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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

EVALUATION OF GENETIC DIVERSITY AND BIOCHEMICAL INDICATORS OF LENTIL (*Lens culinaris* Medik.) AND BEAN (*Phaseolus vulgaris* L.) SPECIMENS

Specialty: 2406.02 – Biochemistry

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Applicant: **Shemsiyye Elkhan Mammadova**

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The dissertation work was performed at the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan and the Laboratory of Enzymology of Photosynthetic Carbon Assimilation of the Institute of Molecular Biology and Biotechnologies.

Scientific supervisors:

Doctor of Biological Sciences, Associate Professor
Haji Mammadtaghi Shikhlinski

PhD in Biology, Associate Professor
Ulduza Ahmad Gurbanova

Official Opponents:

Doctor of Biological Sciences, Professor
Ahad Ali Nabiyev

Doctor of Biological Sciences, Associate Professor
Atabay Aghasoltan Jahangirov

PhD in Biology, Associate Professor
Narmina Fuad Abdullayeva

Dissertation Council ED 1.25 of the Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Molecular Biology and Biotechnologies.

Chairman of the Dissertation Council:

Doctor of Biological Sciences, Professor, Full member of ANAS
Irada Mammad Huseynova

Scientific secretary of the Dissertation Council:

Doctor of Biological Sciences
Yashar Mirza Feyziyev

Chairman of the Scientific Seminar:

Doctor of Biological Sciences, Associate Professor
Shahniyar Mikayil Bayramov

GENERAL CHARACTERISTICS OF THE RESEARCH

Relevance of the topic. Legumes, which constitute the main part of agricultural crops, play an important role in meeting the demand of population for food and other products[1].

Currently, the production of high-quality plant-based protein is one of the pressing issues of the world. In this regard, legumes are intensively cultivated in all countries of the world, their arable land occupies more than 130 million hectares. Legumes are distinguished from other plants by their high protein content (25-45%). The nutritional value of bean seeds is considered equal to meat products. They are eaten boiled or used in the baking industry in the form of flour. Legumes produce more protein than cereals. Their proteins have a very high solubility and are therefore well digested by the body. The content of essential amino acids (lysine, methionine, cystine, tryptophan) in legumes is 2-4 times higher than in cereals. Beans are also a valuable food crop.. In modern times, 22% of plant proteins and 7% of carbohydrates in the human nutrition ration, and 38% of proteins and 5% of carbohydrates in animal feed are provided by legumes[2].

The seeds of legumes are a highly nutritious and concentrated feed for animals. Lentils hold a special place among legumes due to their taste, high protein content, good digestibility by the human body, and many essential amino acids.

Beans are the largest legume in terms of cultivated area and production. The fresh plant is richer in minerals and vitamins, while its dried grains are richer in proteins.

The lentil (*Lens culinaris* Medikk.) and the bean (*Phaseolus vulgaris* L.) have more protein (20-30%) than cereals (10-12%), so they are widely used in the fight against protein deficiency and are considered a source of non-allergenic proteins. On the other hand, since the lentil plant has a low-fat content, it is cholesterol-free and easier to digest than other legumes.

Lentil proteins consist of both basic, alkaline, and amphoteric amino

[1] Qurbanov, F.H. Kənd təsərrüfatı bitkilərinin seleksiya və toxumçuluğu / F.H. Qurbanov.- Bakı: 2011 s.4.

[2] Adak, M.S. Baklagillerin Üretimini Artırma Olanakları / M.S.Adak, M. Güler, N. Kayan// Yemeklik VII. Türkiye Ziraat Mühendisliği Teknik Kongresi, ZMO Yayınları,- ANKARA: 2010, -46 s.

acids[3,4]. Recently, the study of the biochemical composition of grains containing gliadin, gluten, and globulin proteins has been widely used for the evaluation of grain quality indicators. During selection, storage proteins are used as convenient stable markers to characterize grain quality and other indicators[5].

Purpose and tasks of the research. The main purpose of the research was to evaluate the productivity, biochemical and genetic indicators of lentil and bean samples preserved in the National Genebank, to create core collections based on the study of the genetic diversity of the plant samples biomorphologically as well as with reserve proteins and molecular markers, and to prepare the scientific basis for their use in selection. For this purpose, the following tasks were set:

- Cultivation of lentil and bean samples and their evaluation based on yield elements;
- Grouping samples according to productivity indicators and selecting productive forms;
- Evaluation of existing genetic polymorphism according to DNA fragments in lentil and bean genotypes;
- Study of the activities of enzymes involved in amino acid metabolism in lentil and bean plants;
- Grouping genotypes based on molecular markers with ISSR markers and determining the degree of genetic similarity;
- Determination of polymorphism in lentil and bean genotypes through storage proteins;
- Creation of trait and core collections.

Main provisions of the dissertation presented for defense.

- It is possible that there is a correlation between yield elements

[3] Khazaei, H. Seed Protein of Lentils: Current Status, Progress, and Food Applications. Foods / H. Khazaei, M. Subedi, M. Nickerson, C. Martínez-Villaluenga, J. Frias, A. Vandenberg// -2019, 8(9), -p.391. doi: 10.3390/foods8090391.

[4] Sánchez-García, J. Nutritional and antioxidant changes in lentils and quinoa through fungal solid-state fermentation with Pleurotus ostreatus. Bioresour Bioprocess / Sánchez-García, J, Asensio-Grau A, García-Hernández J, Heredia A, Andrés A. // -- 2022, 11;9(1):51.

[5] Sadıqov, H.B. Tetraploid buğda genotiplərinin zülal polimorfizmi və keyfiyyət əlamətlərinin genetik markerlərlə əlaqəsi: / biologoya üzrə elmlər doktoru dissertasiyasının avtoreferatı /- Bakı 2021, -57s.

and productivity of the lentil and bean plants.

➢ The results obtained from determining the main biochemical indicators of the samples provide broad opportunities for explaining the biochemical mechanism of adaptation in the selection process.

➢ The existence of genetic polymorphism between specimens has been proven by ISSR and biochemical globulin markers.

➢ Comparison of the results of cluster analyses based on productivity indicators, biochemical and DNA markers shows that these genotypes have different genetic systems.

Scientific novelty of the research: For the first time, the biodiversity of the lentil and bean collection stored in the National Genebank was comprehensively assessed using morphological, biochemical, and molecular analyses, and samples that differed in terms of phenotype and genotype were identified. Using modern statistical methods, a core collection consisting of 46 lentil and 15 bean samples was created, which differed from each other in terms of biomorphological characteristics and productivity indicators, including genetic profiles, and reflected the genetic diversity in the studied collection.

Theoretical and practical significance of the research. High-yielding genotypes were also selected based on morphological traits and a trait collection was created. For the first time, genetic diversity in both lentil and bean plants was studied using globulin storage proteins as markers, and unique fragments were discovered, which can be used in genetic passportization. At the same time, highly polymorphic markers selected during the study can be used as genetic markers for differentiating lentil and bean specimens. For the lentil, UBC 823, UBC 810, UBC 809, UBC 827 ISSR primers can be used, and for the bean, UBC 826, UBC 817, UBC 888, UBC 868, UBC 843 primers can be used for assessing inter-genotype DNA polymorphism.

Approbation of the research. The results of the research were presented and discussed at the scientific conference dedicated to the 80th anniversary of Baku State University "Development Prospects of Experimental Biology" (2014), International Scientific-Practical Conference on Innovative Development of Agrarian Science and Education: World Experience and Modern Priorities (2015),

International Scientific Conference held at the Ganja State University "Actual Problems of Modern Chemistry and Biology" (2016), “Modern technologies for the production of environmentally friendly products for sustainable development of agriculture”, Tbilisi (2016), International scientific-practical conference organized by the Belgorod Research Institute “Innovative technologies for cultivation of white lupine and other grain crops” (2017), the IV International Scientific-Practical Conference “World plant resources status and development prospects” (Kyiv, 2018). International scientific conference "Actual problems of modern natural and economic sciences", GSU, Ganja (2018), Proceedings of the multidisciplinary international conference “Study of some biochemical parameters of lentil genotypes in the territory of the Republic of Azerbaijan”, Baku (2020), the International Conference " "Biodiversity, soil and water resources of Shusha and its surrounding areas: a vision of the future", Baku (2022), International Conference “Heydar Aliyev and the Nature of Azerbaijan” (2023), in the laboratories and scientific seminars of the Institute of Genetic Resources.

Publications. A total of 21 works are among local and foreign publications on dissertation materials, including 11 articles and 10 theses.

The organization where the dissertation work was performed: The dissertation work was carried out at the Institute of Genetic Resources and at the Laboratory of Enzymology of Photosynthetic Carbon Assimilation of the Institute of Molecular Biology and Biotechnologies of the Ministry of Science and Education of the Republic of Azerbaijan.

Structural sections of the dissertation. The dissertation consists of 185 pages, including an introduction, 6 chapters, conclusions, recommendations, and a list of cited literature, with a total of 271,032 characters. The dissertation is illustrated by 27 tables and 46 figures. An introduction contains 7 pages (12,563 characters), Chapter I - 38pages (70,310 characters), Chapter II-17 pages (24,836), Chapter III-17 pages (23,650), Chapter IV-15 pages (18,453 characters), Chapter- V-21 pages (21,084 characters), Chapter VI-23 pages (25 969 characters), Results, Conclusions and Recommendations are 7 pages (11,189 characters), 256 used literature sources are 31 pages (53,271 characters).

CHAPTER I. MAIN CONTENT OF THE RESEARCH

In this chapter, the origin, distribution and systematics of lentil (*Lens culinaris* Medicus) and bean (*Phaseolus vulgaris* L.) plants, their economic importance, their role in the world's food diet, morpho-physiological and biological characteristics, the role of amino acids in plant metabolism, stress factors and adaptation, the effect of biotic factors on plant growth and development, genetic markers, the role of molecular markers in determining biodiversity in higher plants, and genome maps of lentil and bean plants are extensively analyzed.

CHAPTER II. OBJECTS AND METHODS OF THE RESEARCH

The research objects were 46 lentil and 15 bean samples introduced from the ICARDA international center and originating in Azerbaijan. 45 of the lentil samples are of ICARDA origin, and 1 is of Azerbaijani origin.

Field characterization of lentil and bean genotypes was carried out under special experimental conditions at the Absheron research base of the Institute of Genetic Resources.

Protein was determined by the method of Keldal[6]. Lysine was determined by the method of A.S. Museyko and A.F. Sisiyanova[7]. Tryptophan was determined by the method of A. Yermakov, N.R. Yarosh[8].

A spectrophotometric method was used to determine the activity of NAD-malate dehydrogenase (NAD-MDH, EC 1.1.1.37)[9],

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[6] Kjeldahl, J. "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern" (New method for the determination of nitrogen in organic substances), *Zeitschrift für analytische Chemie*, 1883, 22 (1) : 366-383.

[7] Мусейко, А.С,. Сысоев, А.Ф. Определение лизина в семенах // Доклады ВАСХНИЛ, 1970, 6, -с. 8- 12.

[8] Ермаков, А.И., Ярош, Н.П. Определение триптофана в семенах // Бюл. ВИР, 1969, 14, -356 с.

[9] Scheibe, R. Strategies to maintain redox homeostasis during photosynthesis under changing conditions / R. Scheibe, J. Backhausen, V. Emmerlich, S. Holtgrefe // J. Exp. Bot., -2005, 56, -p. 1481-1489.

aspartate aminotransferase (AsAT, EC 2.6.1.1) and alanine aminotransferase (ALAT, EC 2.6.1.2) enzymes[10]. The results obtained from the extraction of nuclear DNA from lentil and bean samples were statistically analyzed using the SPSS computer program. The degree of variation of yield components among genotypes and the statistical significance of this variation were evaluated using the ANOVA method. Cluster analysis for agronomic indicators was performed using the UPGMA (Unweighted Pair Group Method Using Arithmetic Average) method based on Euclidean genetic distance. Besides, using the UPGMA method, the Jaccard genetic similarity coefficient was calculated with the application of ISSR primers. Electrophoretic analysis of globulin storage proteins in grains of 46 lentil and 15 bean genotypes selected for the study was performed on polyacrylamide gel (A-PAGE) using a new improved method developed by the modification of the Poperelyan's method.

CHAPTER III. EVALUATION OF LENTIL AND BEAN GENOTYPES ACCORDING TO YIELD ELEMENTS

3.1. Comparative structural analysis of lentil genotypes using statistical methods.

46 lentil samples of various origins were cultivated under irrigated conditions at the Absheron research base of the Institute of Genetic Resources of the Republic of Azerbaijan for 3 years.

The structural elements of the samples (plant height, height of the first pod, number of branches, number of grains per plant, number of grains per pod, pod height, number of pods per plant, mass of 100 seeds) were analyzed, and the average indicators for 3 years were calculated based on the results obtained. A sharp variation was recorded in the number of pods and seeds. Thus, the number of pods per plant was 50.0-167.0, and the number of seeds per plant was 50.0-225.7. The mass of 100 seeds varied between 2.5-5.2 g. A significant positive correlation

[10] Alfonso, SU. Photosynthetic responses of a C(3) and three C(4) species of the genus Panicum (s.l.) with different metabolic subtypes to drought stress/ SU Res. - 2012 Sep; v. 112(3): -p.175-91. doi: 10.1007/s11120-012-9763-4.

(P<0.05, P<0.01) was found between the height of the plant and the number of pods per plant, and the number and mass of grains per plant. However, the significance of this correlation varied. While the correlation between the height of the first pod and the number of grains per plant and the number of pods was not statistically significant, there was a moderately significant (P<0.01) positive correlation between this trait and plant height. A positive correlation was recorded between the number of seeds per plant and the number of pods, and a negative correlation (r=-0.093) was recorded between the number of seeds per plant and the mass of 100 seeds. Cluster analysis was based on the number of seeds and pods per plant, which allowed the genotypes to be divided into groups. The first cluster is the largest group, comprising 46% of the studied samples. The samples included in this group can be considered tall and medium-yielding, the samples included in the II cluster are tall and high-yielding, in the III cluster, short and low-yielding, in the IV cluster, tall and high-yielding, and in the V cluster, short and high-yielding. Thus, the analyses revealed a moderate (P < 0.01) statistically significant genetic diversity among 46 lentil genotypes according to individual morphological and quantitative traits. It was found that 21.7% of the studied samples were highly productive, 45.7% were moderately productive, and 32.6% were low-productive. Among the genotypes, Flip 2011-61, Flip 2011-41, Flip 2011-43, 10940, 10939, 10929, and Jasmin were evaluated as highly promising samples.

3.2 Comparative study of yield indicators in bean genotypes using statistical methods.

15 bean samples were cultivated under irrigated conditions at the Absheron research base of the Institute of Genetic Resources of the Republic of Azerbaijan for 2 years, and the structural elements of the samples (plant height, height of the first pod, the number of branches, the number of grains per plant, the number of grains per pod, pod height, the number of pods per plant, mass of 100 seeds) were determined and the results obtained were analyzed. The height of the studied samples varied greatly in both years, ranging from 35-120 cm, the number of grains per plant from 33-114, and the mass of 100 seeds from 23g to 44 g. Path analysis was performed to identify traits that directly or indirectly affect yield. The main yield elements that directly affect seed mass in bean

plants were the number of seeds per plant (1.14-1.11), the number of pods (0.91-0.94), plant height (0.14-0.17), and the number of branches (0.24-0.27). The number of branches and the mass of 100 seeds were evaluated as traits that indirectly affect productivity. To determine the relationship between productivity elements, a correlation analysis was conducted based on the average values of these indicators for two years. In the bean plant, a significant positive correlation was found between the pod size and the height of the first pod (r ~ 0.670**), the mass of 100 seeds and the height of the first pod (r ~ 0.656**), the number of seeds per plant and the height of the plant (r ~ 0.565*), the pod length and the mass of 100 seeds (r ~ 0.646*). Cluster analysis was conducted based on yield elements and the samples were grouped into 3 clusters. It was found that 33.3% of the studied samples were high-yielding, 26.7% were medium-yielding, and 40% were low-yielding. The genotypes, such as Aze PHA- t / 16, K-13038, Aze PHA- t / 15, K-3493, Afgo-2027, Saksa, Aze PHA- t / 18 were evaluated as highly promising samples.

CHAPTER IV. STUDY OF BIOCHEMICAL INDICATORS OF LENTIL AND BEAN GENOTYPES

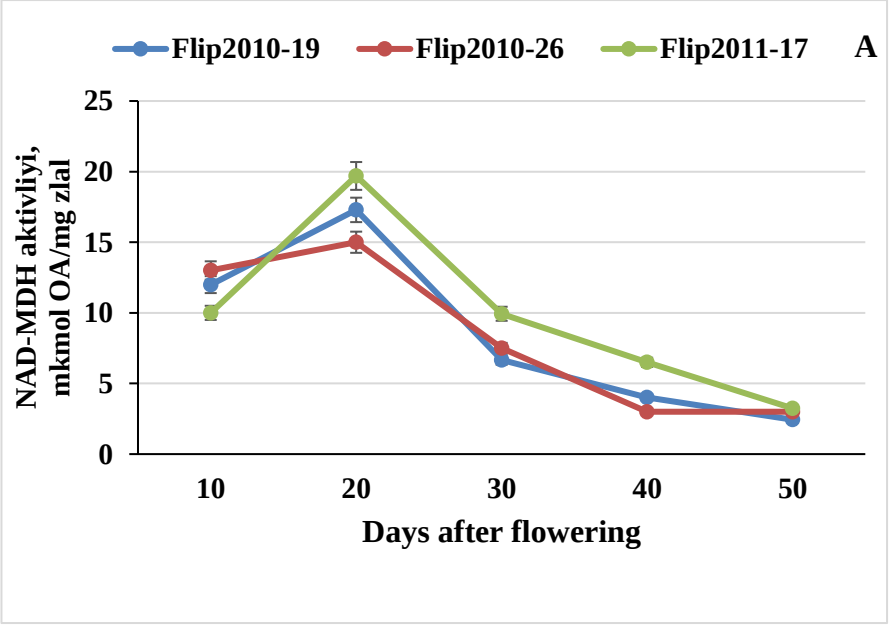
Determination of total protein, lysine, and tryptophan in lentil plants was carried out on lentil samples grown under irrigated conditions between 2013 and 2016. The protein content ranged from 23.5% to 30.4%, the lysine content ranged from 644 to 974 mg, and the tryptophan content ranged from 170 to 242 mg. Among the samples, the genotypes Flip2010-19, Flip 2011-20, Flip 2011-19, 10946, 10930, 10926, and 10934 were distinguished by the high amount of protein. Flip 2010-19, Flip 2011-18, Flip 2011-14, Flip 2011-41 and 10930 were distinguished by the high amount of tryptophan, and Flip 2011-36, Flip 2010-26, Flip 2010-81, 10942, and 10934 were distinguished due to the high amount of lysine.

Determination of total protein, lysine, and tryptophan in bean plants. According to the results of the research we conducted on bean plants under irrigated conditions in 2015-2016, the protein content in bean samples ranged from 22.7 to 29.0%, the lysine content from 633 to 845 mg, and the tryptophan content from 195 to 255 mg. Aze PHA-t/6, Aze PHA-t/15, Aze PHA-t/16, K-3493, Aze PHA-K-37 were distinguished by their high protein content; Aze PHA-t/16, Aze PHA-

t/15, Aze PHA-t/17, Aze PHA-K-37, AFQO 2027, K-13038, K-3493 and St.Yerli piyada were distinguished by their high lysine content; and AzePHA –t/6, AzePHA – 18, K-3493, Aze PHA -t/17, K-13038 and Galibiyet were distinguished by their high tryptophan content.

Study of some carbon and nitrogen enzymes in developing grains of lentil and bean plants. The highest NAD-MDH activity in ripening lentil grains was found in Flip2010-26 lentil samples, which had relatively low protein potential (Figure 4.1).

Unlike the lentil plants, the highest NAD-MDH enzyme activity in bean grains was observed 30 days after flowering in Aze PHA – t/17 and Aze PHA – t/18 samples and 20 days after flowering in Aze PHA – t/16 samples. The highest enzyme activity was recorded in Aze PHA – t/16 samples with high protein potential. Twenty days after flowering, the enzyme activity in Aze PHA – t/18 bean samples with low protein potential was 1.3 times lower than in Aze PHA – t/16 bean samples.



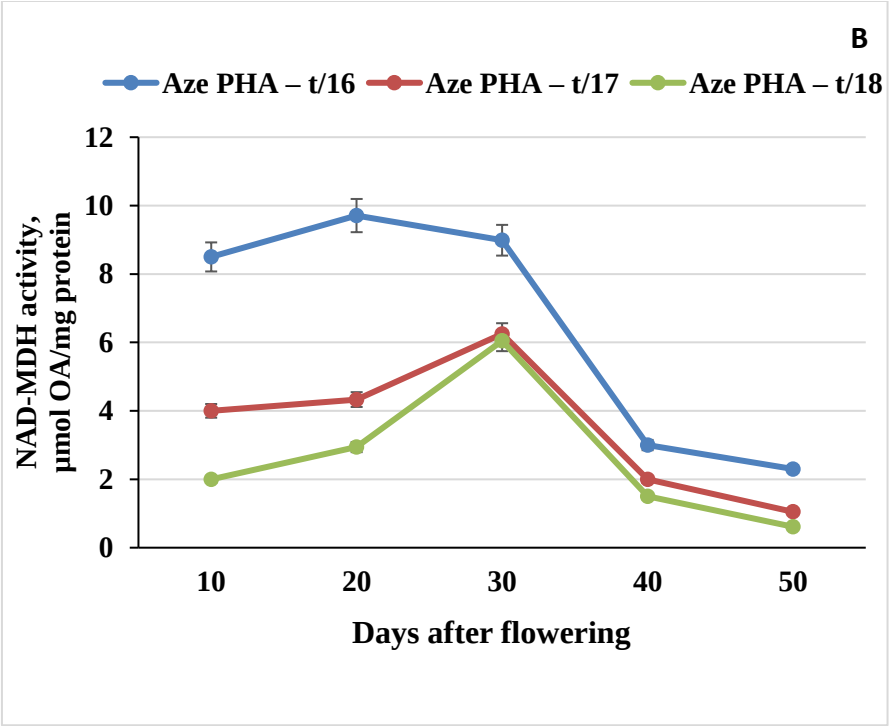


Figure 4.1. Dynamics of time-dependent changes in NAD-MDH activity in ripening grains of lentil and bean plants

In lentil and bean grains, the enzyme activity decreased sharply at the end of grain ripening. The results of some previous studies show that the genesis, activity, and isoenzyme spectrum of MDH in plant cells determine their species characteristics and the main type of metabolism.

AsAT activity was determined spectrophotometrically according to the method of Alfonso et al. AsAT activity varied with the same time pattern in germinating lentil and bean grains, with the highest enzyme activity observed 24 days after flowering. Thus, the highest enzyme activity was observed 24 days after flowering (Figure 4.2).

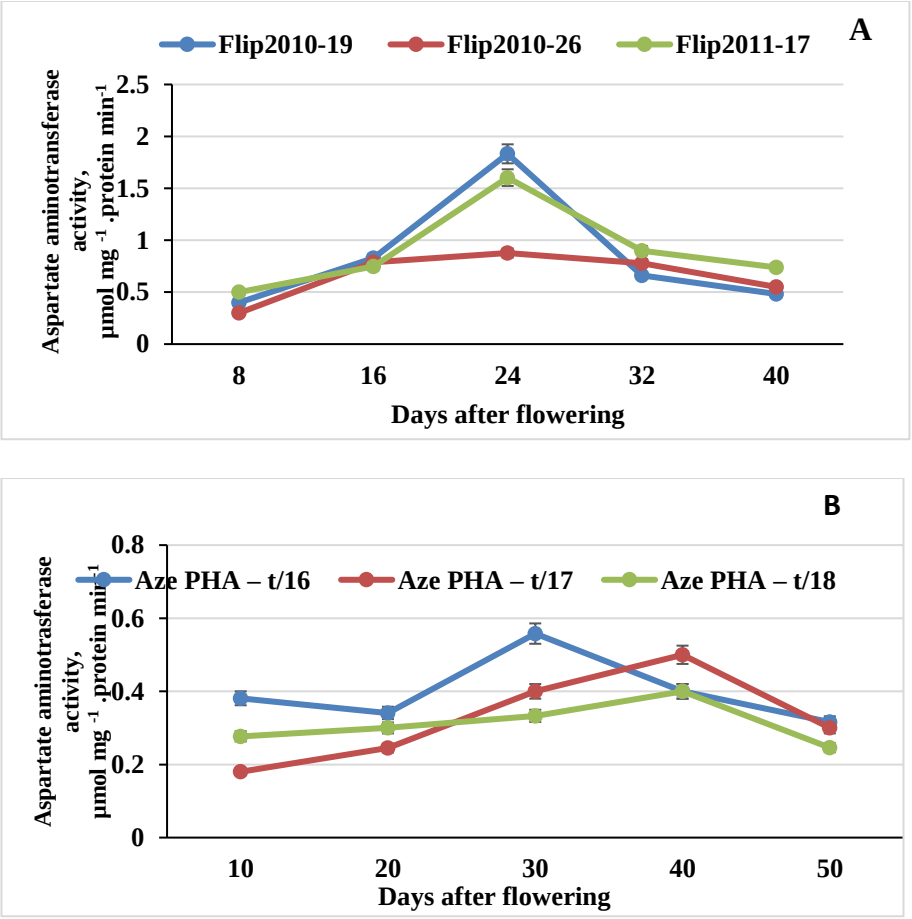


Figure 4.2. Dynamics of time-dependent changes in AsAT activity in ripening grains of lentil and bean samples

The highest activity of the AsAT enzyme in ripening lentil grains was observed 24 days after flowering in all samples. The lowest activity of the enzyme was observed in the Flip2010-26 sample, and the highest activity was detected in the Flip2010-19 sample.In ripening bean grains, the highest activity of AsAT was recorded in Aze PHA – t/16 samples, which had high protein potential, 30 days after flowering, and in Aze PHA – t/17 and Aze PHA – t/18 samples 40 days after flowering. A sharp decline in enzyme activity was observed on the 50th day of

grain ripening.

Different results were obtained when studying the dynamics of time-dependent changes in ALAT activity in ripening lentil and bean grains (Figure 4.3).

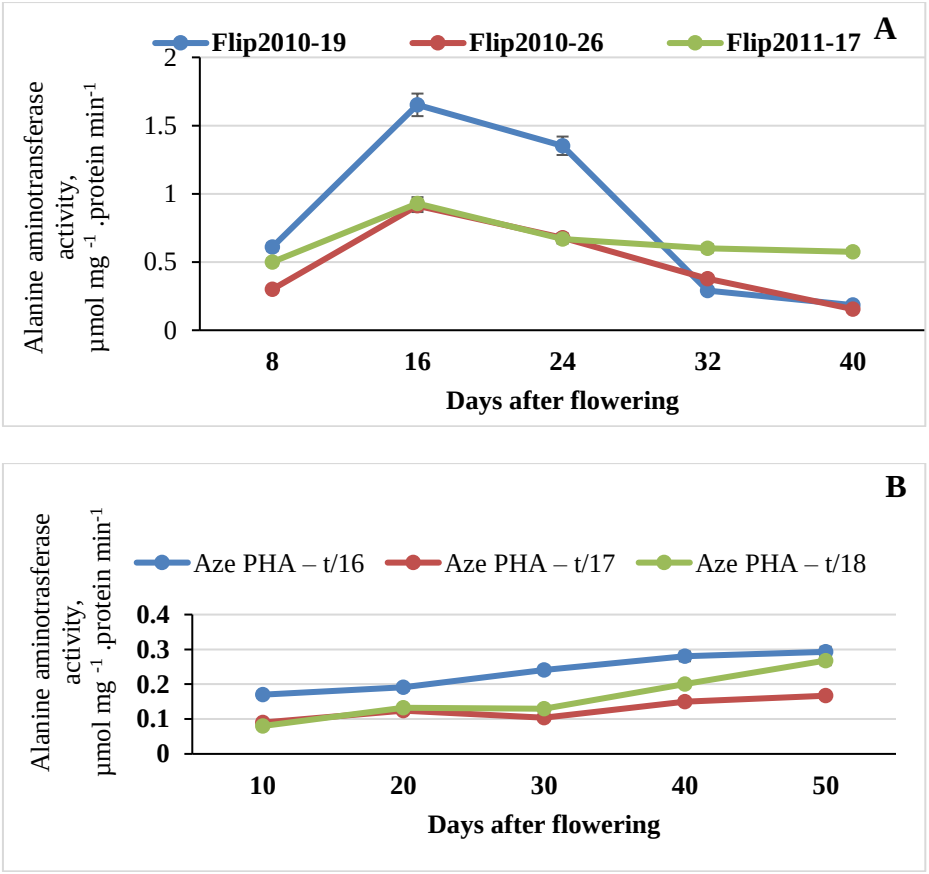


Figure 4.3. Dynamics of time-dependent changes in ALAT activity in ripening lentil and bean grain samples

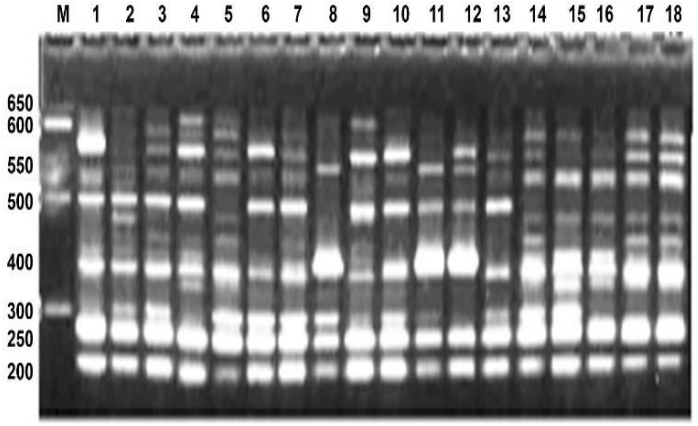
Thus, the highest activity of the ALAT enzyme was observed in ripening lentil grains in all three samples 16 days after flowering. The enzyme activity in the Flip2010-19 sample, which had a high protein potential, was 2 times higher than in the Flip2010-26 and Flip2011-

17 samples 16 days after flowering. As in the case of AsAT and NAD-MDH enzymes, the activity of ALAT in both bean and lentil grains declined sharply at the end of grain ripening.

Similar to NAD-MDH and ASAT, the highest activity was recorded in the Aze PHA – t/16 samples with high protein potential. The results of the study indicate that the mentioned enzymes play an important role in balancing carbon and nitrogen metabolism in lentil and bean plants.

CHAPTER V. STUDY OF INTRASPECIFIC POLYMORPHISM USING DNA MARKERS

In our study, out of 12 ISSR markers used to assess the diversity and relationship between lentil genotypes, 3 were monomorphic and 9 were polymorphic (Figure 5.1). The studies were continued with 9 ISSR markers with polymorphic and clear bands, and 76% polymorphism was recorded. In total, 69 bands were synthesized, of which 52 bands were polymorphic. The value of the GDI (Genetic diversity index) was 0.67, and the values of the EMR (Multiplex Efficiency) and MI (Marker Index) parameters studied to evaluate the discrimination ability of the ISSR marker system varied between 5.54-10.33 and 1.68-3.51, respectively.



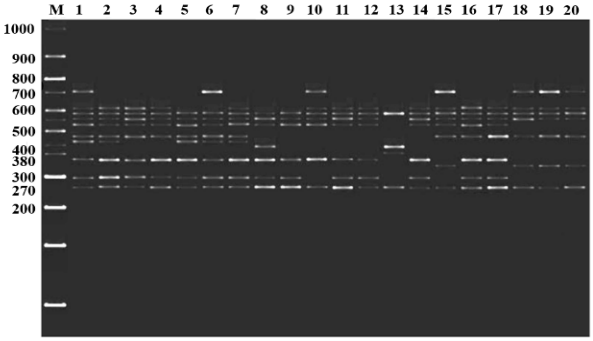


Figure 5.1. Distribution of alleles synthesized with the UBC 818 and UBC835 primers in lentil genotypes

The highest value of the Marker Index (3.51) was observed in the UBC 827 primer, which synthesized 10 bands, and the lowest value (1.68) was observed in the UBC 823 primer, which synthesized 6 bands.To group lentil samples based on genetic polymorphism and estimate the genetic distance between them, a cluster analysis was performed and a dendrogram that divided the genotypes into 6 main groups was compiled (Figure 5.2). Cluster analysis was able to distinguish between closely related genotypes as well as distant genotypes. Thus, the samples Flip 2010-96 and Flip 2011-41, Flip 2011-32 and Flip 2011-97, 10932 and Flip 2011-20, Flip 2010-81 and Flip 2011-19 were evaluated as the most distant genotypes.

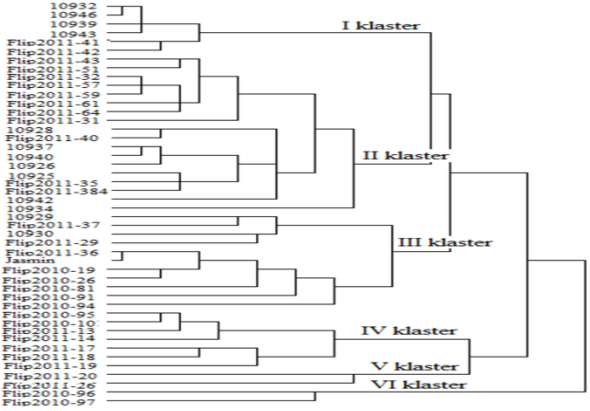


Figure 5.2. Dendrogram depicting the genetic relationship of lentil genotypes based on ISSR markers

Study of intraspecific polymorphism in bean plants using DNA markers.

To determine the polymorphism in bean samples, an analysis of 15 ISSR markers was performed. Among these ISSR primers, 10 polymorphic primers that amplified fragments of the expected length were selected and used in the research. A total of 80 bands were synthesized, of which 70 bands were polymorphic, with 87.5% polymorphism recorded. The average values of GDI and PIC were 0.87 and 0.33, respectively, while the average values of EMR (Multiplex Efficiency) and MI (Marker Index) parameters were 7.4 and 2.44, respectively.

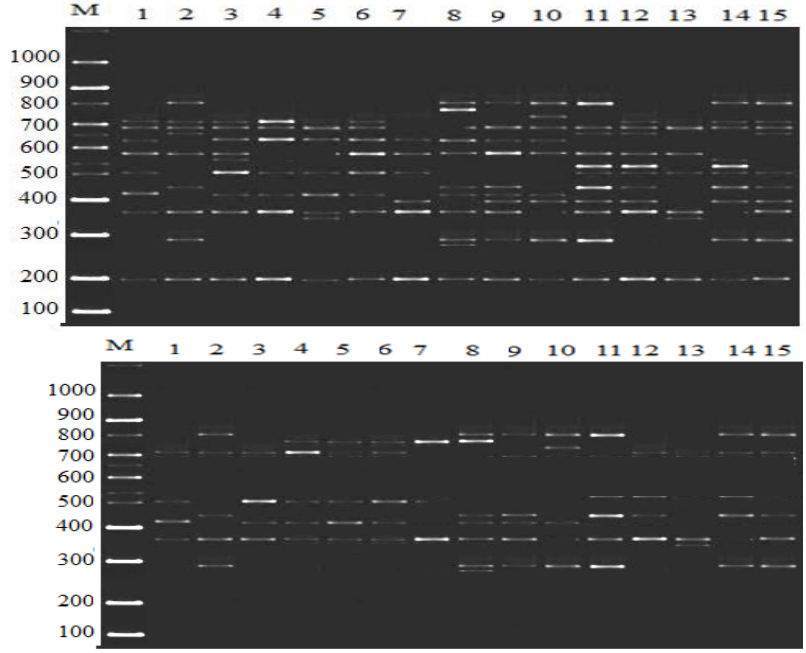


Figure 5.3. Distribution of alleles synthesized with the UBC826 and 817 primers in bean genotypes

Only Afgo-2027 and Aze PHA-t/18, K13038, and K3493 samples had high genetic similarity index values. Cluster analysis further confirmed the high polymorphism in genotypes and grouped the samples into 3 clusters based on Nei's genetic distance index

(Figure 5.4). According to the genetic distance index, the closest genotypes to each other were AZE PHA t/-18 and Afgo 2027, K130-38 and K3493, and the most distant genotypes to each other were AZE PHA T/17 and Aze PHA -18, Galibiyet and St.Yerli piyada samples.

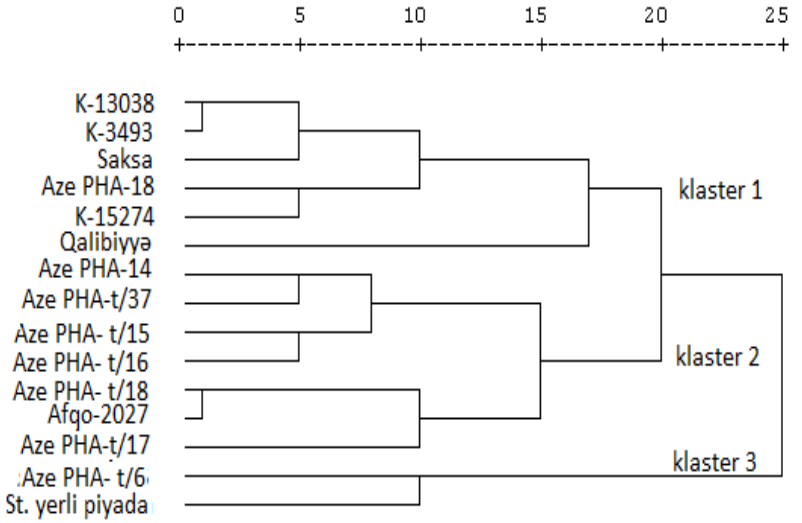


Figure 5.4. Dendrogram depicting the genetic relationship of bean samples based on ISSR markers.

CHAPTER VI. STUDY OF GENETIC DIVERSITY OF LENTIL AND BEAN GENOTYPES BASED ON GLOBULIN PROTEIN

Protein markers are among the main markers used in the genetic identification of plants. In our research, the electrophoregrams of globulin proteins obtained during vertical electrophoretic analysis of legumes using protein markers, which is a modification of the ACID-PAGE method, were conventionally divided into four zones called ω-, ϒ-, β- and α-globulins. The genetic diversity of 46 lentil genotypes was studied using globulin storage proteins, and the electrophoresis patterns obtained as a result of gel electrophoresis analysis are displayed in Figure 6.1. As seen in the figures, the spectra (bands) observed in the studied genotypes were distributed in 4 zones: ω-, γ-,

β-, and α-zones, depending on the molecular mass of the globulins and the speed of movement in polyacrylamide gels.

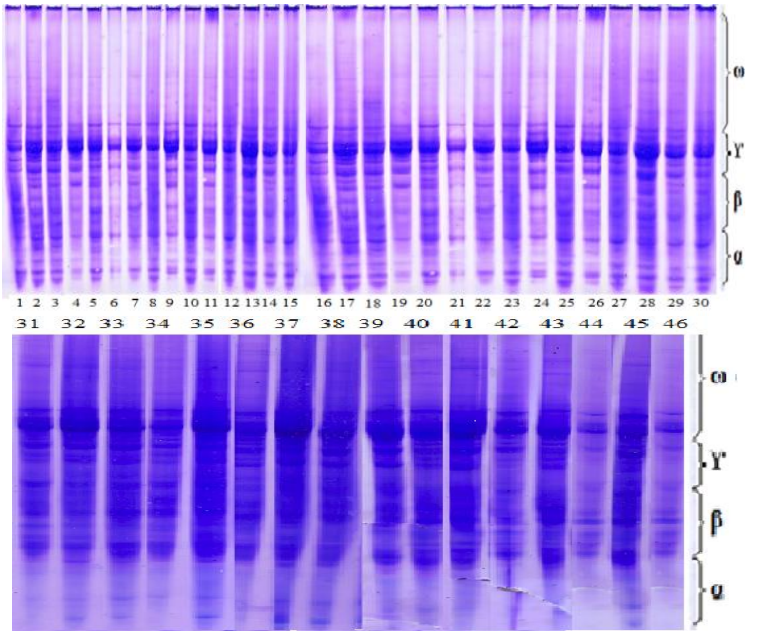


Figure 6.1. Results of electrophoresis of globulin storage proteins in the lentil genotype.

As a result of electrophoretic analysis of globulin proteins, 24 spectra and 55 patterns (combinations of spectra formed in different zones in each genotype) were detected in all zones in 46 lentil genotypes, a relatively large number of spectra were observed in the ω- and γ- zones (7 spectra in each), and 5 spectra were detected in each of the β- and α- zones. 7 different spectra (bands) were found in the ω-zone, of which spectra 1, 6 and 7 were observed in 32, 46 and 41 samples, and were selected as high-frequency, while spectra 2, 3, 4 and 5 were observed in genotypes 13, 19, 12, 18, respectively.

22 different patterns were detected in the ω-zone (Table 6.2.), although only pattern number 1 is found in 7 genotypes, each of the other patterns was observed in three or fewer genotypes.

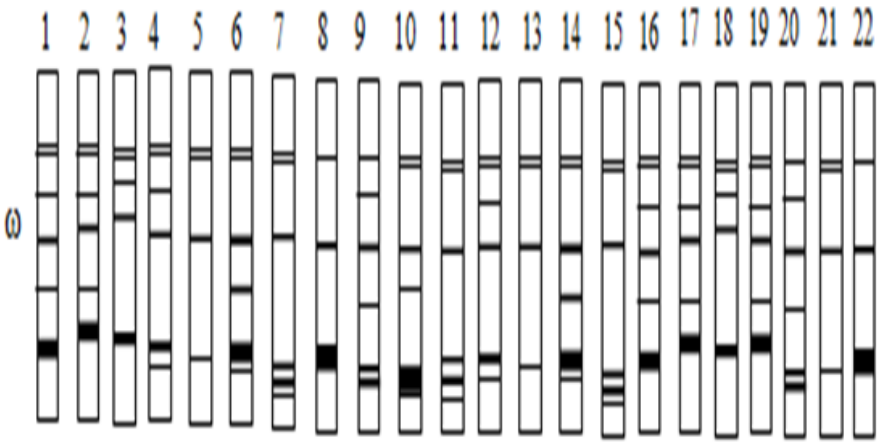


Figure 6.2. Ideogram of patterns observed in the ω-zone of globulin storage proteins.

The value of Nei's genetic diversity index calculated based on the frequencies of occurrence of patterns in the ω-zone of globulin storage proteins was equal to 0.930. Seven spectra and nine different patterns were detected in the γ-zone of globulin storage proteins. Among the observed spectra, spectra 2 and 4 found in 46 genotypes were evaluated as high-frequency spectra, and spectra 1, 5 and 6 recorded in the 44-45-42 sample were evaluated as high-frequency spectra. Spectrum number 3, recorded in 39 samples, was characterized as medium frequency, and spectrum number 7, recorded in 24 samples, was characterized as low frequency. Of the patterns in the γ-band, pattern number 2 was recorded in 17, pattern number 6 in 15, pattern number 3 in 5, pattern number 8 in 3, and pattern number 4 in 2 samples. Each of the other patterns was specific to one sample. The value of the genetic diversity index was calculated to be H= 0.743.

Five spectra and 11 patterns were observed in the β-zone. Of these, spectrum number 3 was observed in 46 genotypes, spectrum number 5 in 40 genotypes, spectrum number 2 in 32 samples, spectrum number 1 in 19 samples, and spectrum number 4 in 23 samples (Table 6.1).

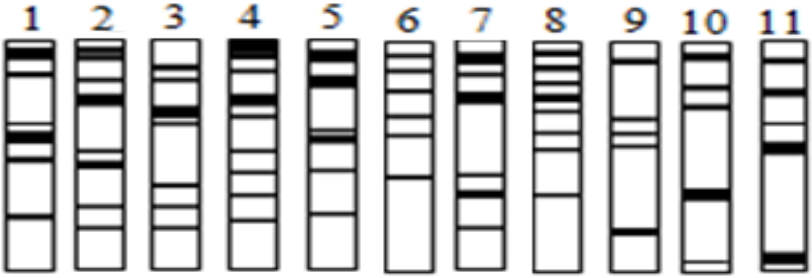


Figure 6.3. Ideogram of patterns observed in the β-zone of globulin storage proteins

As for the patterns, pattern number 5 was recorded in 9 genotypes, patterns number 1, 8 and 9 were recorded in 5 genotypes each, and patterns number 2 and 3 were recorded in 7 genotypes each. Patterns number 4, 10 and 11 were each observed in one genotype, while pattern number 6 was found in only 2 genotypes (Figure 6.1.). The value of Nei's genetic diversity index calculated for the β-zone was equal to 0.872.

Five spectra were recorded in the α-zone of globulin storage proteins, spectrum number 3 was observed in 42 genotypes and had a high frequency, spectrum number 4 was recorded in 37 genotypes and had a medium frequency, spectra 1 and 5 were recorded in 31 genotypes each, and spectrum 2 was recorded in 28 genotypes and characterized by a low frequency. Nei's genetic diversity index calculated for the α-zone was 0.827. Of the 13 different patterns found in the α-zone, pattern 6 was recorded in 9 genotypes, patterns 3 and 5 were recorded in 8 genotypes each, patterns 10 and 11 were recorded in 5 genotypes each, and patterns 2 and 4 were recorded in 2 genotypes each. Each of the patterns 1, 7, 8, 9, 12, and 13 was unique and specific to only one genotype.

Cluster analysis was used to determine the genetic distances between lentil genotypes based on polymorphisms of globulin storage proteins. Jaccard genetic similarity indices in genotypes were determined using a dendrogram (Figure 6.4), which is a graphical representation of the cluster analysis conducted based on the UPGMA method, and the 46 lentil genotypes were grouped into 5 clusters.

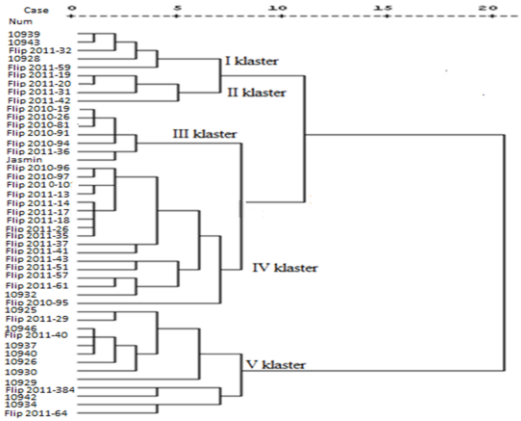
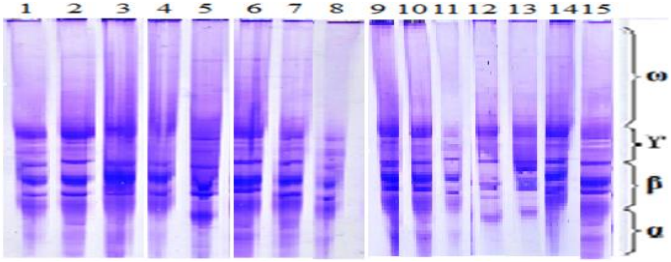


Figure 6.4. Grouping of lentil genotypes based on globulin storage proteins

Study of polymorphism of globulin storage proteins in bean genotypes. In the research, the genetic diversity of 15 bean genotypes was studied based on globulin storage proteins. The observed spectra (bands) were distributed in 4 zones: ω, γ, β, α zones, depending on the molecular mass of the globulins and their migration speed in polyacrylamide gels. As a result of electrophoretic analysis of globulin storage proteins, 16 spectra and 21 patterns were detected across all zones in 15 bean genotypes, with 4 spectra in each zone, and a larger number of their combinations (7 patterns) were observed in the α zone.



K-37,AzePHA-t/16,AzeAPH-t/17,AzePHA-t/18,saksa,AzePHA-18,K-15274,AzePHA-14,Galibiyet,K-3498, K-13038,Afqo-27,AzePHA-t/6, ,AzePHA-t/15,Yerli piyada.

Figure 6.5. Results of electrophoresis of globulin storage proteins in the bean genotype

Four different spectra and five patterns (bands) were detected in the ω zone, and the highest genetic diversity (H=0.993) among the globulin zones of the studied genotypes belonged to the ω zone. 4 spectra and 4 different patterns were detected in the γ-zone of globulin storage proteins, and Nei's genetic diversity index value was equal to 0.580. Four spectra and five patterns were observed in the β zone of globulins, and Nei's genetic diversity index calculated for the β zone was 0.707. Four spectra and seven patterns were detected in the α zone, and Nei's genetic diversity index was 0.837.

To determine the genetic distance between bean genotypes, cluster analysis was conducted based on the UPGMA method and the genotypes were graphically depicted using a dendrogram (Figure 6.6). Based on the polymorphism of globulin storage proteins, Jaccard genetic similarity indices between genotypes were determined, and the 15 studied bean genotypes were grouped into 4 clusters.

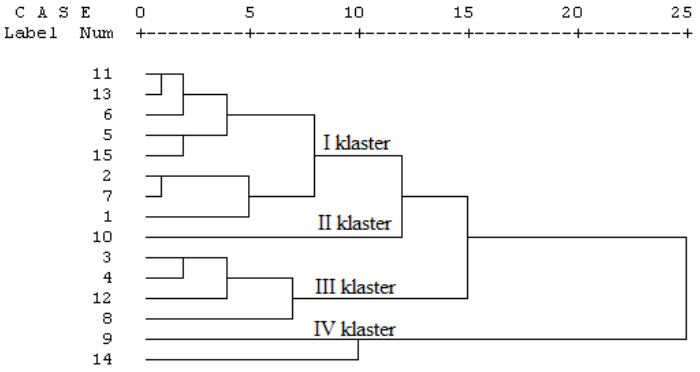


Figure 6.6. Grouping of bean genotypes based on globulin storage proteins

Comparative study of genetic diversity in the lentil plant based on technological indicators, protein and DNA (ISSR) markers.

Biomorphological, biochemical and DNA markers were used in a complex manner to study the genetic structure of lentil samples. As a result of the study, high genetic diversity was revealed through these markers (Figure 6.7). Unlike ISSR analyses, protein marker analysis

showed higher polymorphism. Besides, genetic diversity calculated based on biochemical parameters was higher than that calculated based on morphological traits and ISSR marker analyses.

The globulin marker used in the study belongs to biochemical markers, while ISSR markers belong to DNA markers, and the influence of environmental factors on them is insignificant. In this regard, both globulin and ISSR markers indirectly serve to detect polymorphisms present in the genome, and the existence of a dependence between them is real.

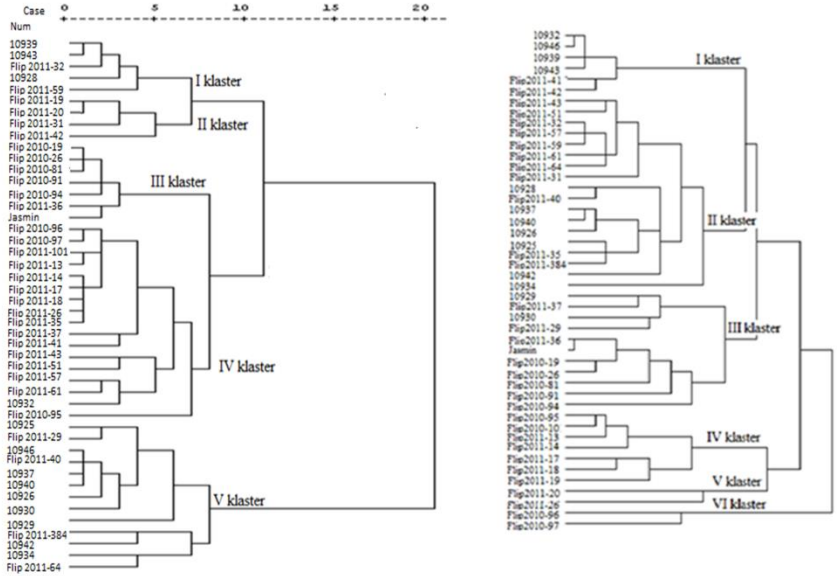


Figure 6.7. Grouping of lentil genotypes based on globulin storage proteins and ISSR markers

Dendrograms obtained from the analysis of genetic diversity at the genome level with ISSR and globulin markers were compared with dendrograms based on quantitative traits and then, correlation analysis was performed. The results obtained when studying the genetic diversity using molecular markers were completely different from the results of morphological analyses. Although no significant correlation was recorded between globulin proteins, ISSR markers, biochemical and morphological traits, some compatibility was

observed. During the study, the analysis of Globulin storage proteins grouped the samples into 5 clusters, and the ISSR marker analysis grouped them into 6 clusters (Figure 6.7, Figure 6.9). Samples 10937, 10940, and 10926, as well as Flip 2011-36 and Jasmin, were located at a close genetic distance in both clusters. Samples 10926, 10928, 10940, and 10937 were grouped in the same cluster as a result of both analyses. The existence of a statistically significant dependence between the genetic similarity indices of markers determining genetic structure at the genome level is completely logical.

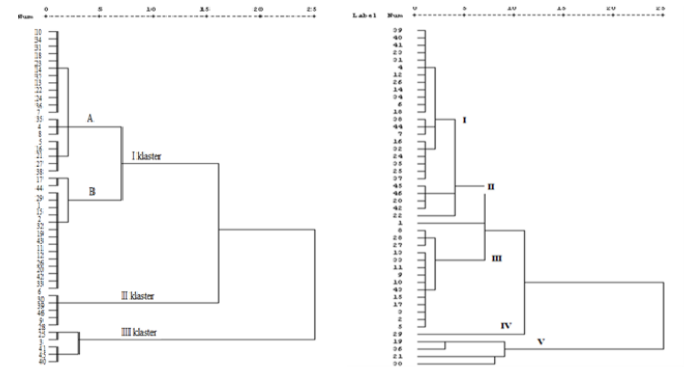


Figure 6.8. Dendrograms displaying the affinity of lentil genotypes based on biochemical parameters and morphological quantitative traits

A correlation of r=0.189 was recorded between productivity indicators and ISSR markers, and a correlation of r=0.194 was recorded between productivity indicators and globulin markers.

Comparative analysis of genetic diversity in lentil plants using technological parameters, protein and DNA (ISSR) markers.

Biomorphological, biochemical and DNA markers are used in a complex way to study the genetic structure of bean plant samples. Genetic similarity indices were compared with each other. As a result of the study, high genetic diversity was revealed based on these markers. In the bean plant, unlike the lentil plant, the genetic diversity index obtained from ISSR marker analysis was higher (H=0.870). The

genetic diversity index calculated based on biochemical parameters (H=0.721) and the genetic diversity index calculated based on morphological traits (H=0.694) was also lower than the genetic diversity index calculated based on globulin marker analyses (H=0.779) (Figure 6.9).

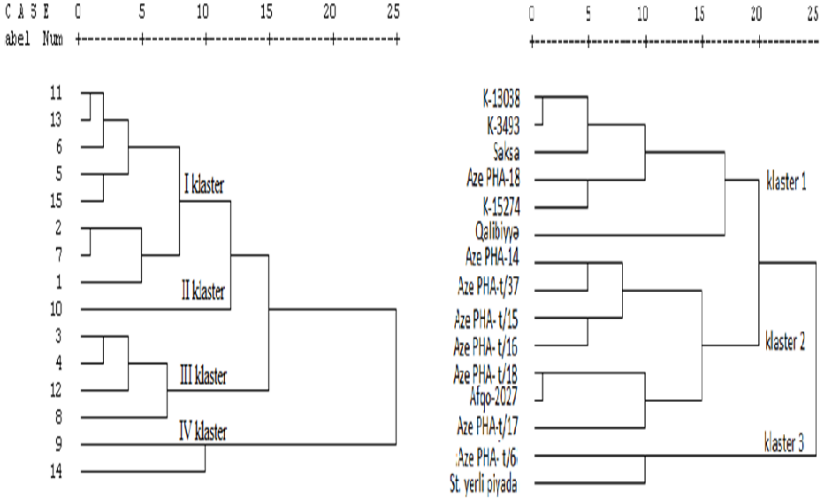


Figure 6.9. Grouping bean genotypes based on globulin storage proteins and ISSR markers

An insignificant correlation was recorded between ISSR and globulin markers (r=0.451). During the study, the analysis of globulin storage proteins divided bean samples into 4 clusters, and ISSR marker analysis into 3 clusters (Figure 6.9). Aze PHA t/18, Aze PHA t/17, and Afgo2027 samples were located in the same group at close genetic distance.

For bean samples, the correlation between morphological traits and ISSR markers was calculated to be 0.341, and the correlation between morphological traits and globulin markers was found to be 0.254. The influence of ecological factors on morphological traits is high, and although the diversity of these traits does not reflect the true genotype of the plant, they play an important role in the study of genetic diversity. Since quantitative indicators and molecular markers each explain the genetic diversity of the studied genotypes in different

ways, it is more appropriate to use them together during the study.

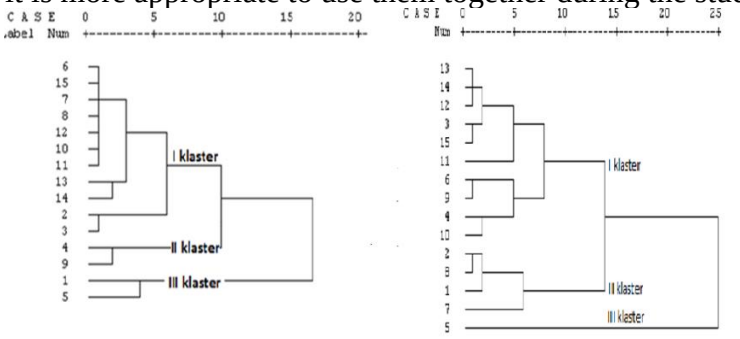


Figure 6.10. Dendrograms displaying the affinity of lentil genotypes based on biochemical parameters and morphological quantitative traits.

An insignificant correlation was recorded between biochemical parameters and morphological traits (r=0.149). The results of both analyses grouped the bean samples into 3 clusters (Figure 6.10).

A more thorough analysis of the genetic diversity of genotypes using ISSR markers, which are DNA markers, revealed that each of the DNA fragments studied and their sizes varied over a wide range for lentil and bean genotypes. In the study of both plants, While some markers in the research of both plants displayed significant polymorphism, some markers displayed low polymorphism. During the investigation of genetic polymorphism of samples with ISSR molecular markers at the DNA level, each marker locus obtained showed a wide variation in their occurrence frequencies and sizes. No specific relationship between the genetic structure and geographical distribution of lentil and bean genotypes was found.

A significant genetic diversity was found among the genotypes we studied, which were distinguished by different DNA fragments. The dendrograms we compiled based on DNA fragments allowed us to distinguish both lentil and bean genotypes from each other and group them according to their degree of affinity.

The value of the genetic distance index between bean genotypes was 0.76-0.95, and between lentil genotypes, it was 0.56-0.81 (for an average of 44% of the total), and during the

characterization of both bean and lentil samples, no genotypes showing 100% genetic similarity were recorded. The greatest similarity among bean genotypes was identified in samples K-13038 and K-3493, AZE PHA-t/18 and Afgo 2027, and among lentil genotypes, Jasmin and Flip 2011-36, 10932 and 10946. In general, the study revealed a high level of genetic diversity among both introduced bean and lentil samples, with average PIC values of 0.33 and 0.34, respectively. Lentil genotypes were grouped into 6 clusters and bean genotypes into 3 clusters, with no relationship between their distribution and the regions where they were collected. This information, which reflects the genetic distance indices of samples located in the same or close clusters, can be used in further studies. This information, which reflects the genetic distance indices of samples located in the same or close clusters, can be used in further studies.

The results of the characterization of genetic diversity among samples show that ISSR markers are suitable and effective for the differentiation of both lentil and bean genotypes. By crossing samples chosen for high productivity indicators between genetically distinct, distant genotypes, it is possible to derive forms of selection importance using the results obtained.

The genetic distance between both lentil and bean genotypes was determined by cluster analysis using the UPGA method, based on the polymorphism of globulin storage proteins. The polymorphism of globulin storage proteins in the grains of the analyzed samples was determined by the spectra and patterns related to the protein components. In the ω-, γ-, β-, and α-zones of the electropherograms of globulin storage proteins, 22, 9, 11, and 13 different electrophoretic patterns were detected in lentil samples, and 5, 4, 5, and 7 in bean samples, respectively, with high polymorphism.

Finally, the degree of relationship between agronomic, molecular, and biochemical indicators was examined using the Mantel test, and statistically insignificant correlations were observed between the matrices. This indicates that the three separate systems assess the genetic relationship between the samples differently. In samples, Flip 2010-97, Flip 2011-31, Flip 2011-31, 10937, 10943,

10942, and 10934 having high productivity and protein content, a γ-2p pattern was observed during globulin marker analysis, which had been also observed in other studies.

Thus, the presented research is the successful results of the joint application of molecular genetics, field experiments, and computer methods, including morphological quantitative indicators, biochemical analyses, and genetic variations in DNA fragments in introduced lentil and bean genotypes.

CONCLUSIONS

1. It was found that among 46 lentil varieties stored in the National Genebank, 21.7% are high-yielding, 45.7% are medium-yielding, and 32.6% are low-yielding. Among 15 bean varieties, 33.3% are classified as high-yielding, 26.7% as medium-yielding, and 40% as low-yielding. Among the lentil genotypes, Flip 2011-61, Flip 2011-41, Flip 2011-43, 10943, 10939, 10929, and Jasmin were evaluated as highly promising samples, while among the bean genotypes, Aze PHA-t/16, K-13038, Aze PHA-t/15, K-3493, Afqo-2027, Saksa, and Aze PHA-t/18 were identified as highly promising samples [1,2,3,7,8,14].
2. Unlike the lentil plant, the highest activity of the NAD-MDH enzyme in bean grains was observed 30 days after flowering in Aze PHA – t/17 and Aze PHA – t/18 samples and 20 days after flowering in Aze PHA – t/16 samples. The highest AsAT activity was recorded in Aze PHA – t/16 samples with high protein potential. It is believed that the increased activity of nitrogen metabolism enzymes in mature grains is related to the role of these enzymes in nitrogen recycling, storage, and accumulation of reserve proteins.
3. In ripening grains of Flip 2010-19 (lentils) and Aze PHA-t/16 (beans) with high protein potential, the enzyme NAD-malate dehydrogenase (NAD-MDH) showed high activity compared to other samples. At the same time, it was found that there was a positive correlation between the dynamics of changes in the

activity of NAD-MDH and aminotransferase enzymes in these genotypes. This correlation can be considered as one of the main mechanisms of carbon and nitrogen metabolism integration to ensure high protein potential.

4. As a result of electrophoretic analysis of globulin storage proteins in the lentil plant, 24 spectra and 55 patterns were detected. The highest (100%) frequency of occurrence among the patterns was recorded in ϒ-2 and ϒ-4. The highest value of the genetic diversity index calculated for the 4 zones was recorded in the ω zone (H=0.930), and the lowest value was recorded in the ϒ-zone (H=0.743). Cluster analysis based on polymorphism of globulin storage proteins grouped 46 lentil genotypes into 5 clusters [4,6,11,13,15,16, 18, 19, 21].
5. As a result of electrophoretic analysis of globulin storage proteins in 15 bean plants, 16 spectra and 21 patterns were detected. The highest (100%) frequency of occurrence among the spectra was found in the ϒ-4 and β-3 spectra. The highest value of the genetic diversity index was detected in the ω (H=0.933) zone, and the lowest value was found in the ϒ (H=0.580) zone. Cluster analysis based on polymorphism of globulin storage proteins grouped the 15 studied bean genotypes into 4 clusters [5, 9, 17].
6. For the first time, electrophoresis analysis was conducted on 46 lentil genotypes with 9 ISSR markers, 69 bands were recorded and 52 bands were polymorphic. The average value of GDI was 0.67. UBC 810, UBC 827, UBC 809, and UBC 823 were evaluated as more effective primers in determining genetic diversity in lentil samples. As a result of cluster analysis, Jasmin and Flip 2011-36 samples, 10932 and 10946 samples were evaluated as the closest genotypes, while the samples Flip 2010-96 and Flip 2011-41, Flip 2011-32 and Flip 2011-97, 10932 and Flip 2011-20, Flip 2010-81 and Flip 2011-19 were evaluated as the most distant genotypes [10].
7. As a result of electrophoresis analysis conducted on 15 bean genotypes with 10 ISSR markers, 80 bands, 70 of which were polymorphic, and 71.4-100% polymorphism were recorded.

Cluster analysis grouped the samples into 3 clusters. The closest genotypes to each other were AZE PHA T-18 and Afgo 2027, K130-38 and K3493, and the most distant genotypes were AZE PHA T/17 and Aze PHA -18, Galibiyet and St. Yerli piyada samples.

RECOMMENDATIONS

1. As a result of the research, 7 lentil genotypes (Flip 2011-61, Flip 2011-41, Flip 2011-43, 10943, 10939, 10929, and Jasmin) and 6 bean genotypes (Aze PHA- t/16, K-13038, Aze PHA- t/15, K-3493, Afqo-2027, Saksa, and Aze PHA-t/18) selected for their productivity traits can be used in targeted selection studies as promising parental forms.

2. The most genetically distant genotypes selected for lentil and bean samples using molecular markers are recommended to be used in the future to obtain lentil and bean hybrids with high yield potential.

3. Using globulin protein markers is recommended to determine the purity of specimens stored ex-situ in enterprises engaged in the production of bean and lentil products.

The publication list pertaining to the research topic

1. Mammadova, Sh.E. Shikhlinski, H.M. Study of some biomorphological parameters in Lentil genotypes / World Journal of pharmacy and pharmaceutıcal sciences, -2015, vol. 4, №12, -p.228-233.
2. Məmmədova, Ş.E., Şıxlinski, H.M. Mərcimək və lobya genotiplərində göbələk xəstəliklərinin biokimyəvi göstəricilərə təsiri / AET Əkinçilik İnstitutunun Elmi əsərləri məcmuəsi, Bakı, -2015, XXVI cild, -s.252-256.
3. Məmmədova, Ş.E., Şıxlinski, H.M. Mərcimək genotiplərində bir sıra biomorfoloji parametrlərin öyrənilməsi / AMEA Əkinçilik İnstitutunun Elmi əsərləri məcmuəsi, Bakı, -2016, XXVII: cild, -s.78-81.

4. Məmmədova, Ş.E. Askoxitoz xəstəliyinin mərcimək genotiplərinin biokimyəvi göstəricilərinə təsirinin tədqiqi / AMEA Genetik Ehtiyatlar İnstitutunun Elmi əsərləri, -2019, VIII cild, №1, -s.88-94.
5. Məmmədova, Ş.E. Lobya nümunələrinin dənlərində biokimyəvi göstəricilərinin tədqiqi / AMEA Naxçıvan Bölməsinin Elmi əsərləri (təbiət və texniki elmlər seriyası), -2020 №4, -s.179-183.
6. Мамедова, Ш. Изучение некоторых биохимических показателей генотипов чечевицы / Science, Education, Innovation:Topical, Issues and modern aspects, -2020, №2, -p. 843-847.
7. Мамедова, Ш.Е. Характеристика элементов структуры урожайности генотипов чечевицы статистическими методами (*Lens culinaris medic*) / «Современная наука: Актуалъные проблемы теории и практики», Москва, -2022 Том 1,-с21-26.
8. Мамедова, Ш.Е., Гасанова, С.К., Агаева, С.А. Сравнительное исследование показателей продуктивности новой коллекции чечевицы на основе структурного анализа / Журнала наука и инновации, Республика Беларусь, -2022, Том 7, - с.68-73.
9. Mammadova, Sh., Study of Genetic diversity of globulin proteins in common bean (*Phaseolus vulgaris* L) genotypes / Nasrullayeva M., Kalbiyeva Y., İsgəndərova R., Huseynova T. // Znanstvena misel, Slovenia, -2023, v. 8, -p 4-9.
10. Mammadova, Sh.E. Aghayeva, S.A. Genetic polymorghism assessment in a new lentil (*Lens culinaris* Medik.,1787) collection using ISSR markers. / ACTA Biologica Sibirica, -2023, v.9, p.1181-1188.
11. Mammadova, Sh.E. A study of the genetic diversity of globulin proteins in (*Lens culinaris* Medik.) genotypes / Бюллетенъ науки и практики, -2024, Т.10, №1 -p. 43-51.
12. Məmmədova, Ş.E. Mərcimək və lobya genotiplərində göbələk xəstəliklərinin biokimyəvi göstəricilərə təsiri / BDU 80 illik yubleyinə həsr olunmuş “Eksperimental biologiyanın inkişaf

perspektivləri" mövzusunda Respublika elmi konfransının materialları, -2014, -s.153-154.
13. Şıxlinski, H.M., Məmmədova, Ş.E. Mərcimək genotiplərində bir sıra biomorfoloji göstəricilərin tədqiqi / Azərbaycan Aqrar elmin və təhsilin innovativ inkişafı, Beynəlxalq Elmi-praktik konfrans materialları, I cild, Gəncə, -2015, -s.103-105.
14. Məmmədova, Ş.E, Şıxlinski, H.M. Mərcimək genotiplərində bir sıra biomorfoloji parametrlərin öyrənilməsi. // GDU-nun "Müasir kimyəvi biologiyanın aktual problemləri" mövzusunda beynəlxalq elmi konfransı, III hissə, Gəncə, -2016,cild III, -s.253-255.
15. Мammедова, Ш.Е, Изучение биохимических показателей в генотипах чечевицы. / С.Дж.Фархадова, У.М.Ахмедли, И.А.Мирзалыева //Современные технологии производства екологически чистых продуктов для устойчивого развития сельского хозяйства, Тбилиси, -2016, -с.219-221.
16. Мамедова, Ш.Е. Анализ некоторых биохимических параметров у генотипов чечевицы / Г.М.Шыхлинский, С.Д.Фархадова // Инновационные технологии возделывания белого люпина и других зерновых культур Белгородского научно-исследовательского института сельского хозяйства, Белгород, 13-15 июня 2017, с.297-299.
17. Memmedova, Sh. Investigation of the effects of Ascochyta (*Ascochyta boltshauseri*) on biochemical parameters of bean samples / S.D.Farhadova, H.M.Shiklinski. //Свитовы рослинны ресурси стан та перспективи розвитку, IVмеждународной научно-практической конференции в Украине, Киев, -2018, -с.84-85.
18. Məmmədova, Ş.E, Mərcimək genotiplərində bir sıra biokimyəvi parametrlərinin tədqiqi / H.M.Şıxlinski, Q.Q.Qasımov // GDU-nun "Müasir təbiət və iqtisad elmlərinin aktual problemləri" mövzusunda beynəlxalq elmi konfransı, II hissə, Gəncə, -2018, cild II ,-s.224-226.
19. Мамедова, Ш. Изучение некоторых виохимических показателей генотипов чечевицы на территории Азербайджанской Республики // Beynəlxalq konfransın

materialları, Bakı 27-28 noyabr, 2020 c.296-299.
20. Məmmədova, Ş. Suvarma şəraitində becərilən mərcimək nümunələrində keyfiyyət göstəricilərinin müqayisəli tədqiqi // Şuşa və ətraf ərazilərin biomqxtəlifliyi, torpaq və su ehtiyatları: gələcəyə baxış. Beynəlxalq konfrans, Bakı, 22-24 sentyabr 2022 s.332.
21. Məmmədova, Ş., Kəlbiyeva Y. Suvarma şəraitində səpilmiş mərcimək (*Lens culinaris* Medik.) genotiplərində qlobulin zülallarının genetik müxtəlifliyinin tədqiqi // Heydər Əliyev və Azərbaycan təbiəti mövzusunda Beynəlxalq konfrans, Bakı, -2023, **-s**. 61.
22. Məmmədova Ş., Mərcimək və lobya bitkilərinin yetişməkdə olan dənlərində bəzi karbon və azot fermentlərinin tədqiqi /U.Ə. Qurbanova // Qlobal iqlim dəyişkənliyi şəraitində müasir əkinçilik: aktual problemlər, inkişaf tendensiyaları, perspektivlər beynəlxalq elmi konfransı, Bakı, -2024,-s. (in print)

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Address: AZ1073, 11 Izzat Nabiyev, Baku, Azerbaijan.

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