



Genetic Relationships Among Wild *Lens* Mill. Species Revealed by SDS –PAGE

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Summary: Seed storage proteins of 18 lentil accessions of the following *Lens* taxa: *L. culinaris*, *L. orientalis*, *L. odemensis*, *L. nigricans*, *L. ervoides*, *L. lamottei*, *L. tomentosus* and *L. lenticula*, were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). All visible polypeptide bands were scored for presence or absence and data were used for dendrogram construction by UPGMA method. Three clusters were formed at the dendrogram: the first one integrated accessions of *L. orientalis* and *L. odemensis*; the second cluster joined *L. culinaris* и *L. tomentosus* accessions, and related to them *L. lamottei* species, the third cluster included *L. nigricans* and *L. ervoides* accessions. Species affiliation of accessions *L. orientalis* k-2861, *L. nigricans* k-2859 and *L. nigricans* k-2860 was confirmed. Taxonomic position of *L. lenticula* k-2858 accession was not determined exactly.

Key words: lentil, SDS-PAGE, seed storage protein, wild species

Introduction

Lentil, as an important legume crop, needs further improvement in order to increase plant productivity, disease resistance and adaptability to machinery cultivation. Wild lentil species can serve as an additional source of genetic diversity for creation of new lentil cultivars.

Taxonomy of genus *Lens* Mill. is still a topic open to discussions. The classification suggested by Barulina (1930) includes 5 species, where one cultivated species is *Lens esculenta* Moench. (a synonym is *L. culinaris* Medik) and four are wild species *L. lenticula* (Schreb) Alef., *L. nigricans* (M.B.) Godr., *L. kotschyana* (Boiss) Alef., *L. orientalis* (Boiss) Hand.-Mazz.

Czefranova (1971) made a critical study of *Lens* species and suggested the system including 9 taxa: *L. montbretii* (Fish. et May.) Davis et Peltn., *L. penduncularis* (Nabel) Czefr., *L. culinaris* Medik., *L. orientalis* (Boiss) Schmalh., *L. cyanea* (Boiss. et Hohen.), *L. nigricans* (Bieb.) Webl et Berth., *L. lamottei* Czefr., *L. ervoides* (Brign) Grande, *L. uniflora* (Ten.) Schur. The species *L. montbretii* is a synonym of *L. kotschyana* species, and *L. ervoides* is the one for *L. lenticula*. As a result there was re-

established authors' priority for *L. orientalis* and *L. nigricans*, renamed and described *L. lamottei*, restored 2 species *L. uniflora* and *L. cyanea*.

Studying wild *Lens* species and making interspecific crossings, Ladizinsky (1984) suggested his own system of *Lens* genus. Due to an interspecific incompatibility and hybrid sterility, different *Lens* accessions were grouped in 2 botanic species: *L. culinaris* and *L. nigricans*. *L. culinaris* and contained 3 subspecies: ssp. *culinaris*, ssp. *orientalis*, ssp. *odemensis*, while *L. nigricans* included ssp. *nigricans* and ssp. *ervoides*. It deserves mentioning that ssp. *odemensis* was excluded by Ladizinsky from *L. nigricans* on the bases of morphological differences of stipules and incompatibility with other forms of *L. nigricans*.

Summing up 15 years of research in wild species collecting, studying and hybridization, Ladizinsky (1993) came to a conclusion that in *Lens* genus there should be isolated such species as *L. culinaris* ssp. *culinaris*, *L. culinaris* ssp. *orientalis*, *L. odemensis*, *L. ervoides*, *L. nigricans*. Taxon *L. montbretii* should be returned to *Vicia* genus as *Vicia montbretii*. This species is different not only morphologically, but in the number of chromosomes ($2n = 12$) whereas the *Lens* genus chromosomes number is $2n = 14$.

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Two more species were later added to *Lens* genus (Oss et al. 1997). The researches admitted *L. lamottei* Czefr. species identified by Czefranova (1971) in herbarium samples of *L. nigricans*. Some known populations of this species came from France, Spain, and Morocco. Ladizinsky isolated *L. tomentosus* Ladiz. species, that was known earlier as a *L. orientalis* variant and was different from the latter by its pubescent pods.

Though there was an opinion that *L. tomentosus* and *L. odemensis* should be reduced to sub-species of *L. culinaris* (Ferguson et al. 2000), data of molecular and cytological analysis (Galasso 2003, Sonnante et al. 2003, Duran 2004) confirmed isolation of *L. lamottei* and *L. tomentosus* to independent species.

So, most contemporary researches recognized the next seven taxa of genus *Lens*: *L. culinaris* Medik. (*L. esculenta* Moench.), *L. orientalis* (Boiss.) Hand.-Mazz., *L. odemensis* Ladiz., *L. nigricans* (M.B.) Godr., *L. ervoides* (Brign.) Grande, *L. lamottei* Czefr., *L. tomentosus* Ladiz. Only *L. culinaris* is a cultivated species, and all the rest are wild ones. In this study we used species definitions for *Lens culinaris* and *Lens orientalis* (Boiss.) Schmalh, accepted by Russian botanist Czefranova (1971).

The objective of this study was to establish phylogenetic relations between the species of genus *Lens* and to clarify systematic position of some lentil accessions on the base of seed storage proteins SDS-PAGE analysis.

Materials and Methods

Materials for research were 18 *Lens* accessions of different origins, covering all up to date known taxa (Tab. 1). Lentil accessions were obtained from N. I. Vavilov Research Institute of Plant Industry (VIR), where part of them that originally belong to VIR collection was marked with letter k, and accessions firstly received from the International Center for Agricultural Research in the Dry Areas (ICARDA) retained their original code. Seed storage proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in gel of 12.5% concentration (Konarev et al. 2000). Electrophoretic spectra were analyzed on the base of presence or absence of all visible protein bands. Dissimilarity value (1-F) was estimated according to Nei and Li (1978) and dendrogram was constructed by UPGMA method.

Table 1. List of lentil accessions used in SDS-PAGE analysis
Tabela 1. Spisak akcesija sočiva korišćenih u SDS-PAGE analizi

Number Broj	Species Vrsta	Accession name Naziv akcesije	Country of origin Zemlja porekla
1	<i>L. orientalis</i>	ILWL 7	Turkey
2	<i>L. orientalis</i>	ILWL 11	Syria
3	<i>L. orientalis</i>	k-2861	Syria
4	<i>L. lenticula</i>	k-2858	Turkey
5	<i>L. tomentosus</i>	ILWL 90	Turkey
6	<i>L. tomentosus</i>	ILWL 120	Syria
7	<i>L. odemensis</i>	ILWL 21	Palestine
8	<i>L. odemensis</i>	ILWL 164	Syria
9	<i>L. lamottei</i>	ILWL 29	Spain
10	<i>L. lamottei</i>	ILWL 428	Spain
11	<i>L. ervoides</i>	ILWL 43	Croatia
12	<i>L. ervoides</i>	ILWL 54	Palestine
13	<i>L. nigricans</i>	ILWL 30	Spain
14	<i>L. nigricans</i>	k-2859	Italy
15	<i>L. nigricans</i>	k-2860	Turkey
16	<i>L. culinaris</i>	Vekhovskaya	Russia
17	<i>L. culinaris</i>	Obraztsov Chiflik 7	Bulgaria
18	<i>L. culinaris</i>	Svetlaya	Russia

Results and Discussion

The Electrophoregram revealed 75 polypeptide bands of different insensitivity; most of them were polymorphic (Fig. 1). The greatest protein diversity was observed in area of 30-65 kDa. Dendrogram, created on the base of seed storage protein electrophoretic spectra, showed that *Lens*

species were quite different from each other and accessions of every species formed independent groups except of *L. tomentosus* samples (Fig. 2).

The intraspecific variation of lentil in seed storage protein spectra was significantly lower than the interspecific one. Nevertheless, high diversity was observed inside of *L. orientalis*, *L.*

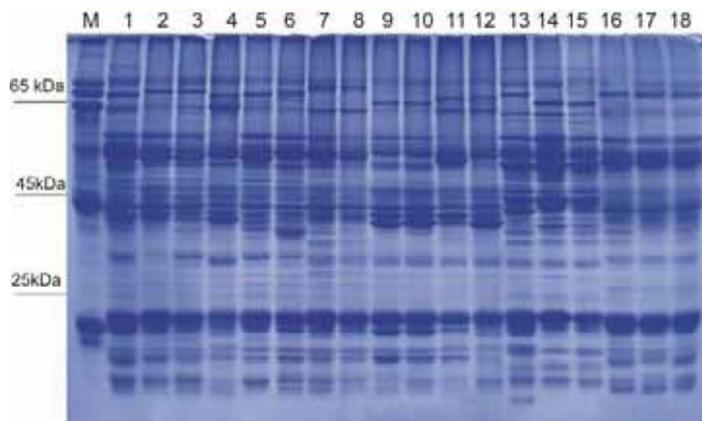


Figure 1. Protein profiles of *Lens* species generated by SDS-PAGE. M - marker of molecular weight (soybean protein). Lanes: 1, 2, 3 - *L. orientalis*; 4 - *L. lenticula*; 5,6 - *L. tomentosus*; 7,8 - *L. odemensis*; 9,10 - *L. lamottei*; 11,12 - *L. ervoides*; 13,14,15 - *L. nigricans*; 16,17,18 - *L. culinaris* (numeration corresponds to Table 1)

Slika 1. Proteinski profili vrsta roda *Lens* species stvoreni pomoću SDS-PAGE. M - marker molekularne mase (protein soje). Trake: 1, 2, 3 - *L. orientalis*; 4 - *L. lenticula*; 5, 6 - *L. tomentosus*; 7, 8 - *L. odemensis*; 9, 10 - *L. lamottei*; 11, 12 - *L. ervoides*; 13, 14, 15 - *L. nigricans*; 16, 17, 18 - *L. culinaris* (brojevi odgovaraju onima u tabeli 1)

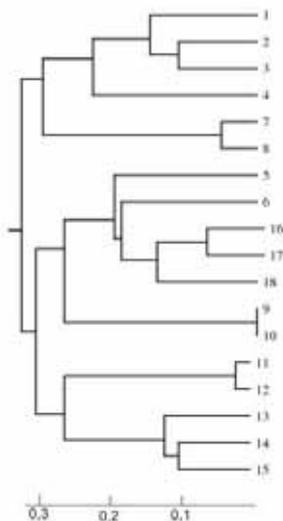


Figure 2. Dendrogram constructed on the base of Nei and Li distance. 1, 2, 3 - *L. orientalis*; 4 - *L. lenticula*; 5,6 - *L. tomentosus*; 7,8 - *L. odemensis*; 9,10 - *L. lamottei*; 11,12 - *L. ervoides*; 13,14,15 - *L. nigricans*; 16,17,18 - *L. culinaris* (numeration corresponds to Table 1)

Slika 2. Dendrogram konstruisan na osnovu udaljenosti Neija i Lija. 1, 2, 3 - *L. orientalis*; 4 - *L. lenticula*; 5, 6 - *L. tomentosus*; 7, 8 - *L. odemensis*; 9, 10 - *L. lamottei*; 11, 12 - *L. ervoides*; 13, 14, 15 - *L. nigricans*; 16, 17, 18 - *L. culinaris* (brojevi odgovaraju onima u tabeli 1)

nigricans, *L. culinaris* species. The accessions of *L. odemensis* and *L. ervoides* were less distinguished and *L. lamottei* samples were characterized by absolutely identical spectra. It was interesting that *L. orientalis* k-2861 accession from VIR collection was quite closely connected to *L. orientalis* ILWL11 accession from ICARDA, *L. nigricans* k-2859 and k-2860 accessions were close to each other and belonged to *L. nigricans* ILWL 30 group that proved their species affiliation. Accession k-2858 known as *L. lenticula* appeared to be in one group with *L. orientalis*, though not very close.

However, in spite of obvious relation of *L. lenticula* k-2858 accession to *L. orientalis*, at the moment there is no sound evidence to determine it as *L. orientalis* species. At the same time, those accessions were located at the dendrogram far enough from *L. ervoides*, species which was supposed to be a synonym of *L. lenticula* by Chefranova (1971). Dissimilarity value between k-2858 and *L. ervoides* accessions was high and means 0.723 and 0.745. On the whole, *L. lenticula* was quite distant from all analyzed species and its dissimilarity coefficient between the nearest *L. orientalis* ILWL7 accession was also at high level (0.422). Therefore, the described situation did not clarify the systematic position of *L. lenticula*.

Presumably three clusters can be isolated on the dendrogram. The first cluster integrates accessions of *L. orientalis* and *L. odemensis* species. The second one joins *L. culinaris* and *L. tomentosus* accessions, and related to them *L. lamottei* species. The third cluster includes *L. nigricans* and *L. ervoides* species. According to electrophoretic spectra of seed storage proteins, it was revealed that *L. orientalis* appeared to be closely related to *L. odemensis* than to other species; *L. culinaris* was found to be closely relative to *L. tomentosus*; *L. nigricans* was supposed to be closely allied to *L. ervoides*. Though *L. lamottei* was grouped into one cluster with *L. culinaris* and *L. tomentosus*, dissimilarity value between *L. lamottei* and other species was not less than 0.522.

SDS-PAGE analysis was applied for seed proteins of wild lentil relative by other researchers (Sammour 1994, Ahmad et al. 1997, Zimniak-Przybylska et al. 2001). Studying five *Lens* taxa, Sammour (1994) and Ahmad et al. (1997) reached a conclusion that *L. orientalis* and *L. odemensis* appeared to be the wild progenitors

of *L. culinaris*. A complete set of taxa including recently recognized *L. tomentosus* and *L. lamottei* was used by Zimniak-Przybylska et al. (2001). As for relationships among studied taxa the most closely relative species were *L. culinaris* and *L. odemensis* from the one side, and *L. orientalis* and *L. tomentosus* from the other side.

Our results are to some extent in contradiction with the above mentioned regarding the nearest relatives of *L. culinaris*. This discrepancy among our result and data of Zimniak-Przybylska (2001) revealed by the same method can be explained by different *Lens* accessions used in analysis.

In contrast, Duran & Peres de la Vega (2004) using RAPD and ISSR markers found that three taxa *L. culinaris*, *L. orientalis* and *L. tomentosus* grouped into one cluster, with *L. tomentosus* being the closest species to cultivated lentil.

Applying multi-target fluorescence in situ hybridization (FISH) on seven *Lens* taxa I. Galasso (2003) concluded that the most similar karyotype to cultivated lentil was that of *L. orientalis*, whereas *L. nigricans* and *L. tomentosus* were two species that showed the most divergent FISH patterns. At the same time, Galasso suggested that two *L. tomentosus* accessions (ILWL 149 and ILWL 120) were probably misclassified based on the fact that their chromosome morphology and FISH karyotypes were more similar to *L. culinaris* than to *L. tomentosus*.

One of the *L. tomentosus* accessions used at the present study was ILWL 120. If the hypothesis of misclassification can be accepted, it explains the fact of close similarity among *L. tomentosus* and *L. culinaris* cultivars revealed in our experiment.

Conclusions

Having performed the SDS-PAGE analysis of lentil seed storage protein of 8 taxa of *Lens* genus, we ascertained genetic relations among the species and reached the conclusion that *L. tomentosus* ILWL 120 is the closest accession to cultivated lentil and confirmed specific affiliation of *L. orientalis* k-2861 and *L. nigricans* k-2859 and k-2860 samples of the collection of N. I. Vavilov Research Institute of Plant Industry. The obtained results can be useful in taxonomic definition of various accessions, interspecific hybridization and breeding programs.

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Genetički odnosi između samoniklih vrsta *Lens* Mill. otkriveni pomoću SDS –PAGE

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Izvod: Izvršena je elektroforeza natrijum-dodecil-sulfatnim poliakrilamidnim gelom (SDS-PAGE) rezervnih proteina semena 18 akcesija sledećih taksona roda *Lens*: *L. culinaris*, *L. orientalis*, *L. odemensis*, *L. nigricans*, *L. ervoides*, *L. lamottei*, *L. tomentosus* i *L. lenticula*. Ispitano je prisustvo svih vidljivih polipeptidnih traka, a podaci su poslužili za pravljenje dendrograma UPGMA metodom. Dendrogram su činila tri klastera: prvi sa združenim akcesijama *L. orientalis* i *L. odemensis*, drugi sa združenim akcesijama *L. culinaris* i *L. tomentosus* i njima srodnom vrstom *L. lamottei* i treći koji je uključio akcesije *L. nigricans* i *L. ervoides*. Bila je potvrđena srodnost akcesija *L. orientalis* k-2861, *L. nigricans* k-2859 i *L. nigricans* k-2860. Taksonomski položaj akcesije *L. lenticula* k-2858 nije bilo moguće precizno odrediti.

Ključne reči: rezervni proteini semena, samonikle vrste, sočivo, SDS-PAGE