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ISSR Analysis to Detect Genetic Variation Among Some Lentil Genotypes in Syria

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Abstract: Lentil (*Lens culinaris* Medik) plays a significant role in human and animal nutrition. It is important to generate baseline information on genetic diversity of local and introduced lentil in Syria. Therefore, this study aimed to assess diversity and relationship among two local varieties and 35 introduced lentil lines from ICARDA using 20 Inter Simple Sequence Repeats (ISSR) primers.18 primers were able to amplify DNA bands with a total of 184 bands. Primers ISSR5, ISSR11, ISSR13, ISSR15 and ISSR16 presented 100% polymorphism percentage. The mean value of polymorphism percentage was 87.31%. ISSR primers could identify 19 unique bands in all genotypes, 12 of them were positive bands (present) and 7 were negative bands (absent). Primer ISSR11 was able to detect 3 positive unique bands in genotype Ln20. The UPGMA (unweighted pair group method with arithmetical averages) clustering based on ISSR data grouped 37 lentil genotypes into 6 clusters. The lowest genetic distance (0.20) was observed between (Ln15 and Ln16, Ln21 and Ln22, Ln27 and Ln28) which proves that they are closely related. While Ln24 presented the highest genetic distance (0.49) with both Ln18 and Idleb3 which refers to their genetic divergence.

استخدام تحليل التكرارات التتابعية الداخلية البسيطة (ISSR) للكشف عن التباين الوراثي لبعض طرز العدس في سوريا

الكلمات المفتاحية :
العدس، أصناف محلية، تحليل التكرارات التتابعية الداخلية البسيطة ISSR، سلالات من ايكاردا، علاقة وراثية

المستخلص : يلعب العدس (*Lens culinaris*) دوراً مهماً في تغذية الإنسان، والحيوان. من المهم إنشاء قاعدة بيانات للتنوع الوراثي بين طرز العدس المحلية، والمدخلة في سوريا لذلك هدفت هذه الدراسة إلى تقييم التنوع الوراثي، والعلاقة بين صنفين محليين، و35 سلالة من العدس مدخلة من ايكاردا باستخدام 20 بادئ لتقانة التكرارات التتابعية البسيطة الداخلية. استطاع 18 بادئ تضخيم 184 حزمة كلية. أعطت البادئات ISSR5، ISSR11، ISSR13، ISSR15 و ISSR16 نسبة تعددية شكلية 100%، بينما بلغ متوسط التعددية الشكلية 87.31%. استطاعت بادئات ال ISSR أن تميز 19 حزمة فريدة، منها 12 حزمة موجبة (موجودة)، و 7 حزم سالبة (غائبة). استطاع البادئ ISSR11 أن يعطي أعلى عدد من الحزم الفريدة الموجبة (3)، والتي كانت جميعها في الطراز الوراثي Ln20. باستخدام طريقة المجموعات الزوجية غير المزانة (UPGMA) المعتمدة على تحليل ISSR تم تقسيم الطرز إلى 6 مجموعات اساسية. لوحظ أقل بعد وراثي (0.2) بين الطرز Ln15 و Ln16، Ln21 و Ln22، Ln27 و Ln28 مما يثبت أنها قريبة وراثياً. بينما لوحظ أن الطراز Ln24 أظهر أكبر بعد وراثي (0.49) مع كل من الطرازين Ln18، و Idleb3 مما يشير إلى أنها متباعدة وراثياً.

INTRODUCTION

Lentil (*Lens culinaris* Medik) is a leguminous crop has an important role in human health and agriculture (Pandey et al., 2018). It belongs to the family *Leguminosae*. It is self-pollinated, diploid plant with 2n=2x= 14 chromosomes (Arumuganathan & Earle, 1991). Lentil ranks seventh among grain legumes and is grown on over 5 million hectars in over 44 countries, with annual

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production and productivity of 6.54 million tons and 13049 kg/ha (FAO., 2021). Canada is the leadingexporting nation, while India is the leading lentil consuming and producing nation (Bedard et al., 2010).

In Syria, the main lentil-production provinces are: Aleppo, Hasakaeh, Idleb, Hama, Dara'a and Sweida. In Hasakeh, farmers have been considering expanding lentil cultivation as it requires less water than wheat and the cultivation season is short making it suitable crop to adopt to climate change. The area planted to food legumes has decreased since the onset of the conflict, but remained relatively stable since then (FAO., 2021). Production of lentil in Syria reached 200218 tonnes with yield of 17772 kg/ha in a harvested area amounted about 112657 ha which ranked the 10th in the world while Canada ranked the 1st with about 1.7 million hectare (FAO., 2021).

The world lentil collection is held by ICARDA which obtained from ICARDA collection missions, donor institutions and ICARDA's breeding programs (Coyne & McGee, 2013). The national program in different lentil growing region including Syria are widely using ICARDA enhanced lentil germplasm.

Knowledge of genetic diversity and population structure is a crucial step for an efficient use of available material in plant breeding and conservation programs (Mbasani-Mansi et al., 2019).Characterization of genetic resource collections has been greatly facilitated by the availability of a number of molecular marker systems. Different types of molecular markers have been used to assess the genetic diversity in crop species, but no single technique is universally ideal. Therefore, the choice of the technique depends on the objective of the study, sensitivity level of the marker system, financial constraints, skills and facilities available (Beyene et al., 2005). There are numerous DNA-based molecular marker systems suitable for genetic diversity assessment. Inter simple sequence repeat (ISSR) marker, in addition to its suitability to genetic diversity study, is highly polymorphic, reproducible, cost effective, it requires no prior information of the sequence (Bornet et al., 2002).

These facts suggest that ISSR could be an unbiased tool to evaluate the changes of diversity in agronomically important crops (Kolodinska Brantestam et al., 2004). It is important to generate baseline information on genetic diversity of Syrian lentils. Therefore, this study aimed to assess diversity and relationship among local varieties and introduced lentil lines from ICARDA using ISSR markers.

MATERIALS AND METHODS

This study was performed during 2019-2021 at Molecular Genetics Laboratory, Department of Biotechnology, General Commission for Scientific Agricultural Research, Damascus, Syria.

Plant material: A set of 35 lines of lentil introduced from ICARDA and 2 local varieties (Idleb3 and Idleb5) were used in this study (Table, 1)

DNA extraction: Young leaves were collected from lentil seedlings and used for genomic DNA isolation using the cetytrimethyl ammonium bromide CTAB method as described by (Lassner et al., 1989).

DNA was quantified using spectrophotometer by taking absorbance at A260 and A280.

DNA quality was checked by agarose gel electrophoresis. DNA bands without smears were considered for PCR amplification.

All genomic DNA samples were uniformed to a final concentration of 50 ng.μl^{-1} and used for PCR amplification reactions.

Table (1). Entries information for 35 lentil lines introduced from ICARDA

Line No.	pedigree	Source
Ln 1	ILL7888/ILL5782	BARI/ICARDA
Ln 2	LC006600899Z×ILL6002	ICARDA
Ln 3	ILL8194×ILL8006	ICARDA
Ln 4	EP35	ICARDA
Ln 5	ILL7012×ILL6994	ICARDA
Ln 6	ILL5888×ILL6002	ICARDA
Ln 7	ILL7616/ILL2501	BARI/ICARDA
Ln 8	ILL7012×ILL6994	ICARDA
Ln 9	ILL6994×ILL9932	ICARDA
Ln 10	ILL6994× ILL9932	ICARDA
Ln 11	ILL88527× Subrata	ICARDA
Ln 12	ILL6994×ILL9932	ICARDA
Ln 13	-	ICARDA
Ln 14	ILL5888× ILL4605	ICARDA
Ln 15	ILL7537× ILL590	ICARDA
Ln 16	Alemaya× ILL8095	ICARDA
Ln 17	ILL6994× ILL5480	ICARDA
Ln 18	ILL6994×ILL9932	ICARDA
Ln 19	ILL6994×ILL9932	ICARDA
Ln 20	ILL6994× ILL5725	ICARDA
Ln 21	ILL6994×ILL9932	ICARDA
Ln 22	ILL6994× ILL5725	ICARDA
Ln 23	ILL6002× ILL4402	ICARDA
Ln 24	Indian landrance	ICARDA
Ln 25	ILL6994× ILL5725	ICARDA
Ln 26	ILL5883× L4147	ICARDA
Ln 27	ILL7723× ILL5883	ICARDA
Ln 28	ILL7616/ILL2501	ICARDA
Ln 29	ILL9848 × ILL8176	ICARDA
Ln 30	Iranian land race(P-604)	ICARDA
Ln 31	Alemaya× ILL8095	ICARDA
Ln 32	PI 379371	ICARDA
Ln 33	P 464	ICARDA
Ln 34	LIP97-33L× ILL8009	ICARDA
Ln 35	ILL6994 × ILL5480	ICARDA

DNA amplification and visualization by ISSR analysis: Molecular polymorphism was assessed by a set of 20 ISSR primers (table, 2). The total reaction volume of PCR amplification was 25 µl containing KAPA Taq ready mix 2X, 20 pM primer and 100 ng of template DNA.

The amplification reaction was carried out in thermocycler (Biometramodell T-1 Thermoblock) under the following conditions: initial denaturation at 95°C for 5 minute; 37 cycles of 1 minute at 94 ºC for denaturation, 1 minute for primer annealing at a (Ta) according to the primer (Table, 2), and 1.30 minute at 72 ºC for extension, with a final extension for 10 minutes at 72 ºC.

Data analysis: ISSR bands were scored in a 0-1 binary format and analyzed using Total Lab 1D software. XLSTAT software (Xlstat, 2017) was used to calculate the Polymorphism Information Content (PIC) for each primer, and to build of the cluster dendrogram based on the UPGMA (unweighted pair group method with arithmetical averages) algorithm.

Table (2). ISSR primers profile (name, sequence and annealing temperature (Ta)

Primer No.	Primer Name	Primer Sequence	Ta (C°)
ISSR1	4	CAC ACA CAC ACA CAC AAC	48
ISSR2	A830241	ACT GAC TGA CTG ACT GAC TG	44
ISSR3	813	CTC TCT CTC TCT CTC TT	50
ISSR4	807	AGA GAG AGA GAG AGA GT	50
ISSR5	8565	GTC ACCACCACCACCACCAC AC	64
ISSR6	866	CTCCTCCTCCTCCTCCTC	53
ISSR7	W814	CTC TCT CTC TCT CTC TTG	45
ISSR8	8	CAC ACA CAC ACA CAC AGA C	48
ISSR9	862	AGCAGCAGCAGCAGCAGC	53
ISSR10	17899B	CAC ACA CAC ACA GG	46
ISSR11	231	GAG TCT CTC TCT CTC TCT C	51
ISSR12	8082	CTC TCT CTC TCT CTC TCT G	51
ISSR13	NLSSR3	CAG CAGCAGCAGCAG	53
ISSR14	17	CAG CAC ACA CAC ACA CAC	51
ISSR15	5	CAC ACA CAC ACA CAC AGT	48
ISSR16	830	TGT GTG TGT GTG TGT GG	44
ISSR17	811	GAG AGA GAG AGA GAG AC	44
ISSR18	812	GAG AGA GAG AGA GAG AA	48
ISSR19	8564	CACCACCACCACCACCACC AC C	48
ISSR20	16	CGT CAC ACA CAC ACA CAC	49

RESULTS

Twenty ISSR primers were used to access the genetic diversity among 37 lentil genotypes.

Out of them, 18 ISSR primers amplified successfully (Fig, 1). A total of 184 bands were generated using 18 ISSR primers with average 10.22 bands/ primer. Out of them, 164 bands were polymorphic with average 9.11 bands/primer, whereas only 20 bands were monomorphic with average 1.11 bands/ primer. The highest number of amplified bands (15) was generated with primer (ISSR18), while the lowest number of amplified bands (6) was generated with primers (ISSR9 and ISSR14).

The size of the amplified bands ranged from 114.4bp in ISSR13 to 1783.78 bp in ISSR1. All bands amplified with primers (ISSR5, ISSR11, ISSR13, ISSR15 and ISSR16) were polymorphic (100%), while primer ISSR14 showed the lowest polymorphism percentage (50%). The mean value of polymorphism percentage was 87.31%.

PIC values ranged from 0.15 in primer ISSR3 to 0.39 in primer ISSR5 with average 0.28 (Table, 3).

The total number of unique bands obtained from all tested genotypes by 18 ISSR primers was 19 bands with average 1.06 bands/primer. Out of them, 12 bands were positive bands (present) with an average of 0.67 bands /primer, and 7 bands were negative bands (absent) with average 0.39 bands/primer. The highest number of unique bands (3) was registered with primer ISSR11 which were all of them positive bands (present), while primer ISSR1 showed the highest number (2) of negative bands (absent). Primers (ISSR2, ISSR6, ISSR13, ISSR14 and ISSR15) did not show any specific band (Table, 3).

Table (3). Molecular size, number of amplified bands (total, polymorphic, monomorphic), number of unique bands (total, positive, negative), polymorphism and PIC values generated with ISSR primers in lentil genotypes**.**

\ISSR primers	MW (bp)	No. of amplicons			Unique bands				
		T	PB	MB	T	+	-	P%	PIC
ISSR1	297.2-1783.78	11	9	2	2	0	2	82	0.25
ISSR2	267.8-743	7	6	1	0	0	0	86	0.33
ISSR3	244.2-1057.6	9	8	1	2	2	0	89	0.15
ISSR4	175.4-784.2	9	7	2	1	0	1	78	0.24
ISSR5	236.8-770.3	9	9	0	1	0	1	100	0.39
ISSR6	191.8-976.9	11	9	2	0	0	0	82	0.38
ISSR8	256.5-716	8	7	1	1	0	1	88	0.35
ISSR9	126.7-436.4	6	4	2	1	1	0	67	0.25
ISSR10	289.4-602.5	13	12	1	1	1	0	92	0.26
ISSR11	253.4-765.3	14	14	0	3	3	0	100	0.30
ISSR12	211.9-1200.1	9	8	1	1	1	0	89	0.27
ISSR13	114.4-867.4	13	13	0	0	0	0	100	0.31
ISSR14	311.4-651.9	6	3	3	0	0	0	50	0.23
ISSR15	334.7-875.5	11	11	0	0	0	0	100	0.33
ISSR16	293.8-1302.7	12	12	0	2	1	1	100	0.27
ISSR17	276.9-1133.1	10	9	1	1	0	1	90	0.30
ISSR18	312.6-1151.6	15	14	1	1	1	0	93.33	0.24
ISSR20	297.6-849.4	11	9	2	2	2	0	82	0.27
SUM		184	164	20	19	12	7		
Average		10.22	9.11	1.11	1.06	0.67	0.39	87.31	0.28

MW: molecular weight, T: total, PB: polymorphic bands, MB: monomorphic bands, +: positive unique band, -: negative unique band, P%: polymorphism, PIC: polymorphism information content

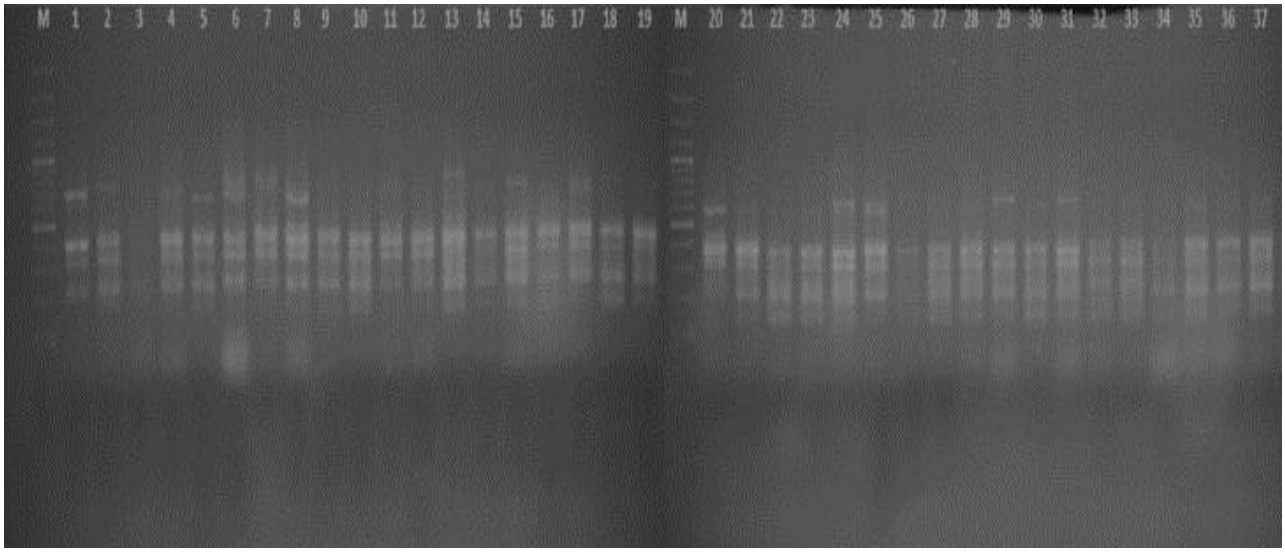

Figure (1). PCR amplification profile of 37 lentil genotypes with ISSR4 marker (M: ladder, lanes 1-35 present lines introduced from ICARDA Ln1 through Ln35, lanes 36-37 present local varieties Idleb3 and Idleb 5).

Out of the 37 tested genotypes, only 12 genotypes (Ln3, Ln10, Ln13, Ln19, Ln20, Ln21, Ln24, Ln26, Ln32, Ln33, Idleb3, Idleb5) showed uniquebands either positive or negative or both. The molecular size of the positive and negative uniquebands illustrated in table 4 and 5 respectively.

Table (4). Positive unique bands generated in lentil genotypes by ISSR primers

Genotype	Primer	molecular size (bp)
Ln3	ISSR9	436.39
Ln19	ISSR20	849.42
Ln20	ISSR11	906.33
Ln20	ISSR11	822.39
Ln20	ISSR11	765.31
Ln21	ISSR16	1302.68
Ln24	ISSR3	655.39
Ln26	ISSR18	154.03
Ln26	ISSR20	420
Ln32	ISSR10	568.78
Ln33	ISSR12	211.94
Idleb3	ISSR3	244.2

The genetic distance of 37 lentil genotypes based on ISSR data revealed a wide range of values. Thelowest genetic distance (0.20) was observed between (Ln15 and Ln16, Ln21 and Ln22, Ln27 and Ln28) which proves that they are closely related. While Ln24 presented the highest genetic distance (0.49) with both Ln18 and Idleb3 (Table, 5) which refers to their genetic divergence.

Table (5). Negative unique bands generated in lentil genotypes by ISSR primers

Genotype	Primer	molecular size (bp)
Ln3	ISSR18	466.2
Ln10	ISSR1	392.07
Ln10	ISSR1	297.21
Ln13	ISSR4	360.15
Ln33	ISSR8	256.53
Idleb3	ISSR17	605.81
Idleb5	ISSR5	374.27

TheUPGMA clustering based on ISSR data grouped 37 lentil genotypes into 2 main clusters. The first cluster made up of group C4 which included two genotypes (Ln23 and Idleb5). The second cluster made up of two subclusters; the first one included two groups (C3 and C6) each of them consist only one genotype (Ln18 and Idleb3, respectively), while the second subcluster divided into two sub sub-clusters; the first sub sub-cluster included three genotypes in one group C2

(Ln6, Ln7 and Ln24) while the second sub sub-cluster also made up of two sub sub sub-clusters; the first sub sub sub-cluster contained group C5(Ln28, Ln29, Ln30, Ln31, Ln32, Ln33, Ln34), while the second sub sub sub-cluster included the remaining genotypes in group C1 (Ln1, Ln2, Ln3, Ln4, Ln5, Ln8, Ln9, Ln10, Ln11, Ln12, Ln13, Ln14, Ln15, Ln16, Ln17, Ln19, Ln20, Ln21, Ln22, Ln25, Ln35) (Fig2).

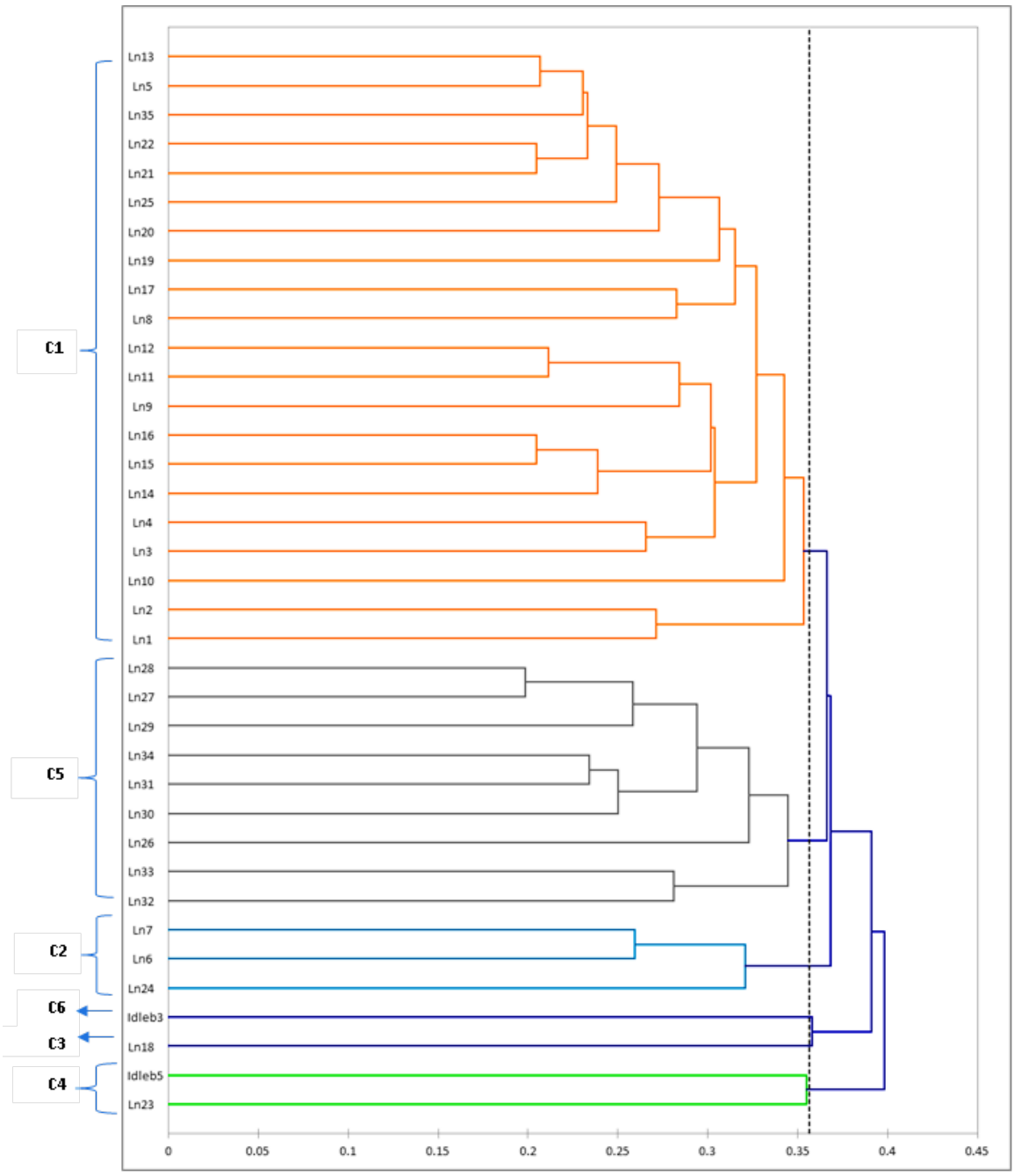

Figure (2). A dendrogram of 37 lentil genotypoes derived by UPGMA based on data generated using ISSR primers.

Table (6). Dissimilarity coefficient matrix of lentil genotypes

	Ln1	Ln2	Ln3	Ln4	Ln5	Ln6	Ln7	Ln8	Ln9	Ln10	Ln11	Ln12	Ln13	Ln14	Ln15	Ln16	Ln17	Ln18	Ln19	Ln20	Ln21	Ln22	Ln23	Ln24	Ln25	Ln26	Ln27	Ln28	Ln29	Ln30	Ln31	Ln32	Ln33	Ln34	Ln35	Ideb3	Ideb5
Ln1	0																																				
Ln2	0.27	0.00																																			
Ln3	0.34	0.28	0.00																																		
Ln4	0.34	0.29	0.27	0.00																																	
Ln5	0.38	0.35	0.34	0.29	0.00																																
Ln6	0.39	0.34	0.32	0.30	0.40	0.00																															
Ln7	0.39	0.37	0.33	0.34	0.41	0.26	0.00																														
Ln8	0.39	0.39	0.39	0.33	0.29	0.29	0.33	0.00																													
Ln9	0.34	0.31	0.33	0.33	0.36	0.38	0.40	0.34	0.00																												
Ln10	0.37	0.38	0.33	0.34	0.35	0.41	0.43	0.35	0.31	0.00																											
Ln11	0.38	0.38	0.33	0.30	0.34	0.33	0.36	0.35	0.30	0.33	0.00																										
Ln12	0.37	0.36	0.28	0.26	0.29	0.28	0.30	0.27	0.27	0.30	0.21	0.00																									
Ln13	0.35	0.32	0.32	0.27	0.21	0.38	0.37	0.29	0.30	0.29	0.29	0.23	0.00																								
Ln14	0.35	0.36	0.37	0.35	0.35	0.36	0.38	0.36	0.36	0.38	0.32	0.34	0.29	0.00																							
Ln15	0.35	0.30	0.29	0.26	0.31	0.27	0.30	0.32	0.32	0.33	0.26	0.24	0.26	0.24	0.00																						
Ln16	0.31	0.30	0.29	0.26	0.33	0.31	0.28	0.32	0.31	0.33	0.30	0.26	0.28	0.23	**0.20**	0.00																					
Ln17	0.38	0.38	0.34	0.33	0.26	0.36	0.37	0.28	0.39	0.33	0.36	0.31	0.28	0.37	0.30	0.32	0.00																				
Ln18	0.39	0.35	0.39	0.36	0.33	0.45	0.44	0.42	0.30	0.35	0.38	0.36	0.31	0.36	0.40	0.39	0.35	0.00																			
Ln19	0.38	0.33	0.34	0.29	0.28	0.39	0.40	0.38	0.37	0.36	0.36	0.33	0.28	0.31	0.31	0.34	0.34	0.31	0.00																		
Ln20	0.41	0.37	0.36	0.34	0.29	0.36	0.39	0.30	0.38	0.39	0.35	0.27	0.27	0.40	0.30	0.36	0.33	0.42	0.37	0.00																	
Ln21	0.38	0.33	0.33	0.31	0.26	0.36	0.37	0.32	0.33	0.38	0.28	0.29	0.25	0.36	0.29	0.35	0.32	0.34	0.32	0.26	0.00																
Ln22	0.37	0.38	0.36	0.36	0.21	0.42	0.40	0.35	0.34	0.31	0.34	0.29	0.21	0.32	0.32	0.33	0.28	0.31	0.29	0.29	**0.20**	0.00															
Ln23	0.40	0.38	0.41	0.35	0.30	0.44	0.44	0.40	0.42	0.37	0.42	0.40	0.30	0.38	0.35	0.35	0.37	0.41	0.39	0.38	0.29	0.27	0.00														
Ln24	0.42	0.41	0.40	0.36	0.42	0.31	0.34	0.35	0.42	0.45	0.33	0.34	0.37	0.35	0.32	0.34	0.39	**0.49**	0.41	0.39	0.34	0.39	0.38	0.00													
Ln25	0.39	0.39	0.40	0.34	0.24	0.44	0.44	0.35	0.40	0.37	0.36	0.32	0.25	0.36	0.32	0.36	0.31	0.33	0.29	0.25	0.28	0.22	0.30	0.43	0.00												
Ln26	0.44	0.36	0.38	0.34	0.37	0.36	0.38	0.33	0.34	0.36	0.35	0.30	0.37	0.40	0.31	0.32	0.43	0.40	0.39	0.36	0.34	0.36	0.39	0.38	0.39	0.00											
Ln27	0.41	0.32	0.34	0.29	0.33	0.35	0.32	0.39	0.37	0.39	0.29	0.32	0.32	0.36	0.25	0.32	0.40	0.43	0.36	0.33	0.33	0.33	0.37	0.34	0.34	0.27	0.00										
Ln28	0.42	0.37	0.34	0.34	0.37	0.39	0.37	0.39	0.37	0.35	0.34	0.36	0.36	0.32	0.28	0.35	0.41	0.43	0.36	0.39	0.37	0.33	0.40	0.37	0.39	0.30	**0.20**	0.00									
Ln29	0.43	0.42	0.43	0.38	0.41	0.43	0.45	0.44	0.43	0.40	0.40	0.43	0.41	0.37	0.36	0.42	0.44	0.46	0.44	0.41	0.42	0.34	0.42	0.40	0.39	0.39	0.27	0.24	0.00								
Ln30	0.34	0.35	0.36	0.35	0.28	0.39	0.38	0.29	0.37	0.36	0.35	0.33	0.31	0.32	0.31	0.32	0.37	0.37	0.36	0.33	0.31	0.25	0.30	0.37	0.30	0.32	0.28	0.28	0.33	0.00							
Ln31	0.37	0.39	0.41	0.35	0.39	0.35	0.38	0.40	0.43	0.43	0.40	0.41	0.40	0.33	0.34	0.38	0.42	0.45	0.41	0.40	0.37	0.33	0.38	0.35	0.38	0.38	0.33	0.32	0.29	0.24	0.00						
Ln32	0.40	0.35	0.41	0.39	0.39	0.33	0.35	0.40	0.43	0.45	0.39	0.40	0.41	0.32	0.32	0.35	0.43	0.47	0.41	0.41	0.38	0.38	0.38	0.37	0.45	0.36	0.29	0.36	0.39	0.33	0.29	0.00					
Ln33	0.38	0.36	0.39	0.37	0.40	0.40	0.38	0.43	0.42	0.47	0.41	0.37	0.39	0.38	0.34	0.35	0.46	0.46	0.44	0.37	0.35	0.40	0.38	0.42	0.39	0.38	0.32	0.41	0.42	0.35	0.37	0.28	0.00				
Ln34	0.39	0.34	0.39	0.33	0.34	0.36	0.34	0.38	0.39	0.38	0.36	0.37	0.34	0.33	0.31	0.34	0.38	0.36	0.38	0.35	0.35	0.32	0.35	0.36	0.33	0.28	0.25	0.26	0.29	0.26	0.23	0.28	0.28	0.00			
Ln35	0.37	0.29	0.30	0.27	0.22	0.35	0.33	0.30	0.34	0.38	0.32	0.28	0.24	0.33	0.28	0.33	0.35	0.36	0.32	0.27	0.22	0.25	0.32	0.38	0.25	0.33	0.27	0.30	0.36	0.25	0.33	0.34	0.29	0.26	0.00		
Ideb3	0.37	0.37	0.42	0.41	0.32	0.47	0.45	0.43	0.37	0.48	0.42	0.43	0.35	0.36	0.42	0.45	0.45	0.36	0.36	0.42	0.35	0.32	0.42	**0.49**	0.34	0.44	0.41	0.41	0.42	0.35	0.39	0.42	0.38	0.36	0.25	0.00	
Ideb5	0.48	0.42	0.45	0.42	0.42	0.48	0.45	0.44	0.47	0.47	0.41	0.41	0.41	0.45	0.44	0.46	0.46	0.48	0.44	0.41	0.34	0.39	0.36	0.44	0.39	0.45	0.41	0.39	0.43	0.38	0.44	0.46	0.40	0.40	0.32	0.38	0

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 (Mbasani-Mansi et al., 2019). ISSR markers are important to study intraspecific variations in plant species, as they are effective in detecting very low levels of genetic variation (Zietkiewicz et al., 1994).

In the present study, ISSR markers were successfully applied to evaluate genetic variation among some lentil lines introduced from ICARDA and two local varieties. These results agree with those obtained by (Fikiru et al., 2007; Toklu et al., 2009) in cultivated lentil and those obtained by (de la Vega & Vinagre, 2004) in wild lens.

Primers used in this study produced high levels of polymorphism with average (87.31%). In accordance with this result, (Babayeva et al., 2018) detected a high degree of polymorphism (84%), while (de la Vega & Vinagre, 2004) observed higher polymorphism rate (98.8%) in a set of lentil material using ISSR markers. Thepresence of a high percentage of polymorphism in this study confirms the high discriminative power of used ISSR markers in the studied lentil collection.

ISSR5, ISSR11, ISSR13, ISSR15 and ISSR16 with repeats (CT)n, (CCA)n, (CAG)n, (CA)n and (TG)n, respectively, produced 100% polymorphism. This result disagreed with previous investigations in lentil (Joshi et al., 2013; Seyedimoradi & Talebi, 2014) who registered the lowest polymorphism percentage (75 and 67%, respectively) using primers with (CA)n repeats.

In the present study, tested ISSR primers yielded high number of polymorphic bands per primer (9.11), which is higher than those resulted by (Seyedimoradi & Talebi, 2014). Similarly, (Babayeva et al., 2018) observed 8.9 polymorphic bands/primer using 7 ISSR primers in 47 lentil genotypes, while (Toklu et al., 2009), using 10 ISSR primers for Turkish lentil landraces and cultivars, observed only 7.5 polymorphic bands per primer. Discrepancies of polymorphism indices among studies could be attributed to the in formativeness level of the selected primers (Mbasani-Mansi et al., 2019).

Among a set of used ISSR primers, ISSR11proved to be the most informative primer based on polymorphism and identifying unique bands. This primer is based on (CT)n repeats. In accordance with this result, (Seyedimoradi & Talebi, 2014) found that primer UBC815 with (CT)n repeats produced a high polymorphism percentage (75%). Primer ISSR11 recommendes to be used for the identification of lentil genotypes with less time and cost.
ISSR dendrogram was able to distinguish the37 lentil genotypes into six groups. Similarly, (Fikiru et al., 2007) analyzed 70 Ethiopean lentil genotypes using ISSR markers and found five groups. Some genotypes showed wide divergence (Ln24 and Ln18; Ln24 and Idleb3), and their favorable characters should be taken into consideration in future breeding programs.

CONCLUSION

In conclusion, large number of genotypes should be analyzed with more numbers of primers to distinguish genetic variation in lentil genotypes, which can be applied in future breeding program for the improvement of lentil crop with respect to yield and different quality traits, to meet the increasing demand of farmers and consumers.

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