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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 culunaris* 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	*Lens culinaris*	Mardin	Geno 48	TR 69971	*Lens culinaris*	Mardin
Geno 2	TR 31727	*Lens culinaris*	Sanliurfa	Geno 49	TR 69974	*Lens culinaris*	Mardin
Geno 3	TR 26217	*Lens culinaris*	Icel	Geno 50	TR 69981	*Lens culinaris*	Kirsehir
Geno 4	TR 26287	*Lens culinaris*	Gaziantep	Geno 51	TR 69986	*Lens culinaris*	Kirsehir
Geno 5	TR 26488	*Lens culinaris*	Manisa	Geno 52	TR 69990	*Lens culinaris*	Kirsehir
Geno 6	TR 26520	*Lens culinaris*	Balikesir	Geno 53	TR 69991	*Lens culinaris*	Kirsehir
Geno 7	TR 28024	*Lens culinaris*	Konya	Geno 54	TR 69993	*Lens culinaris*	Kilis
Geno 8	TR 39574	*Lens culinaris*	Sivas	Geno 55	TR 69997	*Lens culinaris*	Zonguldak
Geno 9	TR 26749	*Lens culinaris*	Bilecik	Geno 56	TR 69999	*Lens culinaris*	Kayseri
Geno 10	TR 40230	*Lens culinaris*	Diyarbakir	Geno 57	TR 70006	*Lens culinaris*	Kayseri
Geno 11	TR 31770	*Lens culinaris*	Gaziantep	Geno 58	TR 70008	*Lens culinaris*	Kirsehir
Geno 12	TR 42162	*Lens culinaris*	Hatay	Geno 59	TR 70009	*Lens culinaris*	Kirklareli
Geno 13	TR 42234	*Lens culinaris*	Sanliurfa	Geno 60	TR 70017	*Lens culinaris*	Corum
Geno 14	TR 42236	*Lens culinaris*	Sanliurfa	Geno 61	TR 70018	*Lens culinaris*	Adiyaman
Geno 15	TR 42240	*Lens culinaris*	Mardin	Geno 62	TR 70030	*Lens culinaris*	Adiyaman
Geno 16	TR 42301	*Lens culinaris*	Nigde	Geno 63	TR 70039	*Lens culinaris*	Kastamonu
Geno 17	TR 42309	*Lens culinaris*	Konya	Geno 64	TR 70058	*Lens culinaris*	Elazig
Geno 18	TR 42347	*Lens culinaris*	Afyon	Geno 65	TR 70080	*Lens culinaris*	Elazig
Geno 19	TR 80028	*Lens culinaris*	Usak	Geno 66	TR 70081	*Lens culinaris*	Bilecik
Geno 20	TR 44539	*Lens culinaris*	Corum	Geno 67	TR 70083	*Lens culinaris*	Denizli
Geno 21	TR 47404	*Lens culinaris*	Gaziantep	Geno 68	TR 70098	*Lens culinaris*	Nigde
Geno 22	TR 47414	*Lens culinaris*	Sanliurfa	Geno 69	TR 70099	*Lens culinaris*	Kayseri
Geno 23	TR 47434	*Lens culinaris*	Sanliurfa	Geno 70	TR 70102	*Lens culinaris*	Tokat
Geno 24	TR 47439	*Lens culinaris*	Sanliurfa	Geno 71	TR 70109	*Lens culinaris*	Tokat
Geno 25	TR 47445	*Lens culinaris*	Sanliurfa	Geno 72	TR 70110	*Lens culinaris*	Tokat
Geno 26	TR 47455	*Lens culinaris*	Sanliurfa	Geno 73	TR 70136	*Lens culinaris*	Gaziantep
Geno 27	TR 47458	*Lens culinaris*	Adiyaman	Geno 74	TR 70137	*Lens culinaris*	Gaziantep
Geno 28	TR 47586	*Lens culinaris*	Ankara	Geno 75	TR 70147	*Lens culinaris*	Gaziantep
Geno 29	TR 51375	*Lens culinaris*	Kastamonu	Geno 76	TR 70156	*Lens culinaris*	Yozgat
Geno 30	TR 51401	*Lens culinaris*	Tokat	Geno 77	TR 70161	*Lens culinaris*	Konya
Geno 31	TR 49399	*Lens culinaris*	Hatay	Geno 78	TR 70167	*Lens culinaris*	Konya
Geno 32	TR 61268	*Lens culinaris*	Tekirdag	Geno 79	TR 70174	*Lens culinaris*	Konya
Geno 33	TR 61271	*Lens culinaris*	Tekirdag	Geno 80	TR 70467	*Lens culinaris*	Eskisehir
Geno 34	TR 61447	*Lens culinaris*	Bursa	Geno 81	TR 70477	*Lens culinaris*	Erzurum
Geno 35	TR 65991	*Lens culinaris*	Afyon	Geno 82	TR 70487	*Lens culinaris*	Ankara
Geno 36	TR 67080	*Lens culinaris*	Afyon	Geno 83	TR 70489	*Lens culinaris*	Sanliurfa
Geno 37	TR 61440	*Lens culinaris*	Bursa	Geno 84	TR 70499	*Lens culinaris*	Sanliurfa
Geno 38	TR 48824	*Lens culinaris*	Adiyaman	Geno 85	TR 70511	*Lens culinaris*	Sanliurfa
Geno 39	TR 68691	*Lens culinaris*	Eskisehir	Geno 86	TR 70545	*Lens culinaris*	Sivas
Geno 40	TR 68970	*Lens culinaris*	Eskisehir	Geno 87	TR 70546	*Lens culinaris*	Sivas
Geno 41	TR 68975	*Lens culinaris*	Eskisehir	Geno 88	TR 70562	*Lens culinaris*	Nevsehir
Geno 42	TR 69021	*Lens culinaris*	Eskisehir	Geno 89	TR 70563	*Lens culinaris*	Nevsehir
Geno 43	TR 69041	*Lens culinaris*	Kutahya	Geno 90	TR 70569	*Lens culinaris*	Siirt
Geno 44	TR 69058	*Lens culinaris*	Eskisehir	Geno 91	TR 70597	*Lens culinaris*	Mugla
Geno 45	TR 69948	*Lens culinaris*	Hatay	Geno 92	TR 70617	*Lens culinaris*	Konya
Geno 46	TR 69952	*Lens culinaris*	Kahramanmaras	Geno 93	TR 70621	*Lens culinaris*	Isparta
Geno 47	TR 69961	*Lens culinaris*	Aksaray	Geno 94	TR 70627	*Lens culinaris*	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).

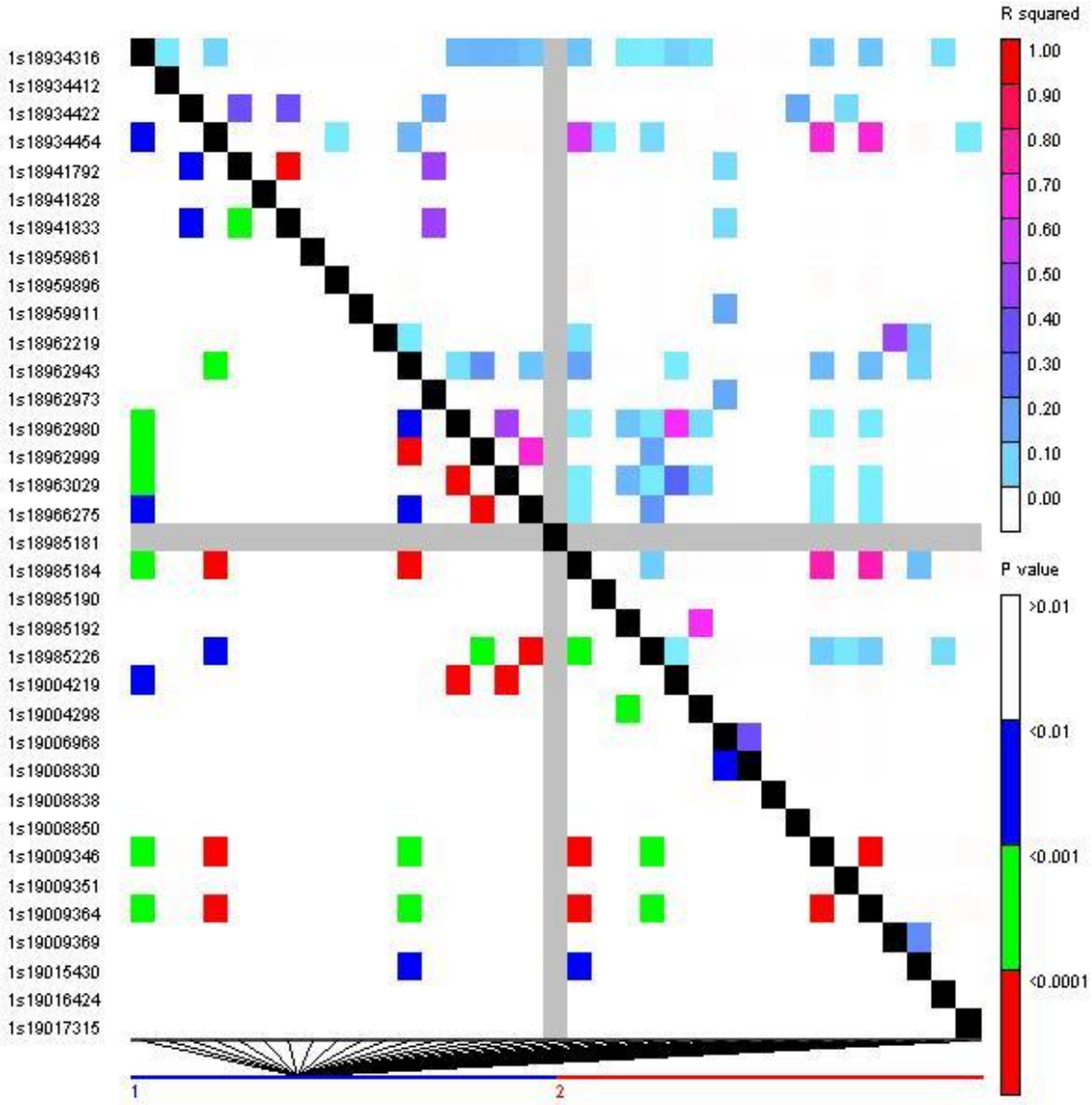

Figure 1. Linkage disequilibrium (LD) decay analysis in 94 Turkish lentil landraces.

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.

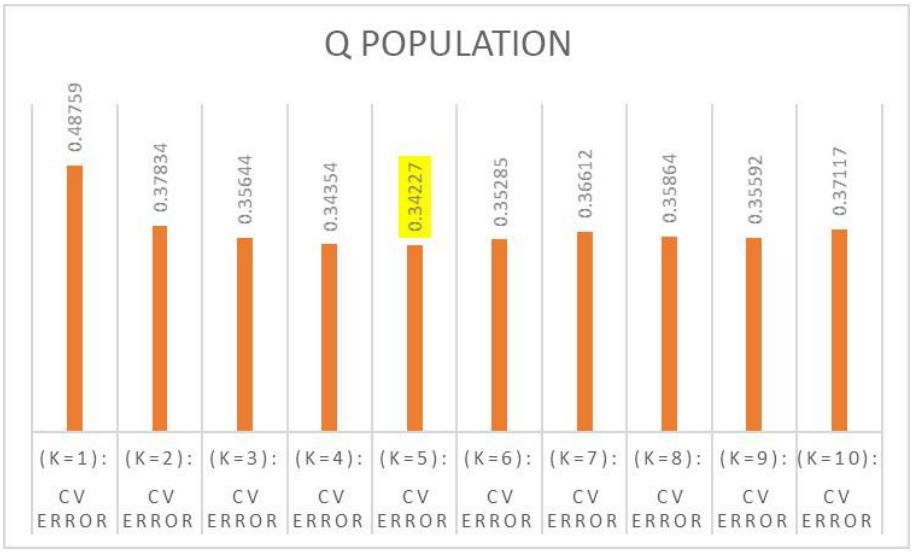

Figure 2. Results of K and CV errors calculation from 1 to 10. The lowest CV error is marked in yellow.

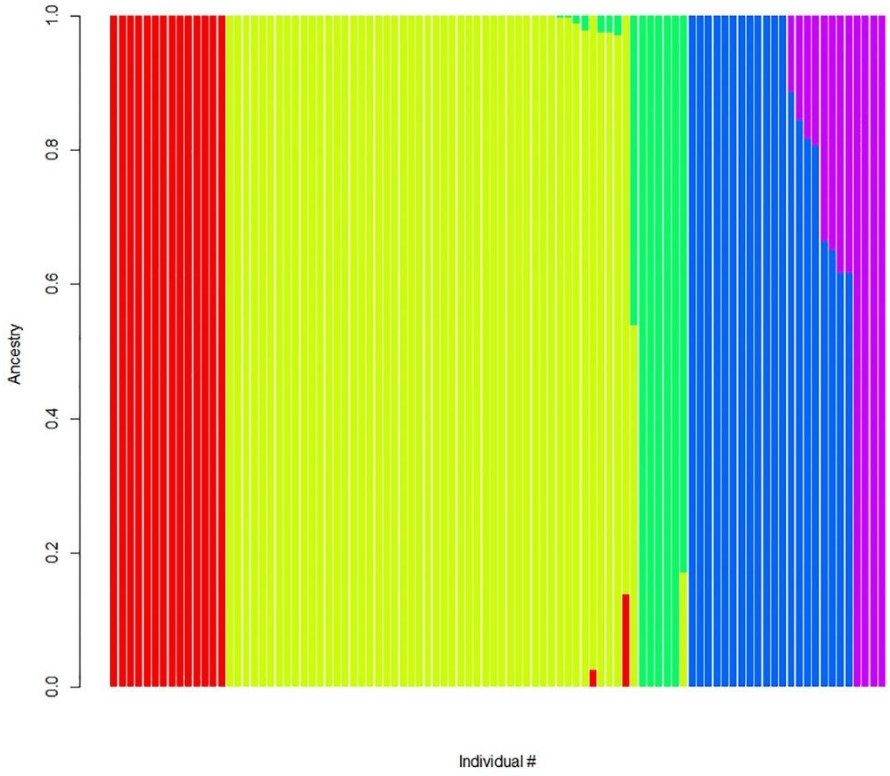

Figure 3. Population structure of 94 Turkish lentil landraces based on SNP data (K=5). Red, yellow, green, blue and purple indicates cluster one, two, three, four and five, respectively.

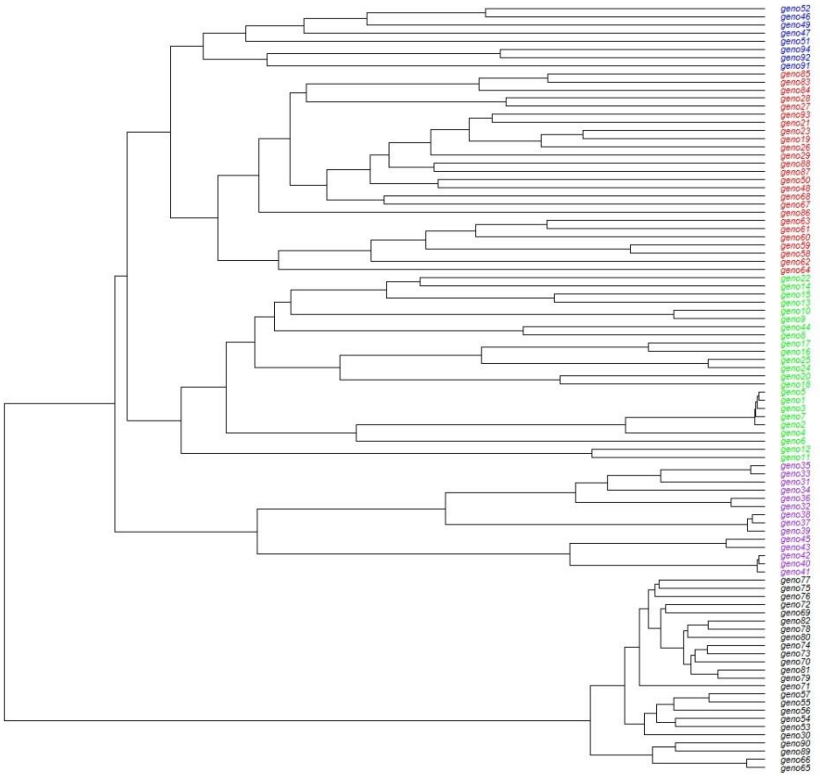

Figure 4. UPGMA dendrogram of 94 Turkish lentil landraces based on SNPs.

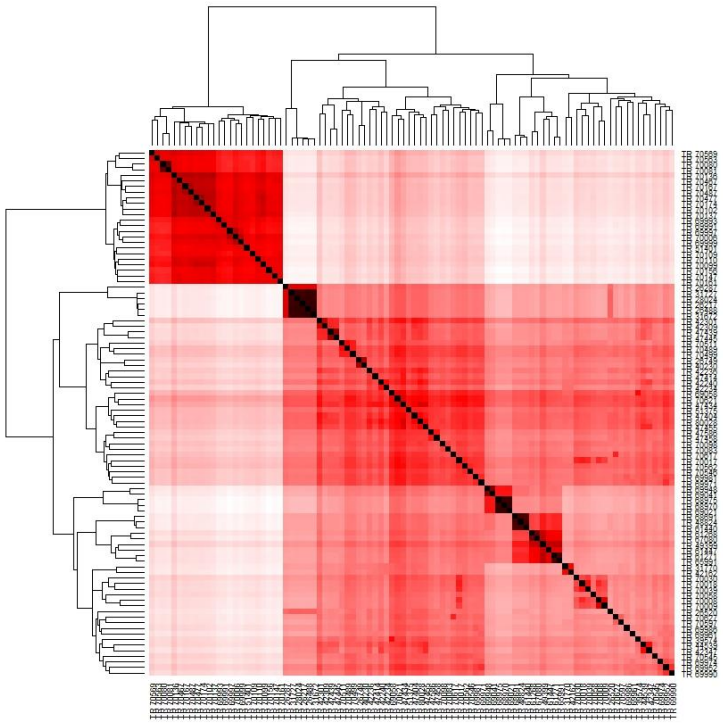

Figure 5. The heat map of 94 Turkish lentil landraces based on SNPs.

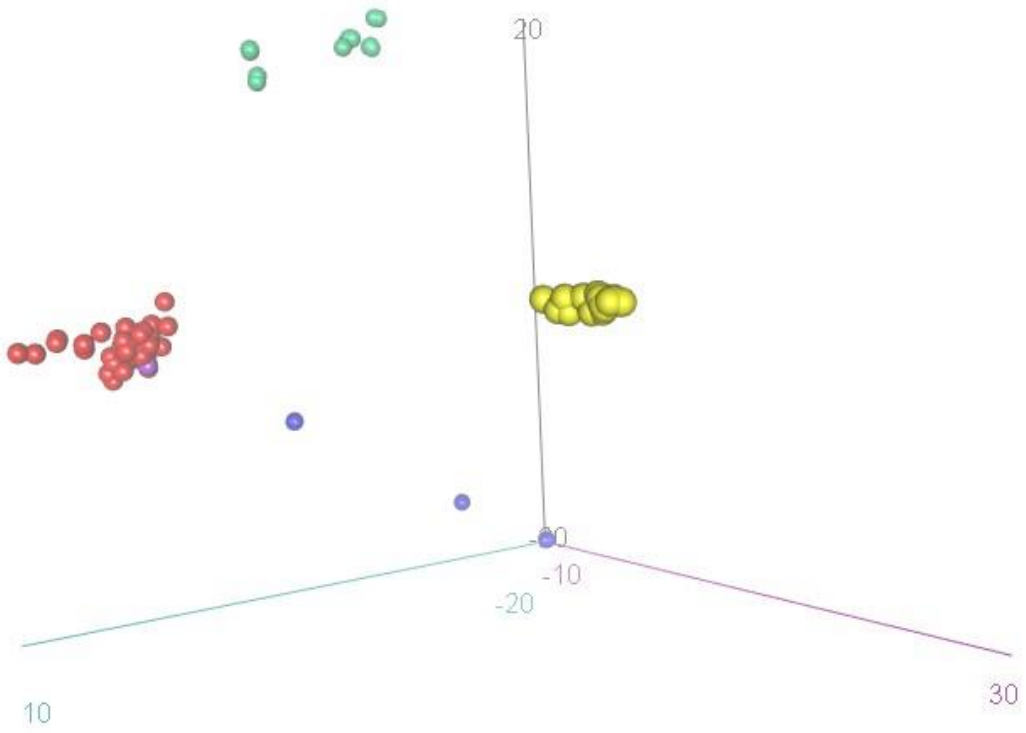

Figure 6. The relationships between the 94 Turkish lentil landraces visualized by PCoA utilizing the SNPs.

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 F_2 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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LITERATURE CITED

Abo-Elwafa, A., K. Murai and T. Shimada. 1995. Intra-and inter-specific variations in Lens revealed by RAPD markers. Theoretical and Applied Genetics 90: 335-40.

Ahamed, K., B. Akhter, M. Islam, M. Humaun and M. Alam. 2014. Morphological characterization and genetic diversity in lentil (*Lens culinaris* medikus ssp. *Culinaris*) germplasm. International Journal of Agricultural Research, Innovation and Technology 4: 70-6.

Ahmad, M., D. Mcneil, A. Fautrier, K. Armstrong and A. Paterson. 1996. Genetic relationships in Lens species and parentage determination of their interspecific hybrids using RAPD markers. Theoretical and Applied Genetics 92: 1091-8.

Alghamdi, S., A. Khan, M. Ammar, E. El-Harty, H. Migdadi, S. El-Khalik, A. Al-Shameri, M. Javed and S. Al-Faifi. 2014. Phenological, nutritional and molecular diversity assessment among 35 introduced lentil (*Lens culinaris* Medik.) genotypes grown in Saudi Arabia. International Journal of Molecular Sciences 15: 277-95.

Ates, D., S. Aldemir, B. Yagmur, A. Kahraman, H. Ozkan, A. Vandenberg and M. B. Tanyolac. 2018a. QTL Mapping of Genome Regions Controlling Manganese Uptake in Lentil Seed. G3: Genes, Genomes, Genetics 8(5):1409-1416.

Ates, D., T. K. Asciogul, S. Nemli, S. Erdogmus, D. Esiyok and M. B. Tanyolac. 2018b. Association mapping of days to flowering in common bean (*Phaseolus vulgaris* L.) revealed by DArT markers. Molecular Breeding 38: 113.

Ates, D., T. Sever, S. Aldemir, B. Yagmur, H. Y. Temel, H. B. Kaya, A. Alsaleh, A. Kahraman, H. Ozkan, A. Vandenberg and B. Tanyolac. 2016. Identification QTLs controlling genes for se uptake in lentil seeds. PLoS One 11: e0149210.

Babayeva, S., Z. Akparov, M. Abbasov, A. Mammadov, M. Zaifizadeh and K. Street. 2009. Diversity analysis of Central Asia and Caucasian lentil (*Lens culinaris* Medik.) germplasm using SSR fingerprinting. Genetic Resources and Crop Evolution 56: 293.

Basheer-Salimia, R., B. Camilli, S. Scacchi, E. Noli and M. Awad. 2015. Assessment of genetic diversity in lentils (*Lens culinaris* Medik.) based on SNPs. Genet. Mol. Res 14: 5870-8.

Bejiga, G., S. Tsegaye, A. Tullu and W. Erskine. 1996. Quantitative evaluation of Ethiopian landraces of lentil (*Lens culinaris*). Genet. Resour. Crop Evol 43: 293-301.

de la Rosa, L. and N. Jouve. 1992. Genetic variation isozyme genes and proteins in Spanish primitive cultivars and wild subspecies of genus Lens. Euphytica 59: 181-187.

Dikshit, H. K., A. Singh, D. Singh, M. S. Aski, P. Prakash, N. Jain, S. Meena, S. Kumar and A. Sarker. 2015. Genetic diversity in Lens species revealed by EST and genomic simple sequence repeat analysis. PLoS One 10: e0138101.

Doyle, J. J. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19: 11-5.

Echeverrigaray, S., A. C. Oliveira, M. T. V. Carvalho and E. Derbyshire. 1998. Evaluation of the relationship between lentil accessions using comparative electrophoresis of seed proteins. J. Genet. Breed 52: 89-94.

El-Nahas, A., H. El-Shazly, S. M. Ahmed and A. Omran. 2011. Molecular and biochemical markers in some lentil (*Lens culinaris* Medik.) genotypes. Annals of Agricultural Sciences 56: 105-12.

Erskine, W., Y. Adham and L. Holly. 1989. Geographic distribution of variation in quantitative traits in a world lentil collection. Euphytica 43: 97-103.

Erskine, W. and M. Choudhary. 1986. Variation between and within lentil landraces from Yemen Arab Republic. Euphytica 35: 695-700.

Erskine, W. and F. Muehlbauer. 1991. Allozyme and morphological variability, outcrossing rate and core collection formation in lentil germplasm. Theoretical and Applied Genetics 83: 119-25.

Ferguson, M. E., L. D. Robertson, B. V. Ford-Lloyd, H. J. Newbury and N. Maxted. 1998. Contrasting genetic variation amongst lentil landraces from different geographical origins. Euphytica 102: 265-73.

Fikiru, E., K. Tesfaye and E. Bekele. 2007. Genetic diversity and population structure of Ethiopian lentil (*Lens culinaris* Medikus) landraces as revealed by ISSR marker. African Journal of Biotechnology 6.

Ford, R., E. Pang and P. Taylor. 1997. Diversity analysis and species identification in Lens using PCR generated markers. Euphytica 96: 247-55.

Havey, M. and F. Muehlbauer. 1989. Variability for restriction fragment lengths and phylogenies in lentil. Theoretical and Applied Genetics 77: 839-43.

Huson, D. H. and D. Bryant. 2006. Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23: 254-67.

Huttner, E., P. Wenzl, M. Akbari, V. Caig, J. Carling, C. Cayla, M. Evers, D. Jaccoud, K. Peng and S. Patarapuwadol. Diversity arrays technology: a novel tool for harnessing the genetic potential of orphan crops. *Proceedings of the Discovery to Delivery: BioVision Alexandria 2004, Proceedings of the 2004 Conference of The World Biological Forum. CABI Publishing: UK, 2005*, 145-55.

Idrissi, O., A. R. Piergiovanni, F. Toklu, C. Houasli, S. M. Udupa, E. De Keyser, P. Van Damme and J. De Riek. 2018. Molecular variance and population structure of lentil (*Lens culinaris* Medik.) landraces from Mediterranean countries as revealed by simple sequence repeat DNA markers: implications for conservation and use. Plant Genetic Resources 16: 249-59.

Idrissi, O., M. S. Udupa, E. De Keyser, P. Van Damme and J. De Riek. 2016. Functional genetic diversity analysis and identification of associated simple sequence repeats and amplified fragment length polymorphism markers to drought tolerance in lentil (*Lens culinaris* ssp. *culinaris* Medicus) Landraces. Plant Molecular Biology Reporter 34: 659-80.

Idrissi, O., S. M. Udupa, C. Houasli, E. De Keyser, P. Van Damme and J. De Riek. 2015. Genetic diversity analysis of Moroccan lentil (*Lens culinaris* Medik.) landraces using Simple Sequence Repeat and Amplified Fragment Length Polymorphisms reveals functional adaptation towards agro-environmental origins. Plant Breeding 134: 322-32.

Jin, L., G. Jian-Ping, X. Dong-Xu, X.-Y. Zhang, G. Jing and Z. Xu-Xiao. 2008. Genetic diversity and population structure in lentil (*Lens culinaris* Medik.) germplasm detected by SSR markers. Acta Agronomica Sinica 34: 1901-9.

Kaur, S., N. O. Cogan, L. W. Pembleton, M. Shinozuka, K. W. Savin, M. Materne and J. W. Forster. 2011. Transcriptome sequencing of lentil based on second-generation technology permits large-scale unigene assembly and SSR marker discovery. BMC Genomics 12: 265.

Khazaei, H., C. T. Caron, M. Fedoruk, M. Diapari, A. Vandenberg, C. J. Coyne, R. Mcgee and K. E. Bett. 2016a. Genetic diversity of cultivated lentil (*Lens culinaris* Medik.) and its relation to the world's agro-ecological zones. Frontiers in Plant Science 7:1093.

Kushwaha, U., S. Ghimire, N. Yadav and B. Ojha. 2013. Genetic relatedness of lentil (*Lens culinaris* L.) germplasm by using SSR markers. International Journal of Applied Sciences and Biotechnology 1: 132-6.

Lazaro, A., M. Ruiz, L. de la Rosa and I. Martin. 2001. Relationship between agro/morphological characters and climatic parameters in Spanish landraces of lentil (*Lens culinaris* Medik.). Genet. Resour. Crop Evol 48: 239-249.

Lombardi, M., M. Materne, N. O. Cogan, M. Rodda, H. D. Daetwyler, A. T. Slater, J. W. Forster and S. Kaur. 2014. Assessment of genetic variation within a global collection of lentil (*Lens culinaris* Medik.) cultivars and landraces using SNP markers. BMC Genetics 15: 150.

Maxted, N. and S. J. Bennett, 2001. Legume diversity in the Mediterranean region. In. *Plant genetic resources of legumes in the Mediterranean.* Springer 51-75.

Nei, M., 1987. Molecular evolutionary genetics. Columbia University press, New York. 1987. 512 pp. Am. J. Phys. Anthropol 75(1988): 428-429.

Nei, M. and W.-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76: 5269-73.

Nemli, S., T. Kianoosh and M. B. Tanyolac. 2015. Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) accessions through retrotransposon-based interprimer binding sites (iPBSs) markers. Turkish Journal of Agriculture and Forestry 39: 940-8.

Ozkuru, E., D. Ates, S. Nemli, S. Erdogmus, N. Karaca, H. Yilmaz, B. Yagmur, C. Kartal, M. Tosun and O. O. Ocak. 2019. Genome-wide association studies of molybdenum and selenium concentrations in *C. arietinum* and *C. reticulatum* seeds. Molecular Breeding 39: 46.

Ozkuru, E., D. Ates, S. Nemli, S. Erdogmus, N. Karaca, H. Yilmaz, B. Yagmur, C. Kartal, M. Tosun and O. Ozdestan. 2018. Association mapping of loci linked to copper, phosphorus, and potassium concentrations in the seeds of *C. arietinum* and *C. reticulatum*. Genomics. https://doi.org/10.1016/j.ygeno.2018.12.010.

Piergiovanni, A. R and G. Taranto. 2003. Geographic distribution of genetic variation in lentil collection as revealed by SDS-PAGE fractionation of seed storage proteins. J. Genet. Breed 57: 39-46.

Raj, A., M. Stephens and J.K. Pritchard. 2014. FastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets. Genetics 197: 573-589.

Sansaloni, C. P., C. D. Petroli, J. Carling, C. J. Hudson, D. A. Steane, A. A. Myburg, D. Grattapaglia, R. E. Vaillancourt and A. Kilian. 2010. A high-density Diversity Arrays Technology (DArT) microarray for genome-wide genotyping in Eucalyptus. Plant Methods 6: 16.

Scippa, G., D. Trupiano, M. Rocco, V. Viscosi, M. Di Michele, A. D'andrea and D. Chiatante. 2008. An integrated approach to the characterization of two autochthonous lentil (*Lens culinaris*) landraces of Molise (south-central Italy). Heredity 101: 136.

Seyedimoradi, H. and R. Talebi. 2014. Detecting DNA polymorphism and genetic diversity in Lentil (*Lens culinaris* Medik.) germplasm: comparison of ISSR and DAMD marker. Physiology and Molecular Biology of Plants 20: 495-500.

Sharma, S., M. Knox and T. N. Ellis. 1996. AFLP analysis of the diversity and phylogeny of Lens and its comparison with RAPD analysis. Theoretical and Applied Genetics 93: 751-8.

Sharpe, A., L. Ramsay, L.A. Sanderson, M. Fedoruk, W. Clarke, R. Li, S. Kagale, P. Vijayan, A. Vandenberg and K. Bett. 2013. Ancient crop joins modern era: gene-based SNP discovery and mapping in lentil. BMC Genomics 14: 192.

Sonnante, G. and D. Pignone. 2001. Assessment of genetic variation in a collection of lentil using molecular tools. Euphytica 120: 301-7.

Sonnante, G. and D. Pignone. 2007. The major Italian landraces of lentil (*Lens culinaris* Medik.): their molecular diversity and possible origin. Genetic Resources and Crop Evolution 54: 1023-31.

Sultana, T., A. Ghafoor and M. Ashraf. 2006. Geographic patterns of diversity of cultivated lentil germplasm collected from Pakistan, as assessed by seed protein assays. Acta Biologica Cracoviensia, Series Botanica, Poland 48: 77-84.

Toklu, F., T. Karakoy, E. Haklı, T. Bicer, A. Brandolini, B. Kilian and H. Ozkan. 2009. Genetic variation among lentil (*Lens culinaris* Medik) landraces from Southeast Turkey. Plant Breeding 128: 178-86.

Tommasini, L., T. Schnurbusch, D. Fossati, F. Mascher and B. Keller. 2007. Association mapping of *Stagonospora nodorum* blotch resistance in modern European winter wheat varieties. Theoretical and Applied Genetics 115:697-708.

Tsanakas, G. F., P. V. Mylona, K. Koura, A. Gleridou and A. N. Polidoros. 2018. Genetic diversity analysis of the Greek lentil (*Lens culinaris*) landrace 'Eglouvis' using morphological and molecular markers. Plant Genetic Resources 16: 469-77.

Varshney, R.K., A. Graner and M.E. Sorrells. 2005. Genomics-assisted breeding for crop improvement. Trends in Plant Science 10:621-630.

Villa, T. C. C., N. Maxted, M. Scholten and B. Ford-Lloyd. 2005. Defining and identifying crop landraces. Plant Genetic Resources 3: 373-84.

Wong, M. M., N. Gujaria-Verma, L. Ramsay, H. Y. Yuan, C. Caron, M. Diapari, A. Vandenberg and K. E. Bett. 2015. Classification and characterization of species within the genus Lens using genotyping-by-sequencing (GBS). PLoS One 10: e0122025.

Yuzbasioglu, E., S. Ozcan and L. Acık. 2006. Analysis of genetic relationships among Turkish cultivars and breeding lines of Lens culinatis Mestile using RAPD markers. Genetic Resources and Crop Evolution 53: 507-14.

Zaccardelli, M., F. Lupo, A. R. Piergiovanni, G. Laghetti, G. Sonnante, M. G. Daminati, F. Sparvoli and L. Lioi. 2012. Characterization of Italian lentil (*Lens culinaris* Medik.) germplasm by agronomic traits, biochemical and molecular markers. Genetic Resources and Crop Evolution 59: 727-38.