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Articles

Genetic polymorphism assessment in a new lentil (Lens culinaris Medik., 1787) collection using ISSR markers

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ISSR markers were used in the study of the genetic diversity of 46 imported lentil varieties. The lentil collection had a strikingly high level of polymorphism (76%). The UBC 810 and UBC 809 primers have the highest polymorphism rates among the primers, exceeding 85.7%. The use of nine ISSR markers resulted in 69 pieces, with 76% displaying polymorphism. The computed average genetic diversity index ranged from 0.56 to 0.81, indicating a wide range of genetic variation among lentil genotypes. Accessions were classified into six unique groups as a consequence of cluster analysis. The most divergent genotypes within their respective clusters were identified as Flip 2010-96 and Flip 2011-41, Flip 2011-32 and Flip 2011-97, 10932 and Flip 2011-20, and Flip 2010-81 and Flip 2011-19. These findings bear noteworthy implications for the future of lentil breeding, cultivation, and protection. The observed genetic diversity imparts valuable insights that can be harnessed to fortify lentil crops, fostering resilience and adaptability. The identified distant genotypes present promising avenues for targeted breeding initiatives, facilitating the development of lentil varieties harboring diverse and desirable traits. In summation, this study contributes pivotal information to the scientific community, establishing a framework for subsequent research and progress in the improvement of lentil crops.

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Keywords

ISSR marker, lentil, genotype, *Lens culinaris* Medik., polymorphism

Introduction

Lentil (*Lens culinaris* Medic.) is one of the main legume crops cultivated in the semi-arid areas of the world and in Azerbaijan. With high levels of protein, minerals and vitamins, this plant meets the nutritional needs of millions of people around the world. Currently, lentils grown in more than

seventy and consumed in more than 120 countries (Erskine et al. 2011). ISSR markers have been used in genetic research of many plants, including legumes (Bornet and Branchard 2001; Rajesh et al. 2003; Tahir et al. 2011; Bhareti et al. 2012 and Wang et al. 2012). Annual diploid lentil is extremely important in human nutrition due to its high protein content (25%), high fiber content (87%) and low fat (Bosmali et al. 2012). Recently, people pay attention to their health and are likely to consume more plant protein than animal proteins. According to FOASTAT 2014, global lentil production increased almost fivefold in the last 50 years (Kumar et al. 2015).

As elsewhere in the world, lentils in Azerbaijan have a special place in providing food security. The main challenges in this area are the creation of new lentil collections with high-quality, resistant to abiotic and biotic stresses and their use in farms, especially in the arid farming systems of the republic.

In the Caucasus region, the first gene pool was established at the Genetic Resources Institute in Azerbaijan, where 256 landraces and introduced breeding species of lentil are mainly conserved (Babayeva et al. 2018). In order to increase the effectiveness of these collections in breeding programs, molecular-genetic characterization is also needed. Molecular markers are an important tool for the detection of polymorphism at the DNA level, for study of nucleotide sequences characteristic for genome analysis, localized in close to genes, managing the phenotype of any trait and not affected by environmental factors.

Study on polymorphism is not only important for determining genetic diversity, but also for finding alleles useful for possible progress in future breeding programs. To date, polymorphism in lentil plants has been investigated by various molecular markers (Fikiru 2007; Gupta et al. 2012; Bosmali 2012; Babayeva 2018). The ISSR (simple sequenced) markers are technically simple and can detect changes in both coding and non-coding sections of the genome (Singh et al. 2002). The study was conducted to evaluate the genetic diversity of landraces and introduced lentil genotypes and to study the accessions important for breeding (Mamedova et al. 2022).

Materials and methods

A total of 46 lentil genotypes, introduced from ICARDA gene bank, were used in this study (Table 1). The rest accessions represent local landraces collected from different regions of Azerbaijan and improved local varieties, which were obtained by selection from ICARDA introductions in different years.

Genomic DNA was extracted from fresh leaves using CTAB protocol by Doyle and Doyle (1987). PCR reactions for ISSR primers were performed in a 20 µl, con- taining 2 µl 10x PCR buffer; 2 µl mixture dNTP (5 mM); 1.5 µl MgCl2 (50mM); 2 µl of each primer (15 pmol/ µl); 0.1 µl of Taq-polymerase enzyme (1 U/ µl) and 2 µl of extracted DNA (50 ng/ µl). The Thermal Cycler (Applied Biosystems, USA) for ISSR markers was programmed as: pre-denaturation at 94°C for 5 minutes; 35 cycles of – denaturation at 94°C for 1 min, annealing for 45 seconds (temperature depended on the primer used), elongation for 5 minutes at 72°C; the final elongation at 72°C for 10 minutes. PCR products were analyzed by agarose gel electrophoresis, following ethidium bromide staining and visualized under UV light using gel documentation system BioRad. The band size was determined by using Photo-Capt version 12.4 with reference to standard 100 bp ladder. ISSR bands were presented in a matrix form of binary data, in which presence or absence of PCR fragments was considered as 1 and 0 respectively. All analysis was performed using the SPSS 16.0 statistical package (SPSS/PC-16, SPSS Inc., Chicago, IL, USA; http://www.spss.com). The genetic diversity index (GDI) (Weir 1990), polymorphism information content (PIC) (Roldan-Ruiz et al. 2000), effective multiplex ratio (EMR), marker index (MI) (Powell et al. 1994), resolution power (RP) and mean resolution power (MRP) (Prevost et al. 1999; El-Nahas et al. 2011) were calculated for the analyses.

№	Name and number	Origin	№	Name and number	Origin

	of Lentil samples according to the catalog			of Lentil samples according to the catalog	
1	Flip2010-19	İCARDA	24	Flip2011-59	İCARDA
2	Flip2010-26	İCARDA	25	Flip2011-61	İCARDA
3	Flip2010-81	İCARDA	26	Flip2011-64	İCARDA
4	Flip2010-91	İCARDA	27	10932	İCARDA
5	Flip2010-94	İCARDA	28	10946	İCARDA
6	Flip2010-95	İCARDA	29	10939	İCARDA
7	Flip2010-96	İCARDA	30	10943	İCARDA
8	Flip2010-97	İCARDA	31	Flip2011-32	İCARDA
9	Flip2010-101	İCARDA	32	Flip2011-31	İCARDA
10	Flip2011-13	İCARDA	33	10928	İCARDA
11	Flip2011-14	İCARDA	34	Flip2011-40	İCARDA
12	Flip2011-17	İCARDA	35	10937	İCARDA
13	Flip2011-18	İCARDA	36	10940	İCARDA
14	Flip2011-19	İCARDA	37	10926	İCARDA
15	Flip2011-20	İCARDA	38	10925	İCARDA
16	Flip2011-26	İCARDA	39	Flip2011-384	İCARDA
17	Flip2011-35	İCARDA	40	10942	İCARDA
18	Flip2011-37	İCARDA	41	10934	İCARDA
19	Flip2011-41	İCARDA	42	10929	İCARDA
20	Flip2011-42	İCARDA	43	10930	İCARDA
21	Flip2011-43	İCARDA	44	Flip2011-29	İCARDA
22	Flip2011-51	İCARDA	45	Flip2011-36	İCARDA
23	Flip2011-57	İCARDA	46	Jasmine	Azerbaijan

Table 1. *Name, origin and catalog numbers of lentil samples*

Results and Discussion

This study used ISSR markers to evaluate the diversity and relationship between lentil genotypes. 3 of the used 15 ISSR markers, were monomorphic. The research was continued with 9 ISSR markers, providing polymorphic and clear bands. A total of 69 bands were created in 46 lentil genotypes using 9 ISSR primers, and 52 of them have been polymorphic (Table 1). The molecular size of the amplicons ranged between 100 and 1000 bp. The number of bands for each primer was between 6 and 10, on the average was 8, the highest number of bands have been observed in UBC 827 and the lowest number of bands have been in UBC 823 and UBC 812 (Table 1). In other studies, ISSR markers have been used successfully to evaluate genetic diversity in lentil accessions (Fikiru et al. 2007).

In all collection, the polymorphism was 76%. The highest polymorphism was recorded in UBC 810 and UBC 809 primers (> 85.7%) (Table 2). For comparison, in studied lentil collections, El-Nahas et al. (2011) reported 46% polymorphism, Babayeva et al. (2018) 84%, Duran et al. (2004) 98.8% polymorphism. The highest value of the Genetic Diversity Index among the 10 markers was recorded in the UBC 809 (0.77) and the lowest GDI was recorded in the UBC 812 (0.56) primer. The average genetic diversity in the collection was 0.67. the PIC values, depending on the number of detected alleles and their frequency distribution ranged between 0.15 and 0.56, the average value was 0.25.

Genetic Distance and Cluster Analysis: Cluster analysis has created a dendrogram that divides genotypes into 6 major groups (Fig. 1). Genetic distance values range between 0.07 (for 44% of the average collection) and 0.05, which supports the narrow genetic database of the collection again. The genotypes showing 100% genetic similarity among themselves, were not recorded. The high

genetic similarity was noted between Arzu and Flip 2011-36 and between 10932 and 10946. Thus, the study has revealed a high genetic similarity between the introduced lentil accessions.

Primer name	Annealing temperature, Ta, °C	Number of total bands	Number of polymorphic bands	Polmorphism ratio, %	GDI
UBC-840	47	8	5	71.4	0.63
UBC-810	41	7	6	85.7	0.74
UBC-827	49	10	8	80.0	0.64
UBC-809	45.5	7	6	85.7	0.77
UBC-818	47	9	7	77.8	0.63
UBC 834	45.5	8	6	75.5	0.74
UBC-835	45	8	5	62.5	0.66
UBC-812	41	6	4	66.7	0.56
UBC-823	45	6	5	83.3	0.81
Total		69	52	-	-
Average		8	5.7	76.0	0.67

Table 2. *ISSR primers, number of total and polymorphic bands, polymorphism ratio and Genetic diversity index (GDI) values*

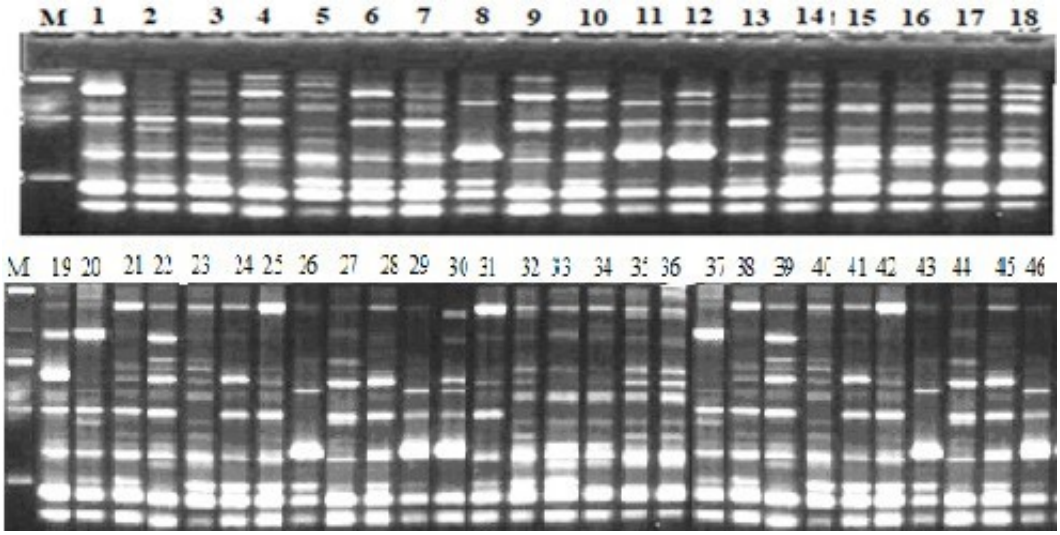

Figure 1. *ISSR profile of 46 lentil genotypes generated by UBC 818.*

The first cluster consists of 6, the second cluster 18, the third cluster 11, the fourth cluster 7, each of the fifth and sixth clusters consist of two genotypes. Six genotypes have been located in group I, and the genetic distance between 10932 and 10946 genotypes is very close (0.25). The cluster II containing the most accessions has been divided into four subgroups, and the 10934 and 10942 genotypes were located in a separate subgroups. The GD value between these genotypes and other accessions was 0.86 and 0.78, respectively. The III cluster consisted of 11 accessions has been also divided into three subclusters. Flip 2011-29, Flip 2011-37, 10929, 10930 have created the sub-cluster A, and Flip 2011-36, Jasmine, F.2011-26, F.2011- 19, F.2010-81, F.2010-91, F.201094 was located in this subcluster (B). Improved variety Jasmine, located with Flip 2011-36 in the third cluster has shown the highest genetic similarity (GD = 0.21). In cluster III, the genetic distance values between Jasmine and the other accessions were 0.68-0.74. The lowest genetic distance value (GD = 0.49) among genotypes in cluster IV consisted of 7 accessions was found between F.2010-95 and F.2010-10 (Fig. 2).

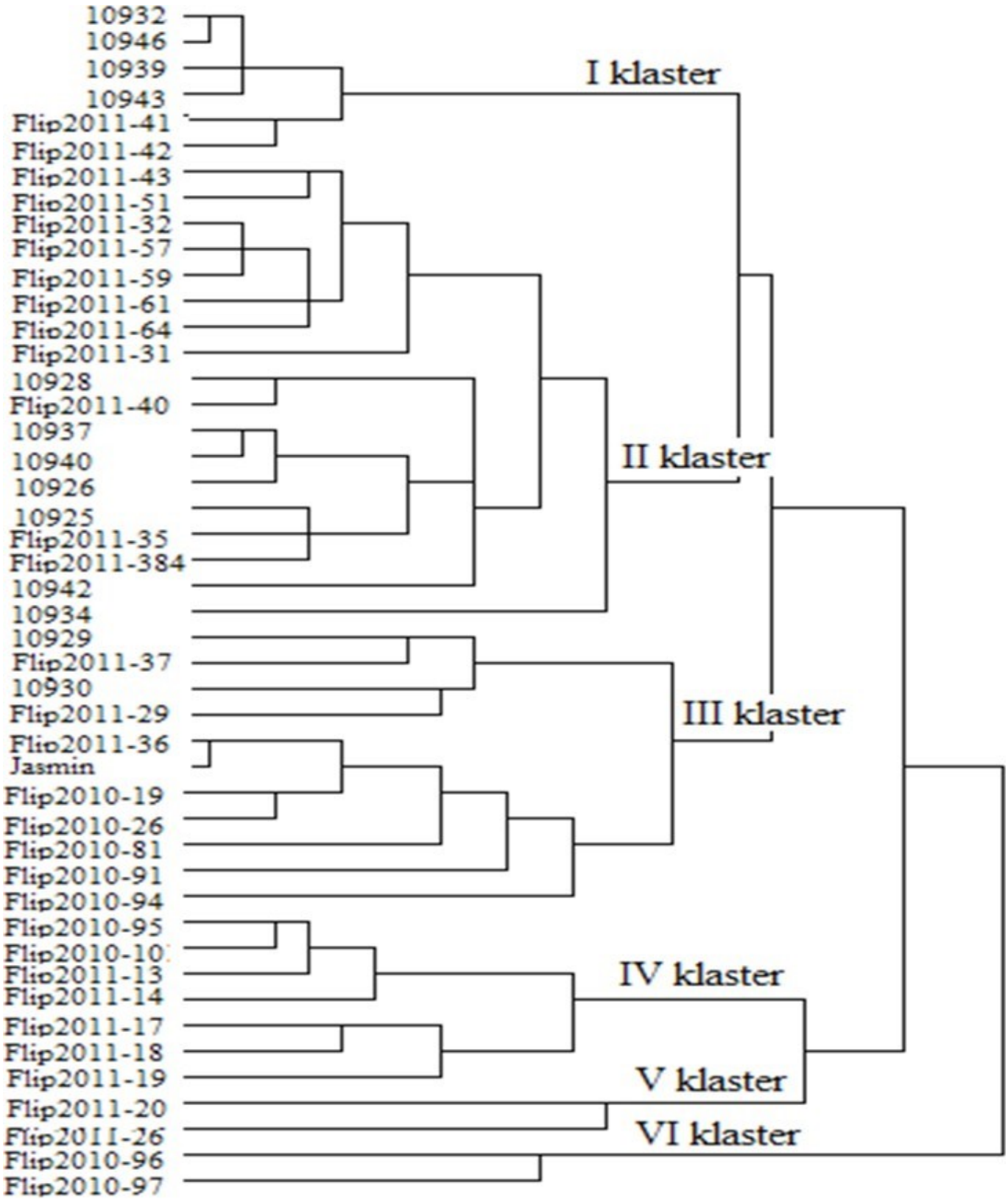

Figure 2. *UPGMA dendrogram based on Jaccard dissimilarity coeficient in 46 lentil genotypes.*

The genetic distance value between genotypes of the fifth and sixth clusters was 0.69, and indicated their difference.

In our study, cluster analysis could distinguish distant genotypes, as well as closely related

genotypes. 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 have been evaluated as the most distant genotypes. The genotypes of Australian origin were grouped into the same group, which was also reported in our previous study (Babayeva et al. 2009). The released variety Jasmine of Azerbaijan and the genotype introduced by ICARDA showed high genetic similarity, which may be explained by the fact that Arzu variety is ICARDA origin.

The study approved the high genetic diversity at the molecular level in the lentil collection. Acquired knowledge about the diversity of genotypes will increase the efficiency of their utilization in the breeding process and accelerate breeding work to create new varieties adapted to the country's environmental and geographical conditions.

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