

GENETIC DIVERSITY IN LENTIL LANDRACES REVEALED BY DIVERSITY ARRAY TECHNOLOGY (DArT)

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ABSTRACT

Lentil is annual, autogamous and diploid ($2n = 2x = 14$) food legume with ~4 Gbp genome in size. Turkey is well known for its species richness with “diversity hot spots” for different legumes including lentil. In previous studies, various DNA markers were utilized but genetic diversity of lentil landraces have not yet been clarified. For this reason, present study aimed to identify genetic diversity of 94 Turkish lentil landraces utilizing 16,383 SNPs based on DArT technology. Results from “fastSTRUCTURE” analysis indicated that the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram including a heat map and also principal component analysis (PCoA) showed that Turkish lentil landraces were classified into five main groups in current study, indicating the existence of a large genetic variation among landraces. Highest genetic variation was between geno34 and geno76 (0.9126) while the lowest genetic variation was between geno7 and geno1 (0.0104) and the average genetic variation among 94 lentil landraces was 0.63. The data obtained from current study can be utilized to increase genetic diversity of cultivated species and establish suitable conservation and breeding strategies of lentil.

Keywords: DArT, genetic diversity, Landrace, *Lens culinaris*, Lentil, SNP

INTRODUCTION

Lentil (*Lens culinaris* Medik.; Fabaceae) is annual, autogamous, diploid ($2n = 2x = 14$) and early domesticated food legume with ~4 Gbp genome size (Toklu et al., 2009; Ates et al., 2016). Its cultivation is largely done in Asia and Mediterranean region and it also has active diffusion in USA. Its annual production is ~5 million ton globally and the top three countries in the production of lentils are Canada, India and Turkey (Dikshit et al., 2015; Ates et al., 2018a). This crop is important for human and animal nourishment and also for soil improvement (Ahamed et al., 2014; Ates et al., 2016; Ates et al., 2018a). Grains of lentil are consumed as staple food and providing rich source of minerals, protein, carbohydrate and micronutrients (Toklu et al., 2009; Ates et al., 2016; Ates et al., 2018a). Therefore, enhancement of lentil production and consumption worldwide could decrease mineral malnutrition influencing majority of the world population (Idrissi et al., 2018). On the other hand, lentil cultivation provides carbon, nitrogen and organic matter to the soil by fixing atmospheric nitrogen (Ahamed et al., 2014).

The Mediterranean region including Turkey is well known for its species richness with “diversity hot spots” for different legumes including lentil (Maxted and Bennett, 2001; Idrissi et al., 2018). This region has a long history of lentil cultivation and domestication. Farmers in this region selected lentil landraces due to adaptation to

stress conditions (abiotic and biotic) over a long time period. Furthermore, owing to edaphic and climatic situations, a broad agro environment diversity occurs (Idrissi et al., 2018). Lentil landraces gathered from these various regions most likely have distinct responses to stress conditions and high genetic diversity (Idrissi et al., 2016; Idrissi et al., 2018). Having detailed information about molecular identification of the population structure and genetic diversity of landraces has been considered a key factor in improvement and breeding program (Ahamed et al., 2014). An influential breeding program of lentil requires usage of efficient and effective genetic resources of lentil in order to develop superior new lentil varieties (Idrissi et al., 2018).

Current agricultural industrialization is based upon high yielding varieties that also resistant to abiotic and biotic stress conditions (Tsanakas et al., 2018). Therefore, in course of time, these new varieties have taken local varieties termed as landraces’ place. Landraces were identified as dissimilar character, dynamic populations of cultivated plant which have their own specific historical data and lacks of crop improvements, have wide range of genetic diversity, and also capable of local adaptation and related with conventional farming systems (Tsanakas et al., 2018). Genetic diversity of lentil landrace provides rich source of beneficial alleles (Villa et al., 2005). When current varieties have taken landraces places, these alleles

of landraces were lost and this situation can create various obstacles during sustainability of agriculture. Therefore, recently, studies of the genetic diversity of lentil landraces utilizing different types of molecular markers has gained much attention (Tsanakas et al., 2018).

Until today, many studies have been conducted on genetic diversity of lentil varieties, species and landraces utilizing several approaches, including physiological and morphological markers (Erskine and Choudhary, 1986; Erskine et al., 1989), isozymes (Erskine and Muehlbauer, 1991), storage proteins of seed (Sultana et al., 2006) and DNA based molecular markers such as restriction fragment length polymorphism (RFLP) (Havey and Muehlbauer, 1989), random amplified polymorphic DNA (RAPD) (Abo-Elwafa et al., 1995; Ahmad et al., 1996; Ford et al., 1997; Ferguson et al., 1998; Sonnante and Pignone, 2001; Yuzbasioglu et al., 2006), amplified fragment length polymorphism (AFLP) (Sharma et al., 1996; Toklu et al., 2009; Alghamdi et al., 2014), inter-simple sequence repeat (ISSR) (Fikiru et al., 2007; Scippa et al., 2008; Toklu et al., 2009; El-Nahas et al., 2011; Seyedimoradi and Talebi, 2014), simple sequence repeat (SSR) (Jin et al., 2008; Babayeva et al., 2009; Kaur et al., 2011; Zaccardelli et al., 2012; Kushwaha et al., 2013; Dikshit et al., 2015; Idrissi et al., 2015; Idrissi et al., 2018; Tsanakas et al., 2018) and single nucleotide polymorphism (SNP) (Lombardi et al., 2014; Basheer-Salimia et al., 2015; Khazaei et al., 2016). On the other hand, Turkish lentil landraces which take a significant role in breeding offer rich genetic sources and farmers in Turkey still cultivated on a small scale landrace preferred for their ability of adaptation to regional environmental conditions (Toklu et al., 2009). However, no comprehensive data is existing on genetic diversity of Turkish lentil landrace, with the exclusion of the studies containing a few number [13 samples (Yuzbasioglu et al., 2006) and 44 samples (Toklu et al., 2009)] of landraces.

Genetic diversity among the Turkish lentil landraces requires further research utilizing latest molecular techniques, such as Diversity Array Technology (DArT) in order to brighten its great potential. This technology is a high-throughput, sequence-independent, DNA hybridization-based method that can develop thousands and thousands of markers in a single test across a whole plant genome (Huttner et al., 2005; Sansaloni et al., 2010). Up to date, a few number of SNPs have been developed in order to detect genetic diversity of lentil landraces (Lombardi et al., 2014; Basheer-Salimia et al., 2015; Khazaei et al., 2016). With this in mind, this study aimed to identify genetic diversity of Turkish lentil landraces based on DArT technologies.

MATERIALS AND METHODS

Plant material and DNA isolation

As a plant material, 94 Turkish lentil landraces, that collected from 39 provinces in Turkey were utilized

in current study (Table 1). The landraces were supplied from the Seed Bank of Aegean Agricultural Research Institute in Izmir, Turkey.

Seeds were sown in a pod (15cm diameter and 15cm high) containing clay+sand and manure (1:1:1 ratio) soil mixture. From four- to six-week-old seedling fresh leaves of each lentil landraces were collected for DNA isolation. All samples (each 100 mg) were labelled, quickly placed in liquid nitrogen and then they were kept in a deep freezer (at -86°C) until utilize for further analyses. Protocol of CTAB (cetyltrimethylammonium bromide) methods (Doyle, 1987) was applied with minor modifications. Qubit® 2.0 Fluorometer (Invitrogen Co., US) was used to detect DNA quantification and DNA purity was controlled by using 1% agarose gel. Finally, 50 ng/μL DNA concentration was used for DArT analysis.

DArT analysis

Procedure of DArT analysis was followed as defined by literature (Nemli et al., 2015). DArT results can be found at the website of <https://www.dropbox.com/h?preview=lentil+accessions+GBS+Results.xlsx>

Population structure, linkage disequilibrium and genetic diversity

Population structure and linkage disequilibrium (LD) analysis were performed as defined by Raj et al. (2014) and Nemli et al. (2015), respectively. Dendrograms were built according to Dice's genetic similarity coefficient (Nei and Li, 1979) by utilizing the unweighted pair-group method with arithmetic averages (UPGMA). Software package of The Splits Tree4 (Huson and Bryant, 2006) was utilized on the binary data in order to obtain Nei's distance coefficient (h) data and then Neighbor-Net tree (Nei, 1987) was built.

RESULTS AND DISCUSSION

Different DNA markers present various efficiencies in lentil genome for appreciating DNA polymorphism. DArT marker system is inexpensive and more flexible compared to other array platforms or marker systems and it has been widely used in genetic diversity studies of various plants since a plenty number of SNPs are existing for plant genomes (Ates et al., 2018b; Ozkuru et al., 2018; Ozkuru et al., 2019). In current study, initially 44,628 SNPs were developed by Diversity Arrays Technology Pty. Ltd. (DArT P/L, Canberra, Australia). After filtering biallelic and missing data rate lower than 80%, remaining 16,383 SNPs were used in order to detect genetic diversity among 94 Turkish lentil landraces (Table 1). Compare to previous studies [1,536 SNPs (Sharpe et al., 2013); 384 SNPs (Lombardi et al., 2014); 5,389 SNPs (Wong et al., 2015) and 1,194 SNPs (Khazaei et al., 2016)], higher number of SNPs were developed in order to detect genetic diversity of lentil in our studies.

Table 1. List of Turkish lentil landraces collected from 39 provinces in Turkey.

| No | Accession no | Taxon | Province/Turkey | No | Accession no | Taxon | Province/Turkey |
|---------|--------------|-----------------------|-----------------|---------|--------------|-----------------------|-----------------|
| Geno 1 | TR 31672 | <i>Lens culinaris</i> | Mardin | Geno 48 | TR 69971 | <i>Lens culinaris</i> | Mardin |
| Geno 2 | TR 31727 | <i>Lens culinaris</i> | Sanliurfa | Geno 49 | TR 69974 | <i>Lens culinaris</i> | Mardin |
| Geno 3 | TR 26217 | <i>Lens culinaris</i> | Icel | Geno 50 | TR 69981 | <i>Lens culinaris</i> | Kirsehir |
| Geno 4 | TR 26287 | <i>Lens culinaris</i> | Gaziantep | Geno 51 | TR 69986 | <i>Lens culinaris</i> | Kirsehir |
| Geno 5 | TR 26488 | <i>Lens culinaris</i> | Manisa | Geno 52 | TR 69990 | <i>Lens culinaris</i> | Kirsehir |
| Geno 6 | TR 26520 | <i>Lens culinaris</i> | Balikesir | Geno 53 | TR 69991 | <i>Lens culinaris</i> | Kirsehir |
| Geno 7 | TR 28024 | <i>Lens culinaris</i> | Konya | Geno 54 | TR 69993 | <i>Lens culinaris</i> | Kilis |
| Geno 8 | TR 39574 | <i>Lens culinaris</i> | Sivas | Geno 55 | TR 69997 | <i>Lens culinaris</i> | Zonguldak |
| Geno 9 | TR 26749 | <i>Lens culinaris</i> | Bilecik | Geno 56 | TR 69999 | <i>Lens culinaris</i> | Kayseri |
| Geno 10 | TR 40230 | <i>Lens culinaris</i> | Diyarbakir | Geno 57 | TR 70006 | <i>Lens culinaris</i> | Kayseri |
| Geno 11 | TR 31770 | <i>Lens culinaris</i> | Gaziantep | Geno 58 | TR 70008 | <i>Lens culinaris</i> | Kirsehir |
| Geno 12 | TR 42162 | <i>Lens culinaris</i> | Hatay | Geno 59 | TR 70009 | <i>Lens culinaris</i> | Kirklareli |
| Geno 13 | TR 42234 | <i>Lens culinaris</i> | Sanliurfa | Geno 60 | TR 70017 | <i>Lens culinaris</i> | Corum |
| Geno 14 | TR 42236 | <i>Lens culinaris</i> | Sanliurfa | Geno 61 | TR 70018 | <i>Lens culinaris</i> | Adiyaman |
| Geno 15 | TR 42240 | <i>Lens culinaris</i> | Mardin | Geno 62 | TR 70030 | <i>Lens culinaris</i> | Adiyaman |
| Geno 16 | TR 42301 | <i>Lens culinaris</i> | Nigde | Geno 63 | TR 70039 | <i>Lens culinaris</i> | Kastamonu |
| Geno 17 | TR 42309 | <i>Lens culinaris</i> | Konya | Geno 64 | TR 70058 | <i>Lens culinaris</i> | Elazig |
| Geno 18 | TR 42347 | <i>Lens culinaris</i> | Afyon | Geno 65 | TR 70080 | <i>Lens culinaris</i> | Elazig |
| Geno 19 | TR 80028 | <i>Lens culinaris</i> | Usak | Geno 66 | TR 70081 | <i>Lens culinaris</i> | Bilecik |
| Geno 20 | TR 44539 | <i>Lens culinaris</i> | Corum | Geno 67 | TR 70083 | <i>Lens culinaris</i> | Denizli |
| Geno 21 | TR 47404 | <i>Lens culinaris</i> | Gaziantep | Geno 68 | TR 70098 | <i>Lens culinaris</i> | Nigde |
| Geno 22 | TR 47414 | <i>Lens culinaris</i> | Sanliurfa | Geno 69 | TR 70099 | <i>Lens culinaris</i> | Kayseri |
| Geno 23 | TR 47434 | <i>Lens culinaris</i> | Sanliurfa | Geno 70 | TR 70102 | <i>Lens culinaris</i> | Tokat |
| Geno 24 | TR 47439 | <i>Lens culinaris</i> | Sanliurfa | Geno 71 | TR 70109 | <i>Lens culinaris</i> | Tokat |
| Geno 25 | TR 47445 | <i>Lens culinaris</i> | Sanliurfa | Geno 72 | TR 70110 | <i>Lens culinaris</i> | Tokat |
| Geno 26 | TR 47455 | <i>Lens culinaris</i> | Sanliurfa | Geno 73 | TR 70136 | <i>Lens culinaris</i> | Gaziantep |
| Geno 27 | TR 47458 | <i>Lens culinaris</i> | Adiyaman | Geno 74 | TR 70137 | <i>Lens culinaris</i> | Gaziantep |
| Geno 28 | TR 47586 | <i>Lens culinaris</i> | Ankara | Geno 75 | TR 70147 | <i>Lens culinaris</i> | Gaziantep |
| Geno 29 | TR 51375 | <i>Lens culinaris</i> | Kastamonu | Geno 76 | TR 70156 | <i>Lens culinaris</i> | Yozgat |
| Geno 30 | TR 51401 | <i>Lens culinaris</i> | Tokat | Geno 77 | TR 70161 | <i>Lens culinaris</i> | Konya |
| Geno 31 | TR 49399 | <i>Lens culinaris</i> | Hatay | Geno 78 | TR 70167 | <i>Lens culinaris</i> | Konya |
| Geno 32 | TR 61268 | <i>Lens culinaris</i> | Tekirdag | Geno 79 | TR 70174 | <i>Lens culinaris</i> | Konya |
| Geno 33 | TR 61271 | <i>Lens culinaris</i> | Tekirdag | Geno 80 | TR 70467 | <i>Lens culinaris</i> | Eskisehir |
| Geno 34 | TR 61447 | <i>Lens culinaris</i> | Bursa | Geno 81 | TR 70477 | <i>Lens culinaris</i> | Erzurum |
| Geno 35 | TR 65991 | <i>Lens culinaris</i> | Afyon | Geno 82 | TR 70487 | <i>Lens culinaris</i> | Ankara |
| Geno 36 | TR 67080 | <i>Lens culinaris</i> | Afyon | Geno 83 | TR 70489 | <i>Lens culinaris</i> | Sanliurfa |
| Geno 37 | TR 61440 | <i>Lens culinaris</i> | Bursa | Geno 84 | TR 70499 | <i>Lens culinaris</i> | Sanliurfa |
| Geno 38 | TR 48824 | <i>Lens culinaris</i> | Adiyaman | Geno 85 | TR 70511 | <i>Lens culinaris</i> | Sanliurfa |
| Geno 39 | TR 68691 | <i>Lens culinaris</i> | Eskisehir | Geno 86 | TR 70545 | <i>Lens culinaris</i> | Sivas |
| Geno 40 | TR 68970 | <i>Lens culinaris</i> | Eskisehir | Geno 87 | TR 70546 | <i>Lens culinaris</i> | Sivas |
| Geno 41 | TR 68975 | <i>Lens culinaris</i> | Eskisehir | Geno 88 | TR 70562 | <i>Lens culinaris</i> | Nevsehir |
| Geno 42 | TR 69021 | <i>Lens culinaris</i> | Eskisehir | Geno 89 | TR 70563 | <i>Lens culinaris</i> | Nevsehir |
| Geno 43 | TR 69041 | <i>Lens culinaris</i> | Kutahya | Geno 90 | TR 70569 | <i>Lens culinaris</i> | Siirt |
| Geno 44 | TR 69058 | <i>Lens culinaris</i> | Eskisehir | Geno 91 | TR 70597 | <i>Lens culinaris</i> | Mugla |
| Geno 45 | TR 69948 | <i>Lens culinaris</i> | Hatay | Geno 92 | TR 70617 | <i>Lens culinaris</i> | Konya |
| Geno 46 | TR 69952 | <i>Lens culinaris</i> | Kahramanmaras | Geno 93 | TR 70621 | <i>Lens culinaris</i> | Isparta |
| Geno 47 | TR 69961 | <i>Lens culinaris</i> | Aksaray | Geno 94 | TR 70627 | <i>Lens culinaris</i> | Isparta |

The main parameter in the LD decay is recombination (Tommasini et al., 2007) and comprehending LD level facilitates the selection of suitable methods (Varshney et al. 2005). A means of low level of LD decay is a greater resolution, while a means of higher level of LD decay is a lower resolution. Many factors such as rates of recombination, mutation, inbreeding amount, population admixtures, subdivision and size of population can affect a level of LD decay (Tommasini et al., 2007). In our study, individuals showed low level of LD decay (Figure 1).

Similar to our results Sharpe et al. (2013) reported low level of LD decay in their lentil study. Wild or natural populations often display low level of LD decay because these populations have gone through little artificial selection pressure. These populations also tend to have more diverse alleles per locus because they have not encounter to the genetic bottlenecks which are occurred during the processes of selection and/or domestication (Sharpe et al., 2013).

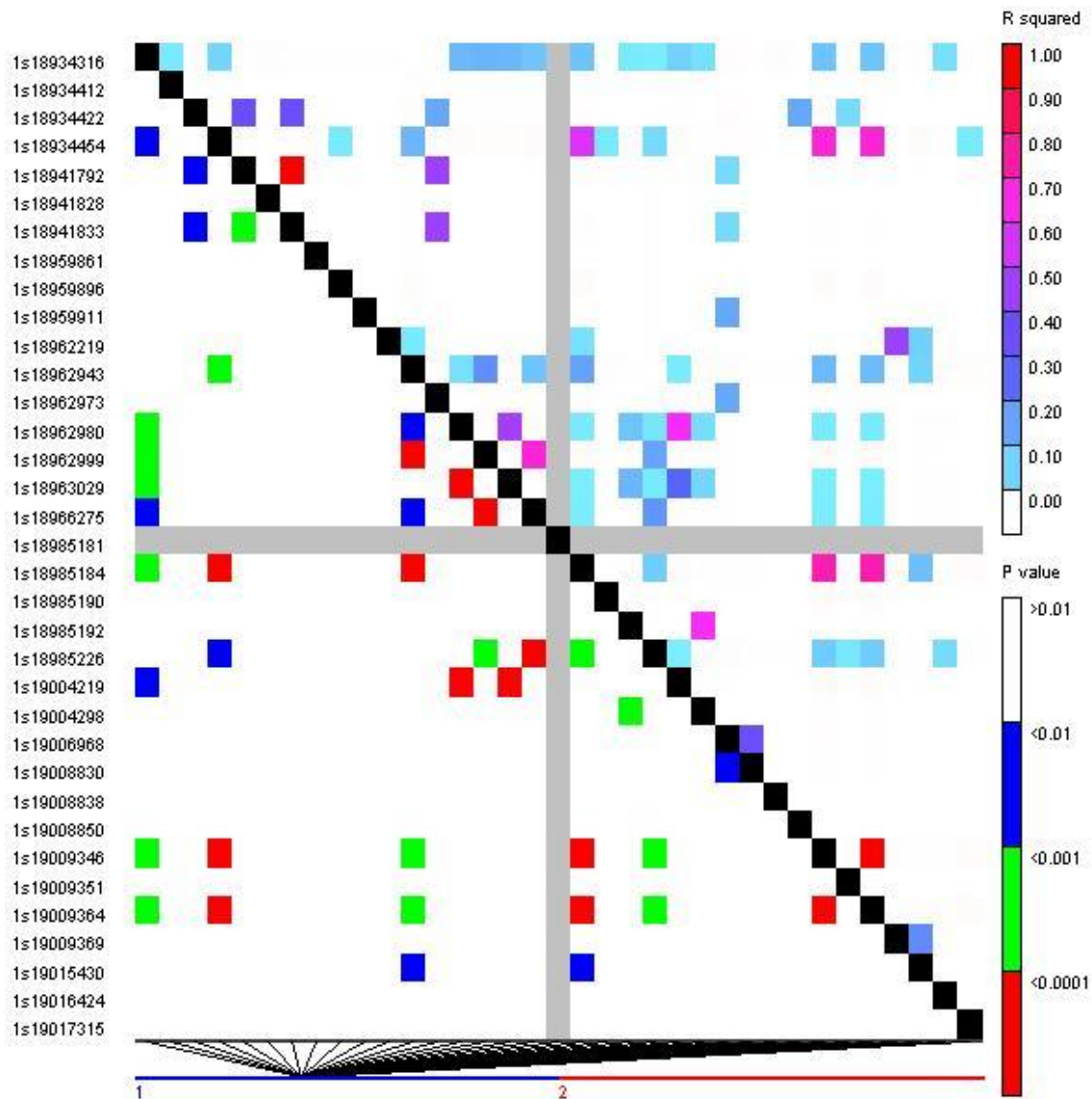


Figure 1. Linkage disequilibrium (LD) decay analysis in 94 Turkish lentil landraces.

The population structure of 94 Turkish lentil landraces was determined in fastSTRUCTURE software (Raj et al., 2014). Figure 2 displays the results of K from 1 to 10 in order to choose the true population number (K) as defined by Raj et al. (2014). K value with the lowest CV error was selected (K=5). This means that the 94 Turkish lentil landraces used in this study were divided into five main clusters, indicating the presence of a large genetic variation in population structures (Figure 3). Based on 16,383 SNPs, the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram including a heat map results also showed that 94 Turkish lentil landraces were classified into five main groups (Figures 4 and 5) in this study. Eight, 25, 23, 14 and 24 lentil landraces took part in first, second, third, fourth and fifth clusters, respectively (Figures 4 and 5). These results showed that there was a clear distinction between Turkish lentil landraces. Also, data from principal component analysis (PCoA) pointed out that five diverse clusters in the spatial representation of the relative genetic distances among 94 Turkish lentil landraces (Figure 6), confirming the data

shown in the structure analysis and UPGMA dendrogram (Figures 3 and 4). On the other hand, classification of the land races was not closely related to the geographic origin. For example, Geno 46, Geno49 and Geno91 collected from Kahramanmaras, Mardin and Mugla, respectively, placed in the same cluster (first cluster, Figures 4 and 5). Meantime, values of Nei's genetic distance indicated sharp genetic variation over close geographic distances, showing that geographically distant lentil landraces were presented genetic similarity, whereas, geographically close lentil landraces were shown genetic dissimilarity. Similar to our results, Lombardi et al. (2014) reported that lentil landraces analysis did not display powerful correlation between genetic diversity and geographical origin and this situation accepted that lentil landraces consist of very diverse mixtures of various genotypes. On the other hand, Toklu et al. (2009) reported, based on the AFLP, ISSR and combined AFLP and ISSR data, that 38 Turkish lentil landraces collected from southeast Turkey were divided into two main clusters and they also noticed that these 38 lentil landraces were not classified into

sampling geographic origin. These genetic classification results suggest that (I) farmers selected lentil landraces due to their ability of specific adaptation to regional environment factors or that (II) farmers moved lentil landraces from one region to another (Toklu et al., 2009).

Migration of lentil landraces into new sites was noticed by Sonnante and Pignone (2007), Sultana et al. (2006) and Toklu et al. (2009), who determined genetic variation of Italian, Pakistani and Turkish lentil landraces, respectively.

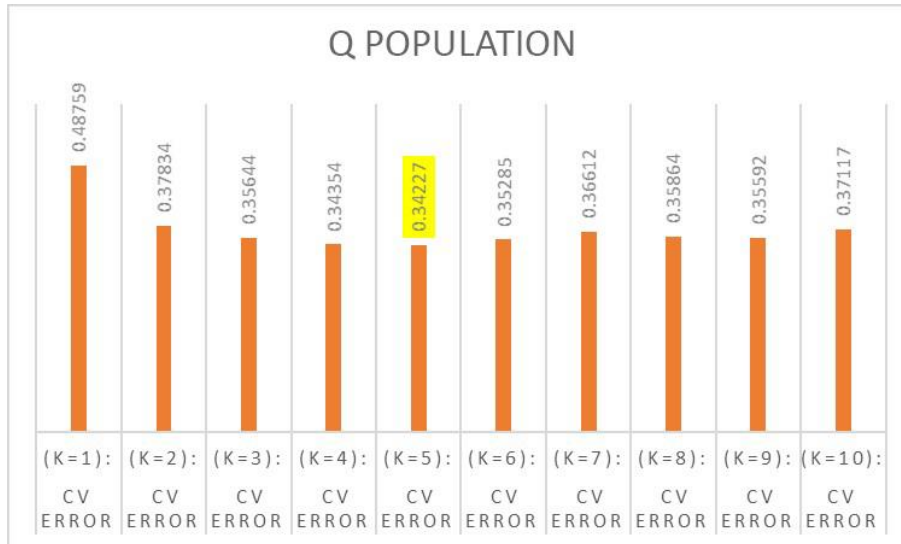


Figure 2. Results of K and CV errors calculation from 1 to 10. The lowest CV error is marked in yellow.

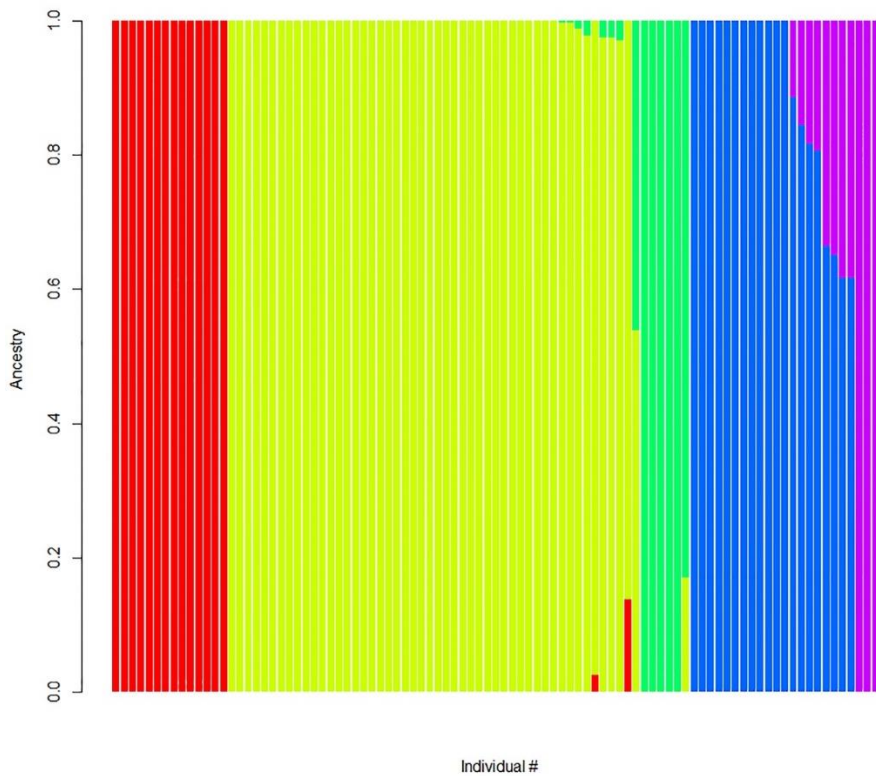


Figure 3. Population structure of 94 Turkish lentil landraces based on SNP data (K=5). Red, yellow, green, blue and purple indicates cluster one, two, three, four and five, respectively.

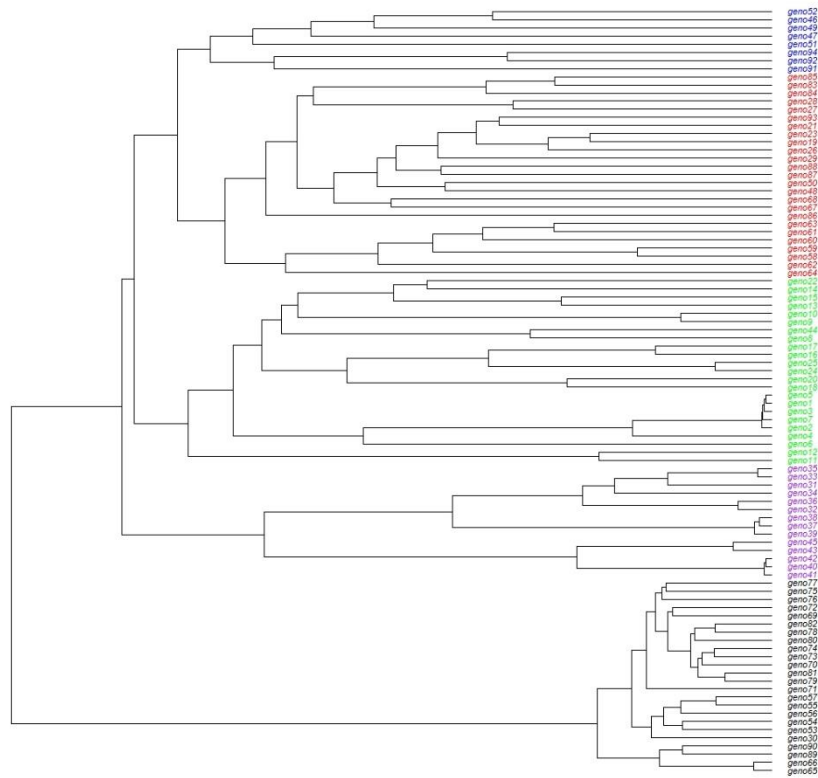


Figure 4. UPGMA dendrogram of 94 Turkish lentil landraces based on SNPs.

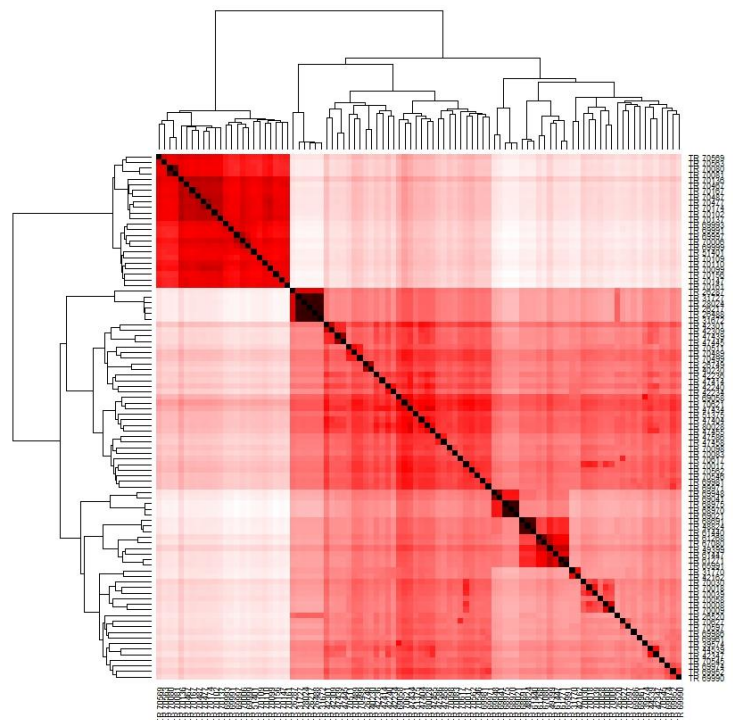


Figure 5. The heat map of 94 Turkish lentil landraces based on SNPs.

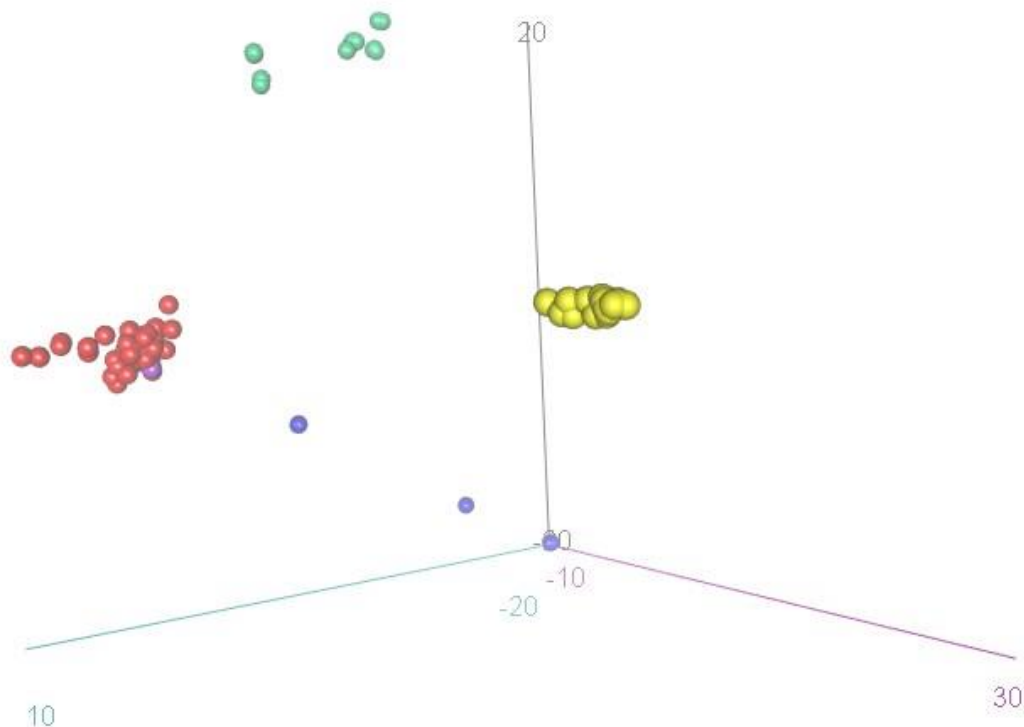


Figure 6. The relationships between the 94 Turkish lentil landraces visualized by PCoA utilizing the SNPs.

A number of previous studies pointed out that lentil landraces from the Mediterranean regions was defined by higher genetic variation than landraces from USA and south Asia (Erskine et al., 1989; Echeverrigaray et al., 1998; Ferguson et al., 1998; Piergiovanni and Taranto et al., 2003; Toklu et al., 2009; Lombardi et al., 2014) but, to date, only the landraces from few countries has been studied in detail (Erskine and Muehlbauer, 1991; de la Rosa and Jouve, 1992; Bejiga et al., 1996; Lazaro et al., 2001; Toklu et al., 2009). In our study, the mean genetic variation among 94 lentil landraces was 0.63 and highest genetic variation was between geno34 and geno76 (0.9126) while the lowest genetic variation was between geno7 and geno1 (0.0104). One of the most important key parameters when preparing new plant breeding strategies is information on genetic relationships and variation between genotypes for choosing of efficient parental genotypes in order to develop new gene combinations. Greater the distance between the two parents, greater the chance to see genetic variation among the genotypes in the F₂ generation (Ates et al., 2018b). Therefore, geno34 and geno76 (Figure 4), widely vary from each other, can be utilized as a parent in further lentil breeding researches.

CONCLUSION

Knowledge of genetic diversity among lentil landraces in germplasm is significant for effective usage of germplasm resources. In the current study, genetic diversity of 94 Turkish lentil landraces was detected based on DArT technologies and results indicating the presence of a large genetic variation in population structures. Comprehension of genetic variation between lentil

landraces is a main parameter for effective characterization and preservation of germplasm. Considering the importance of selecting genetically diverse genotypes as parents in the lentil breeding programs, this phenomenon could help breeders to select desirable genotype from segregating populations.

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