CHARACTERIZATION OF GENETIC DIVERSITY AND RELATIONSHIP IN ALMOND (*PRUNUS DULCIS* [MILL.] D.A. WEBB.) GENOTYPES BY RAPD AND ISSR MARKERS IN SULAIMANI GOVERNORATE

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Abstract. Genetic diversity of 38 almond local genotypes was investigated using RADP and ISSR markers with the analysis of nut morphology. Samples were taken from five locations for this study, including Sharbazher, Mergapan, Qaradakh, Barznja, and Hawraman. Almond nuts width, length and thickness were studied and their mean values were observed to range between 16.18-27.21 mm, 24.18-41.07 mm and 11.49-16.81 mm, respectively. Polymorphic bands of mean values were 9.5 for random amplified polymorphic DNA (RAPD) and 8 for inter-simple sequence repeat (ISSR). The PIC values were recorded for RAPD primes to range between 0.77 to 0.97 and those for ISSR primers were also verified between 0.36 to 0.97. Based on Jaccard similarity coefficients, the genetic distances were recorded between 0.32 (B-G3 vs. B-G4) (M-G2 vs. M-G1) to 0.75 (H-G5 vs. Q-G1) and all genotypes were grouped into 3 major clusters (A, B and C) with a mean dissimilarity 0.535 for 20 RADP markers. In the case of the 15 ISSR markers, a genetic distance between 0.19 (H-G13 vs. H-G12) to 0.78 (H-G5 vs. B-G6) was also observed, with four clades (A, B, C and D) with a mean dissimilarity of 0.485. According to STRUCTURE analysis, all genotypes were divided into two groups. Analysis of molecular variance (AMOVA) demonstrated a high-level genetic differentiation within a population 88% for RAPD and 87% for ISSR.
Keywords: *morphological traits, genetic relationship, genetic structure, random amplified polymorphic DNA, inter-simple sequence repeat*

Introduction

Almond (*Prunus dulcis* (Mill.)) D.A. Webb syn. *P. amygdalus* L. Batsch is a commercially important fruit plant, from the *Rosaceae* family (Zhu, 2014; Sakar et al., 2019). The place of wild almond is originated at the arid mountainous region of Central Asia and deserts of western China, Iran, Turkistan, Afghanistan, Kurdistan, and Southwest Asia with subsequent expansion into European and North African regions (Browicz and Zohary, 1996; Kester and Gradziel, 1996; Xu et al., 2004) and it is also grown commercially around the world. Global almond production for 2018/19 is an estimated 1.4 million metric tons (USDA, 2018). Botanists observed over 30 species, subspecies with ecotype (Grasselly, 1976; Ladizinsky, 1999). Genetically, Almond is diploid, the chromosome number is 2n = 2x = 16 with a genome size approximately 246 Mb (Sánchez-Pérez et al., 2019).

Almond is one of the vital plants that can grow under the rain-fed condition in Iraq, particularly in the Kurdistan region. Therefore, it is important to know the adaptation of this tree that able to tolerate the biotic and abiotic stresses. Almond is a large-sized tree, with long generation time and also has a low level of variability in morphology traits (Casas et al., 1999; Sorkheh et al., 2007, 2009; Zeinalabedini et al., 2008; Bouhadida et al., 2009). To provide information about genetic relationships it is important to study the

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differences at the level of agronomic, morphological and biochemical traits (Khan et al., 2016). Genetic diversity is an important tool that breeders can use to detect and identify with differentiation all genotypes and also it is a useful tool to improve the chances of the selection of better segregates for various characters (Dwevedi and Lal, 2009). Using morphological traits to the identification of almond plants is restricted, because of their environmental variations. However, morphological traits, including seed length and kernel size normally can be used.

In Kurdistan, Iraq, information about almond genotypes has been poorly recognized. Therefore, the modern molecular genetic tool can be applied to identify and characterize the relationships among them. However, several similar studies have been performed regarding genetically recognized cultivars and wild species of almond.

Molecular markers have shown the vital role in crop breeding, particularly in genetic diversity research and gene bank. PCR-based DNA marker systems are generally used, including Random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP) and more recently simple sequence repeats (SSRs) or microsatellites (Gupta et al., 2000).

Since no molecular evidence was obtainable concerning the almond genotypes grown in Sulaimani region, therefore, the consciousness and conception of the genetic diversity in Sulaimani almond accessions are important for the implementation of degree addressed to their usages and preservations. The present study was aimed to estimate the genetic diversity with relatedness among the most important almond genotypes and for the first time their population structure in Sulaimani was developed for almond genotypes by using RAPD and ISSR markers.

Materials and Methods

Locations and plant material

Five locations (*Fig. 1*, *Table 1*) were selected for this study with thirty-eight almond local genotype trees distributed, including Sharbazher (9), Mergapan (3), Qaradakh (5), Barznja (7), and Hawraman (14).

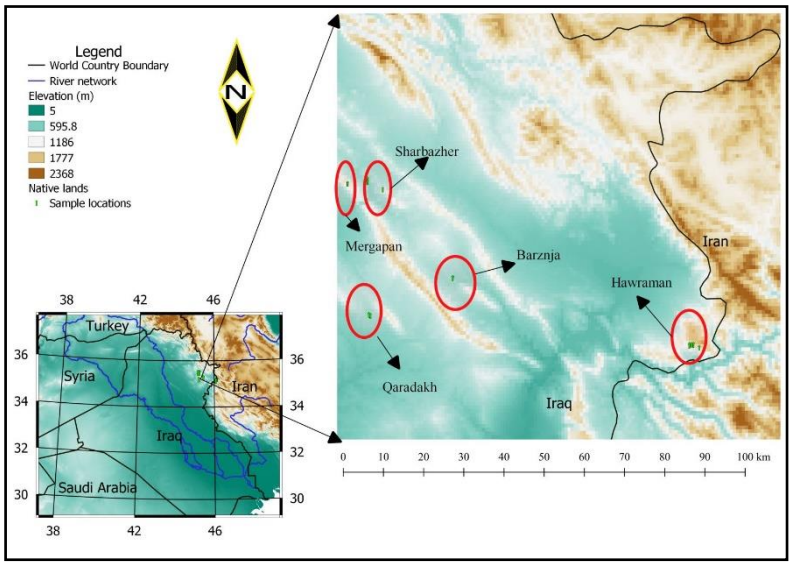

Figure 1. *Distribution of collection sites of study plant materials in Sulaimani governorate*

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Table 1. *Genotypes name, location, latitude and altitude*

Genotypes name	Locations	Latitude		Altitude
		N°	W°	
SH-G1 to SH-G9	Sharbazher	35°49′30″	45°18′93″	997.6
M-G1 to M-G3	Mergapan	35°48′93″	45°13′47″	1148
Q-G1 to Q-G5	Qaradagh	35°19′29″	45°19′69″	925.8
B-G1 to B-G7	Barznja	35°27′69″	45°42′21″	1154
H-G1 to H-G14	Hawraman	35°12′38″	46°07′80″	1402

Morphological study of seeds

Almond nuts were collected from each genotype (*Fig. 2*) Impurities such as damaged or broken nut, dust and dirt have been eliminated. For each nut, weight and size dimensions (the three axial dimensions) including length, width, and thickness of the nuts were measured by using a digital calliper with an accuracy of 0.01mm. The nuts were cracked, then using a hammer and kernel weighted to calculate shelling percentage.

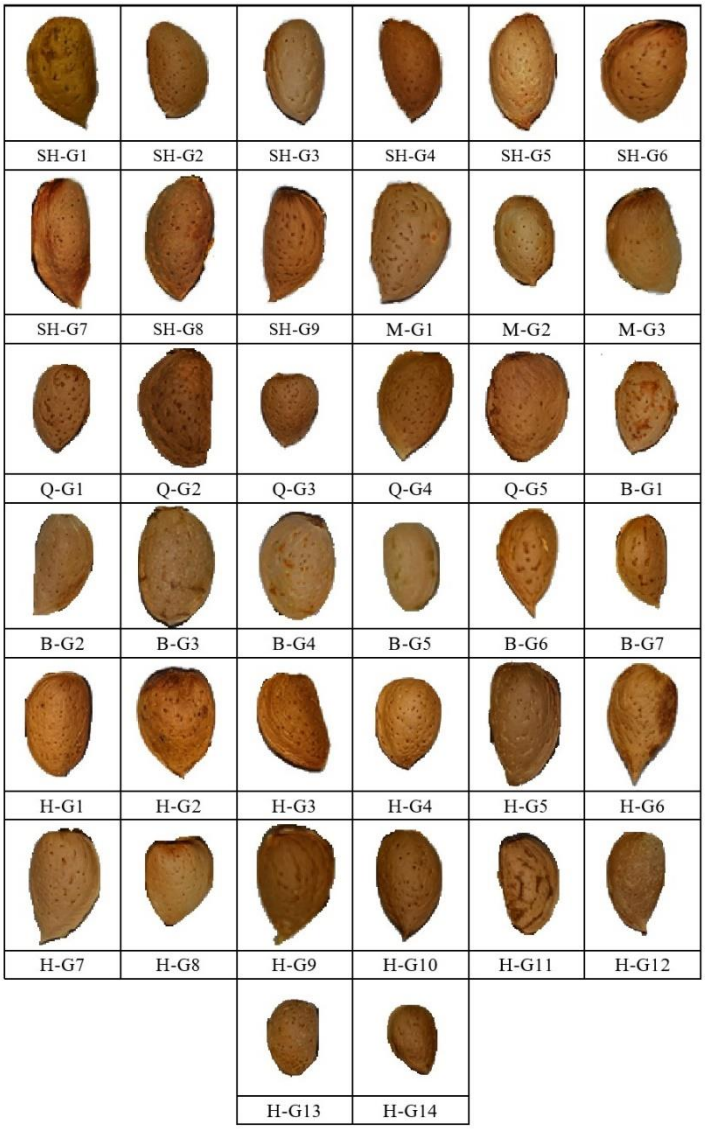

Figure 2. *Shows the different morphological shapes of all almond genotypes nut, (Scale 1:1.75)*

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Genomic DNA extraction and purification

The CTAB DNA extraction protocol with some modifications was used to isolate genomic DNA, according to (Tahir and Hama Karim, 2011). Briefly, 1 gram of fresh leaves was ground and frozen in liquid nitrogen and then 2 ml of CTAB buffer was added and incubated at 60ºC for 60 min. (1 M Tris HCl (pH 8.0), 5 M NaCl, 0.5 M EDTA and 2 g of CTAB (cetyltrimethyl ammonium bromide)), the DNA sample was precipitated with 0.08 volumes of ammonium acetate and 0.54 volumes of ice isopropanol, and then the DNA pellet was washed with 1 ml of ice-cold 70% ethanol and then the dried pellet was resuspended in 50-100 µl of deionized water. DNA was treated with RNase to remove RNA contamination. The DNA concentration was analyzed by electrophoresis using a 1.5% agarose gel.

RAPD analysis

Twenty primers were utilized in this work, (*Table 2*). The reaction mixture (20 µl) was prepared for 1 volume sample (1x) which is 10 µl of master mix buffer with (0.7 µl) primer (20 pmol/ µl), 4 µl genomic DNA (100 ng/ µl) and then the total volume was completed up to 20 µl by distilled water. Amplification was carried out in a thermo-cycler (Master cycler) for 36 cycles, each consisting of an initial denaturation step at 94ºC for 10 minutes, denaturation at 94ºC for 1 minute, annealing temperature at at 36ºC for 1 minute with extension step at 72ºC for 2 minutes and then final synthesis step of 10 minutes at 72ºC, Amplification products were separated on 1.5% agarose gel in 1X TAE (Tris base, acetic acid and EDTA) buffer. Gels were run at a constant voltage of 100V for 60 minutes, then imaged using a UV trans-illuminator. The image was captured by a digital imaging system.

ISSR analysis

Fifteen primers were utilized in this study, (*Table 2*). The reaction mixture (20 µl) was prepared for one volume sample (1x) which is 10 µl of master mix buffer with 0.7 µl primer 20 pmol/ µl, 4 µl genomic DNA (100 ng/ µl) and then the total volume was completed up to 20 µl by distilled water. Amplification was carried out in a thermo-cycler (Master cycler) for 36 cycles, each consisting of an initial denaturation step at 94ºC for ten minutes, denaturation at 94ºC for 1 minute, annealing temperature at 50ºC for 1 minute with extension step at 72ºC for 2 minutes and then final synthesis step of 10 minutes at 72ºC, Amplification products were separated on 1.5% agarose gel in 1X TAE (Tris base, acetic acid and EDTA) buffer.

Design primers

Primers were designed regarding many papers including (Martins et al., 2003; Sharma et al., 2012; Pinar et al., 2015; Abodoma et al., 2017; Saleh et al., 2018).

Statistical analysis data and counting

ANOVA and comparison test among genotypes were performed by XLSTAT software. The scorable bands were coded manually as either present (1) or absent (0) (Tahir et al., 2019) and morphological data were converted to matrix data to create the PCA plot and dendrogram using Euclidean distance and Jaccard methods.

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Table 2. *Primer names, sequences and annealing temperature of RAPD and ISSR markers used in this study*

Primer Number	Primer Name	Primer Sequences 5 ⟶ 3	Annealing Temperature (°C)	Molecular Weight of Bands (pb)
		RAPD		
1	OPA-08	GTGACGTAGG	36	250-1500
2	OPA-10	GTGATCGCAG	36	500-2000
3	OPA-11	CAATCGCCGT	36	500-2000
4	OPA-16	AGCCAGCGAA	36	500-1750
5	OPB-11	GTAGACCCGT	36	260-2200
6	S075	ACGGATCCTG	36	240-2500
7	S084	CAGACAAGCC	36	270-2100
8	S085	CTCTGTTCGG	36	350-2600
9	S081	TCGCCAGCCA	36	250-1750
10	S093	CCACCGCCAG	36	360-1600
11	S078	GGCTGCAGAA	36	250-2100
12	S094	AGAGATGCCC	36	260-1550
13	S087	GGTGCAGTCG	36	270-1600
14	S088	GGTCCTCAGG	36	250-2500
15	S089	CAGTTCGAGG	36	260-1900
16	S090	TACCGACACC	36	265-1850
17	S091	TCGGAGTGGC	36	260-1950
18	S092	ACTCAGGAGC	36	350-2100
19	S095	CAGTTCTGGC	36	1750-380
20	S073	CCAGATGCAC	36	340-1900
		ISSR		
1	807	AGAGAGAGAGAGAGAGT	50	400-1000
2	17898A	CACACACACACAAC	55	260-2200
3	HB04	GACAGACAGACAGACA	60	260-1500
4	HB 8	GAGAGAGAGAGAGG	50	270-1700
5	HB10	GAGAGAGAGAGACC	50	300-1700
6	HB11	GTGTGTGTGTGTCC	50	370-1650
7	HB12	CACCACCACGC	50	275-1850
8	HB15	GTGGTGGTGGC	50	250-1850
9	AG7YC	AGAGAGAGAGAGAGYC	55	450-1400
10	AGC6G	AGCAGCAGCAGCAGCAGCG	55	260-1450
11	IS06	GTGCGTGCGTGCGTGC	60	380-1100
12	IS16	DHBCGACGACGACGACGA	60	280-1500
13	IS17	BDBACAACAACAACAACA	57	300-1700
14	IS19	YHYGTGTGTGTGTG	57	270-1600
15	ISSR.08	ACACACACACACACACYA	52	250-2000

Results and Discussion

Morphological data analysis

Agro-morphological important traits in almond genotypes are nut phenotypic parameters including width, length, thickness, weight, shelling percentage for the economic and health sector. Therefore, identification of morphological traits can be discussed alongside with genetic diversity. To improve the gene pool, the physical traits are inappropriate because environmental factors have a direct influence on developmental stages of the plant with all traits consequently, they demonstrate the diversity among genotypes are just limited (Terzopoulos and Bebeli, 2008). (*Table 3*) shows the mean values of width, length, thickness, weight and shell to the kernel of nuts from thirty-eight almond genotypes, statistical differences were observed. Nut width of the almond genotypes ranged between 16.18 to 27.21 mm, length from 24.18 to 41.07 mm, thickness between 11.49 to 16.81 mm, nut weight from 2.13 to 7.52 g and shell to kernel percentage between 16.39 to 30.84%. It can be seen that our results nearly agree with Esfahlan

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et al., 2012. The values of some almond nut parameters in 40 almond genotypes statistically varied. Nut weight ranged between 3.23 to 8.34 g, nut length from 30.5 to 43.6 mm, nut width from 18.3 to 29.4 mm, and nut thickness 15.00 to 22.33 mm. Kodad et al. (2014) recorded that physical nut traits in 45 almonds with Moroccan genotypes the minimum and maximum nut width were 15.90-27.19 mm, nut length 19.25 to 41.24 mm, nut thickness 11.48-19.61 mm, nut weight 1.15-7.34 g and shelling percentage 19.91-63.79%. In addition, differences in agronomical nut data might be due to the insentience characteristics of genotypes (Kumar and Ahmed, 2015). Furthermore, geographical locations with cross-pollination by insects could be another evidence of almond diversity. (Kester and Gradziel, 1996; Woolley et al., 2000).

Table 3. *Effect of almond tree genotypes on some nut characteristics*

Genotype	Nut Width (mm)		Nut Length (mm)		Nut Thickness (mm)		Nut Weight (g)		Shell to Kernel (%)	
SH-G1	23.41	c-e	38.62	a-d	14.88	c-g	4.94	c-e	19.09	j-m
SH-G2	20.18	h-l	33.35	f-m	14.81	c-g	4.03	e-l	17.93	l-n
SH-G3	19.20	j-o	32.13	j-n	13.87	e-l	4.61	c-h	21.33	e-j
SH-G4	20.61	h-k	34.42	e-l	15.03	c-g	4.98	c-e	19.80	h-l
SH-G5	20.79	g-j	36.82	b-f	15.22	b-e	4.60	c-h	21.21	f-j
SH-G6	20.78	g-j	36.58	c-g	15.61	a-d	4.08	e-l	22.08	e-h
SH-G7	20.39	h-k	39.65	a-c	14.37	c-j	5.00	c-e	22.56	d-f
SH-G8	22.52	d-g	38.54	a-d	15.70	a-c	4.25	e-k	21.90	e-i
SH-G9	17.64	n-q	36.40	c-h	12.98	k-n	3.75	h-m	24.57	cd
M-G1	22.76	d-f	40.39	ab	14.62	c-i	4.91	c-f	16.39	n
M-G2	21.92	e-h	32.86	g-n	15.06	c-g	4.35	c-j	16.49	n
M-G3	23.63	c-e	40.55	ab	14.82	c-g	6.31	b	16.97	mn
Q-G1	21.22	f-i	30.74	l-n	14.95	c-g	4.46	c-i	19.68	h-l
Q-G2	26.65	a	41.07	a	14.60	c-i	4.79	c-g	29.14	ab
Q-G3	20.91	g-j	25.75	o	16.37	ab	3.72	h-m	27.74	b
Q-G4	25.97	ab	35.52	d-k	16.81	a	7.52	a	16.41	n
Q-G5	27.21	a	35.68	d-j	14.57	c-i	6.40	b	17.08	mn
B-G1	19.34	j-n	29.73	mn	12.66	l-o	2.40	op	20.71	f-j
B-G2	19.15	j-o	31.78	k-n	12.28	m-o	3.44	j-n	25.29	c
B-G3	24.56	bc	38.05	a-e	14.61	c-i	5.24	cd	18.22	k-n
B-G4	24.04	cd	32.62	h-n	14.67	c-h	4.48	c-i	20.36	f-k
B-G5	17.49	o-q	28.97	n	12.91	k-n	3.50	i-n	20.36	f-k
B-G6	16.54	pq	32.44	i-n	11.87	no	2.13	p	19.80	h-l
B-G7	16.18	q	30.23	mn	13.38	h-m	2.17	p	30.84	a
H-G1	19.11	j-o	31.54	l-n	12.99	k-n	3.82	g-l	23.58	c-e
H-G2	22.78	d-f	33.02	g-m	15.30	b-d	2.80	m-p	20.57	f-k
H-G3	18.18	m-p	31.55	l-n	11.49	o	3.44	j-n	19.63	i-l
H-G4	22.84	c-f	29.80	mn	15.13	b-f	2.73	n-p	21.29	e-j
H-G5	22.55	d-g	37.19	b-e	13.16	j-n	5.31	c	19.16	j-m
H-G6	19.78	i-m	36.21	c-i	13.27	i-m	3.91	f-l	22.56	d-f
H-G7	24.28	cd	36.20	c-i	14.22	d-k	4.30	d-k	22.00	e-i
H-G8	22.96	c-f	30.42	mn	12.92	k-n	4.07	e-l	19.44	j-l
H-G9	21.50	f-i	39.10	a-d	13.77	f-l	4.06	e-l	20.77	f-j
H-G10	20.72	h-k	32.67	h-n	14.82	c-g	4.01	e-l	20.41	f-k
H-G11	18.56	l-o	31.59	l-n	13.44	h-m	3.42	j-n	25.67	c
H-G12	17.74	n-q	31.80	k-n	13.07	j-n	3.32	k-o	20.16	g-l
H-G13	18.96	k-o	24.88	o	13.74	g-l	3.13	l-o	24.76	c
H-G14	16.81	pq	24.18	o	12.24	m-o	2.48	op	22.45	d-g

Different letters in the same column indicate significant differences between means according to Duncan multiple range test at $P \leq 0.05$.

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The principal component analyses (PCA) plot (*Fig. 3*) showed the distribution of all genotypes and morphological data on the plot. The plot demonstrated a negative relationship between shell to the kernel and nut weight. It displayed also, a positive linkage between nut width and nut thickness.

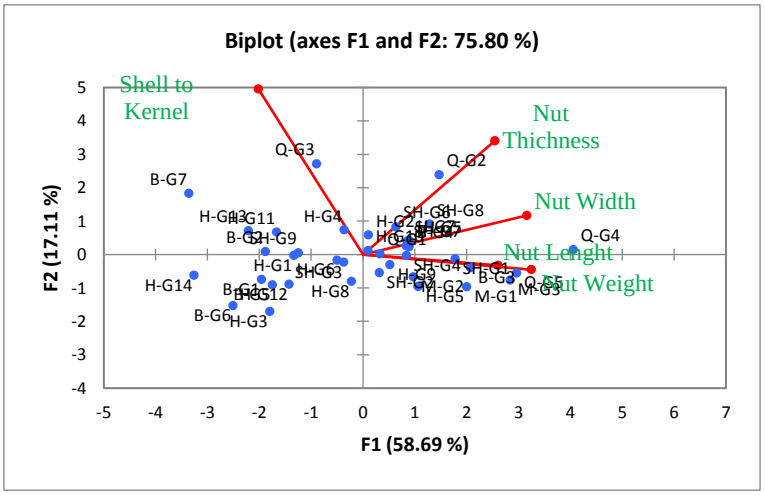

Figure 3. *PCA plot among 38 genotype accessions based on 5 nut characteristics in different locations*

Allelic variation in almond accessions using RAPD and ISSR markers

RAPD and ISSR markers were used to analyze the genetic diversity of almond genotypes. The assessment of genetic diversity and relationships among almond specimens has been more important to improve the chances of better selection segregates for various characters (Pinar et al., 2015). The valuation of genetic diversity is significant not only for plant development but also for efficient management and conservation of germplasm resources. Information about genetic relationships is important to represent the differences at the DNA level and molecular characteristics about all genotypes. It is also significant for the breeding program which detects drought-resistant traits which is a vital trait to improve economically important crops (Khan et al., 2016). In our results of the primers tested, 20 (out of 21) RAPD and 15 (out of 17) ISSR primers were confirmed as amplified fragments for their reproducibility and high polymorphism (*Table 4*). The maximum, minimum and mean values of polymorphic bands were 5, 15 and 9.5 for RAPD and 4, 12 and 8 for ISSR, respectively. The PIC values were recorded for RAPD primes that ranged between 0.77 to 0.97 and for ISSR primers it was also verified between 0.35 to 0.96. The PIC values discovered are nearly similar to those reported such as The PIC values for 16 RAPD primers exhibited by Sharma et al. (2012) ranging from 0.26 to 0.87. Mean value of PIC was reported 0.77 using 80 primers of RAPD to 29 almond cultivars (Sorkheh et al., 2009). Shiran et al., (2007) demonstrated that the PIC values were confirmed as ranged 0.47 to 0.97 using 42 RAPD randomly primer to apply 39 almond varieties. In addition, for ISSR markers, range of PIC was got from (0.59 to 0.69) by using 21 primers that applied to 29 *prunus* species (Sarhan et al., 2015). In another research 9 ISSR primers were used in the peach plant, PIC ranged between 0.71 to 0.88 was documented (Tian et al., 2015). Furthermore, PIC values ranging from 0.13 to 0.47 and 0.12 to 0.47 were verified after using (37 RAPD and 38 ISSR) random primers for

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45 peach cultivars respectively (Sharma and Sharma, 2018). Regarding the polymorphic bands, El Hawary et al. (2014) demonstrated 2.8 mean value of the polymeric band for 10 primers, and also mean of the polymorphic band was recorded as 8.36 (Gouta et al., 2008). Abodoma et al. (2017) also reported that the polymorphic band for using nine ISSR primer was 13.2 and mean value of polymeric band was 5.53 using 13 primers (Cabrita et al., 2014) recorded polymorphic band was 4.23 for 13 RAPD primer (Pinar et al., 2015) but 5 was got for 4 ISSR primers. Moreover, the average allele polymorphism was 18.6 per primer using ISSR primers applied to 29 *prunus* species (Sarhan et al., 2015).

Table 4. *Markers name, number of polymorphic bands, major allele frequency, gene diversity and PIC value of 20 RAPD and 15 ISSR*

Marker	Number of polymorphic bands	Major Allele Frequency	Gene Diversity	PIC
RAPD				
OPA-08	5	0.18	0.89	0.88
OPA-10	11	0.13	0.96	0.96
OPA-11	11	0.05	0.97	0.97
OPA-16	7	0.13	0.94	0.94
OPB-11	12	0.21	0.92	0.92
S075	15	0.08	0.96	0.96
S084	10	0.13	0.94	0.94
S085	12	0.24	0.92	0.92
S081	8	0.32	0.87	0.86
S093	11	0.45	0.78	0.77
S078	12	0.21	0.91	0.91
S094	9	0.08	0.96	0.96
S087	8	0.24	0.87	0.86
S088	10	0.16	0.95	0.95
S089	8	0.08	0.96	0.95
S090	7	0.13	0.93	0.93
S091	7	0.24	0.89	0.88
S092	9	0.16	0.93	0.93
S095	10	0.16	0.93	0.93
S073	8	0.16	0.93	0.92
Mean	9.5	0.18	0.92	0.92
ISSR				
807	7	0.63	0.59	0.58
17898A	11	0.08	0.96	0.96
HB04	10	0.13	0.94	0.93
HB8	7	0.08	0.96	0.96
HB10	4	0.53	0.66	0.62
HB11	7	0.13	0.92	0.91
HB12	12	0.18	0.92	0.91
HB15	4	0.79	0.37	0.35
AG7YC	11	0.08	0.97	0.96
AGC6G	8	0.11	0.95	0.94
IS06	10	0.08	0.96	0.96
IS16	6	0.50	0.69	0.66
IS17	5	0.50	0.71	0.69
IS19	9	0.16	0.92	0.91
ISSR.08	9	0.34	0.86	0.85
Mean	8	0.29	0.82	0.81

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Clustering and AMOVA analysis

Clustering analyses were performed for assessing the connection between Almond genotypes, based on the Jaccard similarity coefficients using the unweighted pair-group method (UPGMA). The dissimilarity coefficients were ranged between 0.32 (B-G3 vs. B-G4) (M-G2 vs. M-G1) to 0.75 (H-G5 vs. Q-G1), all 38 Almond genotypes were clustered into 3 groups (A, B and C) with a mean dissimilarity (0.54) for 20 RADP markers (*Fig. 4*), cluster A include H-G3, SH-G3, SH-G6, H-G4, H-G8, SH-G7, H-G11, H-G5, H-G9, H-G12, H-G7, H-G6, H-G2 and H-G10, only Q-G1 was observed in cluster B, the rest of the genotypes were found in cluster C. In addition, dissimilarity values were also observed between 0.19 (H-G13 vs. H-G12) to 0.78 (H-G5 vs. B-G6) by using 15 ISSR markers which clustered all genotypes into A, B, C and D with a mean dissimilarity of 0.49 (*Fig. 5*). Cluster A includes only H-G7, and group B is consent of all genotypes without cluster A, C, D) group C involves H-G1, SH-G6 and SH-G7, H-G3, H-G5, H-G4, H-G6 and H-G14 belong to cluster D. In addition, both RADP and ISSR markers, exhibited a dissimilarity between 0.32 (B-G3 vs. B-G4) to 0.72 (H-G1 vs. H-G9) and clustered all genotypes into 4 groups (A, B, C and D) with a mean dissimilarity of 0.52 (*Fig. 6*). Cluster A, include H-G7, group B involves H-G4, H-G6, H-G8, H-G5, H-G11, H-G10, H-G12, H-G13, H-G2 and H-G9, cluster C includes all genotypes that are not in cluster A, B, and D and cluster D includes H-G14, H-G3, SH-G3, SH-G6 and SH-G7. Many researchers registered regarding Almond genetic diversity and their relationships. Martins et al. (2003) studied the genetic diversity of Portuguese *Prunus dulcis* cultivars and their relationship using (RAPD) and (ISSR) markers, the UPGMA dendrogram also achieved a good degree of confidence between associations which was a cophenetic correlation higher than 0.80 for 124 amplified fragments. In addition, four main clusters included *P. dulcis* cultivars (cluster I); P5 (cluster II); *P. webbii* (cluster III); and *P. persica* (cluster IV), the outgroup. Pinar et al. (2015) demonstrated that dissimilarity coefficients were 0.90 and clusters and sub-clusters of the dendrogram had values of cophenetic correlation higher than 0.85 between genotypes by using the UPGMA method for the total number of amplified RAPD plus ISSR fragments with a dendrogram consisting of nine main clusters.

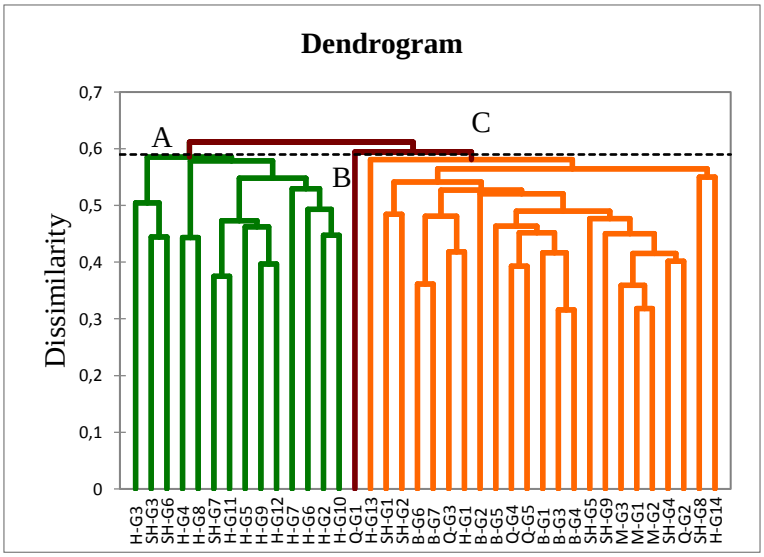

Figure 4. *Cluster tree created by UPGMA method based on 20 RAPD markers among 38 almond genotypes*

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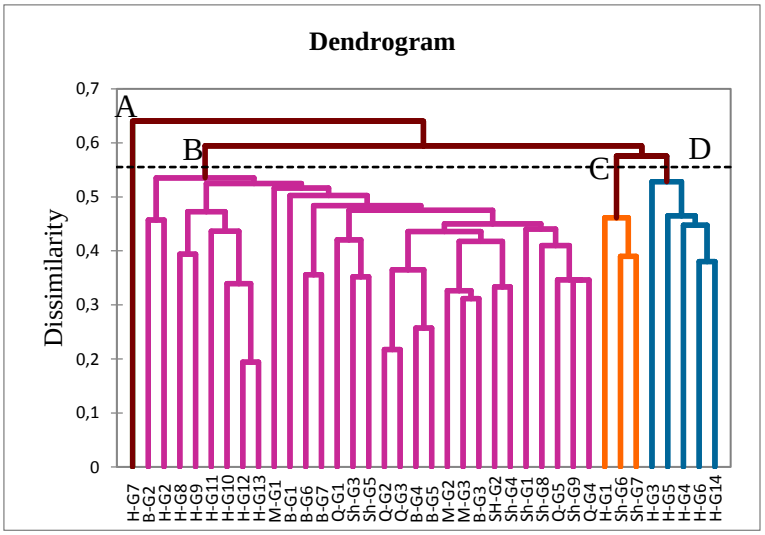

Figure 5. *Cluster tree created by UPGMA method based on 15 ISSR markers among 38 almond genotypes*

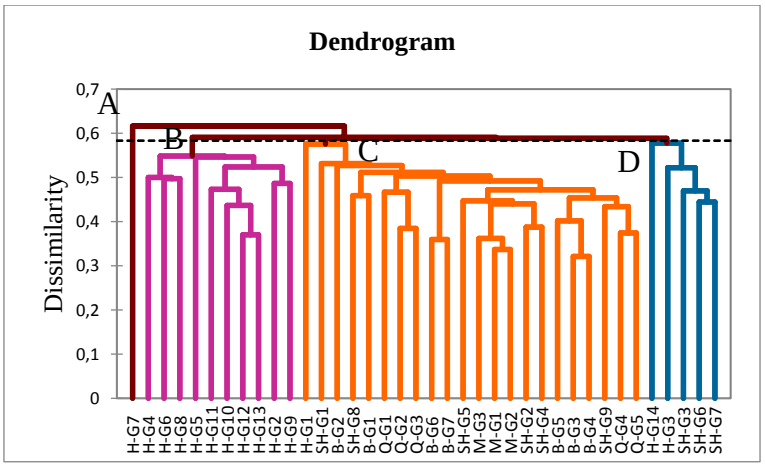

Figure 6. *Cluster tree created by UPGMA method based on 20 RAPD with 15 ISSR markers among 38 almond genotypes*

The genetic variety among the 86 almond cultivars and genotypes were assessed using 15 SSR marker and also UPGMA cluster analysis based on the similarity matrix coefficient was analyzed. Genetic similarities ranged from 0.03 (*P. tenella* and 'Ne Plus Ultra') to 1.00 (three accessions from Akdamar Island and Turkey) with an average of 0.29. Almond genotypes were clustered according to their pedigree and geographic origin. Based on the dendrogram of 86 almond cultivars genotypes, and wild species two groups of different size were formed, with *P. tenella* forming an outgroup and separated from the rest of the genotypes (Halász et al., 2019).

Analysis of molecular variance (AMOVA) of the 38 Almond genotypes in RAPD analysis demonstrated 88% of the total variation within the populations, and 12% was credited to differences between populations (*Table 5*). In addition, the ISSR marker revealed high variance in the intra-populations (87% of the total variation), and merely 13% could be qualified to differences between sub-populations. AMOVA analysis for 86

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genotypes using 15 SSR markers revealed that considerable genetic variation occurred within populations (71.30%), and genetic variation among populations was 28.70% which is a significant reaching value (Halász et al., 2019). This level of variation among populations is much higher than the value estimated for *P. sibirica* (Wang et al., 2014) or *P. mahaleb* (Jordano and Godoy, 2000), and that shown by Fernández i Martí et al. (2015) in almond.

Table 5. *Analysis of molecular variance (AMOVA) of the five populations for 38 Almond genotypes*

Source	df	SS	MS	Est. Var.	%	P-Value
RAPD						
Among Pops	4	307.18	76.80	5.29	12%	0.001
Within Pops	33	1289.06	39.06	39.06	88%	0.001
Total	37	1596.24		44.35	100%	
ISSR						
Among Pops	4	188.16	47.04	3.391	13%	0.001
Within Pops	33	754.31	22.86	22.86	87%	0.001
Total	37	942.47		26.25	100%	

Genetic structure for all genotypes using RAPD and ISSR markers

STRUCTURE method was used to collect evidence about population structure for almond genotypes depending on allele frequencies (Evanno et al., 2005) therefore, in this work, according to Delta K, genotypes were divided into two groups or sub-populations, group 1 (green line) and group 2 (red line). For RAPD and ISSR (*Fig. 7A and B*) clusters were also represented by colour, the red line in RAPD and the green line in ISSR consisted of Hawraman location, but the green line in RAPD and the red line in ISSR conceited other locations including Sharbazher, Mergapan, Qaradakh and Barznja. In addition, a combination class of genotypes may refer to more than one background. For example, samples SH-G3 and Q-G1 in RAPD markers (*Fig. 7A*), and only sample Sh-G1 in ISSR marker (*Fig. 7B*) could possibly have a complicated history linking intercrossing or practicably resulting from the gene flow between taxa, in addition, the high variability between genotypes may be the consequences of the changing climates found within the locations. The true number of clusters (K) in a sample of individuals was observed and determined for 20 RAPD and 15 ISSR markers, that the real highest K value was K= 2 for each marker (*Fig. 7C and D*).

Allele frequencies using STRUCTURE analysis was investigated to determine the genetic constitution of different groups. The Evanno criterion gave a strong signal for K = 9 indicating nine genetically distinct subgroups resided within the studied genotypes (Halász et al., 2019). Our results are assessed depending on genetic diversity and relationships between different accessions in different locations of Sulaimani governorate, that had some morphological similarities within genotypes and molecular studies also have verified it. It can be said that this hypothesis may be conducted the self-incompatible nature of the almond plants. Janick (1990) demonstrated that the high heterosis of the cultivars is mostly due to cross-pollination among them and also it is recorded high genetic variability. In addition, growing almonds in different regions have also been isolated during the time period that has progressed of characteristic ecotypes.

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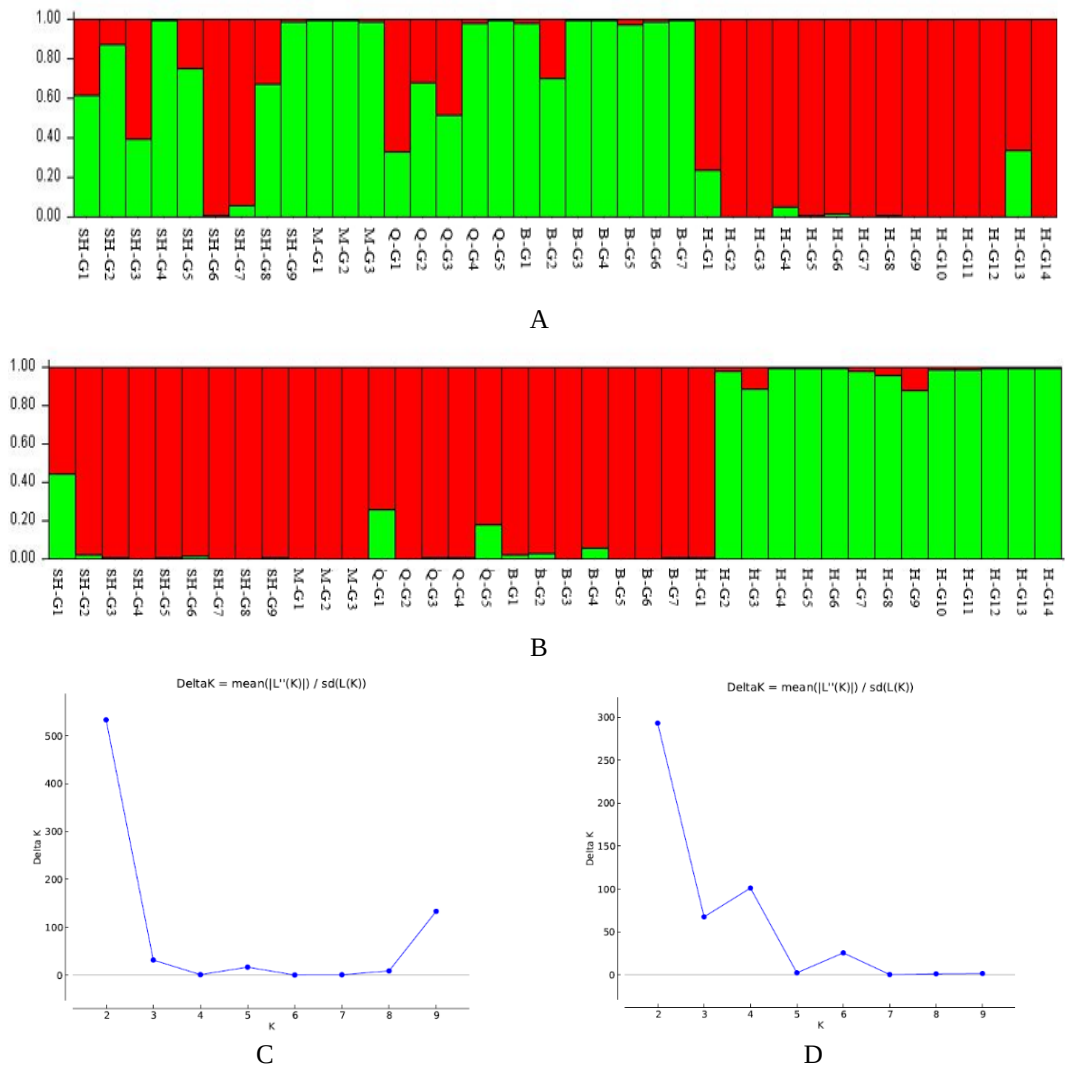

Figure 7. *Thirty-eight almond genotypes clustered into different sub-populations by STRUCTURE software. (A) for RAPD and (B) ISSR. Accessions are coordinated as per estimated membership coefficients (q) in K= 2 clusters. (C) for RAPD and (D) for ISSR, Determining the optimal value of K by the (ΔK) procedure described by Evanno et al., 2005*

Conclusions

This work aimed to assess the genetic diversity using RAPD and ISSR mark with nut agronomical traits to 38 almond accessions grown in Sulaimani Iraq region. According to our research, Agronomical nuts (width, length, thickness, weight and shell to kernel data) were studied for each genotype that mean values of those parameters were significantly documented. Genetically, the number of polymorphic bands, major allele frequency, gene diversity and the polymorphism information content (PIC), were demonstrated. The polymorphic bands of mean value were 9.5 for RAPD and 8 for ISSR. The PIC values were recorded for RAPD primers ranging between (0.77 to 0.97) and for ISSR primers it was also verified between 0.35 to 0.96. Jaccard similarity coefficients were achieved between 0.32 (B-G3 vs. B-G4) (M-G2 vs. M-G1) to 0.75 (H-G5 vs. Q-G1) and clustered into 3 clusters (A, B and C) with a mean similarity of 0.54 for 20 RADP markers. For 15

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ISSR markers, 0.19 (H-G13 vs. H-G12) to 0.78 (H-G5 vs. B-G6) were also observed, and clustered into 4 clusters (A, B, C and D) with a mean similarity of 0.49. In addition, based on the analysis of STRUCTURE software, RAPD and ISSR were analyzed and were divided into two groups, analysis of molecular variance stated a low variation among groups (12%) for RAPD and (13%) for ISSR. Therefore, the consequence of the genetic diversity in Sulaimani Almond accessions is important for breeding as well as to the implementation of degree addressed to their usages and preservations.

Our recommendations for future researches will be conducting quantitative traits loci analysis and genome-wide associated to determine QTL that associated with drought tolerance in almond genotypes, and also to assist future conservation and breeding programs to use different locations and accessions of almonds with using various types of markers including SNIPs, SRAPs, ALFPs, and SSRs.

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