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Electrophoretic seed globulin patterns and species relationships in the genus *Lens* Miller

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Abstract. SDS-PAGE analysis of seed globulins covered 200 accessions of the following *Lens* taxa: *L. culinaris* subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, *L. odemensis*, *L. ervoides*, *L. nigricans*, *L. lamottei* and *L. tomentosus*. The number of polypeptide bands detected in particular taxa varied from 22 in *L. lamottei* to 35 in *L. ervoides*. All the taxa under study showed variation due to differences among accessions and individual variation. Electrophoretic data were subjected to statistical analysis by the UPGMA method based on Euclidean distances. In the case of *L. culinaris* subsp. *culinaris*, some distinctness of the *microsperma* accessions from southern and central Asia was found. As to relationships among the studied taxa, the obtained results showed that *L. culinaris* subsp. *culinaris* appeared to be most closely allied to *L. odemensis*, while *L. culinaris* subsp. *orientalis* was found to be the closest relative of *L. tomentosus*. The four taxa formed one cluster separated from *L. lamottei* and *L. ervoides*. *L. nigricans* was shown to be the most divergent taxon.

Key words: genetic resources, *Lens*, lentil, SDS-PAGE, seed globulins.

Introduction

Lens Miller is a small genus. It comprises the cultivated lentil *L. culinaris* Medik. subsp. *culinaris*, its wild progenitor *L. culinaris* subsp. *orientalis* (Boiss.) Ponert and five additional wild relatives, namely *L. odemensis* Ladiz., *L. ervoides* (Brign.) Grande, *L. nigricans* (M. Bieb.) Godr., *L. tomentosus* Ladiz. and *L. lamottei* Czefr. Taxonomic classification of the genus *Lens* has undergone several modifications and is still controversial. *L. odemensis*, formerly regarded as a subspecies of *L. culinaris* (LADIZINSKY et al. 1984), was later considered a distinct morphological type of *L. nigricans* and described as a new species (LADIZINSKY 1986). *L. lamottei* was first described in Russian by CZEFRANOVA

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(1971) and subsequently recognized as being the same taxon as the differentiated cytotype of *L. nigricans* (LADIZINSKY et al. 1984, van OSS et al. 1997). *L. tomentosus* was newly separated from *L. culinaris* subsp. *orientalis* as a distinctive species (LADIZINSKY 1997). Recently, FERGUSON and coworkers (2000) proposed the classification of *L. tomentosus* as a subspecies of *L. culinaris*.

Wild *Lens* taxa are potential genetic resources for the cultivated lentil, one of the oldest West Asian crops, valued as a high-protein food and of considerable economic importance on the Indian subcontinent, in the Middle East, southern Europe and eastern and northern Africa. So far, mainly landraces were grown and only little attention was given to breeding advanced, resistant and high-yielding cultivars. Recognizing genetic relationships among the *Lens* taxa and getting a better knowledge of genetic variation within the taxa are essential for lentil breeding.

In the last two decades the genus *Lens* was subjected to extensive morphological, cytological and crossability studies as well as to comparative biochemical investigations with the use of protein and DNA markers. The studies, partly reviewed by AHMAD and McNEIL (1996), provided inconsistent results. This paper presents electrophoretic data concerning the diversity of seed globulins in all seven taxa distinguished in the genus *Lens* and is aimed at contributing to *Lens* taxonomy and characterization of genetic resources of the cultivated lentil.

Material and methods

Plant material

The studied material comprised 200 accessions representing the following taxa of the genus *Lens*: *L. culinaris* subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, *L. odemensis*, *L. ervoides*, *L. nigricans*, *L. lamottei* and *L. tomentosus*. As regards *L. culinaris* subsp. *culinaris*, the examined accessions were arbitrarily divided into *microsperma* and *macrosperma* types, with 100-seed weight below 4.5 g and equal to or higher than 4.5 g, respectively (SOLH, ERSKINE 1984).

As many as 191 accessions were obtained from the International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, the seed source designated with the letter A. The remaining seed samples were provided by Prof. A. Slinkard, Crop Development Centre, Saskatoon, Saskatchewan, Canada (B: 5 accessions); Dr. G. Ladizinsky, Hebrew University of Jerusalem, Rehovot, Israel (C: 3 accessions) and Dr. A Börner, Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany (D: 1 accession). General information on the studied accessions is given in Table 1.

Original seed samples were analysed. Five individuals of each accession were examined separately. In the description of the results, some particular accessions

Table 1. Information on the examined *Lens* accessions

Taxon	Abbr.[a]	Accessions			
		Type[b]	Number	Geographical region and country of origin[c]	Source[d]
L. culinaris subsp. *culinaris*	Cu	*Microsperma* (1.0-4.4g)	39	**N. & central Europe**: CSK 1, POL 1; **S. Europe**: GRC 1, ITA 1, PRT 1, YUG 1; **N. Africa**: EGY 1, LBY 1, MAR 1, TUN 1; **E. Africa**: ETH 2, SOM 1; **S.W. Asia**: IRN 2, IRQ 1, JOR 2, SYR 1, TUR 2, YEM 2; **S. & central Asia**: AFG 3, BGD 2, IND 3, NPL 2, PAK 3; **S. America**: CHL 2, URY 1	A, D
		Macrosperma (4.5-7.0g)	18	**N. & central Europe**: DEU 1, FRA 1, GBR 1, ROM 1; **S. Europe:** ALB 1, ESP 1; **N. Africa**: DZA 1; **S.W. Asia**: IRN 2, SYR 2, TUR 1; **S. America**: BOL 1, CHL 3, COL 1, ECV 1	A
L. culinaris subsp. *orientalis*	Or		38	**S.W. Asia**: CYP 4, IRN 5, JOR 2, LBN 3, PAL 3, SYR 10; TRM 2, TUR 3, UZB 3; **S. & central Asia**: TJK 3	A, B,
L. odemensis	Od		26	**S.W. Asia**: PAL 2, SYR 15, TUR 9	A
L. ervoides	Er		29	**S. Europe**: YUG 4; **S.W. Asia**: JOR 3, LBN 3, PAL 3, SYR 8, TUR 8	A
L. nigricans	Ni		30	**N. & central Europe**: FRA 3; **S. Europe**: ESP 3; ITA 3, YUG 3; **S.W. Asia**: TUR 18	A
L. lamottei	La		11	**N. & central Europe**: FRA 1; **S. Europe**: ESP 7; **N. Africa**: MAR 1; **S.W. Asia**: TUR 2	A, C
L. tomentosus	To		9	**S.W. Asia**: SYR 2, TUR 7	A, C

[a] Abbreviations of taxa names.
[b] Ranges of 100-seed weight are given in parentheses.
[c] Numbers of accessions from particular countries are given.
[d] For key to the seed sources see: Plant material.

are indicated by a catalogue number, donor's designation (A-D) and geographical origin. Both in the text and in Table 1 standard country codes are used (LIPMAN et al. 1996).

Analytical techniques

Globulins were extracted from seed cotyledons. Extraction was performed according to PASQUALINI et al. (1991). First, albumins were extracted and discarded. Subsequently, globulins were extracted with 0.5 M KCl in 0.1 M tris-maleate buffer, pH 6.9, containing 1 mM dithiothreitol and 1 mM ethylene-diaminetetraacetic acid.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 12% slab gels in a discontinuous buffer system according to LAEMMLI (1970), as described by ZIMNIAK-PRZYBYLSKA and PRZYBYLSKA (1995). Relative molecular masses (M_rS) of globulin polypeptides were estimated by SDS-PAGE using the following standard proteins: bovine albumin (M_r 66 kDa), egg albumin (M_r 45 kDa), carbonic anhydrase (M_r 29 kDa), β-lactoglobulin (M_r 18.4 kDa) and cytochrome C (M_r 12.4 kDa) from SIGMA.

Band homology within and between taxa was established as described earlier (PRZYBYLSKA, ZIMNIAK-PRZYBYLSKA 1995).

Statistical analysis

Accessions of *L. culinaris* subsp. *culinaris* were classified into groups called populations according to geographical regions and seed weight (*microsperma* and *macrosperma* types). In the statistical analysis only populations consisting of at least three accessions were considered. Coefficients of similarity, based on Euclidean distances, were calculated from frequencies of globulin polypeptide bands for pairs of accessions within taxa, for populations of *L. culinaris* subsp. *culinaris* and for the studied taxa. Similarity coefficients of populations and of the studied taxa were used for UPGMA hierarchical grouping and for constructing dendrograms.

Results

Major and minor, well-defined and reproducible globulin polypeptide bands were recorded. The reported M_r values of the distinguished polypeptides should be regarded as approximate because of errors in M_r estimations by SDS-PAGE (AIKEN, GARDINER 1991). The frequency distribution of the considered globulin polypeptides in particular *Lens* taxa under study is shown in Table 2. Electrophoretic seed globulin patterns produced by some individuals of the examined taxa are presented in Figure 1.

Table 2. Frequency of seed globulin polypeptide bands in the studied *Lens* taxa. The values present percentages of individuals showing particular polypeptide bands

Band		Taxon[a]						
No.	MW(kDa)	Cu	Or	Od	Er	Ni	La	To
1	76.7	0	0	9	0	0	0	0
2	75.2	100	100	100	100	100	91	100
3	73.6	23	0	2	1	0	0	0
4	72.1	9	94	2	0	20	0	78
5	70.6	48	6	0	92	7	35	0
6	70.2	0	0	0	6	70	38	0
7	65.5	100	100	100	96	93	100	100
8	58.7	100	100	89	4	93	100	100
9	54.8	62	70	100	37	0	100	0
10	52.9	90	98	96	81	58	100	100
11	52.5	0	0	0	30	72	0	0
12	51.9	13	0	12	0	0	0	0
13	50.8	82	96	88	39	9	18	80
14	49.8	0	0	0	8	84	0	0
15	49.0	81	0	89	8	75	96	93
16	43.9	0	0	0	3	74	0	0
17	42.9	99	100	100	88	3	100	100
18	42.0	0	32	11	3	91	0	60
19	41.3	0	0	0	3	77	0	0
20	39.6	56	67	78	63	4	100	89
21	39.2	99	100	100	85	85	0	100
22	37.6	56	87	78	44	6	78	100
23	36.4	73	37	62	0	17	100	100
24	35.4	0	0	0	7	67	0	0
25	32.9	0	0	15	3	91	0	0
26	31.4	0	0	59	3	87	0	0
27	28.1	4	0	22	0	0	0	0
28	27.2	0	0	54	99	0	0	0
29	21.7	78	73	87	22	73	100	93
30	21.4	21	20	0	0	82	0	0
31	20.6	86	85	100	99	53	100	13
32	20.0	75	88	58	47	0	82	89
33	19.2	96	97	95	65	100	100	89
34	17.5	100	97	100	94	7	91	100
35	16.9	0	0	0	6	93	0	11
36	16.4	0	0	0	83	100	100	89
37	15.3	86	89	86	100	6	100	100
38	14.6	0	0	87	2	6	0	100
39	14.2	49	33	0	98	94	100	0
40	13.4	39	100	0	2	6	91	100
41	13.2	100	0	89	0	0	0	0
42	13.0	0	0	0	3	94	0	0

[a] Taxa designations as in Table 1.

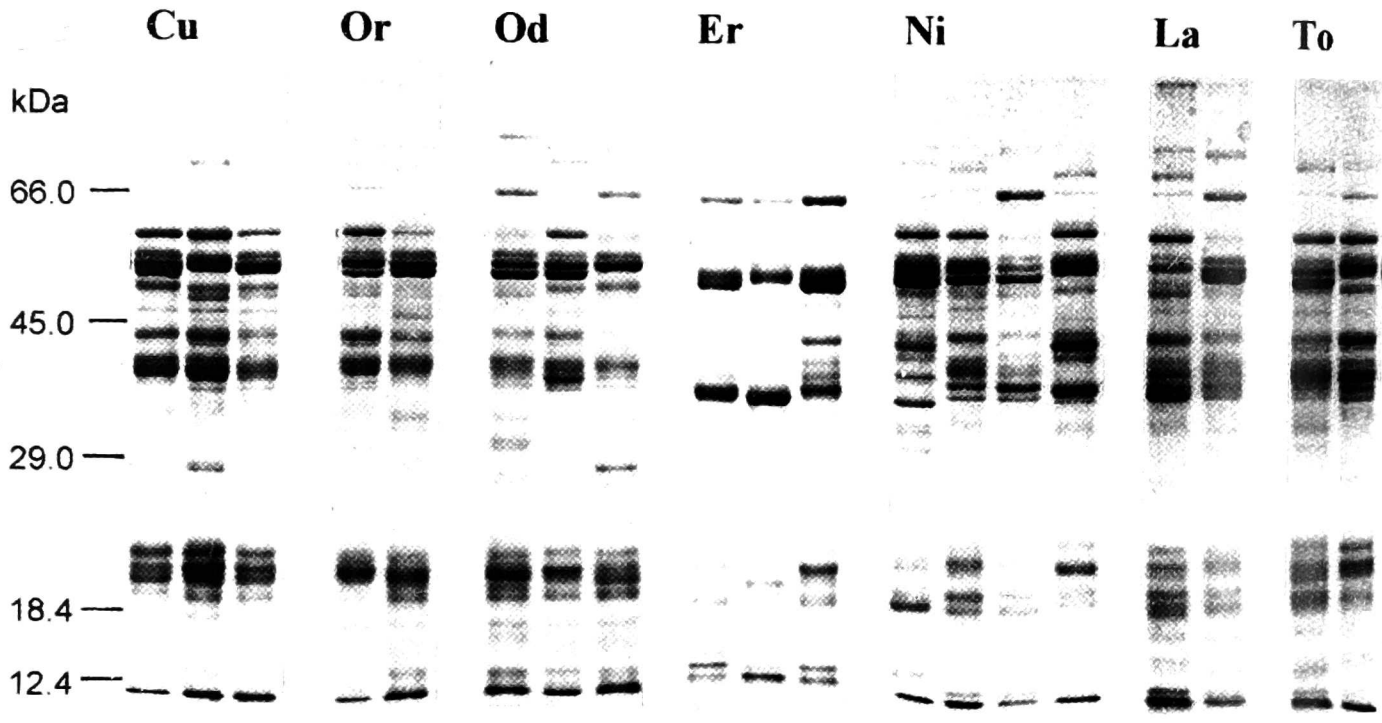

Figure 1. SDS-PAGE patterns of seed globulins illustrating some variation detected in the examined *Lens* taxa. Taxa designations as in Table 1.

Variation within the taxa

Mean genetic similarities within the studied taxa varied from 0.66 in *L. ervoides* to 0.86 in *L. lamottei* (Table 3). As shown in Table 3, genetic similarities within particular taxa varied within wide ranges, which was due to differences between accessions as well as to individual variation. However, it should be added that the similarities among most accessions of particular taxa were close to the mean similarities.

***L. culinaris* subsp. *culinaris*.** Of the 27 globulin polypeptide bands detected in this taxon only five bands were recorded in all examined individuals. However, no accession could be distinguished due to a characteristic band(s).

Table 3. Genetic similarities within the studied *Lens* taxa based on seed globulin data

Taxon[a]	Mean	Range
Cu	0.71	0.39-0.95
Or	0.79	0.62-1.00
Od	0.75	0.52-0.92
Er	0.66	0.35-0.99
Ni	0.70	0.20-1.00
La	0.86	0.68-1.00
To	0.83	0.71-0.95

[a] Taxa designations as in Table 1.

In search for possible correlations between the electrophoretic seed globulin data and geographical origin and/or seed weight of the cultivated lentils, nine populations indicated in Figure 2 were subjected to hierarchical grouping based on the frequency of seed globulin bands. The dendrogram shows that the population comprising the *microsperma* accessions from southern and central Asia

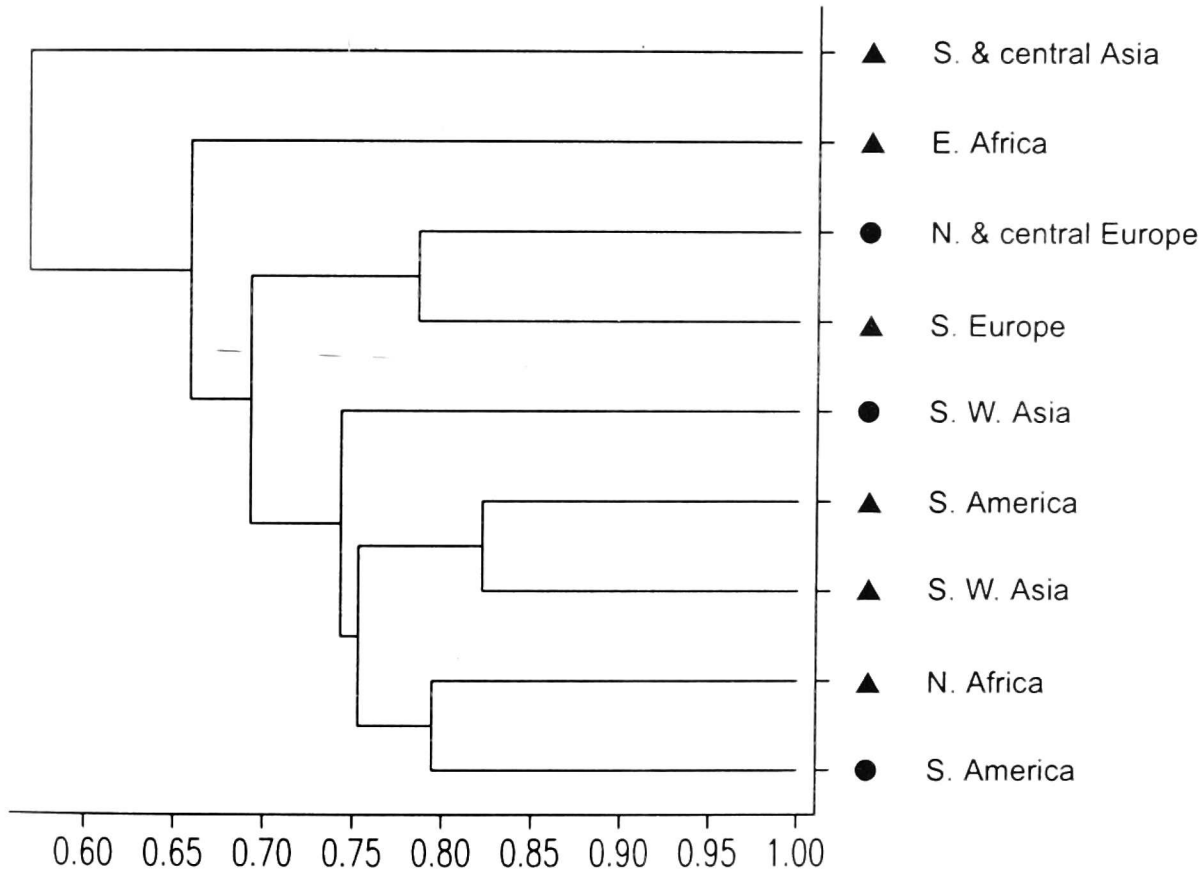

Figure 2. UPGMA dendrogram of *microsperma* (▲) and *macrosperma* (●) *Lens culinaris* subsp. *culinaris* populations from different geographical regions based on frequencies of seed globulin polypeptide bands

forms a peripheral group, joined with the remaining accessions at 0.57 similarity. The population from East Africa, also of *microsperma* type, is a distinctive group, likewise. As regards other populations considered, some distinctness of European populations of both *microsperma* and *macrosperma* types may be observed. The remaining populations under consideration form a group comprising *microsperma* and *macrosperma* accessions from different geographical regions.

L. culinaris* subsp. *orientalis. In total, 23 globulin polypeptide bands were recorded, six of them being observed in all examined individuals. Two accessions, IL 325/A/JOR and IL 76/A/PAL, did not show the polypeptide with M_r 72.1 observed in all individuals of the remaining accessions.

L. odemensis. Of the 29 detected polypeptides, only seven polypeptides were found in all examined individuals. In one accession, IL 21/A/PAL, the commonly occurring polypeptide with M_r 52.9 was not observed.

L. ervoides. Thirty-five polypeptides were recorded in this species, but only two polypeptides were found in all analysed individuals. As many as ten polypeptides were observed in less than 5% individuals. In three accessions, IL 48/A/YUG, IL 52/A/YUG and IL 339/A/JOR, some individuals could be distinguished due to the presence of characteristic bands with M_r values of 32.9, 31.4 and 13.0.

L. nigricans. Thirty-four globulin polypeptides were distinguished in *L. nigricans*, three of them being present in all examined individuals. Of the 30 studied accessions, 12 accessions showed characteristic sets of bands. In general, in *L. nigricans* differences among accessions were most pronounced, which is reflected in the especially wide range of genetic similarities within this taxon (Table 3).

L. lamottei. Of the 22 globulin polypeptide bands detected in this species, 13 bands were recorded in all examined individuals. Some of the commonly occurring polypeptides were not observed in three accessions: IL 29/A/ESP, IL 432/A/ESP and IL 437/A/TUR apparently lacked the polypeptides with M_r values of 75.2, 17.5 and 13.4, respectively.

L. tomentosus. Twenty-three polypeptide bands were distinguished in nine studied accessions; 12 polypeptides were found in all analysed individuals. The polypeptide band with M_r value of 16.9 was recorded only in accession IL 93/A/TUR.

Relationships among the taxa

In total, 42 globulin polypeptide bands were recorded in the studied *Lens* taxa. Particular taxa did not show any characteristic bands. Hierarchical grouping of the taxa, based on frequency of the recorded bands, is presented in Figure 3. The dendrogram shows that *L. nigricans* is the most distinctive taxon, associated with the remaining taxa at 0.31 similarity. *L. lamottei* and *L. ervoides* join the other taxa investigated at ca. 0.6 similarity. Close association (above 0.7 simi-

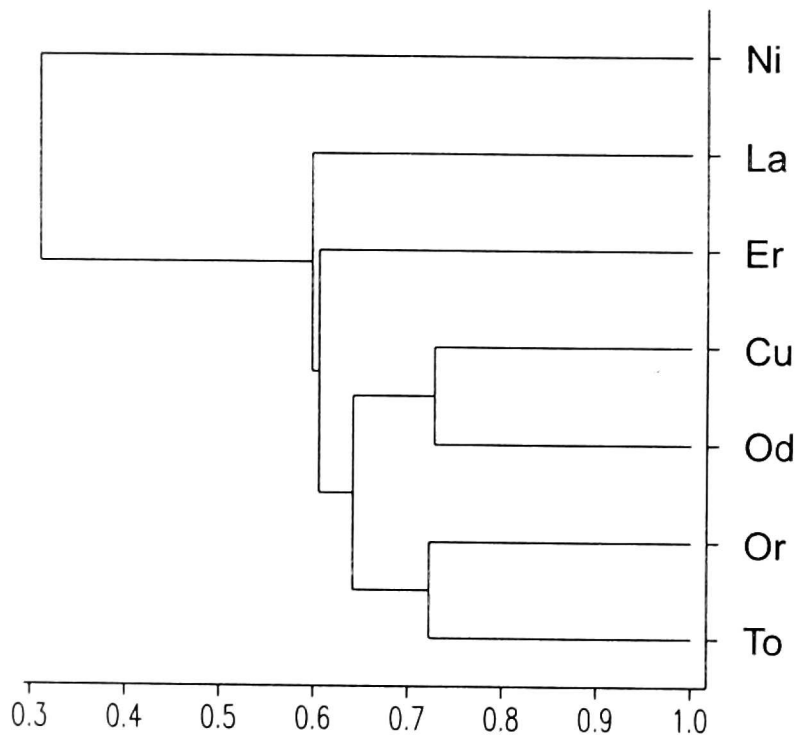

Figure 3. UPGMA dendrogram of *Lens* taxa based on frequencies of seed globulin polypeptide bands. Taxa designations as in Table 1.

larity) of *L. culinaris* subsp. *culinaris* with *L. odemensis* and of *L. culinaris* subsp. *orientalis* with *L. tomentosus* is evident; the two pairs of taxa join at 0.64 similarity.

Discussion

BARULINA (1930) classified cultivated lentil landraces into *microsperma* and *macrosperma* types according to seed size. However, the performance of hybrids between large and small-seeded lentils indicates that lentil should not be subgrouped according to seed size (LADIZINSKY 1979). Moreover, as found by SOLH and ERSKINE (1984), the distribution of accessions differing in seed size or seed weight is continuous and therefore *microsperma* and *macrosperma* types cannot be treated as distinct taxonomic units. Biochemical data provide inconsistent results as to separation of *microsperma* and *macrosperma* types. A significant differentiation between these types was observed by SHARMA and coworkers (1995, 1996) in RAPD and AFLP analyses. On the other hand, ABO-ELWAFA and coworkers (1995) reported that *microsperma* and *macrosperma* cultivars were indistinguishable by the RAPD markers. Seed globulin data presented in this paper support the opinion that the division of the cultivated lentil into *microsperma* and *macrosperma* types is arbitrary. However, it should be mentioned that forms from southern and central Asia, all of *microsperma* type, proved to constitute a distinctive group, which is consistent with the RAPD and isoenzyme data reported by FERGUSON and coworkers (1998b).

In most biochemical investigations performed with the use of protein and DNA markers, the degree of genetic variation in the cultivated lentil was within the range of variation recorded in particular wild taxa (PINKAS et al. 1985, HOFFMAN et al. 1986, ABO-ELWAFA et al. 1995, AHMAD et al. 1996, 1997, FERGUSON, ROBERTSON 1996, van OSS et al. 1997). A similar observation was made in the present study of seed globulins; e.g., genetic diversity in *L. culinaris* subsp. *culinaris* was higher than in *L. lamottei* and in *L. tomentosus,* and lower than in *L. ervoides* and in *L. nigricans.*

Different research teams reported different relative levels of genetic diversity within particular *Lens* taxa. The main reason of the discrepancy is most probably the analysis of different plant samples; even in the case of using the same type of biochemical markers the reported results were sometimes inconsistent. For example, the isoenzyme analyses performed by PINKAS and coworkers (1985) and by FERGUSON and ROBERTSON (1996) showed, respectively, a relatively low and a relatively high polymorphism in *L. nigricans.*

Electrophoretic seed globulin data presented in this paper revealed a marked polymorphism in *L. nigricans,* which is in agreement with the majority of the relevant literature data (ABO-ELWAFA et al. 1995, SHARMA et al. 1995, AHMAD et al.

1996, 1997, FERGUSON, ROBERTSON 1996). In our study, a relatively high polymorphism was found also in *L. ervoides*. This observation is in accordance with only some of the reported isoenzyme and RAPD data (PINKAS et al. 1985, SHARMA et al. 1995). Generally, biochemical markers revealed low variation in *L. ervoides* (ABO-ELWAFA et al. 1995, AHMAD et al. 1996, 1997, FERGUSON, ROBERTSON 1996, VAN OSS et al. 1997, FERGUSON et al. 1998a). A rather low level of seed globulin polymorphism recorded in *L. lamottei* and in *L. tomentosus* may be partly explained by limited numbers of accessions representing these species.

Genetic relationships among the taxa distinguished in the genus *Lens* were investigated with the use of protein and molecular markers by several research groups. The obtained results indicated that the two subspecies of *L. culinaris*, subsp. *culinaris* and subsp. *orientalis*, are close relatives (PINKAS et al. 1985, HOFFMAN et al. 1986, HAVEY, MUEHLBAUER 1989, ABO-ELWAFA et al. 1995, SHARMA et al. 1995, 1996, FERGUSON, ROBERTSON 1996, AHMAD et al. 1996, 1997, FORD et al. 1997, FERGUSON et al. 2000). On the other hand, in most studies a marked distinctness of *L. nigricans* and *L. ervoides* was observed (HAVEY, MUEHLBAUER 1989, ABO-ELWAFA et al., 1995, SHARMA et al. 1995, AHMAD et al. 1996, 1997). As to the remaining taxa, their positions were usually reported to be intermediate between *L. culinaris* and *L. nigricans/L. ervoides*. In general, the presented electrophoretic seed globulin data confirm the relationships among *Lens* taxa reported by other investigators; in our work the distinctness of *L. nigricans* was especially marked, which is consistent with the results of extensive isoenzyme analysis reported by FERGUSON and coworkers (FERGUSON, ROBERTSON 1996, FERGUSON et al. 2000).

It seems interesting that in the comparative biochemical studies of *Lens* taxa including *L. lamottei* (= the differentiated cytotype of *L. nigricans*) and *L. tomentosus*, the former taxon proved to be a distinctive one (HOFFMAN et al. 1986, MUENCH et al. 1991, FERGUSON, ROBERTSON 1996, FERGUSON et al. 2000), while the latter tended to group with *L. culinaris* subsp. *culinaris*, *L. culinaris* subsp. *orientalis* and *L. odemensis* (VAN OSS et al. 1997, FERGUSON et al. 2000). Similar relationships were observed in the present study of seed globulin polymorphism.

As regards taxonomic relationships within the genus *Lens*, some trends observed in biochemical studies are not consistent with the morphological and crossability evidence. Morphologically, only *L. ervoides* is distinct (LADIZINSKY 1993). *L. nigricans*, usually distinguishable in biochemical studies, is morphologically similar to other taxa (LADIZINSKY 1993, FERGUSON et al. 2000) and forms one crossability group with *L. ervoides* and *L. lamottei* (VAN OSS et al. 1997). On the other hand, *L. tomentosus*, related to some other *Lens* taxa according to protein and molecular data, is reproductively isolated from all other *Lens* species (LADIZINSKY 1997, VAN OSS et al. 1997). The discrepancy between crossability and biochemical differentiation of *Lens* taxa was observed in different studies;

PINKAS and coworkers (1985), interpreting their isoenzyme data, suggested that "...the evolution of reproductive barriers does not necessarily follow the divergence of isozyme loci".

Conclusions

The presented electrophoretic seed globulin data reflect the confused *Lens* taxonomy. No species-specific diagnostic globulin subunits were detected and a marked variation in seed globulin patterns was observed in particular taxa. Nevertheless, the frequency distribution of the recorded globulin polypeptides gives some support to the recent suggestion of FERGUSON and coworkers (2000) concerning taxonomic classification of the genus *Lens*. According to the authors only four species should be distinguished in the genus. The species are: *L. culinaris* Medik., *L. ervoides* (Brign.) Grande, *L. nigricans* (M.Bieb.) Godr. and *L. lamottei* Czefr. The remaining taxa should be included in *L. culinaris* as the following subspecies: subsp. *culinaris*, subsp. *orientalis* (Boiss.) Ponert, subsp. *tomentosus* (Ladiz.) M.E. Ferguson et al. and subsp. *odemensis* (Ladiz.) M.E. Ferguson et al.

In view of serious inconsistencies of the literature data concerning biochemical relationships and crossability of the distinguished *Lens* taxa, further taxonomic and genetic studies of the genus are needed. Such studies should cover plant materials properly representing genetic variation within particular taxa.

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