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BRIEF COMMUNICATION

Seed protein diversity among lentil cultivars

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Abstract

Seed protein diversity of fourteen lentil cultivars grown in Turkey was studied by using sodium dodecyl sulfate polyacrylamide gel electrophoresis. A distance matrix was produced based on five polymorphic protein bands, scored for their absence as 0 and presence as 1. Seed protein distances among the cultivars ranged from 0.00 to 0.80. The dendrogram based on the distance matrix indicated two distinct clusters. The first cluster includes the cultivars Sultan 1, Meyveci 2001 and Kayi 91. The second cluster contains the cultivars Pul 11, Ozbek, Emre 20, Malazgirt 89, Ciftci, Seyran 96, AliDayi, Firat 87, Sazak, Erzurum 89 and YerliKirmizi.

Additional key words: *Lens culinaris*, SDS-PAGE, storage proteins.

Lentil is an important seed legume crop, cultivated worldwide as human food. Historically, lentil breeding has received little attention and most cultivars of lentil have been selected from heterogeneous populations, such as land races (Havey and Muehlbauer 1989).

Seed protein patterns obtained by electrophoresis have been successfully used not only to resolve taxonomic and evolutionary problems of several crop species but also to distinguish cultivars of a particular crop species (Ladizindsky and Hymowitz 1979, Ferguson and Grabe 1986, Karihaloo *et al*. 2002). In particular, seed protein patterns produced by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) have been used for the identification of wheat, tiriticale, pepper, bean, date and *Cucurbita* cultivars (Hussain *et al*. 1986, Stegemann *et al*. 1987, Igrejas *et al*. 1999, Vladova *et al*. 2000, Cherdouh *et al*. 2005, Stoilova *et al*. 2006). The aim of this study was to investigate seed protein diversity among lentil cultivars grown in Turkey by using SDS-PAGE.

Seeds of the lentil (*Lens culinaris* Medik., syn. *Lens esculenta* Moench) cultivars, (Malazgirt 89, Ozbek, Ciftci, Seyran 96, Firat 87, Emre 20, Meyveci 2001, Erzurum 89, Kayi 91, Ali Dayi, Sultan 1, Sazak, Yerli Kirmizi and Pul 11), were obtained from the Haymana Research Station of the Central Field Crop Research Institution, Ankara, Turkey. For each population, 10 bulked seeds were used for protein extraction. Cotyledons were pounded to flour and protein extractions were performed with a solution of 25 cm^3 0.5 M Tris/HCL (pH 6.8), 10 cm^3 10 % SDS, 20 cm^3 glycerol, 1 cm^3 2-mercaptoethanol and 44 cm^3 duble distilled H_2O. The extracts were centrifuged at 7426 *g* for 15 min and the supernatants were heated in boiling water for 5 min prior to being loaded on gel. Electrophoresis was performed twice according to Laemmli (1970) and the gels were stained overnight with Coomassie Brillant Blue G-250.

Protein bands were scored for their absence as 0 and presence as 1. A similarity matrix was established using the similarity coefficient (SM; Sokal and Michener 1958) and *NTSYS-pc* (*Version 1.7*, Rohlf 1992). SM = m/n, where m = shared present fragments + shared absent fragments and n = total of obtained fragments. The similarity coefficients were converted into distances (D) using the formula D = 1 - SM. The dendrogram based on

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Abbreviations: SDS-PAGE - sodium dodecyl sulfate polyacrylamide gel electrophoresis; RAPD - randomly amplified polymorphic DNA.
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the distance matrix was constructed using the unweighted pair-group method with arithmetic averages (UPGMA) under the *NJ* subprogram in the *PHYLIP* software package version *3.6a3*.

The band patterns of fourteen lentil cultivars used in this study are shown in Fig. 1. A total of 24 polypeptide bands were detected with molecular masses ranging from 14.4 to 116 kDa. Of the 24 bands, five polypeptide bands with molecular masses ranging from 35 to 116 kDa were polymorphic and used to discriminate the cultivars (Fig. 1, indicated as B1-B5).

The genetic distances based on five SDS-PAGE bands are presented in Table 1. The dendrogram indicated two distinct clusters (Fig. 2). The first cluster with 11.9 % dissimilarity includes the cultivars Sultan, Meyveci and Kayi 91. The second cluster with 8.9 % dissimilarity contains the cultivars Pul11, Ozbek, Emre, Malazgirt, Çiftçi, Seyran, AliDayi, Firat, Sazak, Erzurum and YerliKirmizi. The second cluster was divided into two subclusters: Pul11, Ozbek, Emre 20, Malazgirt 89 and Ciftci cultivars, and Seyran 96, AliDayi, Firat 87, Sazak, Erzurum 89 and YerliKirmizi cultivars. Apart from Erzurum 89, the green-seeded cultivars Sultan 1, Meyveci 2001 and Kayi 91 were grouped in the first cluster, while the red and brown seeded cultivars were grouped in the second.

Seed protein distances among the cultivars ranged from 0.00 to 0.80. Meyveci 2001 revealed 80 %

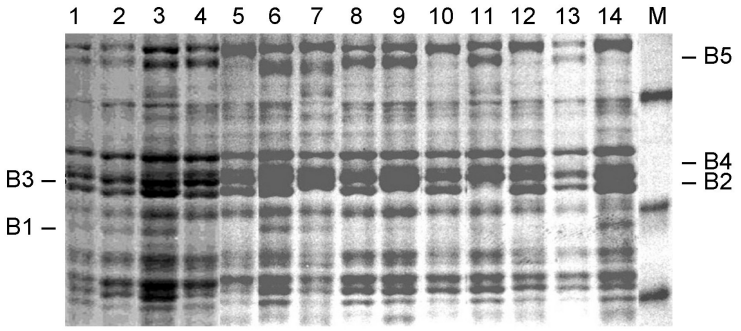

Fig. 1. Electrophoregrams showing seed storage protein banding pattern in 14 lentil cultivars. *Lanes* 1 - 14 refer to the cultivars Malazgirt 89, Ozbek, Ciftci, Seyran 96, Firat 87, Emre 20, Meyveci 2001, Erzurum 89, Kayi 91, Ali Dayi, Sultan 1, Sazak, Yerli Kirmizi and Pul 11, respectively. M - size marker. B1 to B5 - bands used to discriminate the cultivars.

Table 1. Distance matrix based on observed seed protein patterns of 14 lentil cultivars. Numbers 1 - 14 correspond to accession code denoted in Fig. 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-													
2	0.20	-												
3	0.00	0.20	-											
4	0.20	0.40	0.20	-										
5	0.40	0.60	0.40	0.20	-									
6	0.00	0.20	0.00	0.20	0.40	-								
7	0.40	0.60	0.40	0.60	0.80	0.40	-							
8	0.40	0.20	0.40	0.20	0.40	0.40	0.80	-						
9	0.60	0.80	0.60	0.40	0.60	0.60	0.20	0.60	-					
10	0.20	0.40	0.20	0.00	0.20	0.20	0.60	0.20	0.40	-				
11	0.40	0.60	0.40	0.20	0.40	0.40	0.40	0.40	0.20	0.20	-			
12	0.40	0.60	0.40	0.20	0.00	0.40	0.80	0.40	0.60	0.20	0.40	-		
13	0.40	0.20	0.40	0.20	0.40	0.40	0.80	0.00	0.60	0.20	0.40	0.40	-	
14	0.20	0.40	0.20	0.40	0.20	0.20	0.60	0.60	0.80	0.40	0.60	0.20	0.60	-

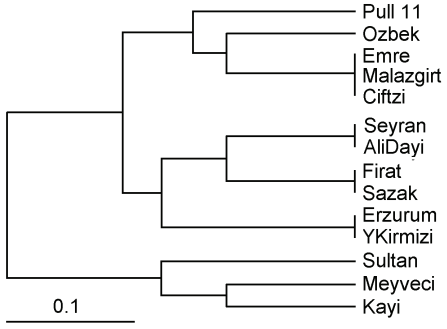

Fig. 2. Dendrogram of 14 lentil cultivars based on dissimilarity matrix of seed storage proteins.

dissimilarity with the cultivars Firat 87, Erzurum 89, Sazak and YerliKirmizi. However, protein patterns observed among the cultivars Emre 20, Malazgirt 89, Ciftci within the first subcluster and the cultivars between Seyran 96 and AliDayi, Firat 87 and Sazak, and Erzurum 89 and YerliKirmizi within the second subcluster were identical in the present study. Therefore, protein profiles obtained from SDS-PAGE were not sufficient in distinguishing all lentil cultivars used in this study, whereas band patterns obtained from the previous study with RAPD (Yüzbaşıoğlu *et al*. 2006) differentiated those indistinguished by SDS-PAGE in the present study. This result is in agreement with the findings of Zavodna *et al*. (2000) which states that the DNA marker systems used differantiated the closely related lentil cultivars. Although Harlan (1951) reported that there is a microcenter of diversity for lentils in Turkey, our findings are in agreement with the study of Ford *et al*. (1999) that states the cultivated lentil has a narrow genetic base due to intensive selection and subsequent exclusion of much genetic variation from germplasm stocks. Based on the SDS-PAGE results, it can be concluded that commercial lentil cultivars grown in Turkey come from a restricted gene pool.

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