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Comparison of crossability, RAPD, SDS-PAGE and morphological markers for revealing genetic relationships within and among *Lens* species

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Abstract The phylogenetic relationships among (sub)-species in the genus *Lens* have been reviewed based on recent published reports. There was both a substantial level of agreement and disagreement between reports based on different analytical procedures and different plant germ plasms. *Lens culinaris* ssp. *orientalis* appeared as the wild progenitor of the cultivated lentils. A gene flow from *L. odemensis* and *L. ervoides* during lentil crop evolution was suggested. Morphological characters (quantitative and qualitative) showed a different taxonomic pattern in the genus *Lens*. The use of nuclear and biochemical markers (RFLPs, RAPDs, seed-protein electrophoresis) appeared to be the most consistent and reliable methods for determining genetic relationships. It is suggested that these techniques be used in combination for taxonomic analysis of the genus *Lens*.

Key words RAPD · SDS-PAGE · Morphological markers · Crossability · Genetic relationships · Genus *Lens*

Introduction

The assessment of genetic variation and genetic similarities is a major concern of plant breeders and population geneticists, because it facilitates the efficient sampling and utilisation of germ plasm resources. The breeder can use genetic-similarity information to make informed decisions regarding the choice of genotypes to cross for the development of populations, or to facilitate the identification of diverse parents to cross in hybrid combinations in order to maximise the expression of heterosis (Smith et al. 1990; Neinluis and Sills 1992).

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In the past, a wide variety of methods have been utilised to develop quantitative estimates of genetic similarities and relationships. Some of these methods and the conclusions drawn with respect to the genus *Lens* are summarised below. Traditionally, morphological and phenological characteristics have been used for these purposes. Since such characteristics are often controlled by multiple genes and subject to varying degrees of environmental modifications and interactions, differences between clones or closely related species are not always absolute. Many of the plant traits are difficult to analyse because they do not have the simple genetic control assumed by many population genetic models (Liu and Furnier 1993). Mac Key (1988) stressed the importance of morphological traits in taxonomic studies of cultivated plants. Morphologically, the wild species *L. c.* ssp. *orientalis* is very close to *L. c.* ssp. *culinaris* and probably was its progenitor, while *L. nigricans* appeared to be somewhat divergent from *L. c.* ssp. *culinaris* and *L. c.* ssp. *orientalis* (Barulina 1930; Zohary 1972; Williams et al. 1974). Hoffman et al. (1988) found that *L. c.* ssp. *orientalis* grouped closest to *L. c.* ssp. *culinaris*, and *L. nigricans* was second closest to *L. c.* ssp. *culinaris* while *L. ervoides* appeared as a distinct species, based on morphological characteristics.

The biological species concept, based on crossability and cytological studies, has been applied to the genus *Lens* and suggested only two species, *L. culinaris* and *L. nigricans* (Ladizinsky et al. 1984). *L. culinaris* contained the (sub)species *culinaris*, *odemensis* and *orientalis* while *L. nigricans* consisted of the (sub)species *nigricans* and *ervoides*. The success rate of interspecific hybridisation in the genus *Lens* is dependent on species genetic affinities and relationships (Ahmad et al. 1995b).

Genetic relationships based on RAPD markers have been demonstrated in the genus *Lens* (Abo-elwafa et al. 1995; Ahmad et al. 1996). A total-seed-protein electrophoretic study (using the PAGE technique) showed a close similarity between *L. c.* ssp. *culinaris*, *L. c.* ssp. *orientalis* and *L. nigricans* (Ladizinsky 1979b). SDS-PAGE results indicated that *L. c.* ssp. *orientalis* and *L.*

odemensis probably represent the gene pool of the wild progenitor of cultivated lentils (Sammour 1994; Ahmad et al. 1995 a). Ladizinsky (1993) has recently revised *Lens* taxonomy according to information derived from studies using isozyme markers (Pinkas et al. 1985; Hoffman et al. 1986) and nuclear DNA restriction fragment length polymorphisms (Havey and Muehlbauer 1989). In his proposal, all subspecies are elevated to species status except for *culinaris* and *orientalis*, which are retained as subspecies under *L. culinaris*.

The objective of the present review was to determine whether genetic relationships of *Lens* species, as reported by a variety of authors, differed based on the form of the analysis used. The methods included in the review were RAPD (random amplified polymorphic DNA), RFLP (restriction fragment length polymorphisms), SDS-PAGE (SDS polyacrylimide-gel electrophoresis), morphological markers and crossability relations. The possible causes of observed differences are discussed.

Materials and methods

The plant germ plasm used for many of the papers reported, is described in Table 1. Experimental procedures, data collection, and analysis for RAPD assays, SDS-PAGE and morphological markers are as described by Ahmad et al (1995 a, b, c). Other methods are reported in the individual papers referred to in the text. The method for establishing the grouping pattern of a species based on crossability was that, if two species crossed with each other, they were placed in one group, while species in different groups did not cross with each other. Similarly, species which were described genetically similar in the original references based on different analytical techniques (RFLP, RAPD, PAGE, SDS-PAGE, isozymes, allozymes, morphology) were incorporated as one group in this article. Four representative figures taken from the original publications based on RAPD, SDS-PAGE and morphological descriptors (quantitative and qualitative) are shown as examples describing phylogenetic relationships of different species of the genus *Lens* and their grouping patterns (see Table 2)

Results and discussion

Two major comparison categories are presented. The first consists of a specific set of lines covering all known *Lens* (sub)species. This sample set was then compared for relationships using five different methods. The results for this group are presented in Table 2 with representative examples shown in Figs. 1–5. The second comparison is based on published reports of random samples of lines from a variety of *Lens* species across specific analytical techniques and the results are illustrated in Table 3. The purpose of the second category is to determine whether different sets of accessions of *Lens* species retained the same grouping pattern as was obtained previously using the same analytical method.

Category 1 relationships

In the experiments testing for crossability among *Lens* species, it was found that *L. c.* ssp. *orientalis* and *L. ervoides* were the most readily crossable with the cultivated *L. c.* ssp. *culinaris*. These were therefore the most closely related (Ahmad et al. 1995b) (Table 2). The second closest group of species consisted of *L. odemensis* and *L. nigricans*, both of which responded similarly in the ease with which they hybridised with cultivated lentils. This analysis produced two major groups. The first consisted of *L. c.* ssp. *culinaris*, *L. c.* ssp. *orientalis* and *L. ervoides*, and second of *L. nigricans* and *L. odemensis* (Table 2).

Within category 1, RAPD markers indicated that *L. c.* ssp. *orientalis* is the most closely related species to the cultivated lentil. *L. c.* ssp. *orientalis* and *L. c.* ssp. *culinaris* formed one group while the other three species, *L. nigricans*, *L. odemensis* and *L. ervoides* formed three separate groups. This is also evident in Fig. 1 from a

Table 1 Cultivated and wild *Lens* accessions used in crossability, RAPD, SDS-PAGE and morphological studies

Number	Species	Subspecies	Type[a]	Cultivar/accession	Source[bc]
1	*Lens odemensis*			W6 3244	Turkey[c]
2	*Lens nigricans*			W6 3208	Italy[c]
3	*Lens odemensis*			W6 3222	Unknown[c]
4	*Lens nigricans*			W6 3210	Yugoslavia (former)[c]
5	*Lens nigricans*			W6 3218	Spain[c]
6	*Lens nigricans*			W6 3221	Russian Federation[c]
7	*Lens ervoides*			W6 3173	Russian Federation[c]
8	*Lens ervoides*			W6 3176	Yugoslavia (former)[c]
9	*Lens ervoides*			W6 3192	Turkey[c]
10	*Lens culinaris*	*orientalis*		W6 3241	Turkey[c]
11	*Lens culinaris*	*orientalis*		W6 3261	Turkey[c]
12	*Lens culinaris*	*orientalis*		W6 3248	Turkey[c]
13	*Lens culinaris*	*culinaris*	Microsperma	Titore	Rakaia, NZ[b]
14	*Lens culinaris*	*culinaris*	Macrosperma	Invincible	Rakaia, NZ[b]
15	*Lens culinaris*	*culinaris*	Macrosperma	Olympic	Rakaia, NZ[b]

[a] Microsperma: small seeded type. Macrosperma: large seeded type
[b] Whenuapai Farm, Rakaia, South Island, New Zealand
[c] Western Regional Plant Introduction Station, Washington, USA

Table 2 Different group formation of *Lens* species as detected by crossability relations, RAPD, SDS-PAGE and morphological markers

Marker	Reference	*Accessions*		Crossability/Cluster				Wild progenitor of cultivated lentils
		Cult.	Wild	Group 1	Group 2	Group 3	Group 4	
Crossability	Ahmad et al. 1995	3	10	*L. c.* ssp. *culinaris* *L. c.* ssp *orientalis* *L. ervoides*	*L. nigricans* *L. odemensis*			*L. c.* ssp. *orientalis* *L. ervoides*
RAPD	Ahmad et al. 1996	3	12	*L. c.* ssp. *culinaris* *L. c.* ssp. *orientalis*	*L. nigricans*	*L. ervoides*	*L. odemensis*	*L. c.* ssp. *orientalis*
SDS-PAGE	Ahmad et al. 1995	3	12	*L. c.* ssp. *orientalis* *L. odemensis* *L. c.* ssp. *culinaris*	*L. nigricans*	*L. ervoides*		*L. c.* ssp. *orientalis* *L. odemensis*
Morphological (quantitative)	Ahmad et al. 1995	3	12	*L. c.* ssp. *culinaris*	*L. ervoides*	*L. c.* ssp. *orientalis* *L. odemensis* *L. nigricans*		
Morphological (quantitative)	Ahmad et al. 1995	3	12	*L. c.* ssp. *culinaris*	*L. nigricans*	*L. c.* ssp. *orientalis* *L. odemensis* *L. ervoides*		

Table 3 Different group of *Lens* species as detected by crossability relations, RAPD, SDS-PAGE and morphological markers

Marker	Reference	Accession		Crossability/Cluster			Wild progenitor of cultivated lentils
		Cult.	Wild	Group 1	Group 2	Group 3	
Crossability and cytogenetics	Ladizinsky 1979a	3	5	*L. c.* ssp. *culinaris* *L. c.* ssp. *orientalis* *L. nigricans*			*L. c.* ssp. *orientalis*
Crossability and cytogenetics	Ladizinsky et al 1984	5	44	*L. c.* ssp *culinaris* *L. c.* ssp. *orientalis* *L. odemensis*	*L. nigricans* *L. ervoides*		*L c* ssp. *orientalis*
RFLP	Rajora and Mahon 1994	3	1	*L. c.* ssp. *culinaris* *L. c.* ssp. *orientalis*			*L c* ssp. *orientalis*
RFLP	Rajora and Mahon 1995	6	1	*L. c.* ssp. *culinaris* *L. c.* ssp. *orientalis*			*L. c.* ssp. *orientalis*
RFLP	Muench et al. 1991	2	10	*L. c.* ssp. *culinaris* *L. c.* ssp. *orientalis* *L. odemensis*	*L. nigricans*		*L. c.* ssp *orientalis*
RFLP	Havey and Muehlbauer 1989	6	25	*L. c.* ssp *culinaris* *L. c.* ssp. *orientalis*	*L odemensis* *L. ervoides*	*L. nigricans*	*L. c.* ssp *orientalis*
RFLP	Mayer and Soltis 1994	114	11	*L. c.* ssp. *culinaris* *L. c.* ssp. *orientalis*	*L. odemensis*	*L. nigricans* *L. ervoides*	*L. c.* ssp. *orientalis*
RAPD	Abo-elwafa et al. 1995	20	16	*L. c.* ssp. *culinaris* *L. c.* ssp. *orientalis* *L. odemensis*	*L. nigricans* *L. ervoides*		*L. c.* ssp. *orientalis*
RAPD	Sharma et al. 1995	26	28	*L. c.* ssp. *culinaris* *L. c.* ssp. *orientalis*	*L. nigricans* *L. ervoides*	*L odemensis*	*L. c.* ssp. *orientalis*
Isozyme	Rosa and Jouve 1992	34	13	*L. odemensis* *L. c.* ssp. *orientalis*	*L. c.* ssp. *culinaris* *L. nigricans*	*L. ervoides*	*L. c.* ssp. *orientalis* *L. odemensis* *L. nigricans*
Allozyme	Pinkas et al. 1985	31	36	*L. c.* ssp. *culinaris* *L. c.* ssp. *orientalis* *L. odemensis* *L ervoides*	*L. nigricans*		*L c.* ssp. *orientalis*
PAGE	Ladizinsky 1979b	15	11	*L. c.* ssp. *culinaris* *L c* ssp. *orientalis* *L. nigricans*	*L. ervoides*		*L. c.* ssp. *orientalis*
SDS-PAGE	Sammour 1994	1	4	*L. c.* ssp. *culinaris* *L c.* ssp. *orientalis* *L odemensis*	*L. nigricans* *L. ervoides*		*L. odemensis* *L. c.* ssp *orientalis*
Morphological	Hoffman et al. 1988	60	90	*L. c* ssp. *culinaris* *L. c.* ssp. *orientalis* *L. nigricans*	*L. ervoides*		*L. c.* ssp *orientalis*

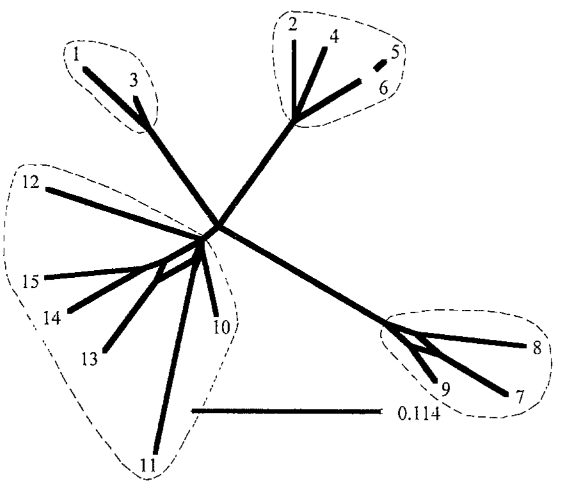

Fig. 1 Tree based on genetic distances measured by RAPD, demonstrating the cluster of 15 lentil lines. Taxon numbers refer to Table 1. Taken from Ahmad et al. 1996

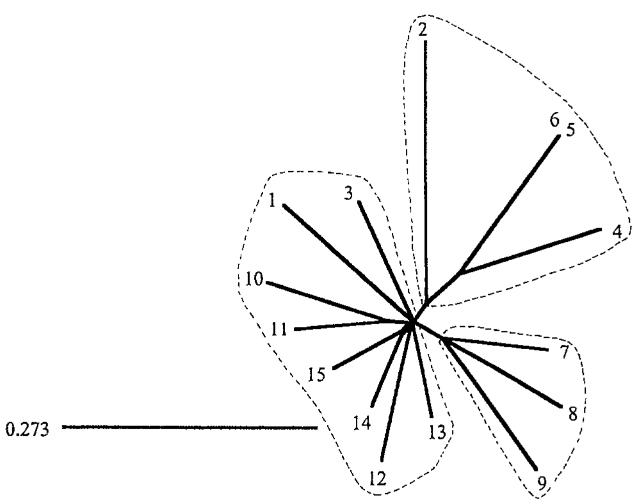

Fig. 2 Tree based on genetic distances measured by SDS-PAGE, demonstrating the cluster of 15 lentil lines. Taxon numbers refer to Table 1. Taken from Ahmad et al. 1995a

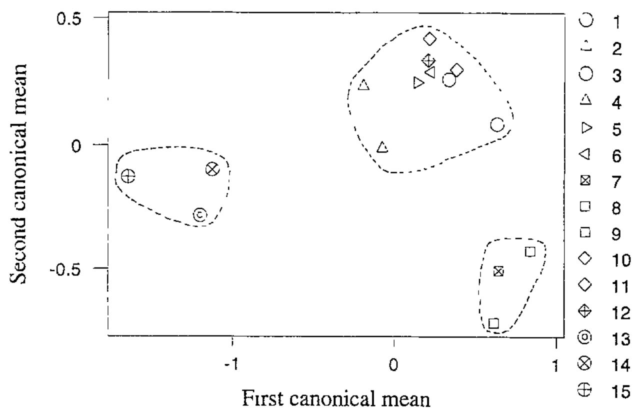

Fig. 3 Canonical variate means for quantitative characters of 15 lentil accessions. Numbers in the legend refer to the taxon numbers listed in Table 1. Graph taken from Ahmad et al. 1995c

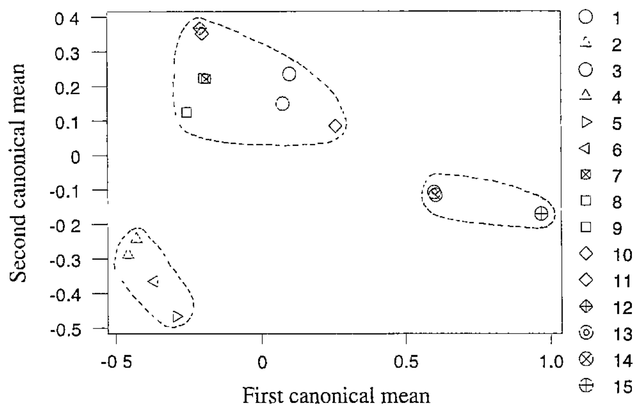

Fig. 4 Canonical variate means for qualitative characters of 15 lentil accessions. Numbers in the legend refer to the taxon numbers listed in Table 1. Graph taken from Ahmad et al. 1995c

cluster analysis of 15 accessions. Table 2 also indicated that *L. c.* ssp. *orientalis* appeared as the wild progenitor of cultivated lentils. *L. ervoides* and *L. nigricans* were the species most distinct from the cultivated lentils (Fig. 1).

The biochemical method, the SDS-PAGE analysis of total seed storage proteins, gave three major groups; one comprised *L. c.* ssp. *orientalis*, *L. odemensis* and *L. c.* ssp. *culinaris*; the second consisted of *L. nigricans*; and the third group contained the species *L. ervoides*. Both *L. c.* ssp. *orientalis* and *L. odemensis* clustered with the cultivated lentil and appeared as the wild progenitor of the cultivated lentil (Fig. 2).

When the same plant material was analysed morphologically (quantitatively), three separate group formations were observed. Accessions of *L. nigricans*, *L. odemensis* and *L. c.* ssp. *orientalis* formed a cohesive group, holding a position closest to each other. Accessions of *L. ervoides* formed a separate group which showed their distintive nature, while the cultivated lentils formed a third separate group. When the same plant material was analysed across qualitative morphological characters, a different pattern of relationships among the *Lens* taxa resulted (Table 2). *L. c.* ssp. *culinaris* and *L. nigricans* formed two groups while the accessions of *L. odemensis*, *L. ervoides* and *L. c.* ssp. *orientalis* formed a large separate group. The complex grouping pattern of *Lens* species across morphological markers (qualitative and quantitave characters) made it difficult to postulate the wild progenitor of cultivated lentils (Table 2) (Figs. 3 and 4).

Category 2 relationships

Based on crossability and cytogenetics, Ladizinsky et al. (1979 a) found *L. c.* ssp. *orientalis* was the wild progenitor of cultivated lentils and observed only one group formation of three *Lens* species (*L. c.* ssp. *culinaris*, *L. c.*

ssp. *orientalis* and *L. nigricans*). Ladizinsky et al. (1984) later proposed *L. c.* ssp. *orientalis* as the closest species to cultivated lentils and *L. nigricans* as the most distinct species. They also observed two groups in the genus *Lens* (Table 3). Therefore, based on crossability relations and cytogenetic studies, there is an agreement that *L. c.* ssp. *orientalis* should be the wild progenitor of cultivated lentils while gene flow from *L. ervoides* to cultivated lentil is also quite possible.

Molecular data using RFLP and RAPD markers of nuclear DNA (Havey and Muehlbauer 1989; Rajora and Mahon 1994, 1995; Abo-elwafa et al. 1995; Sharma et al. 1995) or cp DNA (Muench et al. 1991; Mayer and Soltis 1994) has supported *L. c.* ssp. *culinaris* and *L. c.* ssp. *orientalis* as one group. The genotype composition of group two and three, however, differed among authors. In addition, Muench et al. (1991) and Abo-elwafa et al. (1995) found *L. odemensis* in group one (Table 3).

The results of SDS-PAGE (Sammour 1994), isozymes (Rosa and Jouve 1992), allozymes (Pinkas et al. 1985) and PAGE (Ladizinsky 1979b), have given different grouping patterns of *Lens* species based on random samples of plant germ plasm. These authors all suggested *L. c.* ssp. *orientalis* as the possible wild progenitor of cultivated lentils, though the isozyme data did not group *L. c.* ssp. *culinaris* and *L. c.* ssp. *orientalis* together. The differences found among these results might be due to the use of different plant germ plasm or due to the different geographic situations from where the plant material was collected in assessing the species relationships shown in Table 3.

The morphological affinity between *L. c.* ssp. *culinaris* and *L. c.* ssp. *orientalis* was first described by Barulina et al. (1930) and was later supported by Zohary (1972), Williams et al. (1974) and Hoffman et al. (1988). Such observations led these researchers to postulate that *L. c.* ssp. *culinaris* was derived from *L. c.* ssp. *orientalis*. Similarly, Hoffman et al. (1988) found *L. c.* ssp. *orientalis* as the wild progenitor of cultivated lentils and observed only two groups: the first group was composed of *L. c.* ssp. *culinaris*, *L. c.* ssp. *orientalis* and *L. nigricans* while *L. ervoides* formed the second group (Table 3).

Conclusion

In our studies, we analysed the same plant germ plasm with different analytical techniques, RAPDs, SDS-PAGE, morphological studies and crossability relations. The studies of other investigators used only one analysis method, often with a larger number of plant germ plasm samples for lentil genetic analysis. *L. c.* ssp. *orientalis* appeared as the wild progenitor of cultivated lentils with most of the analytical techniques and with most of the plant germ plasms used. Exchange of genetic material between *L. c.* ssp. *culinaris* and *L. c.* ssp. *orientalis* and within *L. ervoedes* accessions was also observed (Fig. 1). Three or more accessions per species appeared as sufficient plant material for a genetic affinity analysis of *Lens* using DNA techniques (RFLP, RAPD) and storage protein analysis by PAGE and SDS-PAGE. (Tables 2 and 3).

The primary gene pool of cultivated lentils therefore consisted of *L. c.* ssp. *orientalis* and *L. odemensis*, while *L. ervoides* and *L. nigricans* formed a secondary gene pool.

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