Physiol Mol Biol Plants (July–August 2019) 25(4):965–974
https://doi.org/10.1007/s12298-019-00673-5

RESEARCH ARTICLE

Characterization of genetic diversity and population structure of Moroccan lentil cultivars and landraces using molecular markers

Joseph Mbasani-Mansi[1,3] · Mounia Ennami[1] · Fatima Zahra Briache[1,3] · Fatima Gaboun[1] · Nadia Benbrahim[2] · Zine El Abidine Triqui[3] · Rachid Mentag[1]

Received: 1 March 2019 / Revised: 23 April 2019 / Accepted: 1 May 2019 / Published online: 21 May 2019

Abstract Knowledge of genetic diversity and population structure is a crucial step for an efficient use of available material in a plant breeding program and for germplasm conservation strategies. Current study undertakes an assessment of the genetic variations and population structure of Moroccan lentil including nine landraces and eight released varieties using sequence-related amplified polymorphism (SRAP) and random amplified polymorphism DNA (RAPD) markers. Results revealed that the two markers used have a good efficiency to assess genetic diversity in lentil. A total of 115 and 90 bands were respectively scored for SRAP and RAPD, of which 98.3% and 93.3% were polymorphic. The polymorphic information content values were 0.350 with SRAP and 0.326 with RAPD. Analysis of molecular variance based on the combined data sets of both markers revealed lower variations within (35%) than among (65%) landraces (PhiPT = 0.652), implying significant genetic differentiation between landraces. Principal coordinate analysis and the ascendant hierarchical classification clustered samples into groups that were consistent with the geographical origin of the cultivars. Population structure corroborated the main groups and confirmed the high differentiation among them. Moroccan lentil germplasm showed a wide genetic diversity that might be conserved and assessed for tolerance to biotic and abiotic stresses.

Keywords Genetic diversity · *Lens culinaris* Medik. · Morocco · RAPD · SRAP · Population structure

Introduction

Lentil (*Lens culinaris* Medik.) is an annual food legume and one of the four most important legume crop in the world. It is a self-pollinated diploid plant with 2n = 2x = 14 chromosomes and a genome size of 4 Gbp (Arumuganathan and Earle 1991). This species has been cultivated for 10,000 years in several regions worldwide, especially in West Asia, Australia, North and South America, Mediterranean basin, Middle East, and in the Indian sub-continent (Cubero et al. 2009). Lentil seeds are a good source of human nutrient, containing proteins, carbohydrates, fibers, minerals and antioxidant compounds (Solanki et al. 1999; Boye et al. 2010; Alghamdi et al. 2014; Migliozzi et al. 2015). Moreover, lentil straw is used as high-quality animal feed (Erskine et al. 1990; Devendra 1997; Abd El Moneim and Ryan 2004; Lardy and Anderson 2009). In addition, lentil provides interesting possibilities for sustainable agriculture due to its nitrogen fixing capacity that enable low use of fertilizers in cereal-based cropping system (Prakesh et al. 1986; Shah et al. 2003).

In Morocco, lentil is essentially produced in constraining environments, using landraces maintained by farmers. Landraces are characterized by specific and evolutionary

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12298-019-00673-5) contains supplementary material, which is available to authorized users.

✉ Rachid Mentag
rachidmentag@yahoo.ca

[1] Biotechnology Research Unit, National Institute of Agricultural Research, Rabat, Morocco

[2] Plant Breeding, Conservation and Valorization of Phyto-Genetic Resources Research Unit, National Institute of Agricultural Research, Rabat, Morocco

[3] Department of Biology, Faculty of Sciences, Plant and Microbial Biotechnology, Biodiversity and Environment Research Center, Mohammed V University, Rabat, Morocco

adaptation as well as high nutritional and organoleptic qualities (Benbrahim et al. 2017). However, their low yield implies the development of novel cultivars with high yield potential and resistance to biotic and abiotic stresses. Breeding could therefore lead to reduction or loss of lentil genetic diversity over time (Kumar et al. 2018). Thus, an assessment of plant genetic diversity for an efficient use in both breeding and conservation programs is worth carrying out.

Morphological and isozymes markers were first used for lentil genetic diversity assessment (Ladizinsky 1979; Pinkas et al. 1985; Erskine and Muehlbauer 1991; Ahmad and McNeil 1996). However, those markers are influenced by environmental conditions (Marić et al. 2004). For that reason, molecular markers are being largely used for this purpose. These markers are advantageous as they cover the entire genome, hence their high number, and are unaffected either by environmental factors or by plant development stages. Different molecular markers such as random amplified polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), inter simple sequence repeats (ISSR), sequence-related amplified polymorphism (SRAP), and single nucleotide polymorphism (SNP) has been used to assess genetic diversity of lentil (Abo-elwafa et al. 1995; Toklu et al. 2009; Tewari et al. 2012; Zaccardelli et al. 2012; Joshi et al. 2013; Rana et al. 2013; Alghamdi et al. 2014; Bermejo et al. 2014; Lombardi et al. 2014; Seyedimoradi and Talebi 2014; Khazaei et al. 2016). It is noteworthy that the genetic diversity of Moroccan lentil is not yet well studied. Recently, some Moroccan lentil genotypes, including landraces from five regions and two local released varieties, have been assessed by using SSR and AFLP markers (Idrissi et al. 2015, 2016). These studies, which focused merely on diversity among cultivars rather than within cultivars, revealed moderate to high genetic variations (Idrissi et al. 2015). Thus, the principal purpose of this study was to assess genetic variations among and within Moroccan lentil cultivars using SRAP and RAPD markers, as genetic variations within populations is a fundamental component to guide decision-making by breeders. Certain invaluable information on the basis of this investigation regarding breeding programs is expected.

Materials and methods

Plant materials

A set of nine landraces from different origins in Morocco and eight local registered varieties from National Institute of Agricultural Research (INRA) were assessed (Table 1).

DNA extraction

Molecular analyses were performed using young leaves collected from lentil seedlings, placed in liquid nitrogen and stored at − 80 °C before lyophilization. Genomic DNA was extracted according to CTAB protocol, described by Lassner et al. (1989). Extracted DNAs from 89 individual plants were evaluated for quality by electrophoresis in agarose gel, and then quantified using Nano − 100 micro spectrophotometer (Xian Yima Opto-electrical Technology, Shaanxi, China).

SRAP analysis

A total of 30 SRAP combinations of five forward (ME) and six reverse (EM) primers (Table 2) (Li and Quiros 2001), purchased in commercially available kits from Eurogentec, were screened for polymorphism on three randomly chosen lentil genotypes. SRAP fragments were generated in a reaction volume of 20 µl containing 1X PCR buffer (BIOLINE, London, UK), 250 µM of each dNTP, 3 mM of $MgCl_2$, 1 U of Taq™ DNA polymerase (BIOLINE, London, UK), 0.5 µM of each forward and reverse primers, and 50 ng of genomic DNA sample. Amplifications were carried out according to Li and Quiros (2001) PCR program, with an initial denaturation at 94 °C for 5 min, followed by 5 cycles of 94 °C, 35 °C, and 72 °C for 1 min each, as denaturation, annealing and extension respectively. Then the annealing temperature was set to 50 °C for other 35 cycles, followed by 5 min of final extension at 72 °C. PCR products were resolved using 6% polyacrylamide gel. After electrophoresis, the gel was carefully removed from the plates and stained in an ethidium bromide solution for 5 min. It was then rinsed with tap water before viewing the bands under ultraviolet light in Molecular Imager® Gel Doc™ XR System.

RAPD analysis

A set of 62 decamer primers (Online resource 1), purchased in commercially available kits from OPERON (OPERON Technologies, Alameda, CA, USA), were screened on three lentil samples. Primers revealing clear polymorphisms were used on the 89 lentil DNA samples. RAPD-PCR was performed in a final volume of 20 µl containing 25 ng of DNA, 1X buffer (Bioline, London, UK), 250 µM of each dNTP, 3 mM of MgCl2, 1 U of Taq™ DNA polymerase (Bioline, London, UK), and 0.5 µM of primer. PCR amplification was set in a thermocycler Applied Biosystems (Foster City, CA, USA) programmed as followed: 2 min of initial denaturation at 94 °C, followed by 45 cycles of 94 °C for 1 min, 36 °C for 1 min and 72 °C for 1 min with a final elongation at 72 °C for 5 min.

Table 1 List of Moroccan lentil (landraces or varieties) and number of samples used for molecular analysis

N°	Cultivars/Landraces	Code	Status	Number of samples
1	Ain Sbit	LR1	Landrace	9
2	Ait Attab/Beni Mellal	LR2	Landrace	9
3	Ajdir/El Houceima	LR3	Landrace	9
4	Larache	LR4	Landrace	9
5	Moulay Bouslham	LR5	Landrace	9
6	Ouled Lhaj/Fes	LR6	Landrace	9
7	Taza	LR7	Landrace	9
8	Tétouan	LR8	Landrace	9
9	Precoce/Zaer	LR9	Landrace	9
10	Abda	V01	Variety (2004[a])	1
11	Bakria	V02	Variety (1989[a])	1
12	Bichette	V03	Variety (2000[a])	1
13	Chakkouf	V04	Variety (2009[a])	1
14	Hamria	V05	Variety (2003[a])	1
15	L24	V06	Variety (1989[a])	1
16	L56	V07	Variety (1989[a])	1
17	Zaaria	V08	Variety (2003[a])	1

[a]Registration year of variety in the Moroccan national catalogue

Table 2 Forward and reverse primers names and sequences used for SRAP analysis

Forward		Reverse	
Name	Sequence (5′-3′)	Name	Sequence (5′-3′)
ME1	TGAGTCCAAACCGGATA	EM1	GACTGCGTACGAATTAAT
ME2	TGAGTCCAAACCGGAGC	EM2	GACTGCGTACGAATTTGC
ME3	TGAGTCCAAACCGGAAT	EM3	GACTGCGTACGAATTGAC
ME4	TGAGTCCAAACCGGACC	EM4	GACTGCGTACGAATTTGA
ME5	TGAGTCCAAACCGGAAG	EM5	GACTGCGTACGAATTAAC
		EM6	GACTGCGTACGAATTGCA

Amplification products were fractioned by 1.5% agarose gel electrophoresis. Gels were stained with ethidium bromide before the visualization of amplified fragments using Molecular Imager® Gel Doc™ XR System.

Statistical analyses

RAPD and SRAP profiles were scored as present (1) or absent (0) for each primer or primer combination to generate a binary format matrix. Only clearest and reproducible bands were recorded. Polymorphic Information Content value (PICv) was calculated for each primer according to Roldán-Ruiz et al. (2000), in order to measure the efficiency of polymorphic loci. GenAlex ver. 6.5 software (Peakall and Smouse 2006) was used to perform genetic diversity analysis within landraces by calculating: percentage of polymorphic loci (PPL), expected heterozygoty (He), and Shannon's information index (I). Analysis of molecular variance (AMOVA) was performed, using data from SRAP and RAPD, to estimate variations within and among landraces. Comparison of genetic distance from SRAP and RAPD markers of all single DNA samples (landraces and varieties) was computed using Mantel test. For cluster and relationships analysis among cultivars, SRAP and RAPD data of all samples were combined in a single matrix. GenAlEx ver. 6.5 software was used to calculate Nei genetic distance among cultivars. Their relationships were visualized through principal coordinates analysis (PCoA), and a dendrogram based on an unweighted pair-group method with arithmetic averages (UPGMA) was constructed on XLSTAT 5.14 software (XLSTAT 2017) using Jaccard's similarity index. To infer population structure and assign individuals to populations, molecular data were analyzed using STRUCTURE software v.2.3.4 (Pritchard et al. 2000) based on a Bayesian clustering approach. The number of assumed clusters (K) was set from two to eight. Each simulation consisted in 100,000 Monte-Carlo Markov Chain (MCMC) iterations

after a burn-in period length of 50,000. The output of STRUCTURE software was then uploaded in the STRUCTURE HARVESTER online tool (Evanno et al. 2005) implementing the Evanno method to identify the most probable structure with the best K value.

Results

A total of eight SRAP primers pairs (ME1-EM3, ME1-EM4, ME1-EM5, ME2-EM6, ME3-EM4, ME4-EM1, ME5-EM3, and ME5-EM5) and nine RAPD primers (OPD 13, OPL 12, OPL 16, OPL 19, OPX 04, OPX 08, OPX 12, OPX 13, and OPX 17) were selected as they generated robust amplification products and showed clear polymorphism.

Characterization and efficiency of SRAP markers

SRAP primer combinations produced 115 fragments among which 113 (98.3%) were polymorphic. The number of polymorphic bands by primer combination ranged from 11 (ME5-EM5) to 18 (ME2-EM6) with an average of 14.1 bands. Six primer combinations (ME1-EM4, ME1-EM5, ME2-EM6, ME3-EM4, ME5-EM3, and ME5-EM5) presented 100% of polymorphic rates. The PICv varied from 0.31 (ME5-EM3) to 0.39 (ME5-EM5) with an average of 0.35 (Table 3).

Characterization and efficiency of RAPD markers

RAPD primers generated 84 polymorphic bands out of 90 identified (93.3%). The number of fragments amplified by each primer varied from five (OPX 12) to 13 (OPL16). Polymorphism rates generated with RAPD primer ranged from 71.4% (OPX12) to 100% (OPD13, OPL12, OPX04, OPX08, OP13, and OPX17). The PICv ranged from 0.24 (OPL 12) to 0.40 (OPX 08), with an average of 0.33 (Table 3).

Genetic diversity of lentil landraces

SRAP and RAPD markers revealed various levels of genetic diversity within and among lentil landraces (Table 4). Considering SRAP markers, the highest values of number of different alleles (Na), number effective alleles (Ne), Shannon's information index (I), and expected heterozygosity (He) were recorded in LR9, with values 1.26, 1.33, 0.25, and 0.18, respectively. While, the lowest values were observed in LR2 and LR3, with values 1.10, 1.26, 0.20, and 0.14, respectively. Percentage of polymorphic loci (PPL) of landraces with SRAP markers ranged from 31.30 (LR2 and LR3) to 40.0 (LR4). RAPD markers revealed high diversity indexes values (Na, Ne, I, He, and PPL) in LR8 with values 1.23, 1.28, 0.23, 0.16, and 41.1 (Table 4). Combination of data from both markers produced high genetic diversity indices in LR8 and low values in LR2.

Analysis of molecular variance performed with SRAP and RAPD matrix revealed high variance among lentil landraces (61% and 70% respectively) than within landraces (39% and 30% respectively). Combining data from SRAP and RAPD showed 65% of variation among landraces and 35% of variations within landraces, with a significative PhiPT value (PhiPT = 0.652, $p = 0.001$) (Table 5).

Genetic distance

Results of Mantel test showed high correlation among genetic distance revealed by SRAP and RAPD ($r = 0.68$, $p = 0.01$). Thus, the genetic distance of 17 tested lentils, based on combined SRAP and RAPD matrix revealed a wide range of values (Table 6). Genetic distance (GD) varied from 0.149 and 0.630. LR1 and LR9 presented the lowest GD, while LR2 and V05 exhibited the highest GD.

Cluster analysis

The Jaccard's index calculated with data from combined matrix of SRAP and RAPD markers, and used to build an UPGMA tree ranged from 0.503 to 0.969. At 60% of similarity, the dendrogram clustered all cultivars into four main groups, which fit their origin (Online resource 2). Cluster I included four landraces (LR4, LR5, LR9, and LR1) and five varieties (V07, V02, V03, V05, and V04). Cluster II grouped three landraces (LR3, LR6, and LR7) and one variety (V06). Cluster III consisted of individuals from one landrace (LR2) and two varieties (V01 and V08). The last cluster contains only representatives of LR8.

Principal coordinate analysis (PCoA) and population structure

The principal component analysis (PCoA) of 89 lentil samples was performed to establish relationships among individuals. Total variation explained by PCoA was 37.08%, with 14.83% for the first principal component (PCo1), 12.55% for the second (PCo2), and 9.70% for the third (PCo3). Biplot generated by PCoA showed a clear repartition of landraces according to their origins (Fig. 1). Varieties were widely scattered in the plot.

Characterization of genetic structure of 89 individuals using STRUCTURE software, following the Bayesian approach, allowed identification of the best K value. In fact, STRUCTURE output extracted with Structure

Table 3 Bands number, polymorphism rate, and PIC values generated with eight SRAP primers pairs and nine RAPD primers on 17 lentil cultivars

Primers		Total number of bands	Number of polymorphic bands	Polymorphism rate (%)	PICv[a]
SRAP	ME1-EM3	14	13	92.9	0.32
	ME1-EM4	15	15	100.0	0.36
	ME1-EM5	13	13	100.0	0.37
	ME2-EM6	18	18	100.0	0.36
	ME3-EM4	15	15	100.0	0.35
	ME4-EM1	14	13	92.9	0.35
	ME5-EM3	15	15	100.0	0.31
	ME5-EM5	11	11	100.0	0.39
	Total	115	113	–	–
	Mean	14.4	14.1	98.3	0.35
RAPD	OPD 13	6	6	100.0	0.37
	OPL 12	8	8	100.0	0.24
	OPL 16	15	13	86.7	0.30
	OPL 19	11	9	81.8	0.30
	OPX 04	12	12	100.0	0.36
	OPX 08	11	11	100.0	0.40
	OPX 12	7	5	71.4	0.29
	OPX 13	11	11	100.0	0.32
	OPX 17	9	9	100.0	0.36
	Total	90	84	–	–
	Mean	10	9.3	93.3	0.33

[a]*PICv* polymorphic information content values

Table 4 Genetic diversity indices for each lentil landraces revealed by SRAP and RAPD markers

Landraces	SRAP					RAPD				
	Na	Ne	I	He	PPL	Na	Ne	I	He	PPL
LR1	1.20	1.27	0.22	0.15	36.5	1.13	1.22	0.17	0.12	28.8
LR2	1.10	1.26	0.20	0.14	31.3	0.92	1.13	0.10	0.07	15.5
LR3	1.10	1.26	0.20	0.14	31.3	1.04	1.22	0.17	0.12	28.8
LR4	1.23	1.30	0.24	0.17	40.0	0.91	1.15	0.11	0.08	16.6
LR5	1.26	1.27	0.22	0.15	39.1	0.99	1.21	0.16	0.11	25.5
LR6	1.19	1.29	0.22	0.15	33.9	1.04	1.19	0.16	0.11	27.7
LR7	1.20	1.29	0.22	0.15	36.5	0.94	1.19	0.15	0.10	24.4
LR8	1.16	1.27	0.21	0.15	34.8	1.23	1.28	0.23	0.16	41.1
LR9	1.26	1.33	0.25	0.18	39.1	0.96	1.13	0.10	0.07	14.4
Mean	1.19	1.28	0.22	0.15	35.9	1.02	1.19	0.15	0.10	24.8

Na number of different alleles; *Ne* number of effective alleles; *I* Shannon's information index; *He* expected heterozygosity; *PPL* percentage of polymorphic loci

Harvester, detected the highest delta K value with K equals four, suggesting that the most probable structure of tested samples is their distribution into four main populations (Fig. 2). This population structure is associated with a high gene flow within populations and a low gene flow among them.

Individuals are represented by vertical colored columns. Same color in different individuals indicates that they belong to the same genetic group. Different colors in the same individual indicate the posterior probability to belong to different genetic clusters.

Table 5 Analysis of molecular variance (AMOVA) of nine landraces based on data from SRAP, RAPD and combined SRAP and RAPD markers

Marker	Source of variation	Df	SS	VC	%VC
SRAP	Among landraces	8	1015.185	13.179	61
	Within landraces	72	596.667	8.287	39
	Total	80	1611.852	21.466	100
RAPD	Among landraces	8	840.074	11.148	70
	Within landraces	72	336.444	4.673	30
	Total	80	1176.519	15.821	100
Combined SRAP and RAPD	Among landraces	8	1855.259	24.328	65
	Within landraces	72	933.111	12.960	35
	Total	80	2788.370	37.287	100
	Stat	Value	*p*		
	PhiPT	0.652	0.001		

Df degree of freedom; *SS* sum squares; *VC* variance components; *%VC* percentage of variation components

Table 6 Pairwise matrix of Nei genetic distance among 17 lentil cultivars, calculated using SRAP and RAPD data

	LR1	LR2	LR3	LR4	LR5	LR6	LR7	LR8	LR9	V01	V02	V03	V04	V05	V06	V07	V08
LR1	0.000																
LR2	0.368	0.000															
LR3	0.380	0.508	0.000														
LR4	0.291	0.497	0.483	0.000													
LR5	0.323	0.470	0.463	0.168	0.000												
LR6	0.378	0.410	0.324	0.401	0.391	0.000											
LR7	0.377	0.470	0.193	0.343	0.342	0.320	0.000										
LR8	0.482	0.546	0.388	0.417	0.363	0.358	0.368	0.000									
LR9	0.149	0.386	0.410	0.302	0.365	0.376	0.392	0.492	0.000								
V01	0.419	0.386	0.350	0.507	0.485	0.476	0.398	0.489	0.421	0.000							
V02	0.317	0.424	0.460	0.404	0.416	0.390	0.371	0.383	0.342	0.339	0.000						
V03	0.381	0.527	0.501	0.344	0.285	0.430	0.385	0.442	0.373	0.455	0.448	0.000					
V04	0.370	0.497	0.391	0.382	0.411	0.360	0.253	0.317	0.341	0.418	0.299	0.273	0.000				
V05	0.413	0.630	0.439	0.461	0.432	0.408	0.358	0.380	0.448	0.536	0.495	0.418	0.326	0.000			
V06	0.469	0.518	0.370	0.435	0.390	0.381	0.259	0.379	0.431	0.487	0.360	0.448	0.353	0.463	0.000		
V07	0.243	0.469	0.478	0.336	0.369	0.395	0.412	0.456	0.246	0.495	0.367	0.410	0.346	0.440	0.519	0.000	
V08	0.406	0.315	0.346	0.529	0.481	0.374	0.352	0.462	0.385	0.389	0.381	0.587	0.418	0.519	0.367	0.448	0.000

Discussion

Various molecular markers were used in lentil genetic studies, including RFLP, RAPD, SSR, ISSR, AFLP, and SRAP. The choice of molecular marker is a critical step for geneticists and breeders. Our study reports genetic diversity and population structure of Moroccan lentil, including landraces and released varieties, using two PCR-based marker systems. SRAP and RAPD markers cover the entire genome and are easy techniques that can detect DNA polymorphism without knowledge of genome sequence. Whereas RAPD uses a single primer that amplifies DNA arbitrarily (Williams et al. 1990), SRAP detects polymorphism with a primer pair that targets open reading frames (ORFs) (Li and Quiros 2001).

Both molecular markers generated high polymorphism levels, although the average number of fragments per primer, polymorphism rates, and PICv were slightly higher with SRAP primer combinations (14.4, 98.3, and 0.35 respectively) compared to those of RAPD primers (10, 93.3, and 0.33 respectively). This finding is in accordance with several studies reporting the high efficiency of SRAP and RAPD markers (Keify and Beiki 2012; Yin et al. 2014). Polymorphism rates obtained with SRAP primers combinations during this study were consistent with those observed with 35 lentil breeding lines by Alghamdi et al.

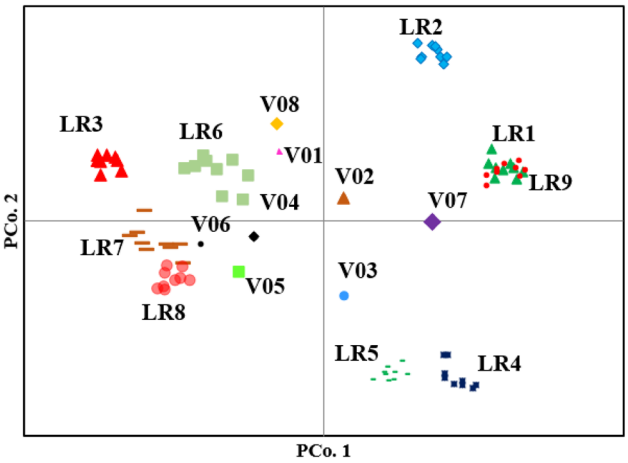

Fig. 1 Principal coordinates analysis (PCoA) of 89 lentil samples from 17 lentil cultivars based on combined data from SRAP and RAPD markers

(2014). However, the polymorphism levels were higher than those obtained by Bermejo et al. (2014) using SRAP on 25 lentil recombinant inbred lines. The average of polymorphism rate (93.3%) generated with RAPD markers was similar to those reported by Tewari et al. (2012) in 83 lentil genotypes and Abo-elwafa et al. (1995) in 36 genotypes of wild and cultivated lentils, reaching 91.3% and 90% respectively. In contrast, low polymorphism rates (54%) were detected in two different studies using RAPD in lentil (Sonnante and Pignone 2001; Yüzbaşıoğlu et al. 2006). In another study including Italian lentil landraces, 57% of bands were polymorphic using AFLP markers (Torricelli et al. 2012). Furthermore, Moroccan lentil expressed moderate polymorphism (54.78%) and PICv (0.3391) with AFLP markers on 51 landraces (Idrissi et al. 2015). Discrepancies of polymorphism indices among studies could be attributed to the informativeness level of selected primers.

Landraces expressed different genetic diversity levels, which varied. The genetic diversity indexes recorded within landraces with SRAP and RAPD markers were relatively close (0.15 and 0.13 respectively). This is in accordance with Idrissi et al. (2015) study in Moroccan landraces where they detected a genetic diversity of 0.13 with AFLP markers. Similarly, Toklu et al. (2009) found a mean of 0.18 after analyzing Turkish lentil landraces using AFLP and ISSR markers. In contrast, higher genetic diversity values were obtained by Tewari et al. (2012) with RAPD and SSR in Indian lentil (0.29, 0.24 respectively) and by Zaccardelli et al. (2012) using SSR markers in some Italian landraces (0.29). Among our landraces, LR2 expressed the lowest genetic diversity with the combined data from SRAP and RAPD markers. This could be the consequence of its geographic origin. LR2 is a landrace collected in Beni Mellal, a region that is geographically distant in comparison with the original regions of other landraces used in this study. Furthermore, this area is characterized by low rainfall which affects negatively the yield. Reduction of the plant yield could probably contribute to the loss of available diversity. This is in accordance with Sonnante and Pignone (2007) who indicated that low genetic diversity of a landrace is a result of a bottleneck effect when grown in an isolated area.

No report of variations within Moroccan landraces is available; previous study focused on variations among landraces rather than within landraces (Idrissi et al. 2015). Results of AMOVA test showed larger variations among than within landraces (65% and 35% respectively). The same pattern was reported by Tewari et al. (2012), who observed that variation among lentil accessions (63%) were higher than within them (37%). This suggests that landraces have a very low overlap, which may lead to a low gene flow among them. Furthermore, since lentil is a self-pollinating plant and farmers generally maintain their own seeds, reduction in variations within population are observed over time, resulting in an increase of inter-population variation. This is generally the case in Moroccan lentil, which is essentially produced by farmers using their own landraces. In this context, LR1 landrace was labelled with a Geographical Indication (GI) in 2015 because of its seeds nutritional quality that is associated to specific environment and agricultural practices of its production (Benbrahim et al. 2017).

Variable genetic distances among lentil cultivars were recorded in this study. Results revealed that landraces with the lowest distance (LR1 and LR9: 0.149; LR4 and LR5: 0.168; LR3 and LR8: 0.193) are cultivated in adjacent geographic regions. This suggests a possible existence of

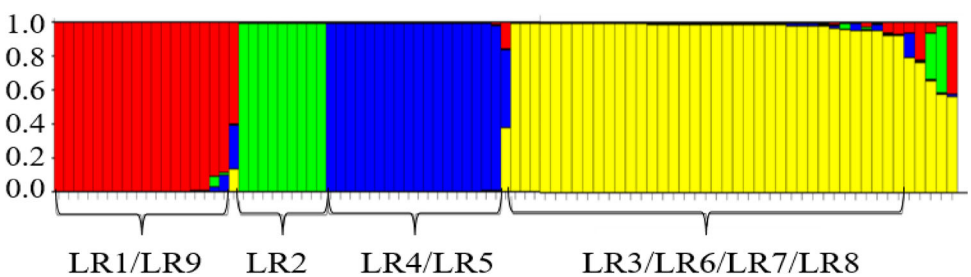

Fig. 2 Genetic assignment of individuals to populations according to the Bayesian method implemented in the program STRUCTURE at K = 4 based on SRAP and RAPD markers

seed movement among these areas, unlike populations of distant geographic origins (LR2 and LR8; LR2 and LR3), which exhibited higher genetic distances (0.546 and 0.508 respectively).

PCoA and cluster analysis displayed relationships among individuals and grouped landraces into four clusters. STRUCTURE analysis consolidated this result, and confirmed that population genetic structure of tested landraces is essentially based on their geographic origin. It also highlighted high gene flow within populations, especially between LR1 and LR9 (from Zaer), LR4 and LR5 (from Gharb), LR3, LR6, LR7 and LR8 (from Rif and Saïs), and a high inter-population differentiation among them. This result is in accordance with the positive and significant correlation between the genetic and the geographic distances, calculated with Mantel test ($r = 0.673$, $p = 0.01$). Separation of landraces could be the result of a long evolution process caused by various climatic conditions of the regions where they are cultivated, associated with agricultural practices. Geographically isolated populations accumulate genetic differences as they acclimatize themselves to different environments (Tewari et al. 2012). This is consistent with the studies that established, immigration rates in plants are inversely proportional to the distance between populations and suggested that the most common gene flow pattern is isolation by distance (IBD) (Sexton et al. 2014). The genetic variation among the plant material studied reflects varied responses observed by Mbasani-Mansi et al. (2019) while assessing its resistance to *Orobanche crenata*, a parasitic plant that is threatening to lentil production. These authors detected some sources of resistance to include in a breeding program.

The limited number of populations revealed by the structure analysis in this study can help to select lentils to include in a core collection for conservation in Moroccan lentil gene bank. A core collection is important, as it allows working with fewer accessions, representative of a crop species with minimum repetitiveness (Arber et al. 1984; Khazaei et al. 2013).

Improved varieties were not affected to a unique cluster; however, most of them were grouped in cluster I. Results of our investigation also confirmed the origin of V07 (L56) that is a released cultivar from LR9 (Precoce), as supposed by Benbrahim et al. (2017). Similar result was reported by Idrissi et al. (2015) who used combined data sets from the SSR and AFLP analyses and clustered this variety into the same group with a landrace from Zaer.

The genetic diversity detected in the plant material studied is consistent with varying responses it expressed to *Orobanche crenata* infection.

Conclusion

This study reports population structure and genetic diversity among and within a Moroccan lentil collection using SRAP and RAPD markers. These makers revealed high polymorphism rates and PIC values. Combination of the two markers targeting different regions of the genome provided adequate information on the genetic diversity among genotypes. Within landraces variation was lower than among landraces. PCoA and cluster analysis displayed groups based on their geographic origins. The genetic diversity and the differentiation among populations associated with characterization of this plant material will offer breeders opportunities to develop new varieties with beneficial agronomic traits to cope with climate changes and biotic stresses. In parallel, ex situ conservation of local landraces should be a priority. In this study, structure analysis indicated a low gene flow among populations. Regrouping the studied genotypes into reduced number of populations consolidates the usefulness of structure analysis while managing conservation strategies and developing core collections.

Acknowledgments This research was supported by National Institute of Agricultural Research (INRA-Morocco) and Ministry of Higher Education, Scientific Research and Professional Training of Morocco (MESRSFC) through funding of MEDILEG Project within the European Union 7th Framework program for research, technological development and demonstration (ERA-Net Project, ARIMNet).

Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

References

Abd El Moneim AM, Ryan J (2004) Forage legumes for dryland agriculture in Central and West Asia and North Africa. In: Rao, Ryan J (eds) Challenges and strategies of dryland agriculture, crop science. Society of America and American Society of Agronomy, Madison, pp 243–256

Abo-elwafa A, Murai K, Shimada T (1995) Intra- and inter-specific variations in Lens revealed by RAPD markers. Theor Appl Genet 90:335–340

Ahmad M, McNeil DL (1996) Comparison of crossability, RAPD, SDS-PAGE and morphological markers for revealing genetic relationships within and among Lens species. Theor Appl Genet 93:788–793

Alghamdi SS, Khan AM, Ammar MH, El-Harty EH, Migdadi HM, El-Khalik SM, Al-Shameri AM, Javed MM, Al-Faifi SA (2014) Phenological, nutritional and molecular diversity assessment among 35 introduced lentil (*Lens culinaris* Medik.) genotypes grown in Saudi Arabia. Int J Mol Sci 15:277–295

Arber W, Illmensee K, Peacock WJ, Starlinger P (1984) Genetic manipulation: impact on man and society. Cambridge University Press, Cambridge

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Benbrahim N, Taghouti M, Zouahri A, Gaboun F (2017) On-farm conservation of Zaer lentil landrace in context of climate change and improved varieties competition. Univ J Agric Res 5:27–38

Bermejo C, Gatti I, Caballero N, Cravero V, Martin E, Cointry E (2014) Study of diversity in a set of lentil RILs using morphological and molecular markers. Aust J Crop Sci 8:689–696

Boye J, Zare F, Pletch A (2010) Pulse proteins: processing, characterization, functional properties and applications in food and feed. Food Res Int 43:414–431

Cubero JI, Pérez de la Vega M, Fratini R (2009) Origin, phylogeny, domestication and spread. In: Erskine W, Muehlbauer FJ, Sarker A, Sharma B (eds) The lentil: botany, production and uses. CAB International, Wallingford, pp 13–33

Devendra C (1997) Crop residues for feeding animals in Asia: technology development and adoption in crop/livestock systems. CAB International, Wallingford

Erskine W, Muehlbauer FJ (1991) Allozyme and morphological variability, outcrossing rate and core collection formation in lentil germplasm. Theor Appl Genet 83:119–125

Erskine W, Rihawi S, Capper BS (1990) Variation in lentil straw quality. Anim Feed Sci Technol 28:61–69

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620

Idrissi O, Udupa SM, Houasli C, De Keyser E, Van Damme P, De Riek J (2015) Genetic diversity analysis of Moroccan lentil (*Lens culinaris* Medik.) landraces using Simple Sequence Repeat and amplified fragment length polymorphisms reveals functional adaptation towards agro-environmental origins. Plant Breed 134:322–332

Idrissi O, Udupa MS, De Keyser E, Van Damme P, De Riek J (2016) Functional genetic diversity analysis and identification of associated simple sequence repeats and amplified fragment length polymorphism markers to drought tolerance in lentil (*Lens culinaris* ssp. *culinaris* Medicus) landraces. Plant Mol Biol Rep 34:659–680

Joshi M, Aldred P, McKnight S, Panozzo JF, Kasapis S, Adhikari R, Adhikari B (2013) Physicochemical and functional characteristics of lentil starch. Carbohydr Polym 92:1484–1496

Keify F, Beiki AH (2012) Exploitation of random amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP) markers for genetic diversity of saffron collection. J Med Plants Res 6:2761–2768

Khazaei H, Street K, Bari A, Mackay M, Stoddard FL (2013) The FIGS (Focused Identification of Germplasm Strategy) approach identifies traits related to drought adaptation in *Vicia faba* genetic resources. PLoS ONE 8:e63107

Khazaei H, Caron CT, Fedoruk M, Diapari M, Vandenberg A, Coyne CJ, McGee R, Bett KE (2016) Genetic diversity of cultivated lentil (*Lens culinaris* Medik.) and its relation to the world's agro-ecological zones. Front Plant Sci 7:1093

Kumar J, Gupta S, Dubey S, Gupta P, Gupta DS, Singh NP (2018) Genetic diversity changes in Indian lentils over the times. J Plant Biochem Biotechnol 27:415–424

Ladizinsky G (1979) Species relationships in the genus *Lens* as indicated by seed-protein electrophoresis. Bot Gaz 140:449–451

Lardy G, Anderson V (2009) Alternative feeds for ruminants. NDSU, Fargo

Lassner MW, Peterson P, Yoder JI (1989) Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. Plant Mol Biol Rep 7:116–128

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor Appl Genet 103:455–461

Lombardi M, Materne M, Cogan NO, Rodda M, Daetwyler HD, Slater AT, Forster JW, Kaur S (2014) Assessment of genetic variation within a global collection of lentil (*Lens culinaris* Medik) cultivars and landraces using SNP markers. BMC Genet 15:150

Marić S, Bolarić S, Martinčić J, Pejić I, Kozumplik V (2004) Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. Plant Breed 123:366–369

Mbasani-Mansi J, Briache FZ, Ennami M, Gaboun F, Benbrahim N, Triqui ZEA, Mentag R (2019) Resistance of Moroccan lentil genotypes to *Orobanche crenata* infestation. J Crop Improv 33(3):306–326

Migliozzi M, Thavarajah D, Thavarajah P, Smith P (2015) Lentil and kale: complementary nutrient-rich whole food sources to combat micronutrient and calorie malnutrition. Nutrients 7:9285–9298

Peakall ROD, Smouse PE (2006) GenAlEx 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Pinkas R, Zamir D, Ladizinsky G (1985) Allozyme divergence and evolution in the genus *Lens*. Plant Syst Evol 151:131–140

Prakesh V, Tandon J, Prasad K (1986) Studies on intercropping rainfed wheat. Ann Agric Sci 7:258–262

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Rana MK, Kumari R, Singh S, Bhat KV (2013) Genetic analysis of indian lentil (*Lens culinaris* Medikus) cultivars and landraces using RAPD and STMS markers. J Plant Biochem Biotechnol 16:53–57

Roldán-Ruiz I, Dendauw J, Van Bockstaele E, Depicker A, De Loose M (2000) AFLP markers reveal high polymorphic rates in ryegrasses (Lolium spp.). Mol Breed 6(2):125–134

Sexton JP, Hangartner SB, Hoffmann AA (2014) Genetic isolation by environment or distance: Which pattern of gene flow is most common? Evolution 68:1–15

Seyedimoradi H, Talebi R (2014) Detecting DNA polymorphism and genetic diversity in lentil (*Lens culinaris* Medik.) germplasm: comparison of ISSR and DAMD marker. Physiol Mol Biol Plants 20:495–500

Shah Z, Shah SH, Peoples MB, Schwenke GD, Herridge DF (2003) Crop residue and fertiliser N effects on nitrogen fixation and yields of legume—cereal rotations and soil organic fertility. Front Plant Sci 83:1–11

Solanki IS, Kapoor AC, Singh U (1999) Nutritional parameters and yield evaluation of newly developed genotypes of lentil (*Lens culinaris* Medik.). Plant Foods Hum Nutr 54:79–87

Sonnante G, Pignone D (2001) Assessment of genetic variation in a collection of lentil using molecular tools. Euphytica 120:301–307

Sonnante G, Pignone D (2007) The major Italian landraces of lentil (*Lens culinaris* Medik.): their molecular diversity and possible origin. Genet Resour Crop Evol 54:1023–1031

Tewari K, Dikshit HK, Jain N, Kumari J, Singh D (2012) Genetic differentiation of wild and cultivated *Lens* based on molecular markers. J Plant Biochem Biotechnol 21:198–204

Toklu F, Karaköy T, Haklı E, Bicer T, Brandolini A, Kilian B, ÖZkan H (2009) Genetic variation among lentil (*Lens culinaris* Medik) landraces from Southeast Turkey. Plant Breed 128:178–186

Torricelli R, Silveri DD, Ferradini N, Venora G, Veronesi F, Russi L (2012) Characterization of the lentil landrace Santo Stefano di Sessanio from Abruzzo, Italy. Genet Resour Crop Evol 59:261–276

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

XLSTAT (2017) Data analysis and statistical solution for microsoft excel. Addinsoft, Paris

Yin Y, Liu Y, Li H, Zhao S, Wang S, Liu Y, Wu D, Xu F (2014) Genetic diversity of *Pleurotus pulmonarius* revealed by RAPD, ISSR, and SRAP fingerprinting. Curr Microbiol 68:397–403

Yüzbaşıoğlu E, Özcan S, Açık L (2006) Analysis of genetic relationships among Turkish cultivars and breeding lines of *Lens culinatis* Medik. using RAPD markers. Genet Resour Crop Evol 53:507–514

Zaccardelli M, Lupo F, Piergiovanni AR, Laghetti G, Sonnante G, Daminati MG, Sparvoli F, Lioi L (2012) Characterization of Italian lentil (*Lens culinaris* Medik.) germplasm by agronomic traits, biochemical and molecular markers. Genet Resour Crop Ev 59:727–738