UDC 575.630
https://doi.org/10.2298/GENSR1802575B
Original scientific paper

GENETIC RELATIONSHIP AMONG INTRODUCED LENTIL GERMPLASM USING AGRONOMIC TRAITS AND ISSR MARKERS

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Lentil (*Lens culinaris* Medik.) is an annual, cool-season grain legume playing an important role in human and animal nutrition, as soil fertility maintenance. National lentil improvement program in Azerbaijan is currently focused on extending the genetic base of the lentil collection through the introduction of new breeding lines from ICARDA and involving them into breeding. The present study was aimed to evaluate the performance of lentil collection, mainly comprised of ICARDA-derived breeding lines for yield traits under Azerbaijan condition and assess genetic diversity among them using inter simple sequence repeats (ISSR) markers. Many breeding lines of ICARDA exhibited agro-morphological performance superior to those of the local improved varieties. Our studies confirmed that the genetic base of the studied lentil collection is quite above board. A total of 71 bands were generated using 7 ISSR primers in 47 lentil genotypes, of which 62 were polymorphic. Genetic diversity values varied from 0.61 (UBC 848) to 0.95 (UBC 835), with a mean of 0.81. ISSR dendrogram was able to clearly distinguish all lentil accessions. Clear tendention was observed on clustering of genotypes according to their pedigree or origin with few exeptions. The results obtained from the Principal Coordinate Analysis were consistent with the results of cluster analysis, with minor differences. Breeding lines with high agronomic performance and sufficient genetic distance from this study can be used as appropriate parents to get more heterotic recombinants. This will accelerate the creation of new varieties well adapted to eco-geographic condition of Azerbaijan with stable and high yield.

Key words: genetic diversity; ISSR; lentil; yield components

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INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an annual, self-pollinated diploid (2x=2n=14) species with a genome size of 4,063 Mbp (ARUMUGANATHAN and EARLE, 1991). It is a cool season grain legume playing an important role in human and animal nutrition, as well as soil fertility maintenance (ABRAHAM, 2015). Lentil has been one of the first crops domesticated in the Fertile Crescent from *Lens culinaris* subsp. *orientalis* Boiss (LADIZINSKY, 1993) since Neolithic times (ZOHARY and HOPF, 2000). Lentil seeds provide the third highest levels of protein after soybeans and hemp (ALGHAMDI *et al*., 2014). Today lentils are cultivated and consumed throughout the world, with Canada, India and Turkey being the top producers (FAOSTAT, 2014).

Central Asia and the Caucasus (CAC) countries, including Azerbaijan also lie within the general region where lentil is thought to have been domesticated and first brought into cultivation (ERSKINE, 1998). Lentils form an important part in the human diet of Azerbaijan for their relatively excellent digestibility when compared to many other legumes (BABAYEVA *et al*., 2014). Although it's important part in consumer basket production of lentil does not meet the annual demand of the population. According to the statistical data of the last 5 years, 52% of demand is covered by imports. The reasons for this are the occupation of most of the lentil area by low productive landraces that are also vulnerable to a range of biotic and abiotic factors and a low number of highly productive modern varieties. The narrow genetic base of cultivated germplasm is another problem for local breeders in Azerbaijan. As for many other crops, intensive selection during domestication and subsequent exclusion of many important traits from germplasm stock has led to a loss of genetic diversity in lentil as well (FORD *et al*., 1997). Thus the introduction of new sources of diversity, their characterization and involvement into breeding programs to widen the genetic base of lentil germplasm in Azerbaijan is needed.

The world lentil collection is held by ICARDA, which obtained from ICARDA collection missions, donor institutions and ICARDA's breeding program (COYNE and MCGEE, 2013). The national programs in different lentil growing regions, including Azerbaijan are widely using ICARDA enhanced lentil germplasm. The first Genebank in the Caucasus region was established in Genetic Resources Institute of Azerbaijan National Academy of Sciences, where 256 accessions of lentil are conserved. Eighty-two of them are local landraces and varieties, while the rest represent accessions introduced from ICARDA. This germplasm is being evaluated across various environments for different morpho-agronomic traits, adaptability and stress resistance. Some lines were selected from the introductions and released by national breeders for general cultivation (/ILL8077 × ILL6994/ /Flip2010-65L/ Jasmin, /ILL 6037/Arzu etc.). They were selected because of their high and stable yield and seed size as well. Despite a large number of field experiments and characterization for traits of agronomic importance, only a few molecular-genetic works were conducted on lentil in the country (BABAYEVA *et al*., 2009).

The molecular characterization of genetic diversity among local and introduced germplasm, using DNA markers provide considerable opportunities for genetic research, construction of core collection and utilization of germplasm in breeding programs (HOU *et al*., 2005; ALIYEV *et al*., 2007). Molecular markers provide a direct measure and go beyond indirect diversity measures based on agronomic traits or geographic origin (KHALED *et al*., 2015). Different molecular markers are employed in genetic diversity analysis of lentil, including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and inter simple sequence repeats (ISSR). Among them, ISSR markers with its ease of application make preliminary studies on genetic variation more

accessible. It is a dominant multi-locus marker system based on PCR amplification of regions in the genome flanked by microsatellite sequences. Higher reproducibility compared to RAPDs and costs less in terms of time and money compared to AFLP make ISSR ideal genetic marker for genetic diversity (WANG *et al*., 2012; IZZATULLAYEVA *et al*., 2014; HASANOVA *et al*., 2017) and DNA fingerprinting (BHAGYAWANT and SRIVASTAVA, 2008; LIU *et al*., 2012) studies. ISSR markers are increasingly applied and detected a sufficient degree of polymorphism in a wide range of crops such as chickpea, barley, faba bean, wheat etc. (FERNANDEZ, 2002; RAO *et al*., 2007; WANG *et al*., 2012; HAJIYEV *et al*., 2015).

The present study was aimed to evaluate the performance of 47 lentil accessions for yield traits under the Absheron region of Azerbaijan as a promising legume crop and assess genetic diversity among them using ISSR markers.

MATERIAL AND METHODS

Plant material and field experiments

A total of 47 lentil genotypes, including 43 breeding lines introduced from ICARDA were used in this study (table 1).

Table 1. List of the lentil genotypes used in the study

N	Genotype	Pedigree	N	Genotype	Pedigree
1	F. 2010-26	ILL 6024 × ILL 0098	25	F. 2012-48	ILL 7948 × ILL 10956
2	F. 2010-94	ILL 7620 × ILL 8113	26	F. 2012-86	ILL 8063 × ILL 590
3	F. 2011-51	ILL 590 × ILL 7979	27	F. 2012-95	ILL 8077 × ILL 5588
4	F. 2011-57	ILL 8090 × ILL 7980	28	F. 2012-99	ILL 6037 × ILL 7979
5	F. 2011-59	ILL 8090 × ILL 7980	29	F. 2012-149	ILL 5883 × ILL 8009
6	F. 2011-40	ILL 6467 × ILL 8009	30	F. 2012-164	ILL 590 × ILL 5883
7	ILL 10937[AU]	CIPAL 401	31	F. 2012-194	ILL 8009 × ILL 7537
8	ILL 10926[AU]	97-039 × 99R060	32	F. 2012-231	ILL 7617 × ILL 5883
9	F. 2011-38	ILL 7949 × ILL 7989	33	F. 2012-244	ILL 7711 × ILL 5480
10	ILL 10942[AU]	97-006 L	34	F. 2012-276	ILL 9977 × ILL 5883
11	ILL 10934[AU]	94-003 L	35	F. 2013-45	ILL 6037 × ILL 7981
12	ILL 10929[AU]	97-001 L	36	F. 2013-47	ILL 8113 × ILL 7537
13	F. 2011-29	ILL 8116 × ILL 5562	37	F. 2013-50	ILL 6212 × ILL 6994
14	F. 2011-36	ILL 7683 × ILL 5562	38	F. 2013-53	ILL 9977 × ILL 5883
15	F. 2010-76	ILL 7617 × ILL 8009	39	F. 2013-54	ILL 9977 × ILL 5883
16	F. 2011-20	ILL 8116 × ILL 5562	40	F. 2013-59	ILL 7934 × ILL 3883
17	F. 2011-49	ILL 7949 × ILL 7686	41	F. 2013-66	ILL 7121 × ILL 1005
18	F. 2010-36		42	F. 2013-68	ILL 7121 × ILL 1005
19	F. 2011-13	ILL 358 × ILL 590	43	F. 2013-69	ILL 7121 × ILL 1005
20	F. 2011-61	ILL 7537 × ILL 590	44	81S15	81S15UJL197 × ILL 4400
21	LECU 355[A]		45	F. 2011-17	ILL 8114 × ILL 590
22	LECU 6[IR]		46	F. 2011-31	ILL 8116 × ILL 5562
23	Jasmin[†; A]	F. 2010-65	47	Arzu[†; A]	ILL 6037
24	F. 2012-32	ILL 8194 × ILL 7706			

[†] Improved variety; AU - Australia; A - Azerbaijan; IR – Iran

Two local improved varieties (Arzu and Jasmin) from Azerbaijan were also included in research material. Local improved varieties were obtained by selecting from ICARDA introductions in different years. Seeds of the accessions were sown at Absheron (40º29'54"N, 49º22'16"E; 30 m a.s.l.) experimental station of Genetic Resources Institute of ANAS with two replications in 2014/2015 growing season. The average rainfall in Absheron is around 200 mm. At the end of growing season 3, single plants from each plot were collected and evaluated based on yield and its components. The following traits were measured: plant height (PH), the height of lowest pod (HLP), number of pods per plant (NPP), 100 seed weight (SW), seed yield per plant (SYP), seed yield per $1m^2$ (SY).

The Pearson correlation coefficient was calculated to determine the relationships between the studied morphological variables. Classification of genotypes according to agro-morphological data was performed by UPGMA (Unweighted Paired Group Method using Arithmetic averages) algorithm based on squared Euclidean distances (SNEATH and SOKAL, 1973). All analysis was performed using the SPSS 16.0 statistical package (SPSS/PC-16, SPSS Inc., Chicago, IL, USA; http://www.spss.com).

DNA extraction and ISSR analysis

Genomic DNA was extracted from fresh leaves using CTAB protocol by DOYLE and DOYLE (1987) with some modifications. For ISSR markers 11 primers were tested, of which 7 markers that produce clear and reproducible bands were used for further experiments (table 4). PCR reaction and agarose gel electrophoresis was performed according to HAJIYEV *et al*. (2015).

Genetic Data analysis

ISSR bands were treated as a binary matrix, where the presence and absence of bands were recorded as (1) and (0), accordingly. Dissimilarity matrix was constructed from the binary data with JACCARD (1908) coefficient. DARwin version 6 software (PERRIER, 2006) was used for dendrogram creation with an unweighted pair group method with arithmetic mean (UPGMA) and Principal Coordinate Analysis (PCoA) (GOWER, 1966). A number of effective alleles, polymorphism percentage and genetic diversity index were calculated for each primer. The genetic diversity index (GDI) was calculated according to WEIR (1990).

The MANTEL test (1967) was adopted to determine the correlation between the Euclidean distance matrix of morphological traits and genetic distance matrix of ISSR markers.

RESULTS AND DISCUSSION

Agro-morphological traits variability

Azerbaijan is a highly import-dependent country in case of most legumes, including lentil. To increase the productivity, the enhancement of national lentil breeding programs is needed. The collection, characterization and conservation of local and introduced germplasm in order to provide the widest range of genetic diversity in breeding programs are the first steps for this purpose.

Forty-seven lentil genotypes mainly introduced from ICARDA were evaluated in the Absheron region of Azerbaijan. Descriptive values of 6 agro-morphological traits analyzed for the 2014/2015 cropping season were given in table 2. Lentil genotypes exhibited a wide range of all traits studied. Considerable variations were recorded in traits important for harvest mechanization, such as plant height and height of the lowest pod. The plant height in the studied

collection varied from 29 to 54 cm, with 7 accessions exceeding 45 cm. The highest values were taken from the newly released Jasmin variety and F. 2012-276 L and the lowest from ILL 10937 and ILL 10929 of Australian origin. In addition, breeding lines F. 2012-244 L, F. 2013-45 L, F. 2013-47 L, F. 2011-57 L etc. were noted as tall genotypes. The range for the height of lowest pod was recorded from 10 to 28 cm, with an average of 17 cm. The height of lowest pod exceeded 20 cm in ten genotypes, making them suitable for mechanical harvesting. LECU 6 of IRAN origin produced the minimum number of pods per plant, while the maximum number was noted for breeding line Flip 2011-40 L (171.0). Twenty-two genotypes produced a higher number of pods per plant than the germplasm collection mean and 15 genotypes gave more than 100 pods per plant.

Table 2. Descriptive values of studied agro-morphological traits in 47 lentil genotype

Characters	Mean±SE	SD	Min	Max
Plant Height	37.4±1.03	7.04	29.0	54.0
First-pod bearing height	16.8±0.62	4.28	10.0	28.0
Number of pods/plant	88.8±4.70	32.2	33.0	171.0
100 seed weight	3.95±0.15	1.05	1.80	6.80
Seed yield/plant	5.18±0.42	2.86	0.90	14.1
Seed yield/1 m^2	264.4±17.0	116.3	78.0	452.0

100 seed weight was found to vary from 1.80 to 6.80 g for F. 2011-51 L and local Arzu variety, respectively. LECU 6 (5.9 g) also had largest seeds followed by LECU 355 (5.2 g) of Azerbaijan origin. The seed yield per plant ranged from 0.90 to 14.1 g and averaged 5.18 g. LECU 6 exhibited low yield per plant, while F. 2013-69 L had the highest value for this trait. For seed yield per $1m^2$ a considerable variation (78-452 g/m^2) was observed, with F. 2012-244 L being the most productive genotype. The average seed yield in the studied germplasm collection was 264.4 g. A higher value of productivity was also revealed for breeding lines F. 2011-61 L, F. 2012-99 L, F. 2012-86 L and F. 2012-231 L. Seed yield per 1 m^2 for these genotypes ranged from 415 to 450 g. LECU 355 and LECU 6 showed the lowest productivity among 47 genotypes studied. Productivity for genotypes F. 2010-94 L, F. 2011-51 L, F. 2011-59 L, F. 2011-38 L, ILL 10942 was also low (82-91 g/m^2) compared to other genotypes.

In general, the range and mean value of yield parameters obtained in the present study for 47 lentil genotypes were comparable with values observed by SINGH and SINGH (2014) and TYAGI and KHAN (2011).

The correlation analysis revealed significant positive correlations between some of the yield traits (table 3). For example, seed yield per plant had a positive correlation with a number of pods per plant and 100 seed weight ($P < 0.01$). Significant positive correlation occurred between plant height and height of lowest pod. The plant height also showed a positive correlation with seed yield/m^2 ($r=0.39$; $P < 0.01$). The results were in agreement with those of SINHA and SINGH (2002) who reported strong and positive associations among seed weight per plant, the number of seeds and pods per plant in lentil. Similar results were also observed by BABAYEVA *et al*. (2014). A negative, but insignificant correlation was only observed for the height of lowest pod and number of pods per plant. Seed yield/m^2 appeared to be positively correlated with all traits, except a number of pods per plant. In contrast to our findings many

scientists (TUBA and SAKAR, 2008; YOUNIS *et al*., 2008; KARADAVUT, 2009) observed a strong positive correlation between seed yield and pod number. The lack of correlation between these traits in our experiment could be due to the empty pods. The correlation between SY and SW was low (r = 0.37) and had significance at the 5% level probability. Strong and positive correlations were found between seed yield/m^2 and seed yield per plant (r = 0.50; P < 0.01). The results were in accordance with MALHOTRA *et al*. (2004).

Table 3. Pearson correlation coefficients for yield traits in lentil genotypes

	PH	HLP	NPP	SW	SYP
PH	1				
HLP	0.679**	1			
NPP	0.030	-0.184	1		
SW	0.275	0.270	0.131	1	
SYP	0.069	0.229	0.426**	0.391**	1
SY	0.387**	0.533**	0.239	0.367*	0.500**

** and * Significant at the 0.01 and 0.05 levels, respectively

Cluster analysis based on agronomic traits grouped 47 lentil genotypes into 3 main clusters, two of which were further divided into subclusters (figure 1). UPGMA dendrogram was able to differentiate accessions according to their productivity.

Cluster I was comprised of 9 genotypes. The genotypes were characterized by low productivity and low values of yield components, except 100 seed weight. So, some genotypes from cluster I exhibited nearly maximum values for SW (5.9 g). Seed yield/m^2 for this group of genotypes ranged from 78 to 117 g/m^2. Genotypes of IRAN and Azerbaijan origin and 2 breeding lines from Australia were also placed in this cluster. Cluster II comprised of 39.5% of genotypes, was characterized by moderate productivity. Seed yiled/m^2 in this group of genotypes, including 3 breeding lines of Australian origin ranged from 150-310 g/m2. The average productivity for cluster II was 223 g/m2, which was less than the germplasm collection mean (264 g/m2). Nineteen accessions with productivity value between 288-452 g/m^2 assembled in Cluster III. Most of the genotypes from this cluster were also characterized with high plant height, 100 seed weight and seed yield per plant. Two local improved varieties (Arzu and Jasmin) also fell into cluster III, suggesting higher yield potentials of these genotypes. This was expected, as these varieties were selected from ICARDA material for their higher productivity and adaptability to agro-climatic conditions of Azerbaijan. Cluster III was in turn divided into 3 subgroups, two of which (A, B) exhibited the highest productivity with a mean of 412.5 g/m2. Seed yield per plant in these genotypes ranged from 3.8 to 10.3 g (average 6.9 g) and 100 seed weight from 3.0 to 5.2 g (average 4.1 g), which was quite high. Thus, these subgroups could be considered as the most favorable genotypes. Five breeding lines of Australian origin fell into I or II clusters, indicating low or moderate productivity for these genotypes. Two genotypes of Azerbaijan and IRAN origin also showed low productivity, while 2 improved varieties proved to be highly productive.

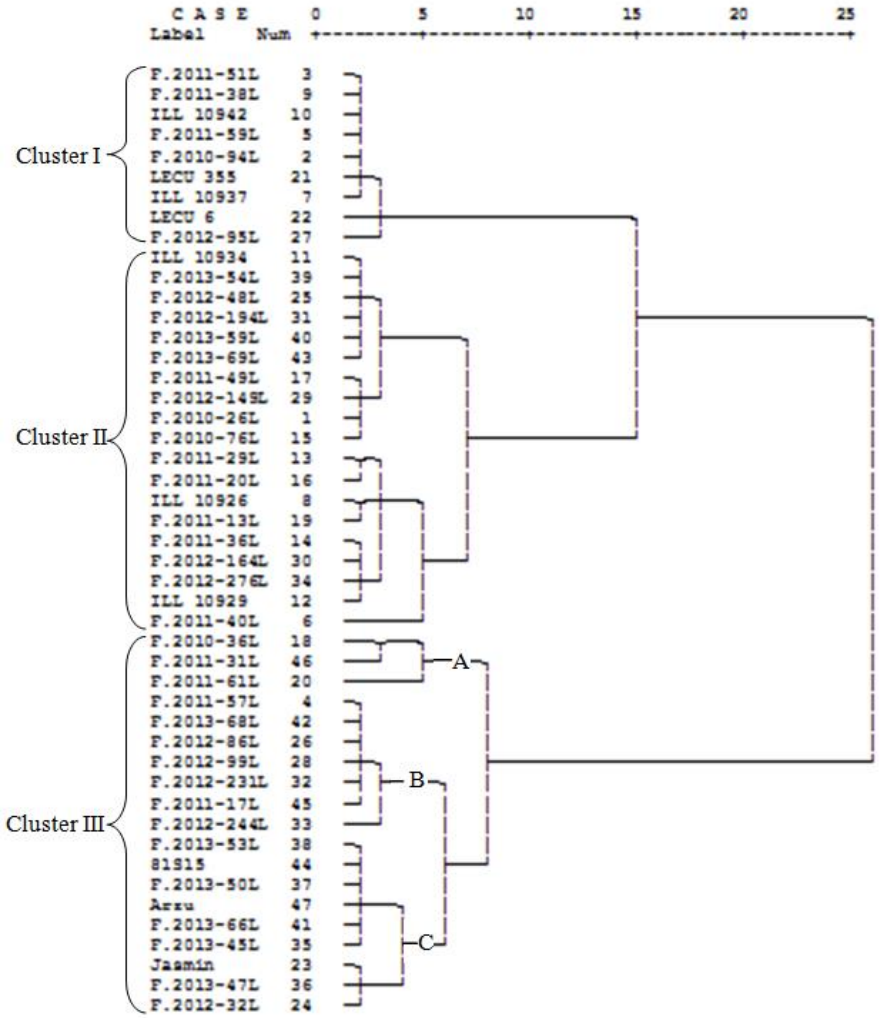

Fig. 1 Dendrogram of 47 lentil genotypes based on agro-morphological data

The present study showed that there is a considerable variation among 47 genotypes for all characters studied. Seed yield/m2 was found to be positively correlated with most of the parameters. The results shows that for the improvement of seed yield in lentil, consideration must be given to traits such as plant height, 100 seed weight and seed yield per plant. However, to confirm this fact, it is desirable to study more genotypes over locations and years. Ten breeding lines developed at ICARDA and characterized with higher productivity and yield parameters from cluster III could be recommended as favorable genotypes for further breeding programs as donors.

ISSR polymorphism and diversity analysis

Genetic diversity is the basis for plant improvement and breeding strategies. National lentil improvement program in Azerbaijan is currently focused on extending the genetic base of the lentil collection through the introduction of new breeding lines from ICARDA. Knowledge on genetic structure and diversity of this material will increase the efficiency of their utilization in the breeding process. A good genetic marker, in diversity studies, is defined by high polymorphism and the ability to generate multilocus data from the genome under study (ANNE, 2006). In this study ISSR markers were used to assess diversity and relationship among local and introduced lentil genotypes.

A total of 71 bands were generated using 7 ISSR primers in 47 lentil genotypes, of which 62 were polymorphic (table 4). The molecular size of the amplicons varied between 250-1500 bp. The number of polymorphic bands per primer ranged from 4 to 17, with an average of 8.9. The highest value was obtained by UBC 835, while UBC 848 produced the minimum number of polymorphic bands (figure 2). ISSR markers have been successfully applied to evaluate genetic variation in cultivated lentils (FIKIRU *et al*., 2007; TOKLU *et al*., 2009) and wild Lens materials (DURAN and PEREZ, 2004). TOKLU *et al.* (2009), using 10 ISSR primers for Turkish lentil landraces and cultivars, observed 7.5 polymorphic bands per primer. Similar to our results, UBC 835 and UBC 810 amplified the higher number of bands.

Table 4. ISSR primers, number of total and polymorphic bands, polymorphism ratio and GDI values

Primer name	Annealing temperature, T_a, ^0C	Number of total bands	Number of polymorphic bands	Polmorphism ratio, %	Genetic diversity index
UBC 810	41	12	11	92	0.89
UBC 812	41	9	8	89	0.82
UBC 818	47	12	11	92	0.91
UBC 827	49	7	6	86	0.74
UBC 834	45.5	7	5	71	0.74
UBC 835	46.5	18	17	94	0.95
UBC 848	49	6	4	67	0.61
Total		71	62		
Average		10.1	8.9	84	0.81

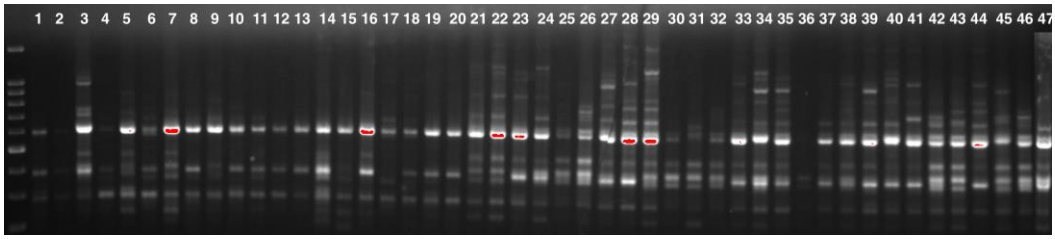

Fig. 2 ISSR profile of 47 lentil genotypes generated by UBC 835

In our study, the average polymorphism in the whole collection was 84%, which is quite higher than the value described by RUISI *et al*. (2015). Out of 7 ISSR primers, three primers (UBC 810, UBC 818, UBC 835) showed the highest level (>90%) of polymorphism (table 4). Compared to this study DURAN *et al*. (2004) detected a high degree of polymorphism (98.8%) in a set of lentil material using ISSR markers. On average ISSR markers produced more useful bands and polymorphism than RAPD markers. High polymorphism of ISSR markers is expected, since microsatellite sequences are highly variable and ubiquitously distributed across the genome (NG and TAN, 2015). Furthermore, di-nucleotide ISSR primers produce more polymorphism as reported in previous studies (CHIN *et al*., 1996; MAROTTI *et al*., 2007).

No accession specific band was identified. However, ISSR primers generated different band combinations (patterns) in most of the studied genotypes. The highest number of patterns was obtained by UBC 818 and UBC 835. In general, 7 ISSR primers were able to differentiate each lentil genotype included in the current study. In agreement with this result ISSR method has been reported to produce more complex patterns than RAPD markers (GHOWDHURY *et al*., 2002), which can be used in fingerprinting of varieties. Other studies showed similar high polymorphism of ISSR markers and their suitability for DNA fingerprinting in different crops (GOLDMAN, 2008; AYTEKIN *et al*., 2011). The presence of a high percentage of polymorphism in our study confirms the high discriminative power of used ISSR markers in the studied lentil collection. It should be also noted that high variability and diversity revealed by us is not only due to the multilocus ISSR technique used but also to the rich diversity among the lentil germplasm collection that was studied. The same set of primers revealed very low diversity (54%) in the chickpea collection (data not shown). Among a set of used ISSR primers UBC 835, UBC 818 and UBC 810 proved to be the most informative primers based on polymorphism and diversity parameters.

Genetic diversity index (GDI) depends on the number of detectable bands and the distribution of their frequency. In our study, genetic diversity values varied from 0.61 (UBC 848) to 0.95 (UBC 835), with a mean of 0.81. This was higher than the results obtained by TOKLU *et al*., (2009) who studied genetic diversity in 44 Turkish lentil cultivars and landraces. The higher value of genetic diversity index once again indicates that lentil breeding lines developed at ICARDA contain a great diversity, which may be used in breeding for the development of new varieties with various traits.

Genetic distance and cluster analysis

The UPGMA clustering based on ISSR data grouped 47 lentil genotypes into 8 clusters (figure 3). ISSR dendrogram was able to clearly distinguish all lentil accessions. The values of genetic distance (GD) coefficients among the all pair-wise combinations ranged from 0.25 to 1.0. In the present investigation the mean dissimilarity index was 0.85 which is quite high as compared to TOKLU *et al*. (2009) who reported high similarity of 44 lentil genotypes based on ISSR (GD=0.43) and AFLP (GD=0.29) data.

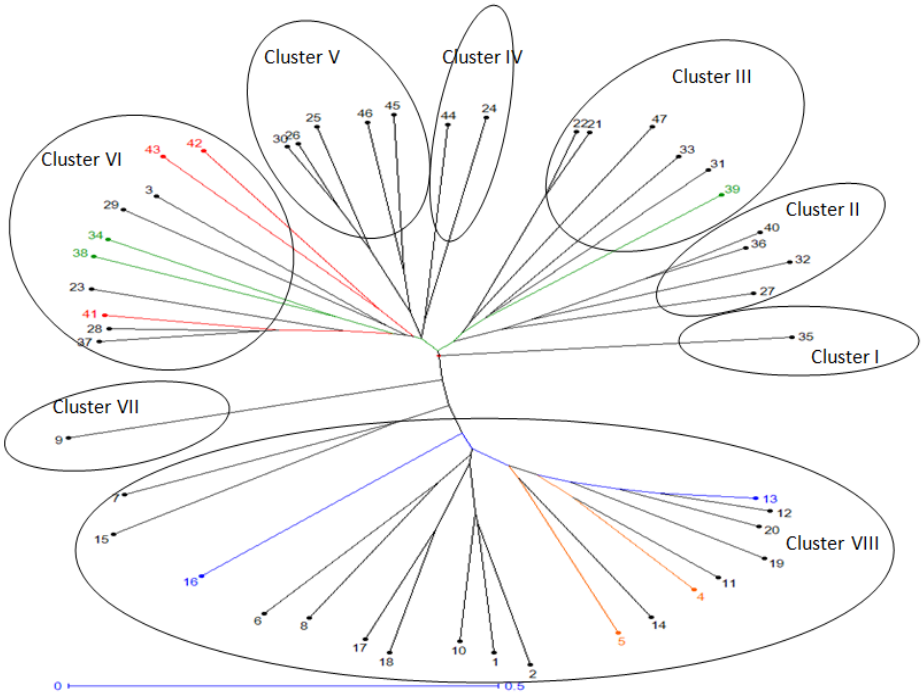

Fig. 3 UPGMA dendrogram based on Jaccard dissimilarity coeficient in 47 lentil genotypes

Two clusters (I, VII) contained a single genotype each (35 and 9, respectively), while cluster IV consisted of two genotypes; F. 2012-32 L and 81 S15. However, the genetic distance value between them was 0.72, indicating that they were still quite dissimilar. The number of genotypes in the remaining groups varied from 4 to 18. The cluster II contained 4 genotypes. Breeding lines F. 2013-47 L and F. 2013-59 L (36 and 40, respectively) showed the highest similarity (GD=0.28). Six genotypes fell into the cluster III. Two accessions of Azerbaijan and Iran origin (21 and 22) included in the study were placed in the same subcluster and were noted as genetically most similar with GD equal to 0.25. Improved variety Arzu also fell into cluster III. The genetic distance values between Arzu and the rest of the accessions in the cluster III were in the range of 0.72-0.92. Five breeding lines were grouped into cluster V. The lowest distance in this cluster was revealed between F. 2012-86 L and F. 2012-164 L (26 and 30, respectively) with GD=0.25. The cluster VI contained 9 breeding lines of ICARDA and one improved variety Jasmin. Lines 28, 37 and 41 formed one subcluster, showing the highest similarity among each other, while the rest accessions were found as genetically dissimilar with a GD range of 0.6-0.92. Most of the accessions fell into the last cluster VIII. In cluster VIII, three subclusters were formed. The breeding line of Australian origin ILL 10973 formed subcluster A together with F. 2010-76 L, while line F. 2011-20 L clustered one subcluster (B) independently. Subcluster C was further divided into 2 clear groups. In the first group lentil line from Australia

ILL 10926 clustered closely to F. 2011-40 L, while another Australian genotype ILL 10942 had a similar genetic background with F. 2010-26 L and F. 2010-94 L. Two breeding lines introduced from ICARDA (17 and 18) with GD=0.44 were also placed in this group. Eight breeding lines belonged to another group of subcluster C. Molecular characterization through ISSR data indicated low genetic variation among the breeding lines of ICARDA 12, 13 and 20, as they were all in one group (GD range: 0.25-044). Australian breeding line ILL 10934 was close to F. 2011-57 L with dissimilarity index equal to 0.44.

Numerous tendencies clearly appeared on clustering of genotypes according to their genetic background. For example, breeding lines F. 2013-66 L, F. 2013-68 L and F. 2013-69 L obtained from a cross between ILL 7221 × ILL 1005 clustered together in cluster VI, with F. 2013-68 L (42) and F. 2013-69 L (43) being in the same subcluster. The same correlation was noted for genotypes 34 and 38. Two breeding lines (4 and 5) derived from a cross ILL 8090 × ILL 7980 fell into the same subcluster, however the genetic distance between this pair was 0.6. F. 2011-13 L and F. 2011-61 L grouped together which had ILL 590 as a parent. Breeding lines F. 2012-86 L and F. 2012-164 from cluster V which had the highest similarity also clustered based on parental lineage. The results agree with ALGHAMDI *et al.* (2014) who also reported the clustering of lentil genotypes from ICARDA and Australia according to their origin and pedigree.

In our study, two genotypes from Azerbaijan and Iran exhibited the highest similarity, which was not expected. This fact may be explained by material exchange and germplasm migration by farmers. These genotypes also showed a similarity on qualitative traits. On the contrary, the genetic distance index between two local improved varieties Arzu and Jasmin was 0.92, indicating that they were quite remote in relationship. All breeding lines of Australian origin were placed in the same cluster VIII; indicating that grouping based on the genetic structure was related to the geographic origin. However, they fell into different subclusters and subgroups with high genetic distance values.

To give an overall representation of diversity Principal Coordinate Analysis (PCoA) (GOWER, 1966) was done using DARwin 6.0 programme. The PCoA analysis confirmed the positions and grouping of lentil genotypes (figure 4). The first and the second axes explained 23.3% and 8.7% of the total variance, while the first five most informative components accounted for 50.3% of the variation. Almost all lentil genotypes derived from the same cross (genotypes with same pedigree were presented by the same color) were clustered in the same quadrant. The only exception was breeding line F. 2013-53 L (38), which was localized in another quadrant. However, its position was closer to F. 2012-276 L (34). In addition, the first axis could separate most breeding lines obtained from a cross with ILL 5883. In general, the results from the Principal Coordinate Analysis were consistent with the results obtained from Jaccards's Genetic Distance estimates, with minor differences. Similar to UPGMA clustering pattern, the closest genotype pairs, such as LECU 355 (21) and LECU 6 (22); F. 2012-86 L (26) and F. 2012-164 L (30); F. 2013-47 L (36) and F. 2013-59 L (40) etc. were in the same quadrant in close positions. The breeding lines of Australian origin 11 and 12 fell into one quadrant and 7, 8 and 10 grouped in another quadrant, with 7 being most divergent. RUISI *et al.* (2015) also used PCoA to visualise 12 lentil agro-ecotypes from different areas of Sicily. Lentil accessions were clearly clustered into five groups based on both the phylogenetic tree and the PCoA. These two ways of studying the genetic diversity were considered complementary and advised to be used together.

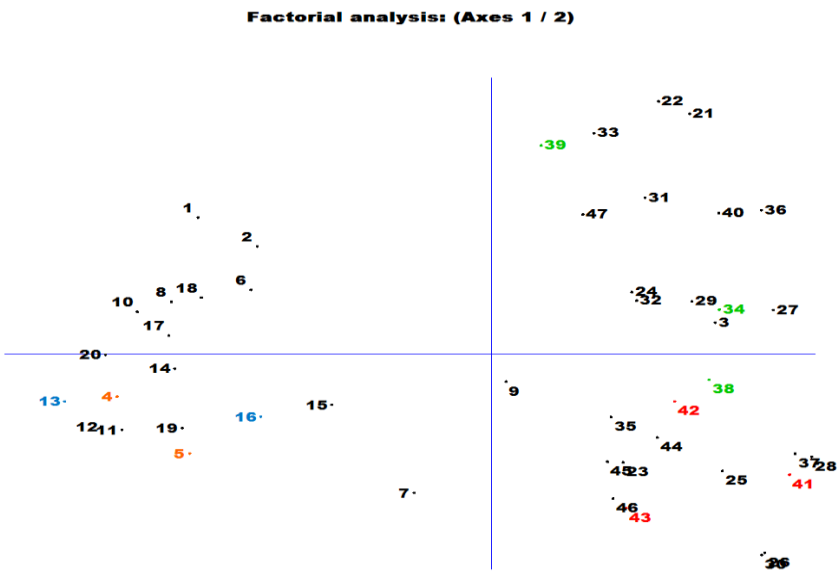

Fig. 4 Scatter plot of lentil genotypes using principle coordinate analysis based on ISSR data

In summing things up, clear tendention was observed on clustering of genotypes according to their pedigree or origin with some exceptions. Lentil genotypes did not show a significant association between agronomic and molecular data. The correlation between these two matrices was non-significant as revealed by Mantel's Z test. This can be explained by the fact that molecular diversity is invisible and unselected by breeders, while agronomic performance is subject to selection (KOENBER *et al*., 2002).

The best hybridisation programme should be initiated involving the genotypes belonging to divergent clusters with high means for yield component traits (KUMAR *et al*., 2012). Results of the current study can be used in the planning of different crosses. For example, in the dendrogram based on agronomic traits, 10 breeding lines from ICARDA germplasm pool were distinguished by high indices of yield traits. These lines fell into different groups based on ISSR data; F. 2011-57 L, F. 2011-61 L and F. 2010-36 L were allocated in the same subcluster of cluster VIII, F. 2012-86 L, F. 2011-17 L, F. 2011-31 L were placed in the cluster V, F. 2012-99 L, F. 2013-68 L in the cluster VI and F. 2012-231 L and F. 2012-244 L in clusters II and III respectively. Thus the hybridisation programme involving genotypes from these different clusters can give high yielding segregates due to non-allelic interaction (SINGH and SINGH, 2014). In other words, by crossing these superior genotypes with high genetic divergence, it is expected to obtain a combination of favorable alleles in the genes that contribute to the traits of interest. This can be also applied to other genotypes from agronomic cluster III and II with high to moderate productivity. In addition, breeding lines F. 2013-45 L and F.2011-38 L that were most divergent genotypes and formed separate clusters were also suggested for crossbreeding.

Our studies confirmed the existence of a considerable amount of genetic variability in the lentil collection at the molecular level. Provision of the molecular and agronomic database

will support the effective utilization of current lentil collection through different breeding strategies. Thus breeding lines with high agronomic performance and sufficient genetic distance from divergent clusters can be used as appropriate parents to get more heterotic recombinants, while those placed closely should be avoided to prevent inbreeding depression. This will accelerate the creation of new varieties as well adapted to eco-geographic condition of the country with stable and high yield.

ACKNOWLEDGEMENTS

The current work was done within the research program of the National Academy of Sciences of Azerbaijan "Identification of plant genetic resources at the genomic and transcriptional levels and creation of sustainable system for their conservation and sustainable use"

Received, November 22nd, 2017
Accepted April 18th, 2018

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GENETIČKE RELACIJE INTRODUKOVANE GERMLAZME SOČIVA NA OSNOVU AGRONOMSKIH SVOJSTAVA I ISSR MARKERA

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Izvod

Sočivo (*Lens culinaris* Medik.) je jednogodišnja, leguminoza za hladnu sezonu koja igra važnu ulogu u ishrani ljudi i životinja, kao i održavanje plodnosti zemljišta. Nacionalni program poboljšanja sočiva u Azerbejdžanu trenutno se fokusira na proširenje genetičke osnove kolekcije sočiva sakupljanjem novih linija iz ICARDA-e i njihovog uključivanja u oplemenjivanje. Cilj ovog rada bio je ocena performansi kolekcije sočiva, koja se uglavnom sastoji od linija dobijenih iz ICARDA-e, za osobine prinosa u uslovima Azerbejdžana, kao i ocena njihovog genetičkog diverziteta korišćenjem ISSR markera. Mnoge linije iz ICARDA-e pokazale su superiornije agro-morfološke performanse u odnosu na lokalne poboljšane sorte. Ukupno 71 traka je dobijena korišćenjem 7 ISSR prajmera kod 47 genotipova sočiva, od kojih su 62 bile polimorfne. Opseg genetičkog diverziteta je bio od 0.61(UBC 848) do 0.95 (UBC 835), sa prosekom od 0.81. ISSR dendrogram je jasno razdvojio uzorke sočiva. Uočena je jasna tendencija grupisanja uzoraka u klastere na osnovu porekla, osim nekoliko izuzetaka. Rezultati dobijeni PCA analizom bili su saglasni rezultatima klaster anlize. Proučavane linijesu sa dobrim agronomskim performansama i dovoljno genetički udaljene da mogu biti korišćene kao roditelji za dobijanje još više heterotičnih rekombinacija. Ovo će ubrzati stvaranje novih sortata dobro prilagođenih eko-geografskim uslovima Azerbejdžana sa stabilnim i visokim prinosom.

Primljeno 22.XI.2017.
Odobreno 18. IV. 2018.