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A STUDY OF THE GENETIC DIVERSITY OF GLOBULIN PROTEINS IN *Lens culinaris* Medik. GENOTYPES

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ИЗУЧЕНИЕ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ ГЛОБУЛИНОВЫХ БЕЛКОВ ГЕНОТИПОВ *Lens culinaris* Medik.

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Abstract. Electrophoretic analysis of globulin storage proteins in the seeds of 46 lentil accessions introduced from ICARDA was carried out. The aim of the work was to identify, certification and study the genetic diversity of lentil genotypes. In addition, a genetic diversity index (H) was calculated for the zones (ω-, γ-, β- and α-) based on the frequency of patterns on electropherograms of spare globulin proteins in seeds of lentil accessions. Twenty-two spectra and 55 patterns were identified in lentil accessions, most of which polymorphism was observed. 7 spectra and 22 patterns were observed in the ω-zone, 7 spectra and 9 patterns in the γ-zone, 5 spectra and 11 patterns in the β-zone, and 5 spectra and 13 patterns in the α-zone. The genetic diversity index was calculated for each of the 4 zones (ω, γ, β and α) based on Nei formula. According to the calculations, more genetic diversity was observed in ω-zone (H=0.930), slightly less in β-zone (H=0.872), α (H=0.827) and the least in γ-zone (H=0.743). Based on cluster analysis, the genotypes were divided into 5 groups and subgroups. Based on these results, an electrophoretic analysis of globulin storage proteins in polyacrylamide gel (A-PAAG) was performed for the first time and polymorphism between lentil genotypes was identified.

Аннотация. Проведен электрофоретический анализ запасных белков-глобулинов в семенах 46 образцов чечевицы, интродуцированных из ICARDA. Целью работы было выявление, паспортизация и изучение генетического разнообразия генотипов чечевицы. Кроме того, для зон (ω -, γ -, β - и α -) рассчитывали индекс генетического разнообразия (H) на основании частоты закономерностей на электрофореграммах запасных белков-глобулинов в семенах образцов чечевицы. У образцов чечевицы идентифицировано 22 спектра и 55 закономерностей, в большинстве из которых наблюдался полиморфизм. В ω -зоне наблюдалось 7 спектров и 22 картины, в γ -зоне — 7 спектров и 9 картин, в β -зоне — 5 спектров и 11 картин, в α -зоне — 5 спектров и 13 картин. Индекс генетического разнообразия рассчитывали для каждой из 4 зон (ω , γ , β и α) по формуле Нея. Согласно расчетам, большее генетическое разнообразие наблюдалось в ω -зоне (H=0,930), несколько меньшее в β -зоне (H=0,872), α (H=0,827) и наименьшее в γ -зоне (H=0,743). На основе кластерного анализа генотипы были разделены на 5 групп и подгрупп. На основании этих результатов впервые проведен электрофоретический анализ запасных белков глобулинов в полиакриламидном геле (А-ПААГ) и выявлен полиморфизм между генотипами чечевицы.

Keywords: Lens culinaris, genotypes, seeds, globulins, storage proteins, genes, patterns, electrophoresis, clusters.

Ключевые слова: чечевица, генотипы, семена, глобулины, запасные белки, гены, паттерны, электрофорез, кластеры.

Known as the Fabaceae family, the largest family of flowering plants, legumes have been cultivated by humans for over 3,000 years and include 650 genera and over 18,000 species. From perennial woody forms to annual herbaceous forms, different species are widely distributed throughout the world. Lentils (*Lens culinaris* Medik.) are a nutritious food, rich in protein, easily digestible carbohydrates, minerals and vitamins. Lentils are an easily digestible food compared to other legumes. The genus includes *L. culinaris*, *L. ervoides*, *L. nigricans* and *L. lamottei*.

L. culinaris is further subdivided into four taxa: L. culinaris ssp. culinaris, L. culinaris subsp. orientalis, L. culinaris subsp. tomentosus and L. culinaris ssp. odemensis [1].

L. culinaris ssp. is cultivated on 5.01 million ha worldwide with an annual production of 6.54 million plants. Canada is the leading producer, producing about 44% of the world's lentils; other major lentil-producing countries are India, the United States of America (USA), Turkey, Australia, Nepal and Bangladesh (https://www.fao.org/faostat/en/#compare).

Nutrient imbalances and deficiencies lead to a range of nutrition-related and non-communicable diseases (NCDs) in humans. Poor diets that are deficient in macro- and micronutrients such as proteins, low-digestible carbohydrates (LDC \rightarrow LDCs), fats, vitamins and minerals lead to protein and micronutrient deficiencies. LDCs are also known as prebiotic carbohydrates and are defined as "substrates selectively used by host microorganisms that confer health benefits" [2].

These dietary prebiotic carbohydrates pass through the upper digestive tract undigested and are fermented by microorganisms in the colon to improve gut health. The most common health effects of diet are increased growth, digestive problems, obesity, overweight and increased risk of diet related NCDs [3].

Major life-threatening NCDs associated with poor diet include cardiovascular disease, cancer, chronic respiratory disease and diabetes. Key foods rich in macronutrients and micronutrients can reduce the risk of malnutrition. Legumes, including lentils (*Lens culinaris* Medik.), have a higher concentration of protein concentration (20-30%) than cereals (10-12%), have the potential to combat protein deficiency and serve as an allergen-free protein. Lentils are highly nutritious, affordable and have a faster cooking time, high protein concentration, low digestible carbohydrates, minerals, vitamins and low concentration of phytic acid than other legumes [4].

Lentils are not a source of cholesterol and due to their low-fat content, they are easier to digest than other legumes. Lentil proteins include both essential and nonessential amino acids, but sulfur-containing amino acids methionine (Met) and cysteine are particularly low [5]. Lentil proteins are stored in membrane protein bodies called 'storage proteins' in seed cells [6]. These seed proteins provide carbon (C), nitrogen (N) and Sulphur (S) and account for 80% of the total protein available for germination, subsequent plant growth and disease resistance [5].

These proteins are divided into four types: globulins (soluble), albumins (water-soluble), prolamins (alcohol-soluble) and glutelins (acid-soluble). Like other legumes, lentils are rich in globulins and albumin, whereas prolamines and glutelins are more prominent in the grain [7].

Globulins are the major proteins that are the first stored proteins in Lentils and account for about 44-70% of all stored proteins [8].

Two subclasses of globulins have also been identified, namely type 11s (Legumin) and type 7s (vicilin/convicilin) [9]. Albumin account for 26-61% of lentil proteins, whereas prolamins and glutelins account for only a small proportion [10].

The amount of storage proteins shows high variability due to the quantitative nature of the genes that regulate protein synthesis in seeds [11]. Finally, the protein content of lentil seeds contributes to human health by providing essential amino acids required for metabolic processes and nutritional balance in the human body.

Albumin and globulin together account for 63-90% of all seed proteins. The soluble fraction (globulins) accounts for 45-50.3% of the total mass. Soluble proteins with an average value of 47.7% make up the major protein fraction. The pea protein under study belongs to the water-soluble fraction. Albumins account for 31.2-35.5% of the sum of soluble proteins with an average va3e. The third most common seed protein is glutelins, whose content ranges from 15.1% to 20.5% [14].

It is clear that the study of morphological traits that are sensitive to the influence of environmental factors requires a long period of time covering several months and large financial resources. However, electrophoresis of reserve proteins is an easy, low-cost and time-consuming method. Environmental factors may have an effect on the total amount of storage proteins in the endosperm of grain, but they have no effect on the molecular structure of these structural proteins. Reserve proteins are genetically determined, that is, protein polypeptides encoded by allelic genes of albumin and globulin coding loci and passed from generation to generation [12].

Differences in molecular weights of protein bands using electrophoresis in polyacrylamide gel with SDS were observed in different variants of seed proteins, namely globulins, glutelins, albumin and prolaminins as reported by Gupta. Polyacrylamide gel electrophoresis (SDS-PAAG) is used to separate proteins [14].

SRAP has been used in the evaluation of legume crops [15-17].

SRAP markers were first used in the study of genetic variability in lentils. These markers are used to assess genetic variability in Lentils. Polymorphisms of globulins and prolamines in lentil genotypes were determined using SDS-PAAG.

Material and Methods

A total of 46 lentil samples imported from ICARDA were used in the research work. In 2013-2016, 46 samples were sown at the Absheron experimental base of the Genetic Resources Institute and field experiments were carried out in the II-III decade of November.

Electrophoretic analysis of the globulin protein was carried out in the Department of "Biochemical Genetics and Technology" of the Genetic Resources Institute. Extraction and electrophoretic analysis of globulin storage proteins in grain of lentil samples in polyacrylamide gel (Acid-PAAG) by F. A. Poperel et al [18], was carried out according to the method of V. Bushuk and R. Zillman [19].

The lentil sample was grinded and extracted twice with 500 µl of 70% alcohol, each time centrifuged at 3500 rpm and then twice with 500 µl of 0.03% alcohol, then it was washed with vinegar and acetone solution and, after dissolution, centrifuged at 3500 rpm each time using a mechanical stirrer. After the fourth time, 500 µl of a 9-molar acetic-urea solution was added to the extract and analyzed in a vertical electrophoresis apparatus in glycine acetate buffer (pH 3.5). The numbering of the patterns was done by comparing them to each other in each zone and then numbering all patterns without regard to repetition. Thus, if a repeated pattern is observed, no new number was assigned to this pattern and all patterns were recognized according to this rule. The frequency of occurrence of each sample of lentil samples was calculated using the following

formula based on the Ney genetic diversity index for all zones [20]:

$$H=1-\sum P_i^2$$

Here H — is the genetic diversity index; Pi — the frequency of each pattern in the zones [21]. The cluster analysis was constructed using the UPGMA method.

Results and Discussion

Protein markers are among the main markers used in genetic identification of plants. For the first time in Azerbaijan, electropherograms of globulin proteins obtained by vertical electrophoretic analysis of legume plants, modified by A-PAAG method and carried out by new methodology, were conditionally divided into 4 zones: they were called — ω -, γ -, β - and α -globulins. Proteins with a large molecular weight are localized in the ω -zone and those with a light molecular weight in the α -zone. The globulin proteins in the grains of lentil samples were more polymorphic and their spectra were more intense than in beans and soybeans. However, globulin proteins were sharply differentiated by the polymorphism of gliadin and gluten storage proteins (Figures 1-2).

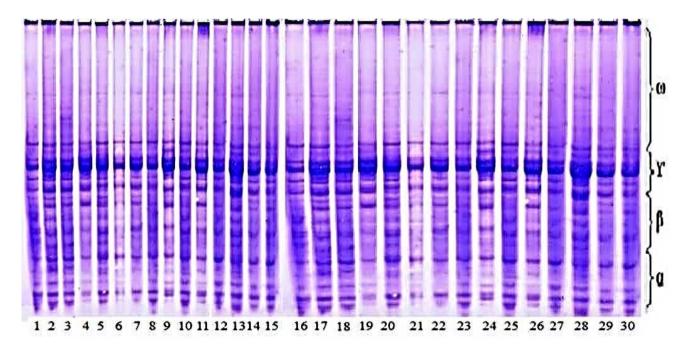


Figure 1. Globulin protein isolated from lentil sample grains electropherograms

1.Flip2010-19, Flip2010-26, Flip2010-81, Flip2010-91, Flip2010-94, Flip2010-95, Flip2010-96, Jasmine, Flip2010-97, Flip2011-101, Flip2011-13, Flip2011-14, Flip 2011- 17, Flip2011-18, Flip2011-19, Flip2011-20, Flip2011-26, , Flip2011-35, Flip2011-37, Flip2011-41, Flip2011-42, Flip2011-43, Flip2011-51, Flip2011-57, Flip201 1-59, Flip2011-61, Flip2011-64, 10932, 10946, 10939

A total of 24 spectra and 55 patterns were identified among the lentil samples studied, among which polymorphism was determined by the frequency of patterns formed by electrophoretic spectra.

7 spectra and 22 different patterns in the ω -zone of globulin storage proteins electropherograms were investigated. In this zone the ω — 1 pattern was detected in 15% of 7 samples, ω — 12 in 11% of 5 samples and ω — 2 in 1 sample with a frequency of 2.0%. Among the spectra ω_{6s} corresponds to 100% high frequency, spectrum ω_1 corresponds to 70% medium frequency and spectrum ω_4 corresponds to 26% low frequency. γ -zone electropherograms of

globulin storage proteins observed 7 spectra and 9 patterns, γ — 2 patterns had a frequency of 37 %, γ — 3 pattern 11% and γ — 5 pattern had a frequency of 1%. γ ₄₈ with between spectra 100% high, γ ₆₈ 87% medium and γ ₇₈ with working 52% had a low frequency.

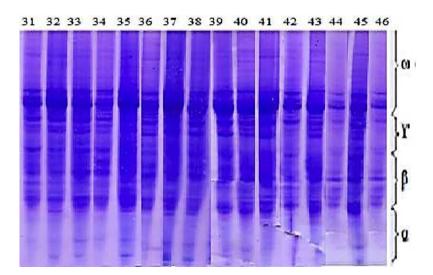


Figure 2. Electropherograms of globulin proteins isolated from seeds of lentil genotypes

10943, Flip-2011-32, Flip-2011-31, 10928, Flip 2011-40, 10937, 10940, 10926, 10925, Flip 2011-384, 10942, 10934, 10929, 10930, Flip-2011-29, Flip 2011 1-36.

In the β -zone electropherograms, 5 spectra and 11 patterns were identified. The least frequent were β — 5 patterns 20%, β — 8 patterns 11% and β — 11 patterns 2%. The frequency of β_{3s} 100% high, β_{2s} 70% medium and β_{1s} 41%. In the α -zone of the electropherograms 5 spectra and 13 patterns were recorded. The α -6 pattern had a frequency of 20%, the α -10 pattern 11% and the α -12 pattern 2%. α_3 n 91% maximum, α_{4s} 80% medium and α_{2s} 61% low frequency. The genetic diversity index was calculated for all 4 zones using Nei's formula between genotypes.

As a result of the calculations, ω (H=0.930) was high, β (H=0.872) and α -zones (H=0.827) had relatively low diversity, and the lowest genetic diversity was observed in Υ -zone (H=0.743) (Figure 3).

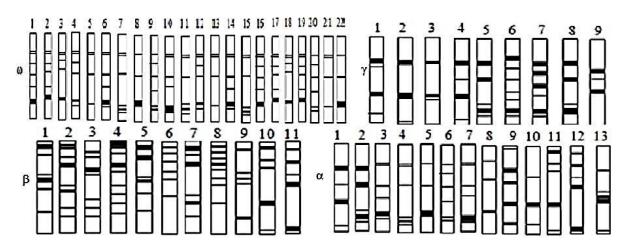


Figure 3. Idiogram of the different patterns in the ω -, Υ -, β - and α zones observed in lentil samples

After isolation and electrophoretic analysis of spare globulin proteins from grains of female lentil samples (electrophoretic spectrum) were numbered using the numbering method "0" and "1"

between genotypes. Subjects standing in the same place, "1" is subjects that are not in the corresponding region "0", is numbered according to the binary nomenclature.

Using the UPGMA computer program, the genetic proximity of the samples was determined, a dendrogram was constructed and the genetic proximity of lentil genotypes was studied using globulin protein markers. As can be seen in Figure 4, the dendrogram shows 5 samples in cluster 1, 4 samples in cluster 2, 7 samples in cluster 3, 17 samples in cluster 4, the most samples in cluster 4 and 13 samples in cluster 5 are grouped into a cluster.

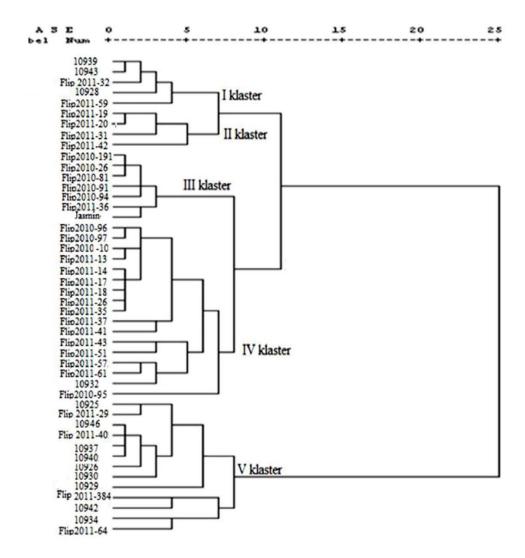


Figure 4. Dendrogram showing the genetic distance between different lentil samples based on the polymorphism of globulin protein electropherograms

Based on the results obtained, it is advisable to use hybridization of samples distant in genetic distance between lentil samples when selecting parental forms in marker breeding and accelerate the selection process.

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