RESEARCH

# Genetic characterization and cotyledon color in lentil

**Cahit Erdoğan**[1*]

Genetic characterization of lentil (*Lens culinaris* Medik.) cultivars is important for lentil breeding. Therefore, random amplified polymorphic DNA (RAPD) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis were carried out to evaluate genetic diversity in 13 Turkish lentil cultivars. A total of eight RAPD primers were used in this study; 61 bands were produced and 55 of them were polymorphic (89.78%). The RAPD primers OPA-10, OPB-11, and OPI-13 had the highest polymorphism ratio (100%). As a result of SDS-PAGE analysis, variations in the seed protein pattern were observed among the lentil cultivars being studied. The SDS-PAGE similarity matrices indicated higher genetic similarity estimates among the lentil cultivars than RAPD. In addition, principal components analysis (PCA) was performed for both SDS-PAGE and RAPD where the first three components accounted for 75.760% and 68.121% of the total variation for SDS-PAGE and RAPD analysis, respectively. It was noted that the lentil cultivars with factor loadings greater than 0.5 for each principal component (PC) were also grouped together in the SDS-PAGE and RAPD dendrogram. In addition to genetic diversity, cotyledon color (an important market criterion) values were measured for lightness (L*), redness (a*), and yellowness (b*). As for cotyledon color, values for brightness, redness, and yellowness varied significantly among lentil cultivars. Among the red lentil cultivars, 'Çağıl' and 'Yerli Kırmızı' had the highest cotyledon L* values of 70.83 and 70.74, respectively. The results of both genetic diversity analyses and cotyledon color of lentil cultivars would help in planning future breeding programs to improve high yielding marketable lentil cultivars.

**Key words:** Cotyledon color, genetic diversity, lentil, RAPD, SDS-page.

## INTRODUCTION

Lentil (*Lens culinaris* Medik.) is one of the oldest crops which originated in the Near East around the Fertile Crescent; it is presently an important grain legume in the agriculture of many countries worldwide. Its seed is rich in proteins, minerals, vitamins, dietary fiber, and high levels of natural antioxidants for human nutrition (Rehman and Shah, 2004; Amarowicz et al., 2009). It also plays an important role in animal nutrition and soil improvement. Lentil is now mainly grown in Asia, but it is produced in the Americas where Canada is the leader. Global production of lentil is approximately 4.9 million tons of which Canada produces 1.8 million tons (FAO, 2013). Turkey has been a conventional and considerable lentil producer for many years. Lentil production and yield in Turkey was 417 000 t and 1483 kg $ha^{-1}$, respectively (TUIK, 2014). Although lentil yield in Turkey is the highest in the world (1070 kg $ha^{-1}$), its yield potential is not completely revealed because of some biotic and abiotic stresses. Variations in environmental conditions stimulate plant breeders to develop new lentil cultivars that are more resistant and tolerant; there are therefore many registered lentil cultivars in Turkey.

Selection is the backbone of crop breeding (Murphy, 2007). There must therefore be a sufficient degree of genetic variation or genetic diversity in crops for selection. Genetic diversity can be mainly revealed by various markers. Of these, morphological and biological markers have been used for a long time (Ferguson and Robertson, 1999). Because these markers are affected by environmental conditions and have some limitations (Lee, 2006), biochemical or protein markers are widely used in plant breeding and identification (Abdel-Hady and El-Naggar, 2007; Ford et al., 2007; Sipahi et al., 2010). Seed storage protein markers, a type of biochemical marker, can be used for selection (Sönmezoğlu et al., 2010) and genetic diversity analysis (Javaid et al., 2004; Ali et al., 2007; Yüzbaşıoğlu et al., 2008; Arslan, 2012) of lentil and other crops. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which requires protein markers, is an easy, safe, and efficient method to discriminate lentil cultivars. On the other hand, DNA markers provide polymorphism information at the DNA level (Kumar, 1999). Random amplified polymorphic DNA (RAPD), based on polymerase chain reaction (PCR) and a DNA marker, is one of the ways to determine genetic diversity in pulses (Kumar et al., 2011). This molecular method has also been widely used in the construction of genetic maps, cultivar identification, and phylogenetic

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analysis since it require relatively small amounts of DNA, does not require sequence information, and is quick and simple (Xu, 2010). Therefore, it has occupied an important place in breeding of grain legumes (Ahmad, 1999).

The cotyledon or flower color is one of the most important market criteria in the red lentil market. Cultivars with deep bright red cotyledons are preferred over those that are dull pale red. Some wholesalers use red dye to paint cotyledons of de-hulled red lentils to increase their marketing value. Red dye, used to paint de-hulled cotyledons, may produce toxicity problems for lentil consumers. Cotyledon color of lentil cultivars could be improved by breeding cultivars with a deeper bright red cotyledon. Cotyledon color is currently among the important quality criteria in some breeding programs.

Information on genetic diversity and cotyledon color characteristics of lentil cultivars are essential for their use in breeding programs aimed at improving the market share of lentil cultivars (Anjam et al., 2005). The purposes of this study were to reveal genetic diversity by using RAPD and SDS-PAGE methods and determine the cotyledon color in 13 Turkish lentil cultivars.

## MATERIALS AND METHODS

Thirteen lentil cultivars (Sakar, Çağıl, Altıntoprak, Meyveci 2001, Özbek, Kafkas, Çiftçi, Seyran 96, Sultan 1, Yerli Kırmızı, Kayi 91, Firat 87, and Erzurum 89) were sown in 4 m rows with 30 cm row spacing and 5 cm intra-row spacing on 17 November 2010. Plants were harvested on 22 May 2011.

The lentil cultivars were used to perform RAPD analysis and determine seed storage protein patterns and cotyledon colors.

### DNA isolation and amplification of RAPDs

Genomic DNA was isolated from bulked fresh leaf samples from each lentil cultivar according to the method by Khan et al. (2004) with minor modifications. Samples of 5 to 7 leaves were ground with a mortar and pestle and liquid nitrogen. After transferring the powder to 1.5 mL microcentrifuge tubes, it was suspended in 600 $\mu$L of 2 × CTAB extraction buffer 1.4 M NaCl, 2% hexadecyltrimethylammonium bromide (Sigma, St. Louis, Missouri, USA), 1% β-mercaptoethanol, 100 mM Tris-HCl, pH 8, and incubated in a water bath at 65 °C for 60 to 80 min. An equal volume of chloroform/isoamyl alcohol (24:1) was added to the mixture, inverted a few times, and slowly shaken at room temperature for 30 min. The emulsion was centrifuged at 10 000 g for 10 min and the supernatant was transferred to a fresh tube before DNA was precipitated by adding a 0.6 volume of isopropyl alcohol and inverting it several times. The DNA pellet was precipitated with a brief spin and washed twice with 70% ethanol. The DNA pellet was air-dried and dissolved in an appropriate volume of sterile double-distilled ($ddH_2O$). Concentrations of DNA samples were quantified on nanodrop (ACTGene UVS-99, Piscataway, New Jersey, USA) at $A_{260/280}$ nm.

Eight 10-mer RAPD primers were used to amplify genomic DNA. The amplification reaction volume was 25 $\mu$L, which contained 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl^2$, 100 $\mu$M each of dATP, dCTP, dGTP, and dTTP, 2 $\mu$M primer, 0.8 units of Taq DNA polymerase, and 30 ng of genomic DNA. Polymerase chain reactions were performed in MultiGene Thermal Cycler (Labnet International, Edison, New Jersey, USA) programmed for an initial denaturation at 95 °C for 5 min, then 40 cycles of 1 min denaturation at 94 °C and 1 min annealing at 37 °C, 1.5 min extension at 72 °C, and the final extension step at 72 °C for 10 min. Polymerase chain reaction products were subjected to electrophoresis in 1.5% agarose gels and visualized by staining with ethidium bromide (0.4 $\mu$g $mL^{-1}$) and UV illumination. Molecular weight markers were used to estimate the amplification sizes of products and compare duplicate reactions.

### SDS-PAGE electrophoresis

Lentil cultivar seeds were ground into a fine powder. To perform SDS-PAGE analysis, 400 $\mu$L of protein extraction buffer (0.05 M Tris-HCI, pH 8, 0.02% SDS, 30.3% urea and 1% β-mercaptoethanol) was added to the Eppendorf tube after placing 10 mg flower seed sample. Tubes were mixed well by vortex and centrifuged at 13 000 rpm at room temperature for 10 min. The extracted crude proteins were then obtained as a clear supernatant and stored at -20 °C. Seed protein extracts were analyzed by a vertical slab gel in a discontinuous buffer system according to the method by Laemmli (1970), and to which 10 µL protein extract solution in 9.5% polyacrylamide gel was loaded. Proteins in the gels were stained with Coomassie brilliant blue according to Hames and Rickwood (1990). Molecular weights of the dissociated polypeptides were determined with molecular weight protein standards in the PageRuler Unstained Protein Ladder #SM0661 (Fermentas International, Burlington, Ontario, Canada).

### Cotyledon color

The color of lentil cotyledons was quantified 30 d after harvest with a chroma meter (CR-400, Minolta, Osaka, Japan) using ground seeds. Color measurements were performed in accordance with standards developed by the Commission Internationale de l'Éclairage (CIE, 1986). Brightness, redness, and yellowness values were measured to describe three-dimensional color space and interpreted as follows: L* is brightness ranging from no reflection for black (L* = 0) to perfect diffuse reflection for white (L* = 100); a* is redness ranging from negative values for green to positive values for red; and b* is yellowness ranging from negative values for blue and positive values for yellow.

**Data analysis**

After staining and destaining gels, protein bands with unequivocal views were scored 1 or 0 depending on their presence or absence in each lentil cultivar by SDS-PAGE analysis. Likewise, RAPD bands were also scored as binary data. The pair-wise similarity matrices were built with simple matching coefficients calculated according to the formula $SM_{ij} = (a + d)/(a + b + c + d)$ where $SM_{ij}$ is the similarity between two individuals i and j, $a$ is the number of bands present in both individuals, $b$ is the number of bands observed only in individual i, $c$ is the number of bands observed only in individual j, and $d$ is the number of bands absent in both individuals (Rohlf, 2000). A dendrogram was constructed by the unweighted pair group method with arithmetic averages (UPGMA) method (Sneath and Sokal, 1973) with NTSYSpc v2.1 software (Exeter Software, Setauket, New York, USA). Nei's gene diversity index of the primers (Nei, 1973) was calculated with the PowerMarker v3.25 program (Liu and Muse, 2005). The RAPD and SDS-PAGE data were subjected to principal component analysis (PCA). The principal axis method was used to extract the components followed by a varimax rotation. Only the components with eigenvalues > 1 were retained for rotation using STATISTICA v.10 software (StatSoft, Tulsa, Oklahoma, USA). On the basis of four replicates, the color parameters of the lentil cotyledons were analyzed with one-factor ANOVA, and the comparison of means was performed by least significance difference (LSD) at the 5% level of probability ($P < 0.05$) using SAS v.9.1 statistical software (SAS Institute, Cary, North Carolina, USA).

## RESULTS

**SDS-PAGE analysis**

The protein band patterns obtained from the 13 lentil cultivars are shown in Figure 1. Only clearly distinguishable protein bands were visually scored. A total of 22 polypeptide bands were detected and molecular weight ranged from 10 to 200 kDa.

Similarity matrices, estimated from simple matching coefficients, were used for building a dendrogram by the UPGMA method with 22 SDS-PAGE bands (Table 1). Genetic similarity coefficients varied among lentil cultivars (Table 1). The dendrogram (Figure 2) clearly revealed two separate main clusters at the 0.75 similarity coefficient level of. The first main and larger cluster was divided into two subclusters called PC1 and PC2. The clusters were named in accordance with the PCA result (Table 2). The highest similarity coefficient (0.96) was observed between 'Seyran 96' and 'Fırat 87', 'Seyran 96' and 'Kayı 91', and 'Seyran 96' and 'Çiftçi' in the PC1

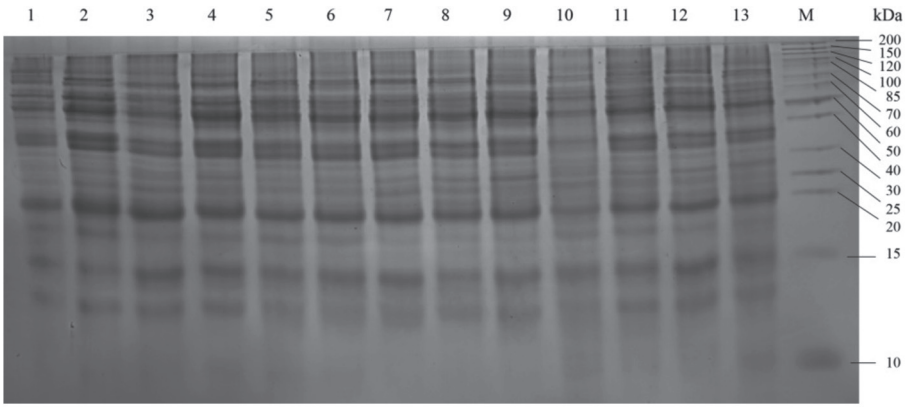


**Figure 1. Electropherograms showing seed storage protein banding patterns in 13 lentil cultivars. Lanes 1 to 13 refer to 'Şakar', 'Çağıl', 'Altıntoprak', 'Meyveci 2001', 'Özbek', 'Kafkas', 'Çiftçi', 'Seyran 96', 'Sultan 1', 'Yerli Kırmızı', 'Kayı 91', 'Fırat 87', 'Erzurum 89', respectively, and M-size marker.**

**Table 1. Similarity matrices in 13 lentil cultivars based on observed seed protein patterns produced by SDS-PAGE.**

| Cultivars | Erzurum 89 | Fırat 87 | Kayı 91 | Yerli Kırmızı | Sultan 1 | Seyran 96 | Çiftçi | Kafkas | Özbek | Meyveci 2001 | Altıntoprak | Çağıl | Şakar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Erzurum 89 | 1.00 | | | | | | | | | | | | |
| Fırat 87 | 0.82 | 1.00 | | | | | | | | | | | |
| Kayı 91 | 0.82 | 0.91 | 1.00 | | | | | | | | | | |
| Yerli Kırmızı | 0.82 | 0.82 | 0.82 | 1.00 | | | | | | | | | |
| Sultan 1 | 0.68 | 0.68 | 0.77 | 0.68 | 1.00 | | | | | | | | |
| Seyran 96 | 0.77 | 0.96 | 0.96 | 0.77 | 0.73 | 1.00 | | | | | | | |
| Çiftçi | 0.73 | 0.91 | 0.91 | 0.73 | 0.77 | 0.96 | 1.00 | | | | | | |
| Kafkas | 0.86 | 0.86 | 0.77 | 0.77 | 0.73 | 0.82 | 0.77 | 1.00 | | | | | |
| Özbek | 0.68 | 0.77 | 0.68 | 0.77 | 0.64 | 0.73 | 0.68 | 0.82 | 1.00 | | | | |
| Meyveci 2001 | 0.77 | 0.77 | 0.86 | 0.77 | 0.91 | 0.82 | 0.77 | 0.82 | 0.73 | 1.00 | | | |
| Altıntoprak | 0.64 | 0.73 | 0.82 | 0.64 | 0.77 | 0.77 | 0.73 | 0.68 | 0.68 | 0.86 | 1.00 | | |
| Çağıl | 0.77 | 0.77 | 0.86 | 0.77 | 0.82 | 0.82 | 0.77 | 0.82 | 0.73 | 0.91 | 0.86 | 1.00 | |
| Şakar | 0.86 | 0.77 | 0.77 | 0.77 | 0.73 | 0.73 | 0.68 | 0.82 | 0.82 | 0.82 | 0.68 | 0.82 | 1.00 |

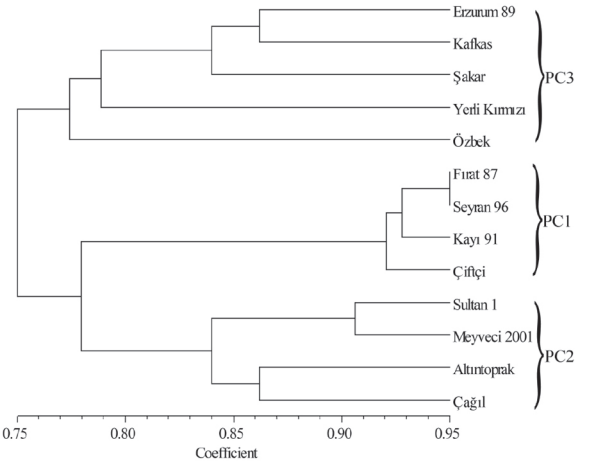


**Figure 2. UPGMA dendrogram of 13 lentil cultivars based on SDS-PAGE similarity matrix.**

**Table 2. Component loadings (Varimax) in 13 lentil cultivars on the first three principal components (PC) by SDS-PAGE and RAPD.**

| | PC1 | | PC2 | | PC3 | |
|---|---|---|---|---|---|---|
| Cultivars | SDS-PAGE | RAPD | SDS-PAGE | RAPD | SDS-PAGE | RAPD |
| Erzurum 89 | 0.119 | 0.299 | -0.067 | **0.585** | **0.859** | 0.179 |
| Fırat 87 | **0.850** | **0.798** | -0.004 | 0.320 | 0.385 | 0.178 |
| Kayı 91 | **0.793** | 0.454 | 0.329 | **0.567** | 0.159 | -0.076 |
| Yerli Kırmızı | 0.356 | -0.112 | 0.114 | **0.843** | **0.631** | 0.210 |
| Sultan 1 | 0.243 | 0.183 | **0.773** | 0.131 | 0.108 | **0.834** |
| Seyran 96 | **0.953** | **0.757** | 0.145 | 0.379 | 0.095 | 0.060 |
| Çiftçi | **0.903** | 0.483 | 0.130 | **0.665** | -0.040 | 0.074 |
| Kafkas | 0.459 | 0.080 | 0.311 | **0.761** | **0.688** | 0.184 |
| Özbek | 0.353 | 0.297 | 0.332 | **0.741** | **0.596** | 0.064 |
| Meyveci 2001 | 0.258 | 0.051 | **0.846** | 0.146 | 0.309 | **0.863** |
| Altıntoprak | 0.069 | **0.857** | **0.814** | -0.072 | -0.206 | 0.167 |
| Çağıl | 0.061 | **0.746** | **0.804** | 0.197 | 0.326 | 0.331 |
| Şakar | -0.033 | **0.800** | 0.282 | -0.028 | **0.827** | -0.176 |

Loadings in bold face are > 0.500.

cluster. The PC2 cluster had the second highest similarity coefficient (0.91). The second main cluster, PC3, consisted of 'Erzurum 89', 'Kafkas', 'Şakar', 'Yerli' 'Kırmızı', and 'Özbek'. In this cluster, 'Özbek' was positioned alone as a subcluster. Similarity values of the PC3 cluster ranged from 0.68 to 0.86.

## RAPD analysis

Eight RAPD primers were tested in 13 lentil cultivars, which generated clear amplifications; of the 61 amplified bands, 55 were polymorphic (89.78%). The number of bands per primer ranged from 5 (OPA-11) to 10 (OPA-20) with a mean of 7.63 bands per primer. The primers OPA-10, OPB-11, and OPI-13 had the highest polymorphism ratio at 100%. The polymorphic information content (PIC) ranged from 0.20 for primer OPA-11 to 0.36 for primer OPH-17 and a mean of 0.33. Both Shannon's information (SI) and Nei's gene diversity (H) indices were also calculated for each primer. The primers OPH-17 and OPA-10 had the highest gene diversity (H) and Shannon's information index (SI) values, which made them the most informative loci (Table 3).

Similarity matrices based on RAPD analysis of 13 lentil cultivars are given in Table 4, and the dendrogram constructed by the UPGMA method is shown in Figure 3. Clusters were named in accordance with the result of the PCA and SDS-PAGE (Table 2). The RAPD dendrogram illustrates that three main clusters (PC1, PC2, and PC3) are built. Based on simple matching coefficients, 'Seyran 96' was noted as being closely related to 'Fırat 87', 'Yerli Kırmızı' to 'Kafkas' and each had a value of 0.89 (Table 4). This relatedness is also clearly and visually shown in the dendrogram (Figure 3). On the other hand, the smallest genetic similarity (0.47) was observed between 'Altıntoprak' in cluster PC1 and 'Yerli Kırmızı' in cluster PC2.

## Cotyledon color

The L* (brightness), a* (redness), and b* (yellowness) values for lentil cotyledons are displayed in Table 5. There were significant differences ($p < 0.05$) in the color parameters of lentil cotyledons. Significant differences were found in L* values among red (orange) and yellow lentil cultivars. Cotyledons of 'Çağıl' and 'Yerli Kırmızı' had the highest L* values at 70.83 and 70.74, respectively, among the red lentil cultivars, whereas 'Erzurum 89' had the highest L* value for cotyledon at 72.12 among the yellow lentil cultivars. The a* value also changed significantly among red and yellow lentil cultivars. It ranged from 7.96 for 'Çağıl' to 11.75 for 'Özbek' among the red lentil cultivars. However, the a* value ranged from -0.90 for 'Erzurum 89' to -0.37 for 'Meyveci 2001' among the yellow lentil cultivars. Furthermore, 'Özbek' had the highest b* value of 18.20 among red lentil cultivars. In the case of yellow lentil cultivars, 'Kayı 91' had the highest b* value (19.44).

**Table 3. RAPD primers and their sequences, number of polymorphic loci, total number of loci, polymorphism ratio, H, PIC, and SI values in 13 lentil cultivars.**

| Primer | Sequence (5'-3') | Number of polymorphic loci | Total number of loci | Polymorphism ratio (%) | H | PIC | SI |
|---|---|---|---|---|---|---|---|
| OPA-07 | GAAACGGGTG | 7 | 8 | 87.5 | 0.37 | 0.29 | 0.48 |
| OPA-10 | GTGATCGCAG | 9 | 9 | 100.0 | 0.40 | 0.32 | 0.59 |
| OPA-08 | GTGACGTAGG | 6 | 8 | 75.0 | 0.25 | 0.21 | 0.41 |
| OPA-11 | CAATCGCCGT | 4 | 5 | 80.0 | 0.23 | 0.20 | 0.40 |
| OPA-20 | GTTGCGATCC | 9 | 10 | 90.0 | 0.31 | 0.25 | 0.48 |
| OPB-11 | GTAGACCCGT | 8 | 8 | 100.0 | 0.34 | 0.27 | 0.51 |
| OPH-17 | CACTCTCCTC | 6 | 7 | 85.7 | 0.47 | 0.36 | 0.66 |
| OPI-13 | CTGGGGCTGA | 6 | 6 | 100.0 | 0.28 | 0.23 | 0.44 |
| Total | | 55 | 61 | | | | |
| Mean | | 6.88 | 7.63 | 89.78 | 0.33 | 0.27 | 0.50 |

H: Nei's gene diversity, PIC: polymorphism information content, SI: Shannon's information index.

**Table 4. Similarity matrix based on RAPD patterns in 13 lentil cultivars.**

| Cultivars | Erzurum 89 | Fırat 87 | Kayı 91 | Yerli Kırmızı | Sultan 1 | Seyran 96 | Çiftçi | Kafkas | Özbek | Meyveci 2001 | Altıntoprak | Çağıl | Şakar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Erzurum 89 | 1.00 | | | | | | | | | | | | |
| Fırat 87 | 0.69 | 1.00 | | | | | | | | | | | |
| Kayı 91 | 0.76 | 0.75 | 1.00 | | | | | | | | | | |
| Yerli Kırmızı | 0.73 | 0.59 | 0.66 | 1.00 | | | | | | | | | |
| Sultan 1 | 0.77 | 0.71 | 0.63 | 0.61 | 1.00 | | | | | | | | |
| Seyran 96 | 0.67 | 0.89 | 0.77 | 0.61 | 0.64 | 1.00 | | | | | | | |
| Çiftçi | 0.69 | 0.81 | 0.75 | 0.69 | 0.64 | 0.83 | 1.00 | | | | | | |
| Kafkas | 0.73 | 0.67 | 0.64 | 0.89 | 0.63 | 0.69 | 0.73 | 1.00 | | | | | |
| Özbek | 0.73 | 0.72 | 0.75 | 0.75 | 0.61 | 0.70 | 0.88 | 0.70 | 1.00 | | | | |
| Meyveci 2001 | 0.67 | 0.56 | 0.53 | 0.66 | 0.80 | 0.55 | 0.59 | 0.61 | 0.63 | 1.00 | | | |
| Altıntoprak | 0.64 | 0.81 | 0.66 | 0.47 | 0.59 | 0.77 | 0.69 | 0.55 | 0.63 | 0.59 | 1.00 | | |
| Çağıl | 0.66 | 0.86 | 0.67 | 0.61 | 0.68 | 0.81 | 0.73 | 0.69 | 0.67 | 0.64 | 0.83 | 1.00 | |
| Şakar | 0.55 | 0.73 | 0.64 | 0.48 | 0.48 | 0.75 | 0.64 | 0.56 | 0.61 | 0.48 | 0.83 | 0.75 | 1.00 |

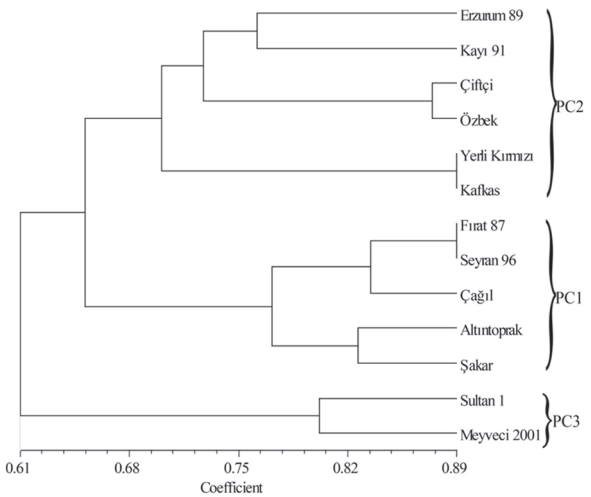


**Figure 3. UPGMA dendrogram of 13 lentil cultivars based on RAPD similarity matrix.**

**Table 5. Cotyledon color parameters in red and yellow lentil cultivars.**

| Cotyledon color | Cultivars | L* | a* | b* |
|---|---|---|---|---|
| Red lentil cultivar | Altıntoprak | 69.75b† | 9.09d | 15.71e |
| | Çiftçi | 67.74e | 11.02b | 17.44b |
| | Çağıl | 70.83a | 7.96f | 14.88f |
| | Fırat 87 | 69.32cd | 9.84c | 16.21d |
| | Kafkas | 69.59bc | 9.33d | 16.10d |
| | Özbek | 67.78e | 11.75a | 18.20a |
| | Şakar | 69.18d | 8.73e | 16.09d |
| | Seyran 96 | 67.82e | 11.01b | 16.89c |
| | Yerli Kırmızı | 70.74a | 8.53e | 14.20g |
| | LSD (0.05) | 0.33 | 0.29 | 0.21 |
| Yellow lentil cultivar | Erzurum 89 | 72.12a | -0.90b | 14.89d |
| | Kayı 91 | 70.91b | -0.81b | 19.44a |
| | Meyveci 2001 | 71.21b | -0.37a | 16.36c |
| | Sultan 1 | 70.96b | -0.84b | 16.73b |
| | LSD (0.05) | 0.50 | 0.17 | 0.36 |

Cultivar means followed by different letters in the same column are significantly different ($P < 0.05$).
$L^*$ is brightness ranging from no reflection for black ($L = 0$) to perfect diffuse reflection for white ($L = 100$).
$a^*$ is redness ranging from negative values for green to positive values for red.
$b^*$ is yellowness ranging from negative values for blue and positive values for yellow.

## DISCUSSION

Lentil is an important food legume and it has been cultivated for centuries in Turkey. Therefore, improving new lentil cultivars is essential for sustainable production.

**Table 6. Individual and cumulative eigenvalues and variance explained by the first three principal components (PC) by SDS-PAGE and RAPD in lentil cultivars.**

| | PC | Eigenvalue | Variance (%) | Cumulative eigenvalue | Cumulative variance (%) |
|---|---|---|---|---|---|
| SDS-PAGE | 1 | 5.92901 | 45.608 | 5.92901 | 45.608 |
| | 2 | 2.03737 | 15.672 | 7.96639 | 61.280 |
| | 3 | 1.88238 | 14.480 | 9.84877 | 75.760 |
| RAPD | 1 | 5.46150 | 42.012 | 5.46150 | 42.012 |
| | 2 | 2.00694 | 15.438 | 7.46844 | 57.450 |
| | 3 | 1.38730 | 10.672 | 8.85575 | 68.121 |

We need the revelation of genetic variation among lentil genotypes to breed a new variety with the desired agronomic and commercial characters. In the present study, the genetic diversity and cotyledon color of 13 Turkish lentil cultivars were investigated. This may be the first report about cotyledon color and one of a few studies on molecular assessment of genetic diversity in Turkish lentil cultivars. The RAPD markers, largely used in genetic studies because they are simple, fast, and inexpensive (Welsh and McClelland, 1990), were applied to identify the lentil cultivars as well as SDS-PAGE.

As a result of SDS-PAGE analysis, variations in seed protein patterns were observed among the lentil cultivars being studied. There was a high degree of homogeneity in the six major protein bands (Figure 1). Uniformity in the major bands among various cultivars indicates that these proteins are conserved by gene coding (Javaid et al., 2004). However, there were variations in the minor bands.

The similarity matrices of SDS-PAGE (Table 1) indicated higher genetic similarity estimates among lentil cultivars than by RAPD (Table 4) although the overall estimates of genetic similarities in both analyses were high. This may be because plant breeders narrow genetic diversity in their breeding populations by selecting the required trait combinations for outputs of improved cultivars (Redden et al., 2007) and suggest the similarity of genes responsible for seed storage proteins (Ali et al., 2007). This result was in accordance with findings by El-Nahas et al. (2011), who investigated molecular and biochemical markers in some lentil genotypes and concluded that SDS-PAGE produced low levels of genetic diversity. On the contrary, using inter simple sequence repeat (ISSR) and amplified fragment length

polymorphism (AFLP), Toklu et al. (2009) reported that Turkish lentil landraces have high genetic variability; their study included 38 lentil landraces collected from southeast Turkey as well as six cultivars.

The SDS-PAGE analysis revealed that all the cultivars with yellow cotyledons, namely 'Kayı 91', 'Sultan 1', and 'Meyveci 2001' except 'Erzurum 89' fell into the first subcluster, which was further divided into PC1 and PC2 (Figure 2). Yüzbaşıoğlu et al. (2008), who studied seed protein diversity in lentil by SDS-PAGE, also reported that these cultivars were grouped in the same cluster. On the other hand, RAPD analysis more clearly separated cultivars with yellow cotyledons (Figure 3). Both SDS-PAGE and RAPD analysis were effective in discriminating lentil cultivars originating from the same places. For example, 'Kafkas', 'Yerli Kırmızı', 'Özbek', and 'Erzurum 89' were grouped together in both dendrograms. With the exception of 'Erzurum 89', the remaining three lentil cultivars originated from the same province of Şanlıurfa, Turkey (Yüzbaşıoğlu et al., 2006). The two dendrograms were not fully overlapped because some lentil cultivars moved from one cluster to another and changed the dendrogram. Sonnante and Pignone (2001), who compared RAPD and ISSR markers in the clustering of lentil genotypes, also reported that the two markers produced dissimilar clusters.

The present study also indicated that RAPD markers were effectively used in determining polymorphism in lentil cultivars. The PCR amplification with eight RAPD primers resulted in reproducible bands of which 89.78% were polymorphic. This polymorphism ratio was high in comparison with RAPD studies by Yüzbaşıoğlu et al. (2006), Alabboud et al. (2009), Hoque and Hasan (2012), and Sonnante and Pignone (2001) with values of 54%, 62.7%, 60.37%, and 54%, respectively, in lentil. However, Sheikh et al. (2011) stated a polymorphism ratio of 90.6% in their study of five lentil accessions and one RAPD primer. The reason for this discrepancy in the studies might be due to the different RAPD primers that were used.

Principal component analysis was performed for both SDS-PAGE and RAPD to reduce the variables which accounted for most of the variance. The PCA indicated three principle components (PC) with a higher eigenvalue than the one extracted for both the SDS-PAGE and RAPD analysis. The first three components accounted for 75.760% and 68.121% of the total variation for SDS-PAGE and RAPD analysis, respectively (Table 6). Cultivars with component loadings greater than 0.5 for each PC (Table 2) were also grouped together in the SDS-PAGE and RAPD dendrogram (Figures 2 and 3).

Cotyledon color is important, and it is a commercial characteristic of the Turkish lentil market just as in some other countries (Ashraf, 2008). Lentil breeders take care of this attribute because of its market value (Sharma, 2009). The L*, a*, and b* values in the present study differed from those found in a study by Zhao et al. (2005), who reported an L* value of 85.47, a* value of -2.99, and b* value of 23.16 for yellow lentil 'Richlea'. Xu et al. (2007) reported higher L* (ranging between 79.2 and 83.5) and a* values (ranging between -3.0 and 5.6), and similar b* values (ranging between 15.4 and 18.8) compared with the present study.

Genetic similarity coefficients obtained by SDS-PAGE and RAPD marker analyses showed higher genetic similarity among lentil cultivars. The highest similarity coefficients were observed between 'Seyran 96' and 'Fırat 87'; 'Seyran 96' and 'Kayı 91'; and 'Seyran 96' and 'Çiftçi' in the PC1 cluster. The overall grouping pattern of clustering corresponds well with PCA and confirms patterns of genetic similarity observed among cultivars. The result provides valid guidelines for breeding high-yielding marketable cultivars.

## CONCLUSIONS

A number of lentil cultivars were registered in Turkey. However, both consumer and farmer preference encourage lentil breeders to breed more attractive lentil cultivars. It is therefore important to know about the genetic diversity and cotyledon color characteristics of current lentil cultivars to improve new lentil cultivars that have both high yield and quality. The results of this study would help in planning future lentil breeding programs to improve high-yielding marketable lentil cultivars.

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