

Study of Genetic Polymorphism Using ISSR Markers in the Lentil Collection

Mammadova Sh.E.

Genetic Resources Institute, Baku, Azerbaijan shamsiye@bk.ru

Kalbiyeva Y.E.

Genetic Resources Institute, Baku, Azerbaijan

Aghayeva S.A

*Genetic Resources Institute, Baku, Azerbaijan
Western Caspian University, Baku, Azerbaijan*

Sevin Teoman Duran

*Department of Crop and Animal Production, Organic Agriculture Programme,
Karacabey Vocational School, Bursa Uludağ University, Bursa, Turkey*

İsmayilova V.M.

*Genetic Resources Institute, Baku, Azerbaijan
Institute of Dendrology, Baku, Azerbaijan*

Mammadov A.M.

Khazar University, Baku, Azerbaijan

Hasanova M.Y.

Institute of Dendrology, Baku, Azerbaijan

Abstract

Genetic diversity of the 46 introduced lentil genotypes was studied using ISSR markers. As a result, in the lentil collection was recorded high polymorphism (76%). The highest polymorphism was recorded in UBC 810 and UBC 809 (> 85.7%) primers. A total of 69 fragments were obtained using 9 ISSR marker and 75,3% of them were polymorphic. The average genetic diversity index ranged between 0.54 to 0.81. Cluster analysis accessions were grouped into 6 clusters, Flip 2010-96 and Flip 2011-41, Flip 2011-32 and Flip 2011-97, ILL 10932 and Flip 2011-20, Flip 2010-81 and Flip 2011-19 were evaluated as the most distant genotypes. The results can be recommended for the breeding, cultivation and protection of lentil crop in the future.

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

INTRODUCTION

Lentil (*Lens culinaris* Medik.) 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 of the National Academy of Sciences of Azerbaijan, where 256 landraces and introduced breeding species of lentil are mainly conserved (S. 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 (EK Fikiru, 2007; M. Gupta, et al., 2012; M. Gupta; 2012; I. Bosmali, 2012; S. 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.

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, containing 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 and Wilkinson, 1999) were calculated for the analyses.

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 4). 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 7.3, 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 (Meenakshi et al., 2013; Fikiru et al., 2007).

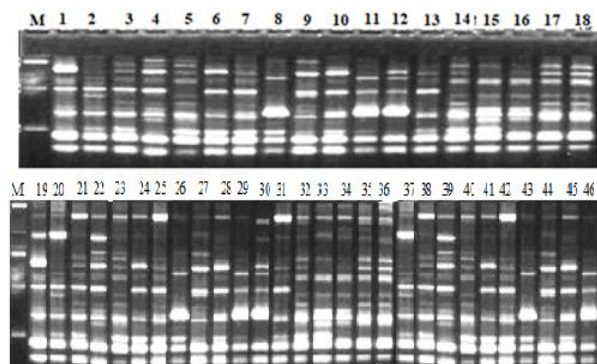
Table 1. ISSR primers, number of total and polymorphic bands, polymorphism ratio and Genetic diversity index (GDI) values

Primer name	Annealing temperature, Ta, 0C	Number of total bands	Number of polymorphic bands	Polymorphism ratio, %	GDI
UBC-840	47	7	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	0.61
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		7.3	5.7	76.0	0,67

In all collection, the polymorphism was 76%. The highest polymorphism was recorded in UBC 810 and UBC 809 primers (> 85.7%) (Table 1). For comparison, in studied lentil collections, H.H. El-Nahas (2011) reported 46% polymorphism, S. Babayeva and colleagues (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 (0.74) 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.

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

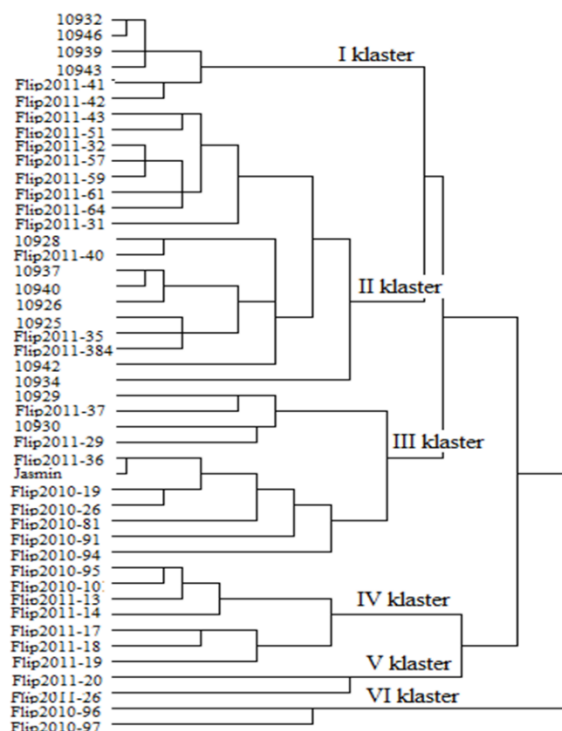


Genetic Distance and Cluster Analysis: Cluster analysis has created a dendrogram that divides genotypes into 6 major groups (Figure 1). Genetic distance values range between 0.07 (for 44% of the average collection) and 0.05, which supports the narrow genetic database of the collection again. The genotypes 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. GDI and PIC were 67% and 0.59, respectively.

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 ILL 10932 and ILL 10946 genotypes is very close (0.25). The cluster II containing the most accessions has been divided into four subgroups, and the ILL 10934 and ILL 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, ILL 10929, ILL 10930 have created the sub-cluster

A, and Flip 2011-36, Arzu, F.2011-26, F.2011-19, F.2010-81, F.2010- 91, F.201094 was located in this subcluster (B). Improved variety Arzu, 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 Arzu 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.3. UPGMA dendrogram based on Jaccard dissimilarity coefficient 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, ILL 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 (S. Babayeva, et al, 2004). The released variety Arzu 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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