Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2024, 17(1), 80-90
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2024, 17(1), 80-90
DOI: 10.18185/erzifbed.1323490
Research Article

Investigation of Genetic Diversity in Lentil Genotypes Obtain from Different Countries Using SDS-PAGE Methodology

Mehmet Zahit YEKEN[1*], Faheem Shehzad BALOCH[2],
Muhammad Azhar NADEEM[2], Muhammad SAMEEULLAH[1], Ekrem GÜREL[3]

[1]Department of Field Crops, Faculty of Agriculture, Bolu Abant Izzet Baysal University, Bolu, Türkiye
[2]Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Sivas University of Science and Technology, Sivas, Türkiye
[3]Department of Biology, Faculty of Science and Literature, Bolu Abant Izzet Baysal University, Bolu, Türkiye

Received: 06/07/2023, **Revised:** 12/12/2023, **Accepted:** 12/12/2023, **Published:** 28/03/2024

Abstract
In the current study, the characterization of 44 lentil genotypes was performed using SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis) technique. The protein bands were scored according to a binary system as the present (1) or absent (0) that ranged 12-20 bands in lentil genotypes. The Jaccard's coefficient of genetic dissimilarity (GD) was measured, and the mean GD was 0.216. Maximum genetic distance was found as 0.526 between Syria2 with Iraq3 and these genotypes might be recommended for future lentil breeding. AMOVA explored the presence of higher genetic variety within genotypes (83%) than among genotypes (17% variations). STRUCTURE algorithm separated lentil genotypes into two groups mainly on the basis of their geographic. The UPGMA clustering separated lentil genotypes into two main clusters A and B. The PCoA was also conducted to confirm the results of structure and UPGMA analyses. Findings from the PCoA and structure analyses were in full agreement with those obtained by UPGMA. The results might be useful for researchers worldwide who are interested in lentil breeding.

Keywords: Genetic diversity, germplasm characterization, population structure, protein

Farklı Ülkelerden Elde Edilen Mercimek Genotiplerinde Genetik Çeşitliliğinin SDS-PAGE Yöntemi ile Araştırılması

Öz
Bu çalışmada, 44 mercimek genotipinin karakterizasyonu, SDS-PAGE kullanılarak gerçekleştirilmiştir. Protein bantları, var (1) veya yok (0) şeklinde puanlanmış ve mercimek genotiplerinin bant aralığının 12-20 bant arasında olduğu belirlenmiştir. Jaccard'ın genetik benzerlik katsayısı (GD), ortalama 0.216 olarak tespit edilmiştir. Suriye2 ile Irak3 genotipleri arasında maksimum genetik uzaklık 0.526 olarak bulunmuş ve gelecekteki mercimek ıslahı çalışmalarında kullanılabileceği belirlenmiştir. AMOVA mercimek genotiplerindeki yüksek genetik çeşitliliğini varlığını araştırmış ve popülasyon içindeki (%83) varyasyonun popülasyonlar arasındaki (%17) varyasyona kıyasla yüksek olduğunu göstermiştir. STRUCTURE algoritması, mercimek genotiplerini coğrafi bölgelerine göre iki gruba ayırmıştır. UPGMA kümelemesi, mercimek genotiplerini iki ana kümeye (A ve B) ayırmıştır. STRUCTURE ve UPGMA analizlerinin sonuçlarını doğrulamak için PCoA gerçekleştirilmiş ve sonuçların uyum içerisinde olduğu belirlenmiştir. Araştırma sonuçları dünyanın farklı yerlerinde mercimek ıslahı ile ilgilenen araştırmacılara önemli katkılar sağlayacaktır.

Anahtar Kelimeler: Genetik çeşitlilik, germplazm karakterizasyonu, popülasyon yapısı, protein.

*Corresponding Author: yekenmehmetzahit@gmail.com
Mehmet Zahit YEKEN, https://orcid.org/0000-0003-0490-371X
Faheem Shehzad BALOCH, https://orcid.org/0000-0002-7470-0080
Muhammad Azhar NADEEM, https://orcid.org/0000-0002-0637-9619
Muhammad SAMEEULLAH, https://orcid.org/0000-0001-6767-2539
Ekrem GÜREL, https://orcid.org/0000-0001-6262-2866

1. Introduction

Pulses are considered the best source of plant-based protein and other essential nutrients like zinc, iron and vitamins required for the development and growth of the human body [1]. Besides their importance for human health, pulses are also a valuable resource employed for fodder or pasture [2]. Among the pulse crops, lentil (*Lens culinaris* Medik.) is a self-pollinating plant with diploid (2n = 2x = 14) chromosomes [3]. It existed around 7000–10,000 years ago in the Eastern Mediterranean region [4]. This crop is produced in more than 58 countries worldwide, and its seeds are high in protein content, vitamins, and essential nutrients [5,6]. As a legume crop, lentil contributes significantly to a sustainable agriculture production system through nitrogen fixation capacity and lowers the application of fertilizer in cereal-based cropping system [7]. According to FAO [8], lentil was grown in a 5.58 million ha area having a production of 5.61 million tons in 2021.

Germplasm characterization is crucial because it provides a source of variations that can be employed for breeding purposes [9]. Similarly, investigation of the relationship among the genotypes is very important for efficient breeding activities to develop modern varieties having high yield, high nutritional value and resistance to biotic and abiotic stress [10]. In the last two decades, genetic diversity has gained great attention and importance [11,12]. It is critical for successful plant breeding studies to discover and characterize novel genes/alleles [13,14].

Numerous research have been undertaken to investigate genetic diversity in various lentil germplasm using DNA-based markers such as RFLP (Restriction fragment length polymorphism) [15], RAPD (Random amplified polymorphic DNA) [16], AFLP (Amplified fragment length polymorphism) [17], ISSR (Inter-simple sequence repeat [18], SSR (Simple sequence repeat) [19], DArT (Diversity Array Technology) [20], SNP (Single Nucleotide Polymorphism technique [13,21,22]. To investigate genetic differences among plant species/subspecies, biochemical markers or seed protein patterns are widely performed [23]. SDS-PAGE technique has mainly been applied for the differentiation of seed protein [24,25]. This technique was used previously to determine genetic diversity in various crop species such as common bean [26,27], coriander [28] and lentil [5,29,30]. In this study, genetic diversity among forty-four lentil genotypes originating from thirteen counties was investigated using seed storage protein pattern. The outputs of this study would contribute an influential source for lentil genetic programs in the future, and support lentil breeders to make successful selections in the near future.

2. Material and Methods

2.1. Plant material

As plant material, the 44 genotypes of lentils from 13 different countries were employed (Table 1). These genotypes were supplied by the USDA (United States Department of Agriculture).

Table 1. The passport information of the lentil genotypes employed in this study.

No	ID	Plant Name	Taxon	Collected Place
1	PI 320946	ILL 513	*Lens culinaris* subsp. *culinaris*	Ancient Palestine
2	PI 193550	ILL 207	*Lens culinaris* subsp. *culinaris*	Ethiopia
3	PI 193549	ILL 206	*Lens culinaris* subsp. *culinaris*	Ethiopia
4	PI 193817	ILL 208	*Lens culinaris* subsp. *culinaris*	Ethiopia
5	PI 193548	ILL 205	*Lens culinaris* subsp. *culinaris*	Ethiopia
6	PI 273664	ILL 247	*Lens culinaris* subsp. *culinaris*	Ethiopia
7	PI 297772	ILL 304	*Lens culinaris* subsp. *culinaris*	Greece
8	PI 297773	ILL 305	*Lens culinaris* subsp. *culinaris*	Greece
9	PI 297765	ILL 297	*Lens culinaris* subsp. *culinaris*	Greece
10	PI 297770	ILL 302	*Lens culinaris* subsp. *culinaris*	Greece
11	PI 297774	ILL 306	*Lens culinaris* subsp. *culinaris*	Greece
12	PI 472365	33-069-00299	*Lens culinaris* subsp. *culinaris*	India
13	PI 472360	33-069-00294	*Lens culinaris* subsp. *culinaris*	India
14	PI 472355	33-069-00288	*Lens culinaris* subsp. *culinaris*	India
15	PI 472370	33-069-00304	*Lens culinaris* subsp. *culinaris*	India
16	PI 472600	33-071-10647	*Lens culinaris* subsp. *culinaris*	Iran
17	PI 472605	33-071-10792	*Lens culinaris* subsp. *culinaris*	Iran
18	PI 472630	33-071-11104	*Lens culinaris* subsp. *culinaris*	Iran
19	PI 472620	33-071-11053	*Lens culinaris* subsp. *culinaris*	Iran
20	PI 472580	33-071-10475	*Lens culinaris* subsp. *culinaris*	Iran
21	PI 577163	W6 8376	*Lens culinaris* subsp. *culinaris*	Iraq
22	PI 577162	W6 8375	*Lens culinaris* subsp. *culinaris*	Iraq
23	PI 577161	W6 8374	*Lens culinaris* subsp. *culinaris*	Iraq
24	PI 577160	W6 8372	*Lens culinaris* subsp. *culinaris*	Iraq
25	PI 577159	W6 8371	*Lens culinaris* subsp. *culinaris*	Iraq
26	PI 612301	Jordan 3	*Lens culinaris* subsp. *culinaris*	Jordan
27	PI 612302	Jordan 1	*Lens culinaris* subsp. *culinaris*	Jordan
28	PI 612303	Jordan 2	*Lens culinaris* subsp. *culinaris*	Jordan
29	PI 302398	ILL 486	*Lens culinaris* subsp. *culinaris*	Jordan
30	PI 420926	11	*Lens culinaris* subsp. *culinaris*	Jordan
31	PI 300559	ILL 479	*Lens culinaris* subsp. *culinaris*	Lebanon
32	PI 300561	ILL 481	*Lens culinaris* subsp. *culinaris*	Lebanon
33	PI 300560	ILL 480	*Lens culinaris* subsp. *culinaris*	Lebanon
34	PI 432253	RPIP 33-085-10610	*Lens culinaris* subsp. *culinaris*	Lebanon
35	PI 432251	RPIP 33-085-10607	*Lens culinaris* subsp. *culinaris*	Lebanon
36	PI 577147	2340	*Lens culinaris* subsp. *culinaris*	Nepal
37	PI 477290	-	*Lens culinaris* subsp. *culinaris*	Pakistan
38	PI 606650	Spanish Brown	*Lens culinaris* subsp. *culinaris*	Spain
39	PI 643449	HALA	*Lens culinaris* subsp. *culinaris*	Syria
40	PI 643450	RACHAYYA	*Lens culinaris* subsp. *culinaris*	Syria
41	PI 643448	KEF	*Lens culinaris* subsp. *culinaris*	Syria
42	PI 644221	TESHALE	*Lens culinaris* subsp. *culinaris*	Syria
43	PI 635040	OZBEK	*Lens culinaris* subsp. *culinaris*	Syria
44	PI 176604	ILL 142	*Lens culinaris* subsp. *culinaris*	Türkiye

2.2. Buffers and reagents

The analysis was carried out in the Biotechnology lab., Department of Biology, Bolu Abant İzzet Baysal University, Bolu, Türkiye. The chemicals used in the study were obtained from Merck. with some modifications of Ghafoor et al. [31], the extraction buffer was arranged using Tris-Hydrochloride 62.5 mM (pH 6.8), SDS 2%, Dithiothreitol (DTT) 10 mM, glycerol 10%, and urea 5M. To obtain seed flour of lentil and extraction of proteins, sterilized microcentrifuge tubes were employed in the study.

2.3. Lentil protein extraction and SDS-PAGE analysis

Twenty seeds were selected for each genotype and bulked. Then, the seeds were grounded using mortar and pestle, and 10 mg powder was weighed in 1.5 ml micro-centrifuge tubes. The proteins were extracted from finely ground lentil seed flour (10 mg) using 400 µL extraction buffer. Following complete mixing by vortex, the micro-centrifuge tubes were heated on a heat block at 40°C for one hour. Following that, homogenates were vortexed for a brief period. The mixtures were centrifuged at 13.000 rpm for 5 minutes. After the centrifuge process, soluble proteins as supernatant were carried to new sterile micro-centrifuge tubes. Total protein concentration was determined as described earlier [32]. Then, the tubes were stored at 4ºC in the fridge. The following day, 20 µg protein of each genotype was mixed with 2X bromophenol blue loading dye and heated at 95 ºC for five minutes. A total of 20 µL denatured protein was loaded into the wells of the polyacrylamide-based discontinuous gel. SDS-PAGE was carried out using the method previously reported by Laemmli [33]. SDS-PAGE was arranged with 4.5% stacking gel and 15% resolving gel. Using the P9DS electrophoresis apparatus (Thermo Scientific, USA), the proteins were electrophoresed at 230 voltage for 4 hours at 4ºC. The gels were then stained using Bio-Safe Coomassie stain (Bio-Rad, Hercules, California, USA) in accordance with the manufacturer's instructions.

2.4. Data analysis

The clear, strong, unambiguous bands were selected for analysis. We scored the strong, clear, and unambiguous protein bands according to a binary system as the present (1) or absent (0). A pairwise genetic distance (GDj) matrix between lentil genotypes was performed according to Jaccard's coefficient [34]. The UPGMA (Unweighted Pair-group Method with Arithmetic Means) clustering analysis and PCoA (Principal Coordinate Analysis) were performed to visualize the pattern of genetic diversity among the lentil genotypes. The UPGMA clustering was constructed via the Jaccard coefficient among the lentil genotypes with Pop Gene v.1.32 software. According to Evanno et al. [35], the number of clusters was found using STRUCTURE software. AMOVA (Analysis of molecular variance) was performed with the vegan library Ver. 2.4.4 in R statistical software.

3. Results and Disscussion

Composed of acidic and basic subunits linked by disulfide bonds, 7S (vicilins and convicilins) and 11S (legumins) globulins represent important storage proteins in lentil seeds [29,36]. The amount of protein bands in the electropherogram was determined to range from 12 to 20 bands in 44 lentil genotypes in SDS. The protein bands obtained from different genotypes were

presented in Figure 1. Using R statistical software, a pairwise genetic distance (GD) matrix among forty-four lentil genotypes was determined, and the maximum GD was found as 0.526 between Syria2 and Iraq3, while the mean GD was 0.216. AMOVA explored the existence of higher genetic diversity within genotypes (83%) compared to among genotypes accounting 0.166% variations (Table 2). The STRUCTURE algorithm separated 44 lentil genotypes into two groups (Figure 2 and Figure 3).

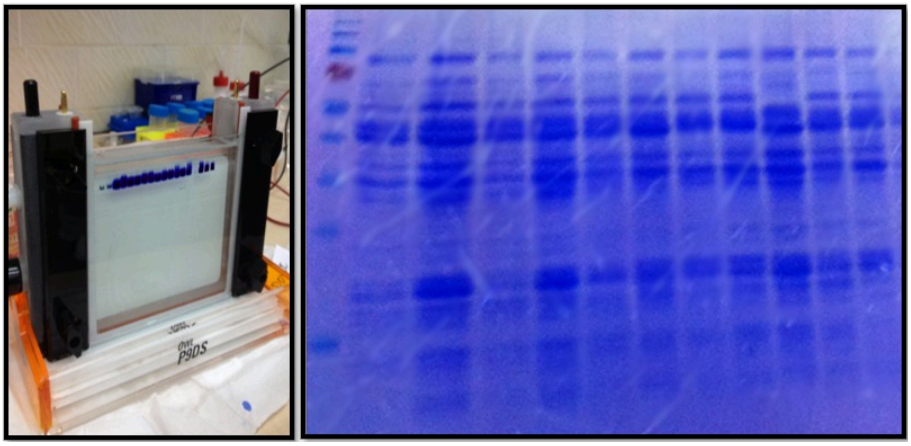

Figure 1. P9DS device used in the study, and some protein bands from different genotypes.

Table 2. Results for AMOVA among lentil genotypes from the different countries as grouped by the clustering algorithm.

Source	df	SS	MS	Estimated Variance	% Variation
Among Population	7	11.305	1.615	0.166	17
Within Population	31	24.952	0.805	0.805	83
Total	38	36.257		0.971	100

MS: Mean square, SS: Sum of squares, df: degrees of freedom,

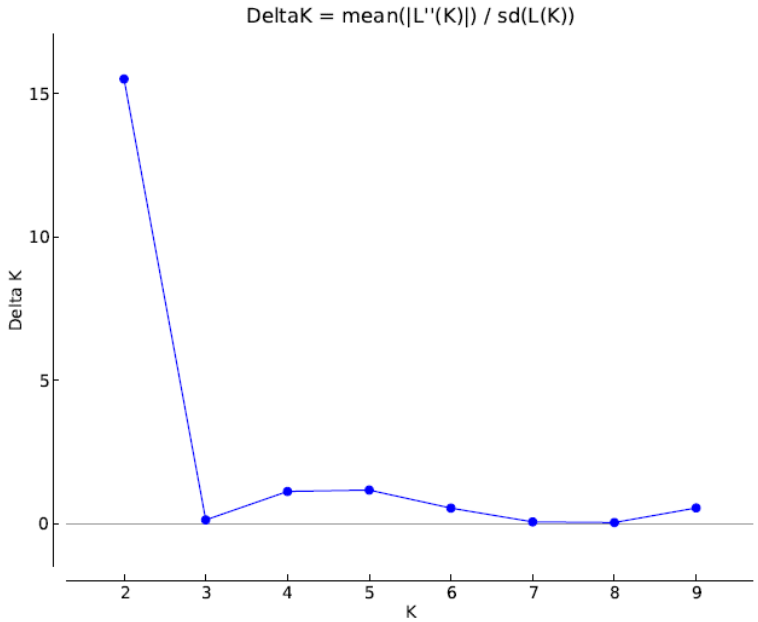

Figure 2. Valued amount of K for the lentil genotypes structure analysis.

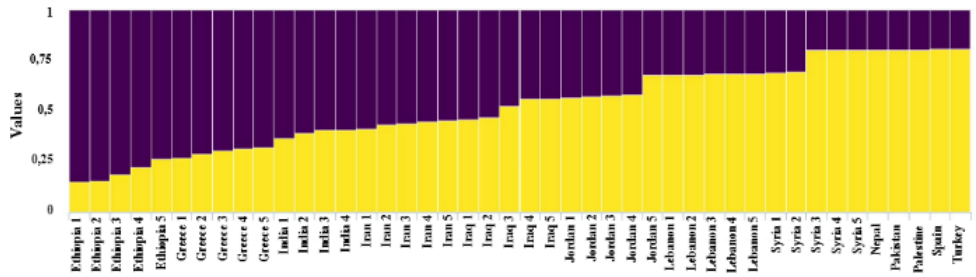

Figure 3. Structure based clustering of lentil genotypes using SDS-PAGE analysis.

The UPGMA clustering was carried out to reveal the pattern of differences among the 44 lentil genotypes. Using the Jaccard genetic distance coefficient, the UPGMA tree was constructed (Figure 4). The studied lentil genotypes were separated into two main clusters A and B. The Cluster A was further grouped in to A1 and A2 by clustering Syria2 and Syria1; Ethiopia5 genotypes, respectively. Cluster B was determined larger than A by clustering a total of 41 lentil genotypes. The main group B cluster was further divided into B1 and B2 subgroups. Subgroup B1 included Iran1, Pakistan and Türkiye lentil genotypes. Results of the UPGMA analysis and principal coordinate analysis, all lentil genotypes were not separated on the basis of their provenance. Interestingly, geographically near genotypes were found genetically distant. For instance, the genotypes from Syria1 and Ethiopia5 which are geographically located in different regions were in group A1. On the other hand, subgroup B2 contained 38 lentil genotypes. The PCoA was also conducted to confirm the results of structure and UPGMA analyses (Figure 5). Results from the PCoA and Structure analyses were in full agreement with those obtained by UPGMA.

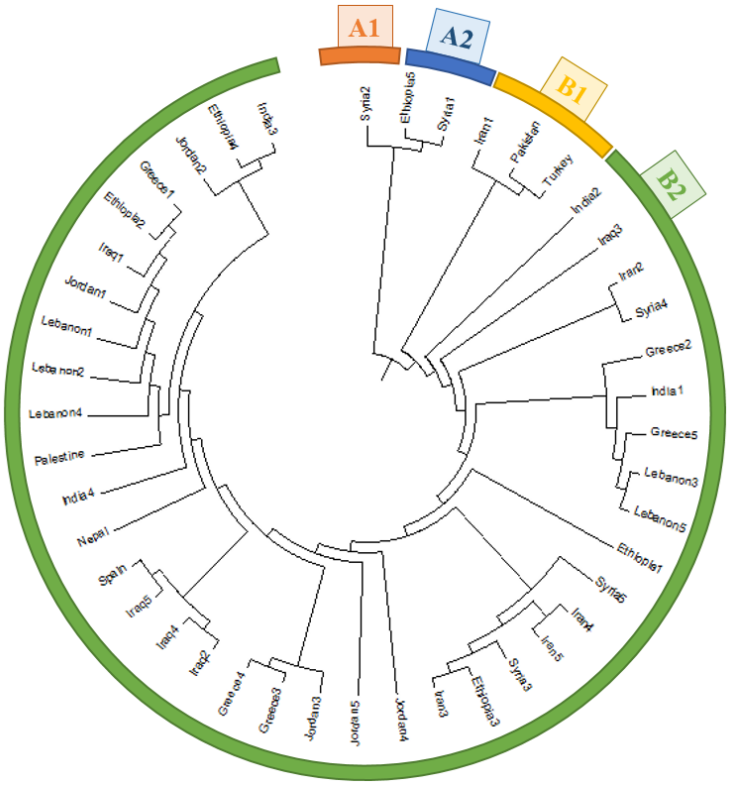

Figure 4. UPGMA clustering analysis of lentil genotypes.

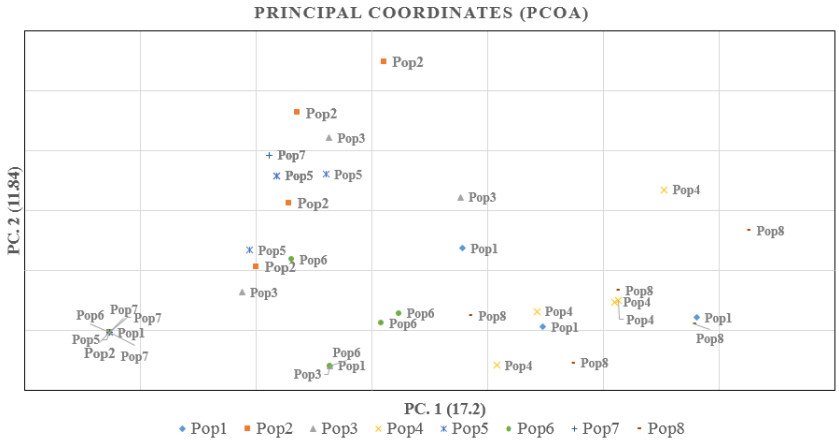

Figure 5. Principal coordinates analysis result of lentil genotypes.

Genetic resources showing the highest level of genetic distance are very important and should be conserved and utilized for future breeding activities. The results of UPGMA, PCoA, and STRUCTURE methods revealed a clear separation of all the lentil genotypes. Various studies were previously performed to explore the genetic variation of different lentil germplasms. For instance, using ISSR markers, Fikiru et al. [37] explored the genetic diversity and population structure of 70 Ethiopian landraces. They reported the presence of higher (56.28%) with the genotypes. In another study, Yüzbaşıoğlu et al. [38] separated 14 lentil cultivars into two main cluster groups using SDS-PAGE analysis. Toklu et al. [39] investigated genetic diversity using molecular markers (AFLP, ISSR, combined AFLP/ISSR) data. They determined that Turkish lentil landraces were separated into two main clusters. Kushwaha et al. [40] determined the highest level of genetic distance in lentil germplasm as 0.027273 using SSR markers. Khazaei et al. [13] used to characterize a total of 352 lentil genotypes with SNP markers and reported that higher genetic variations in their studied material are due to differences within groups (86%). Tahir and Omer [41] explored the genetic variations in lentil genotypes using RAPD markers and morpho-agronomic traits. They reported the existence of higher variation (60%) within lentil genotypes. Lombardi et al. [21], Khazaei et al. [13] and Dissanayake et al. [6] found a weak correlation between clusters and geographical origins in lentil germplasm. Throughout history, the Mediterranean region has been a junction of cultures. It is thought that immigrants and traders played a significant role in the distribution of lentil resources. The genetically different groups determined herein can be evaluated to develop parental lines to initiate lentil breeding programs.

4. Conclusion

A high level of diversity was determined in a panel of 44 lentil genotypes obtained from different countries using SDS-PAGE analysis. The findings have increased the knowledge of the literature on the SDS-PAGE technique in lentil. The results might be useful for breeders/researchers in various parts of the world who are interested in the lentil.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Resources: FSB, Investigation: MZY and MS, Formal analysis: MAN and MZY, Writing-original draft: MZY, MAN, MS, Writing- review & editing: FSB and EG, Supervision: FSB and EG.

References

[1] Maphosa, Y., Jideani, V.A., (2017) Functional Food. María Chávarri Hueda, The Role of Legumes in Human Nutrition (pp: 1-13) IntechOpen: London, UK.

[2] Atnaf, M., Tesfaye, K., Dagne, K., (2015) The importance of legumes in the Ethiopian farming system and overall economy: An overview, American J. Exp. Agric., 7, 347-358.

[3] Singh, M., Sharma, S. K., Singh, B., Malhotra, N., Chandora, R., Sarker, A., Singh, K., Gupta, D., (2018) Widening the genetic base of cultivated gene pool following introgression from wild *Lens* taxa, Plant Breed., 137, 470-485.

[4] Ford-Lloyd, B., (2011) Wild Crop Relatives. Genomic and Breeding Resources. Legume Crops and Forages. *Experimental Agriculture*, 48(1), 152-153. Springer, Heidelberg, Dordrecht, London, New York.

[5] Pal, T., Ghosh, S., Mondal, A., De, K. K., (2016) Evaluation of genetic diversity in some promising varieties of lentil using karyological characters and protein profiling, J. Genetic Eng. Biotechnol., 14, 39-48.

[6] Dissanayake, R., Braich, S., Cogan, N. O., Smith, K., Kaur, S., (2020) Characterization of genetic and allelic diversity amongst cultivated and wild lentil accessions for germplasm enhancement, Front. Gen., 11, 546.

[7] Shah, Z., Shah, S., Peoples, M., Schwenke, G., Herridge, D., (2003) Crop residue and fertiliser N effects on nitrogen fixation and yields of legume–cereal rotations and soil organic fertility, Field Crops Res., 83, 1-11.

[8] FAO (2023). Food and Agriculture Statistics. Retrieved October 06, 2023 from: https:// www.fao.org/faostat/en/#home

[9] Nadeem, M. A., Yeken, M. Z., Shahid, M. Q., Habyarimana, E., Yılmaz, H., Alsaleh, A., Hatipoğlu, R., Çilesiz, Y., Khawar, K.M., Ludidi, N., (2021) Common bean as a potential crop for future food security: an overview of past, current and future contributions in genomics, transcriptomics, transgenics and proteomics, Biotechnol. Biotechnol. Equip., 35, 759-787.

[10] Yeken, M. Z., Emiralioğlu, O., Çiftçi, V., Bayraktar, H., Palacioğlu, G., Özer, G. (2022). Analysis of genetic diversity among common bean germplasm by start codon targeted (SCoT) markers. Mol.Biol. Rep., 49(5), 3839-3847.

[11] Shah, M. A., Khan, A. I., Awan, F. S., Sadaqat, H. A., Bahadur, S., Baloch, F. S., (2015) Genetic diversity of some tomato cultivars and breeding lines commonly used in Pakistani breeding program, Turk J Agric For., 3, 126-132.

[12] Nadeem, M. A., Karaköy, T., Yeken, M. Z., Habyarimana, E., Hatipoğlu, R., Çiftçi, V., Nawaz, M. A., Sönmez, F., Shahid, M. Q., Yang, S. H., (2020) Phenotypic characterization of 183 Turkish common bean accessions for agronomic, trading, and consumer-preferred plant characteristics for breeding purposes, Agronomy., 10, 272.

[13] Khazaei, H., Caron, C. T., Fedoruk, M., Diapari, M., Vandenberg, A., Coyne, C.J. McGee, R. Bett, K.E. (2016) Genetic diversity of cultivated lentil (*Lens culinaris* Medik.) and its relation to the world's agro-ecological zones, Front. Plant Sci., 7, 1093.

[14] Ali, A. Altaf, M. T., Nadeem, M. A., Karaköy, T., Shah, A. N., Azeem, H., Baloch, F. S. Baran, N., Hussain, T., (2022) Duangpan, S. Recent advancement in OMICS approaches to enhance abiotic stress tolerance in legumes, Front. Plant Sci., 13, 952759.

[15] Havey, M., Muehlbauer, F., (1989) Variability for restriction fragment lengths and phylogenies in lentil, Theo. Appl. Genet., 77, 839-843.

[16] Yüzbaşıoğlu, E., Özcan, S., Açık, L., (2006) Analysis of genetic relationships among Turkish cultivars and breeding lines of *Lens culinatis* Mestile using RAPD markers, Genet. Resou. Crop Evo., 53, 507-514.

[17] Alghamdi, S. S., Khan, A. M., Ammar, M. H., El-Harty, E. H., Migdadi, H. M., Abd El-Khalik, S. M., Al-Shameri, A. M., Javed, M. M., Al-Faifi, S. A., (2013) Phenological, nutritional and molecular diversity assessment among 35 introduced lentil (*Lens culinaris* Medik.) genotypes grown in Saudi Arabia, Inter. J. Mol Sci., 15, 277-295.

[18] Seyedimoradi, H., Talebi, R., (2014) Detecting DNA polymorphism and genetic diversity in Lentil (*Lens culinaris* Medik.) germplasm: comparison of ISSR and DAMD marker, Physiol. Mol. Biol. Plant., 20, 495-500.

[19] Tsanakas, G. F., Mylona, P. V., Koura, K., Gleridou, A., Polidoros, A. N., (2018) Genetic diversity analysis of the Greek lentil (*Lens culinaris*) landrace 'Eglouvis' using morphological and molecular markers, Plant Gen. Res., 16, 469-477.

[20] Duygu, A., (2019) Genetic diversity in lentil landraces revealed by diversity array technology (DArT), Turkish J. Field Crop., 24, 252-260.

[21] Lombardi, M., Materne, M., Cogan, N. O., Rodda, M., Daetwyler, H. D., Slater, A. T., Forster, J. W., Kaur, S., (2014) Assessment of genetic variation within a global collection of lentil (*Lens culinaris* Medik.) cultivars and landraces using SNP markers, BMC Gen., 15, 1-10.

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

[23] Jan, S. A., Shinwari, Z. K., Rabbani, M. A., Shah, S. H., Ibrahim, M. I., Ilyas, M., (2016) Optimization of an efficient SDS-PAGE protocol for rapid protein analysis of *Brassica rapa*., J. Bio. Env. Sci*.,* 9, 17-24.

[24] Kakaei, M., Kahrizi, D., (2011) Study of seed proteins pattern of *Brassica napus* varieties via sodium dodecyl sulfate polyacrylamid gel electrophoresis, Inter. Res. J. Biotech*., 2,* 026-028.

[25] Javaid, A., Ghafoor, A., Anwar, R., (2004) Seed storage protein electrophoresis in groundnut for evaluating genetic diversity, Pak. J. Bot., 36, 25-30.

[26] Berber, İ., Yaşar, F., (2011) Characterization of Bean (*Phaseolus vulgaris* L.) cultivars grown in Turkey by SDS-PAGE of seed proteins, Pak. J. Bot*.,* 43, 1085-1090.

[27] Sher, K., Nisar, M., Subhan, M., Hazrat, A., Muhammad, A., Hancı, F., Fazal, Z., (2020) Protein Profiling of Indegnous Common Bean Through SDS-PAGE, Biosci. Res*.,* 17, 688-898.

[28] Yaldiz, G., Sameeullah, M., Çamlıca, M., Baloch, F. S., (2016) Lack of population structure in coriander populations based on SDS (Seed Storage Protein) page analysis, Turkish J.Agric. Food Sci.Tech*.,* 4, 656-661.

[29] Zaccardelli, M., Lupo, F., Piergiovanni, A. R., Laghetti, G., Sonnante, G., Daminati, M. G., Sparvoli, F., Lioi, L., (2012) Characterization of Italian lentil (*Lens culinaris* Medik.) germplasm by agronomic traits, biochemical and molecular markers, Genet. Resou. Crop Evo., 59, 727-738.

[30] Kumar, P., Trivedi, A., Adarsh, A., Verma, R., Kumar, R., Vyas, R., Yadav, M., (2018) Genetic characterization of lentil genotypes based on SDS-PAGE, J. Pharm. Phytochem*.,* 7, 274-278.

[31] Ghafoor, A., Ahmad, Z., Qureshi, A. S., Bashir, M., (2002) Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek based on morphological traits and SDS-PAGE, Euphytica*.,* 123, 367-388.

[32] Sameeullah, M., Sasaki, T., Yamamoto, Y., (2013) Sucrose transporter NtSUT1 confers aluminum tolerance on cultured cells of tobacco (*Nicotiana tabacum* L.), Soil Sci. Plant Nut*.,* 59, 756-770.

[33] Laemmli, U. K., (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature., 227, 680-685.

[34] Jaccard, P., (1908) Nouvelles recherches sur la distribution florale, Bull. Soc. Vaud. Sci. Nat*.,* 44, 223-270.

[35] Evanno, G., Regnaut, S., Goudet, J., (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study, Mol. Ecol., 14, 2611-2620.

[36] Scippa, G. S., Rocco, M., Ialicicco, M., Trupiano, D., Viscosi, V., Di Michele, M., Arena, S., Chiatante, D., Scaloni, A., (2010) The proteome of lentil (*Lens culinaris* Medik.) seeds: discriminating between landraces, Electrophoresis., 31, 497-506.

[37] Fikiru, E., Tesfaye, K., Bekele, E., (2007) Genetic diversity and population structure of Ethiopian lentil (*Lens culinaris* Medikus) landraces as revealed by ISSR marker, African J. Biotechnol., 6, 1460-1468.

[38] Yüzbaşioğlu, E., Acik, L., Özcan, S., (2008) Seed protein diversity among lentil cultivars, Biologia Plantarum**.,** 52, 126-128.

[39] Toklu, F., Karaköy, T., Haklı, E., Bicer, T., Brandolini, A., Kilian, B., Özkan, H., (2009) Genetic variation among lentil *(Lens culinaris* Medik) landraces from Southeast Turkey, Plant Breed., 128, 178-186.

[40] Kushwaha, U., Ghimire, S., Yadav, N., Ojha, B., (2013) Genetic relatedness of lentil (*Lens culinaris* L.) germplasm by using SSR markers, Int. J. Appl. Sci. Biotechnol., 1, 132-136.

[41] Tahir, N. A. R., Omer, D., (2017) Genetic variation in lentil genotypes by morpho-agronomic traits and RAPD-PCR, J Anim. Plant Sci. 27, 468-480.