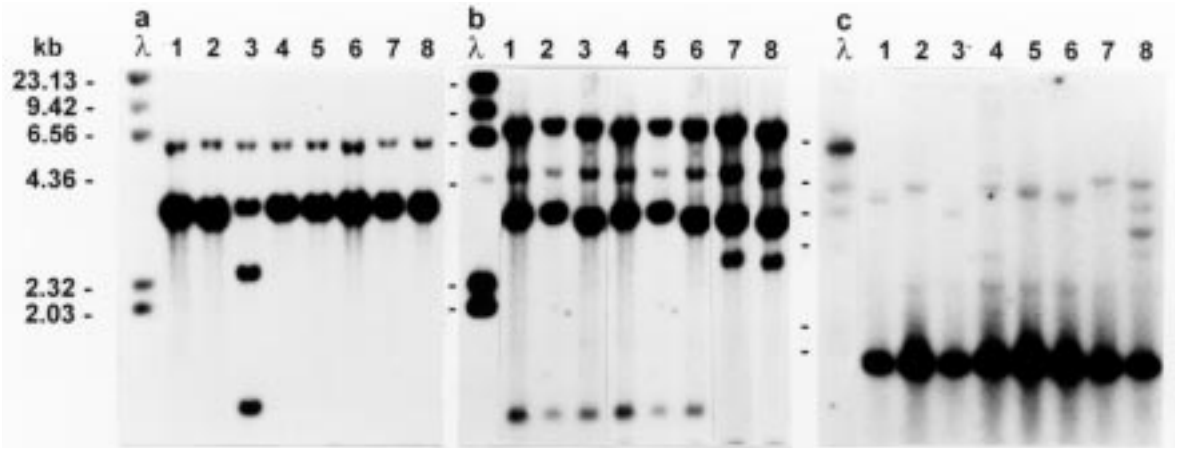

Fig. 1. Restriction fragment variation of mtDNA in eight accessions of *Lens culinaris*. Southern blots of (a) *Bgl*II with maize *Cox*I probe, (b) *Hin*dIII with maize *Cox*I probe, (c) *Bcl*I with wheat *Orf*25 probe. 1 = Laird, 2 = Eston, 3 = ILL-5588, 4 = ILL-5684, 5 = Brewer, 6 = Redchief, 7 = LO4, 8 = LO24.

Table 3. Similarities (*F* values) between accessions of *Lens culinaris* for mitochondrial (above diagonal) and nuclear (below diagonal) DNA restriction fragments

	L.c. ssp. culinaris						L.c. spp. orientalis	
	Laird	Eston	ILL-5588	ILL-5684	Brewer	Redchief	LO4	LO24
Laird		0.977	0.977	0.977	0.989	0.977	0.980	0.961
Eston	0.667		0.961	0.978	0.972	0.961	0.969	0.950
ILL-5588	0.821	0.590		0.961	0.972	0.961	0.963	0.944
LL-5684	0.692	0.923	0.615		0.983	0.972	0.980	0.966
Brewer	0.974	0.641	0.795	0.718		0.983	0.986	0.966
Redchief	0.987	0.658	0.810	0.709	0.987		0.974	0.955
LO4	0.684	0.582	0.810	0.608	0.658	0.675		0.975

Table 4. Restriction fragment polymorphisms of nuclear DNA observed in lentil accessions

		Nuclear DNA fragment variants (size in kb)						
Region	Enzyme	Laird	Eston	ILL-5588	ILL-5684	Brewer	Redchief	LO4
CMH-34	*Bam*HI	20.5	10.3	20.5	10.3	20.5	20.5	20.5
	*Bgl*II	17.2, 3.2	4.1, 12.1	17.2, 3.2	4.1, 12.1	17.2, 3.2	17.2, 3.2	17.2, 3.2
	*Bcl*I	5.5	13.9	5.5	13.9	5.5	5.5	5.5
	*Eco*RI	2.8	10.1	2.8	10.1	2.8	2.8	10.1, 2.8
	*Eco*RV	4.4	5.9	4.4	5.9	4.4	4.4	5.9
	*Hin*dIII	16.5	6.6	16.5	6.6	16.5	16.5	6.6
	*Kpn*I	21.1	21.1	21.1	21.1	21.1	21.1	13.6
	*Sca*I	12.3	12.3	12.3	12.3	12.3	12.3	18.3
CMH-52	*Bcl*I	4.5	4.5	4.2	4.5	4.5	4.5	4.2
	*Bgl*II	22.0	21.2	19.0	21.2	22.0	22.0	19.0
	*Dra*I	2.19	2.11	1.89	2.11	2.19	2.19	1.89
	*Hin*dIII	5.7, 2.40	5.7, 2.32	5.7, 2.10	7.9, 2.32	7.9, 2.40	7.9, 5.7, 2.40	5.7, 2.10
	*Kpn*I	7.9	7.9	6.8	7.9	7.9	7.9	6.8
	*Xba*I	2.90	2.82	2.60	2.82	2.90	2.90	2.60
CMH-69	*Bcl*I	8.7	7.0	8.7	8.7	8.7	8.7	8.7
	*Eco*RI	14.6	14.6	14.6	14.6	14.6	14.6	18.9
	*Kpn*I	11.5	16.5	11.5	11.5	11.5	11.5	11.5
EMH-1	*Bcl*I	8.5	8.5	8.5	8.5	8.5	8.5	6.4
	*Dra*I	3.1	3.1	3.6	3.1	3.1	3.1	3.1

two combinations, CMH-34/*Bgl*II and CMH-52/*Hin*dIII, either two or three fragments were visible in each lane (Table 4: Fig. 2 d). Some probes revealed very similar fragment patterns for the genotypes even with different restriction digests (Fig. 2), although the actual fragment sizes were not the same and the patterns with other restriction digests and the same probe produced different genotype patterns (Table 4). The LO4 (*L.c. orientalis*) accession could be distinguished from all the other accessions by four unique nuclear DNA fragments, and the presence of the two *Eco*RI-CMH-34 fragments that were found only singly in each of the other accessions (Table 4; Fig. 2b). At the other extreme, Redchief could only be distinguished from Laird and Brewer with *Hin*dIII cut DNA using the CMH-52 probe, as

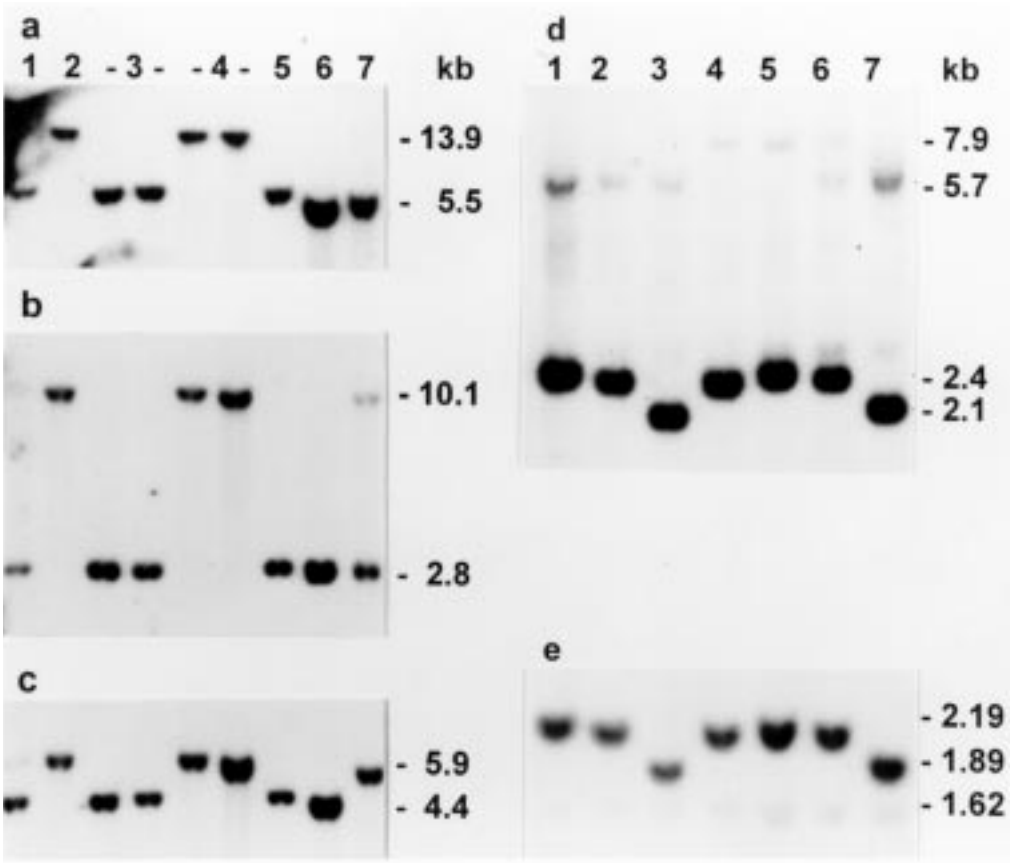

Fig. 2. Restriction fragment variation of nuDNA in seven accessions of *Lens culinaris*. Southern blots of (a) *Bcl*I with lentil cDNA CMH-34 probe, (b) *Eco*RI with lentil cDNA CMH-34 probe, (c) *Eco*RV with lentil cDNA CMH-34 probe, (d) *Hind*III with lentil cDNA CMH-52 probe, (e) *Dra*I with lentil cDNA CMH-52 probe. 1 = Laird, 2 = Eston, 3 = ILL-5588, 4 = ILL-5684, 5 = Brewer, 6 = Redchief, 7 = LO4. Paired samples of 3 and 4 are from different single-seed descent lines.

it showed both the 5.7 and 7.9 kb fragments that were seen singly in Laird and Brewer respectively (Table 4; Fig. 2d).

Nuclear DNA restriction fragment similarities (*F*) averaged 0.743, with a standard deviation of 0.132 (range 0.582–0.987). Brewer, Laird and Redchief were the most similar to each other and ILL-5588 and Eston were the most divergent (Table 3). Redchief was most similar (0.804) to all other accessions and LO24 had the least mean similarity (0.670) to the other accessions. The mean nucleotide divergence per site among the lentil accessions was 0.0169 with a range from 0.00071 between Laird and Redchief and between Redchief and Brewer to 0.03132 between Eston and LO4.

Cluster Analysis of Genetic Relationships

Unweighted pair-group with arithmetic mean (UPGMA) cluster analysis, based on mtDNA *F* values (Table 3), did not indicate any clear clustering of the *L. culinaris* accessions, but rather a sequential addition of individual accessions at decreasing levels of similarity (Fig. 3a). UPGMA analysis, based on nuDNA *F* values (Table 3), identified three main clusters within these seven lentil accessions (Fig. 3b). Laird, Redchief and Brewer clustered at an extremely high level of similarity (mean = 0.983). Eston and ILL-5684 formed a second group with a similarity of 0.923. ILL-5588 and LO4 comprised the third cluster with a similarity of 0.810. The group of Eston and ILL-5684 joined the other accessions only at a mean similarity of 0.648.

DISCUSSION

This study used restriction fragment polymorphisms in both mitochondrial and nuclear DNA to examine the genetic variability among six accessions of *Lens culinaris* ssp. *culinaris* and two (mtDNA) or one (nuDNA) accession(s) of *Lens culinaris* ssp. *orientalis*. Although only six mtDNA-specific probes were used and some would be expected to have considerable homology (maize and wheat derived *Cox*I and *Atp*6), genotype polymorphisms were revealed by all of them. The combined results from 16 enzyme/probe combinations show that each accession has a unique mitochondrial genome. However, because the accessions also share many common restriction fragments, estimates of genetic similarity (Nei 1987) are very high, with an overall mean value of 0.97 ± 0.01(SD) for all pairs of mitochondrial genomes. Nineteen combinations of four nuDNA probes and 10 restriction enzymes also revealed genetic polymorphisms among the seven lentils tested and no two accessions were identical. All of the nuDNA RFLPs seem to represent

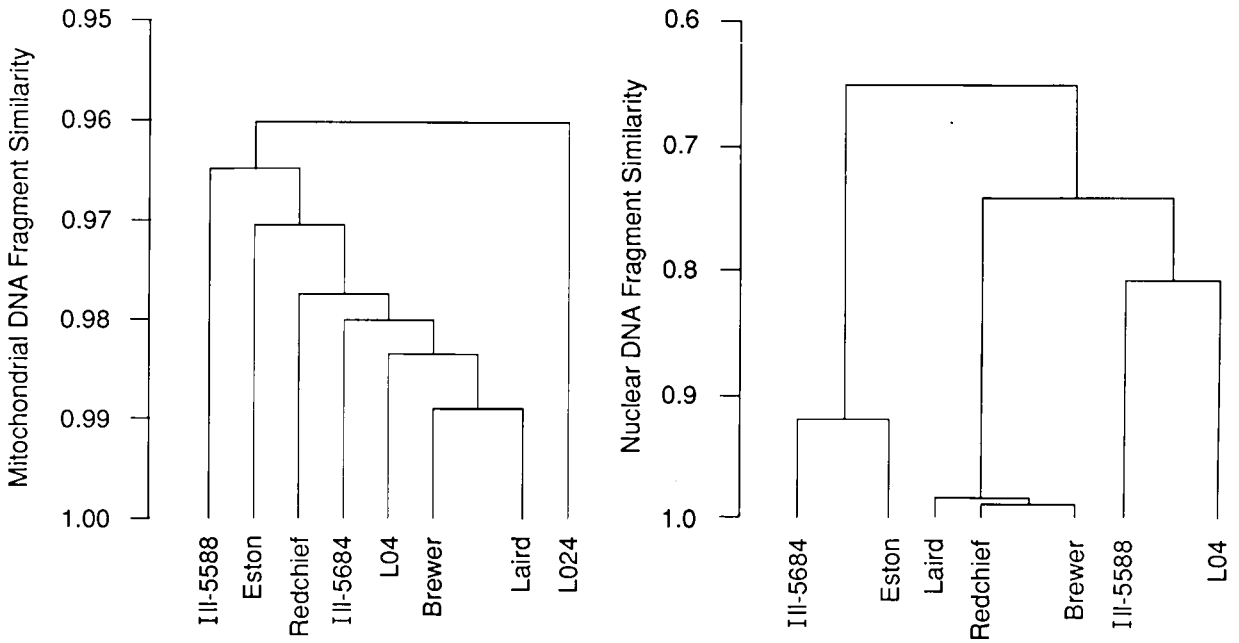

Fig. 3. Apparent relationships among lentil accessions based on UPGMA analysis of mitochondrial (a) and nuclear (b) DNA restriction fragment similarities.

allelic differences at the loci detected. Estimates of nuclear genome similarity between pairs of accessions were generally lower and more variable than those from mtDNA (Table 3), averaging 0.74 ± 0.13 (SD).

The apparent genetic relationships among the lentil accessions differed according to the source of the DNA analyzed (Fig. 3). The dendrogram derived from mtDNA fragment similarities (Fig. 3a) shows the very high similarity of all accessions as well as a pattern of sequential addition of individual accessions, with no real clustering. When based on nuDNA fragment similarity, distinct clusters are apparent. Laird, Redchief, and Brewer comprise a group with very high similarity, and the three could be distinguished by only a single probe/enzyme combination. However, despite the obvious differences in both the pattern of genetic relationships (Fig. 3) and the overall magnitude and variability of genetic similarities (Table 3), the mean genetic similarities for these three accessions were numerically identical for mtDNA (0.983 ± 0.006) and nuDNA (0.983 ± 0.007). A similar pattern of genetic relationships among Laird, Brewer and Redchief for both the mtDNA and nuDNA suggests a parallel evolution of the nuclear and mitochondrial genomes of these lentil cultivars in the regions of the probes used. Nuclear DNA analysis also identified significant similarities between ILL-5684 and Eston (0.923) and between ILL-5588 and LO4 (0.810), but these groupings were not apparent from the analysis of mtDNA.

In addition to providing information on genetic relationships, RFLPs can also be used for differentiating the specific genotypes, both nuclear and cytoplasmic, of the lentil cultivars studied. Nevertheless, examination of a wide range of samples from each accession would further strengthen this conclusion. Despite the relatively small numbers of genotypes and probes used in these experiments, each accession studied was unique in both its mitochondrial and nuclear genomes. Although the mtDNA fragment similarity of the accessions was generally greater than that of the nuDNA, this difference was associated with a greater number of common bands hybridizing to the mtDNA probes and not a lack of discriminating bands. Sixteen mtDNA probe/enzyme combinations detected 34 fragments of diagnostic value, producing 41 distinct band patterns in the eight accessions. With nuDNA probes, 19 probe/enzyme combinations revealed 46 distinguishing fragments that showed a total of 46 different band patterns in seven accessions. Thus, although only 12% of the bands detected with mtDNA probes differed among the accessions, as compared with 71% with nuDNA probes, there appears to be sufficient genetic variability to find diagnostic fragments in both mitochondrial and nuclear genomes for differentiating the cultivars studied. Even the three very similar varieties Laird, Brewer and Redchief could be distinguished from each other on the basis of both mitochondrial and nuclear DNA, although for nuDNA only by a single probe (CMH-52)-restriction enzyme (*Hind*III) combination.

The results of this RFLP analysis showed that lentils have considerable variability in both mitochondrial and nuclear DNA. Each of the accessions tested was unequivocally identified using a relatively small number of restriction enzymes and either mitochondrial or nuclear probes. However, with the exception of three cultivars (Laird, Redchief, Brewer), which have very high similarities in both mitochondrial and nuclear restriction fragments, the apparent phylogenetic relationships among the lentil accessions differed according

to the genomic source of DNA analyzed. Even the *Lens culinaris* ssp. *orientalis* subspecies was not consistently separated from the *Lens culinaris* ssp. culinaris accessions.

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