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Genetic Variability of Different Jordanian Almond Prunus Amygdalus L. Landraces Revealed by Morphological Traits and RAPD Markers

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ABSTRACT

The relationship among six Jordanian almond landraces was studied using morphological traits and RAPD analyses. Across six almond landraces studied, nut shape, kernel size, nut length, nut size, and shell length That showed a high level of variation (CV>30%), while kernel length, shell width, internodes length, and nut width showed comparatively low values (CV<20%). Principal component analysis showed that the first five components explained all morphological variation among the landraces investigated. Kernel and nut traits were predominant in the first three components contributing to most of the total variation that existed among landraces. Euclidean distance was used to construct clusters from morphological data which allocated individuals into two main groups with a distance ranging from 5.5 to 10.14. Hajari, Hami Hallo, and Mukhmaly with small fruit sizes composed one main cluster, while the other three landraces (Oga, Fark, and Abu Dabos) with large fruit sizes composed the other main cluster. Out of 62 pre-screened RAPD primers, 12 with reproducible bands and maximum polymorphism were selected for diversity analysis. Seventy-one bands were scored with 28 of them being the polymorphic. Average value of polymorphism/primer ranged from 20% to 74.2%. Nei's genetic distance coefficient ranged from 0.5 to 0.85 with an average of 0.70. Molecular analysis revealed inconsistent separation among the landraces compared with that based on morphological traits. Although landraces found during the screening in the Ajloun area are limited in number, but considerable variation was observed both at morphological and DNA levels indicating that Jordanian almond landraces are rich and valuable genetic materials for almond improvement.

Keywords: Almond, Prunus amygdalus, landraces, genetic variability, morphology, RAPD, Jordan.

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INTRODUCTION

The aim of the traditional evaluation of genetic diversity and the relationship among almond accessions is to determine the variability among economic traits for almond improvement (Hend et al., 2009; Tahan et al., 2009). However, traditional approaches based on morphological observations are time-consuming, affected by environmental conditions and required a long generation time for adult trees for evaluation (Bouhadida et al., 2009; Khan et al., 2016; Sakar et al., 2019). Moreover, less variability was observed in morphological traits compared to DNA markers (Casas et al., 1999). Molecular tools supplement the information that came from morphology-based data. Several molecular techniques including isozyme (Vezvaei, 2003), DNA-based markers (i.e., ISSRs) (Mahood and Hama-Salih, 2020), SSRs (Bouhadida et al., 2009), AFLPs (Sorkheh et al., 2007), and RAPD (Mahood and Hama-Salih, 2020) which are not affected by environmental changes have been used for describing diversity and genetic characterizations of Prunus international germplasm collection.

In general, landraces are considered as rich source of variation for different desirable traits, which could help almond breeders to plan crosses to incorporate this variability into the genetic background of modern almond cultivars. Local almond landraces are known by farmers with different local names. Their names came from their special nut characteristics. The famous local names are Hajari (hard shell), Mukhmaly (high pubescence), Oga (bent nut), Hami Hallo (bitter taste when the nut is green but becomes sweet when it is dry), Fark (soft shell), and Abu Dabos (mall pin at the top of the nut). Nevertheless, to the best of our knowledge, no comprehensive studies are available in literature aim at investigating morphological and molecular characterizations of almond landraces. Therefore, the present study was conducted to assess the genetic diversity in six different almond landraces from Jordan using morphological traits and RAPD markers, to compare the discriminating power of morphological and RAPD markers to differentiate between six Jordanian common almond landraces, and to estimate the level of polymorphism in economically important traits in Jordanian almond landraces.

MATERIALS AND METHODS

Plant Materials and Variability in Quantitative and Qualitative Traits

Six almond landraces were collected in order to conduct the experiments, namely; Hajari, Oga, Mukhmaly, Hami Hallo, Fark and Abu Dabos from Ajloun Governorate (Figure 1). The morphological characterizations of leaves, nuts, kernel, external tree features and RAPD markers were used to estimate the genetic variability among the six almond landraces. Common almond landraces were characterized for 34 morphological traits during March-August, 2011 according to guidelines provided by the International Plant Genetic Resources Institute, FAO (IPGRI-FAO, 1985) in the descriptors of almond. This guideline manual includes over 120 morphopomological and morphological traits. All fruit measurements were recorded at fruit maturity stage. Characterizations of nuts and kernels were recorded for four accessions (replications) of each almond landrace. In total, 19 metric and 15 scoring traits were recorded in study. The determined metric traits were leaf length (cm), leaf width (cm), petiole length (mm), nut and kernel weight (g) and dimension (width and length) (cm). Other traits such as shell thickness and weight were also determined. Kernel and nut traits were determined for mature summer crop drupes in July and August. All leaf related traits were recorded from the fifth fully expanded leaves. Data were recorded from 25 randomly selected nuts or leaves from each almond accession. Scoring scale was employed for recording scoring traits following almond descriptor guidelines (IPGRI-FAO, 1985).

Genomic DNA Extraction and its Characterizations

By using CTAB DNA extraction protocol with some modifications to isolate genomic DNA, according to

(Tahir and Hama Karim, 2011). The total DNA was extracted from young leaves collected at early spring from the six common almond landraces and stored at -20ºC. Concisely, 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)), then the DNA sample was precipitated with 0.08 volumes of ammonium acetate and 0.54 volumes of ice isopropanol, 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. The high intensity sharp DNA bands with no smear indicated the high molecular weight of extracted genomic DNA samples.

RAPD analysis

Initial screening was done using 62 RAPD primers using the six almond landraces. Twelve RAPD primers out of finally selected for this study with best PCR amplification profile, reproducible sharp bands and maximum polymorphism were shown in Table 1. A series of optimization experiments using the 6 genomic DNA samples was carried out to determine the optimum annealing temperature for RAPD-PCR. The optimum annealing temperature was found to be 37ºC. The amplification reaction for RAPD was performed in 25 µl volumes containing 1 PCR buffer, 1.5 mM MgCl¬¬¬2, 200 mM deoxynucleotide triphosphates (dNTPs), 15 ng decamer primers, 0.7 unit Taq DNA polymerase, and 50 ng of total genomic DNA. DNA amplification reaction was performed in an Mj research gradient programmed as follows: initial pre-denaturation step at 94ºC for 4 min, followed by 35 cycles at 92ºC for 1 min, 37ºC for 1 min, 72ºC for 2 min and a final extension at 72ºC for 10 min. Following amplification, the samples were stored at 4ºC prior to electrophoresis. 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.

To better understand the patterns of variations among landraces, distance matrices generated from RAPD data were used as input data for cluster analysis based on unweighted pair-group method of arithmetic average (UPGMA), and to compute PCA with SPSS software (SPSS, 1997). The Polymorphic Information Content (PIC) for each selected RAPD was calculated with formula described by Roland-Ruiz et al. (2001): PICi=2fi(1-fi), Where PICi is the polymorphic information content of primer I; fi is the frequency of the marker bands which were present, and 1-fi is the frequency of the marker bands were absent. The discriminating power (D value) of each RAPD primer used in this study varied from 0.28 in p17 and p52 to 0.5in p21 (Table 1).

Table 1. RAPD primer sequences used in almond DNA genetic diversity analysis, range of size, polymorphic bands, percentage of polymorphic bands and PIC values.

No	Primer' abbreviation	Primer' name	Sequence (5'-3')	Range of size	No. of bands	Polymorphic bands	Polymorphic bands (%)	D value
1	P52	OPC-16	CACACTCCAG	470-3470	7	3	42.86	0.28
2	P54	OPZ-01	TCTGTGCCAC	430-960	5	2	40.00	0.44
3	P59	OPZ-06	GTGCCGTTCA	1470-5360	5	2	40.00	0.44
4	P62	OPZ-18	AGGGTCTGTG	720-3660	8	2	25.00	0.36
5	P50	OPC-10	TGTCTGGGTG	390-960	6	3	50.00	0.46
6	P41	OPB-09	TGGGGGACTC	2280-5810	7	5	71.43	0.41
7	P40	OPB-08	GTCCACACGG	680-3840	9	4	44.44	0.47
8	P22	OPR-04	CCCGTAGCAC	246-4450	5	1	20.00	0.44
9	P21	OPF-16	GGAGTACTGG	2330-6280	4	1	25.00	0.50

10	**P18**	**OPA-12**	**TCGGCGATAG**	**880-2730**	**5**	**1**	**20.00**	**0.44**
11	**P17**	**OPZ-11**	**CTCAGTCGCA**	**2480-6350**	**5**	**2**	**40.00**	**0.28**
12	**P6**	**UBC-493**	**TGATGCTGTC**	**2220-5550**	**5**	**2**	**40.00**	**0.47**
	Mean				**5.91**	**2.33**	**34.48**	

Statistical Analysis

In order to affirm the basic assumptions of the data to be analyzed, they were firstly tested for the normal distribution and the homogeneity of variance using the Barlett-test (Kohler et al., 2002). As a first step of the statistical analysis of the data after fulfilling the fore-mentioned assumptions, analysis of variance (ANOVA) was conducted (Zar, 1999) through the Proc GLM of the Statistical Package SigmaStat version 22.0 (SPSS, 1997) to detect differences among means of the metric traits. In case of differences among means were detected, the second step was to determine the significant differences among the means using least significant differences (LSD) at a probability level of 0.05 (Abacus Concepts, 1991). Scoring data was described as discrete variables for each trait. Morphological variables were normalized prior to cluster analyses using Z-scores. Thereafter, Euclidean distance coefficient for pairs of landraces was computed using NTSYSY-PC (Numerical Taxonomy and Multivariate Analysis for Personal Computer) software program version 2.00 (Rohlf, 1998). To better understand the patterns of variation among genotypes, distance matrix generated from morphological data was used as input for cluster analysis based on unweighted pair-group method of arithmetic average (UPGMA), and to compute principle component analysis (PCA) with NTSYSY-PC. The PCA was performed for all morphological data together and then separately for metric and scoring traits.

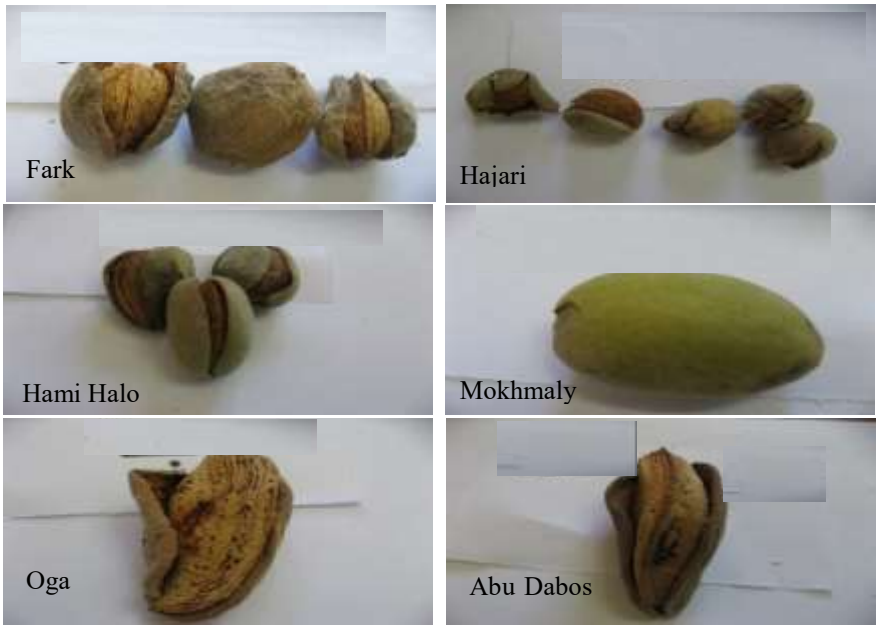

Figure 1. Fruits of the six Jordanian almond landraces from Ajloun District of Jordan used in the study.

RESULTS

Analysis of Variance for Metric Traits

The analysis of variance of the 19 metric traits is presented in table 2. There were high significant (P<0.01) differences among almond landraces in leaves length, petiole length, internodes length, shell length, shell width, shell shape, shell thickness, nut thickness, nut length, nut shape, pores number, nut size, kernel size and kernel width. Other traits such as kernel length, kernel thickness, double kernel (%) showed significant differences (P<0.05). Mean values and CV for metric traits were wide for nut shape, kernel size, nut length, nut size, and shell length. Across 6 almond landraces, nut shape, kernel size, nut length, nut size, shell length were traits with high level of variation (CV>30%), while kernel length, shell width, internodes length, nut width showed low values (CV<20%). Higher CV values indicated a higher selection possibility for those traits

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Table 2. Mean, maximum, minimum, standard deviation (SD) and coefficient of variation (CV) for 19 metric traits recorded for six almond landraces.

Traits	Mean*	SD	CV	Max	Min.
Leave length	5.38	1.12	0.21	6.96	4.02
Petiole length	1.77	0.47	0.27	2.37	1.06
Interned length	1.48	0.26	0.18	1.71	1.02
Shell width	2.20	0.39	0.18	2.66	1.70
Shell length	3.87	1.37	0.35	5.76	2.33
Shell shape	6.07	1.68	0.28	8.42	4.29
Shell thickness	0.20	0.06	0.28	0.26	0.11
Nut width	2.39	0.29	0.13	2.88	2.07
Nut length	3.91	1.54	0.39	5.97	2.06
Nut shape	2.48	3.13	1.26	8.19	0.48
Pores number	104.75	22.10	0.21	121.92	63.08
Nut size	0.70	0.25	0.36	1.17	0.38
kernel length	2.24	0.41	0.18	2.89	1.65
Kernel size	0.22	0.11	0.49	0.40	0.10
kernel thickness	0.68	0.17	0.25	0.88	0.48
weight of kernel	1.143	0.33	0.29	1.48	0.80

*Means covers all the six almond landraces tested in the study.

Leaf, Nut and Kernel Variables

Abu Dabos landrace showed significantly the tallest leaf length, while Fark and Hajari were the shortest. Three landraces (Oga, Abu Dabos, Hami Halo) significantly (P>0.01) showed long petioles with values ranging from 1.91 cm to 2.4 cm, while the other three landraces showed stunted petiole with values ranging from 1.06 to 1.56 cm. Fark and Hami Halo showed long internodes, while Oga showed short internodes. For leave width Mukhmaly showed the maximum value, while lowest was recorded for Fark (Table 3). The longest shell length was observed in Oga and Abu Dabos, whereas the lowest values were obtained in Mukhmaly. Shell width was the lowest Hajari and the highest values were observed in Oga and Abu Dabos. Shell shape for Oga showed the highest value, while the lowest shell shape was found in Hajari. For shell thickness, three landraces (Oga, Abu Dabos and Fark)

gave the highest shell thickness, while the other three shows thin shell (Table 3). Nut width showed the maximum value in Oga and the minimum for Hajari. For nut length, two landraces (Oga and Abu Dabos) gave long nut, while Hajari showed short nut. The maximum value for nut shape was observed in Abu Dabos, while the minimum value detected in Oga. For pores number, five landraces (Oga, Abu Dabos, Hajari, Hami Halo and Mukhmaly) had high pores number, while Fark had the lowest number of pores. Nut size was highest for Oga followed by Abu Dabos, Mukhmaly, Hami Halo, Hajari, while the smallest nut size was for Hajari. The kernel length was longest in Oga, Abu Dabos and Fark, and shortest in Hajari. The largest kernels were observed in Oga, while Abu Dabos had the smallest kernel size. The highest kernel thickness values were observed in Oga and Fark, while the lowest values were detected in Hami Halo and Abu Dabos. Three landraces (Oga, Abu Dabos and Fark) showed higher kernel weight; while the other landraces showed light kernels weight (Table 3).

Table 3. Means of 19 different metric traits for six studied almond landraces.

Trait	Oga	Abu Dabos	Hajari	Hami Halo	Mukhmaly	Fark	LSD
Leave Length	5.49 b	6.96 a	4.02 c	5.98 b	5.65 b	4.18 c	0.54
Leave width	1.78 b	2.56 a	1.99 b	2.04 a	2.63 a	1.64 bc	0.47
Petiole length	1.91 b	2.12 b	1.06 d	2.38 a	1.56 c	1.58 c	0.21
Internodes length	1.02 d	1.47 bc	1.38 c	1.70 a	1.47 bc	1.71 a	0.17
Shell width	5.76 a	5.38 a	2.33 c	3.27 b	3.21 b	3.25 b	0.57
Shell length	2.66 a	2.49 a	1.97 b	1.88 b	1.70 b	2.12 a	0.33
Shell shape	0.84 a	0.79 a	0.43 c	0.52 bc	0.49 bc	0.58 bc	0.87
Shell thickness	0.26 a	0.25 ab	0.11 d	0.187 c	0.187 c	0.24 b	0.02
Nut width	2.88 a	2.62 ab	2.07 c	2.23 c	2.25 c	2.32 bc	0.31
Nut length	5.97 a	5.58 a	2.06 c	3.61 b	3.1 b	3.10 b	0.66
Nut shape	0.48 c	0.82 a	0.41 c	0.62 c	0.73 b	0.75 b	0.88
Pores number	121.92 a	116.04 a	109.88 a	119.36 a	98.20 a	63.08 b	24.7
Nut size	1.17 a	0.69 b	0.38 c	0.66 b	0.66 b	0.66 b	0.25
Kernel width	1.78 b	2.56 a	1.99 b	2.04 a	2.63 a	1.64 bc	0.47
Kernel length	2.89 a	2.37 ab	1.65 c	2.13 bc	2.10 bc	2.32 ab	0.60
Kernel shape	0.48 c	0.62 b	0.99 a	0.66 b	0.59 bc	0.55 bc	0.79
Kernel size	0.40 a	0.10 c	0.16 bc	0.23 bc	0.16 bc	0.28 ab	0.13
Kernel thickness	0.88 a	0.48 b	0.80 a	0.52 b	0.62 ab	0.81 a	0.27
Kernel weight	1.60 a	1.12 b	0.80 b	0.88 b	0.96 b	1.48 a	0.33

Different small letters are significantly different within the same raw among the different landraces.

Variability in Scoring Traits

Duration of flowering was early in 4 almond landraces, namely; Oga, Hajari, Mukhmaly and Fark, respectively, while Hami Halo was late in flowering (Table 4). Petals color is white in Oga and Fark, while the other four landraces had light pink petals. Most flower buds were located in 1-year-old shoots in Oga, Abu Dabos, Hami Halo and Fark. There was no anthocyanin coloration on shoot tip in Oga, Hajari and Fark, while Abu Dabos, Hami Halo and Mukhmaly showed low anthocyanin coloration on shoot tip. Oga, Abu Dabos, Mukhmaly and Fark had dense ramification, while Hajari had extremely dense ramification. Tree habit was spreading in Abu Dabos, Hajari, Hami Halo, Mukhmaly and Fark. Shell color was dark in Oga, Abu Dabos, Hajari and Fark, while in Hami Halo and Mukhmaly it was light colored. Outer shell marking was scribed in Oga, Abu Dabos, Hajari, Hami Halo and Fark. The kernel taste was sweet taste in Oga, Abu Dabos, Hajari, Mukhmaly and Fark, while the taste was medium bitter in Hami Halo. Softness of shell was paper in Hajari, Hami Halo and

Mukhmaly. Oga, Abu Dabos and Fark had a large percentage of dropped fruits, while Hami Halo and Mukhmaly showed low drop fruits. Kernel color intensity was dark brown in Oga and Abu Dabos, while in Hajari and Hami Halo and Fark were light brown. Shriveling of kernel in Oga and Abu Dabos showed wrinkled, while in Mukhmaly and Fark had slightly wrinkled kernel. Pubescence in Oga, Hajari and Hami Halo was low, while it was very dense in Fark kernels. Oga, Hami Halo and Fark had showed double kernel while Abu Dabos, Hajari and Mukhmaly had single kernels (Table 4).

Table 4. Scoring traits for six studied almond landraces.

Trait	Oga	Abu Dabos	Hajari	Hami Halo	Mukhmaly	Fark
Duration of flowering	Early	Intermediate	Early	Late	Early	Early
Color of petals	White	Light pink	Light pink	Light pink	Light pink	White
Location of flower buds	Most on one year old shoot	Most on one year old shoot	Mixed	Most on one year old shoot	Mixed	Most on one year old shoot
Coloration of shoot tip	No anthocyanin coloration	Low	No anthocyanin coloration	Low	Low	No anthocyanin coloration
Ramification	Dense	Dense	Extremely dense	Intermediate	Dense	Dense
Tree habit	Weeping	Spreading	Spreading	Spreading	Spreading	Spreading
Shell color intensity	Dark	Dark	Dark	Light	Light	Dark
Marking of outer shell	Scribed	Scribed	Scribed	Sparsely	Without pored	Scribed
Kernel taste	Sweet	Sweet	Sweet	Intermediate	Sweet	Sweet
Softness of shell	Intermediate	Intermediate	Paper	Paper	Paper	Intermediate
Ease of harvesting	High	High	Intermediate	Low	Low	High
Kernel color intensity	Dark brown	Dark brown	Light brown	Light brown	Brown	Light Brown
Shriveling of kernel	Wrinkled	Wrinkled	Intermediate	Intermediate	Slightly wrinkled	Slightly wrinkled
Kernel pubescence	Low	Intermediate	Low	Low	Intermediate	High
Percentage of double kernel	Double	Single	Single	Double	Single	Double

Multivariate Analysis Based on all Traits Together

For all morphological traits including 19 metric and 15 scoring traits, the first five principal components (PCs) explained all the variation existed among almond landraces (Table 5). The relative magnitudes of eigenvectors for the first principal component was 44.79%, explained mostly by shell related traits (shell width, length, shape, thickness and softness of shell), nut size and nut width, weight of kernel and kernel color intensity. From the second and third principal component, which contributed 24.6% and 15.2% of the total variation, the most predominant characters were leave related traits (length of leave and its petiole), kernel weight, shoot tips coloration and ramification, while the variation in the third component explained by nut shape and percentage of double kernel.

Table 5. Loading values of the 33 metric and scoring traits on the first five principle components for the 6 almond landraces from Ajloun district

	Function				
Trait	PC1	PC2	PC3	PC4	PC5
Leave length	.147	.921	.305	.146	-.128

Leave width	.152	.761	.206	.143	-.321
Petiole length	.237	.918	-.252	.077	.176
Interned length	-.676	.114	-.346	.619	.162
Shell width	.917	-.087	.056	.180	.340
Shell length	.847	.468	.243	.007	-.073
Shell shape	.907	.360	.211	.048	.021
Shell Thickness	.815	.366	-.162	.415	-.065
Nut width	.932	.317	.115	-.047	-.125
Nut length	.806	.558	.189	-.019	-.042
Nut shape	.000	.167	.879	.226	. 385
Pores no.	.061	.590	.384	-.708	-.003
Nut size	.849	.299	-.241	-.190	-.308
kernel length	.907	.315	-.212	.050	-.175
Kernel width	.937	.432	-.324	.453	-.764
Kernel shape	.975	.543	.431	-.321	-.235
Kernel size	.669	-.168	-.633	-.345	-.067
kernel thickness	.449	-.794	-.237	-.326	-.077
weight of kernel	.916	-.169	-.296	.202	-.054
Duration of flowering	-.506	.671	-.343	-.074	.412
Colour of petals	-.779	.386	.486	-.088	-.024
Location of flower buds	-.862	-.322	.300	.137	-.210
Coloration of shoot tip	-.432	.814	.153	.273	-.229
Ramification	.003	-.786	.602	-.136	-.035
Tree habit	.787	-.389	.261	-.165	-.367
Shell colour intensity	.620	-.503	.395	.047	.452
Marking of outer shell	.620	-.503	.395	.047	.452
Softness of shell	-.886	. 024	-.068	-.401	-.220
Ease of harvesting	.650	-.656	-.341	-.106	.140
kernel colour intensity	.834	.209	.320	.296	-.265
shriveling of kernel	.554	.432	.482	-.429	.301
kernel pubescence	.090	-.261	-.129	.951	-.050
kernel taste	-.409	.614	-.525	-.267	.331
percentage of double	.429	.093	-.849	-.116	.270
% of variance	44.797	24.556	15.179	9.890	5.578
Cumulative %	44.79	69.35	84.532	94.422	100.000

The fourth component explained 9.9% of total variation explains mainly by kernel pubescence. Euclidian distance coefficients were calculated for almond landraces based on their metric and scoring traits (Figure 2). Cluster analysis placed the six almond landraces into two main groups. The 1st cluster consisted of Hajari, Hami Halo and Mukhmaly with small fruits in terms of kernel size and nut dimensions, whereas the 2nd cluster included the other three landraces (Oga, Fark and Abu Dabos), which had large sized fruits. In general, clustering of landraces based on plant morphology was not consistent with the genetic background based on RAPD analysis. The mean value of genetic distance was 7.7 ranging from 5.5 to 10.14. The highest genetic distance (10.1) was obtained between Oga and Hami Hallo landrace, while the most similar landraces were Mukhmaly with each of Hami Hallo and Hajari (5.5) (Table 6).

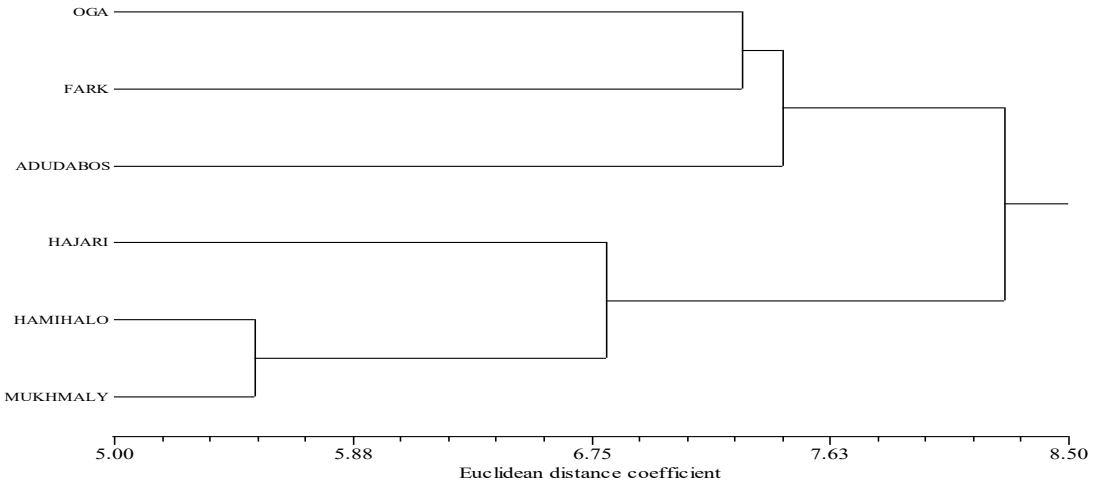


Figure 2. Dendogram using Euclidean distance coefficient for six almond landraces collected from Ajloun District based on 19 metric traits and 15 scoring traits together.

Table 6. Euclidean distance based on 34 scoring and metric traits for six almond landraces collected from Ajloun District.

	Oga	Abu Dabos	Hajari	Hami Halo	Mukhmaly	Fark
Oga	0.00					
Abu Dabos	7.33	0.00				
Hajari	10.02	8.31	0.00			
Hami Halo	10.14	7.54	8.02	0.00		
Mukhmaly	9.53	6.67	5.59	5.51	0.00	
Fark	7.30	7.57	7.16	8.26	6.54	0.00

Multivariate Analysis Based on Metric and Scoring Traits

The Euclidian distance coefficients were calculated for six almond landraces based on 19 metric traits (Table 7). Euclidian distances ranged from 2.3 to 8.9, with a mean of 5.4. Hami Hallo and Mukhmaly were the closest genotypes (D=2.31), while Oga and Hajari were the least similar genotypes (D=8.64). A UPGMA dendrogram was created based on Euclidean genetic distances to estimate the level of relatedness among almond landraces (Figure 3). The cluster analysis based on 17 metric traits placed the 6 almond landraces in 2 main groups. The 1st main group consisted of Oga in 1 isolated group, while the other 5 landraces (Abu Dabos, Hajari, Hami Halo and Mukhmaly) were clustered together in another group. The 2nd main cluster included 2 sub clusters: Abu Dabos in the 1st sub cluster and 4 other landraces in 2nd sub cluster. In the 2nd sub cluster contained 2 sub-sub clusters, the 1st sub-sub cluster observed Hajari, Hami Halo and Mukhmaly were in 1 sub-sub cluster. In the 2nd sub-sub cluster contained of Fark. In Oga has a unique trait such as short internodes length, high shell width, high shell shape, high nut width, low nut shape, high nut length, high pores number, high nut size and large kernel size. The 1st

subgroup in the 2nd main cluster, Abu Dabos has unique traits such as the longest leaves, short internodes, longest shell length and width, high shell shape, thick shell, high nut width, long nut, high nut shape, high pores numbers, small size for kernel, intermediate kernel thickness and kernel width. In the 2nd sub cluster, in the 1st sub-sub cluster Hajari, Hami Halo and Mukhmaly had intermediate leaves length, while in Hajari has the smallest leaf length, intermediate shell length, intermediate shell width, small shell shape, thin shell, low nut width, intermediate nut length, small nut shape, intermediate nut size, intermediate kernel length, intermediate kernel size, thick kernel, and intermediate kernel width. In the 2nd sub-sub cluster, Fark had unique traits such i.e., short leaves, short petiole, longest internodes, long shell, thick kernel, high kernel width. The Euclidian distance coefficients were calculated for almond landraces based on 15 scoring traits (Table 8), and it ranged from 4.09 to 7.5. Oga and Fark were the closest genotypes (D=4.09), while Oga and Hami Halo were the least similar genotypes (D=7.5). A UPGMA dendrogram was created based on Euclidean genetic distances to estimate the level of relatedness among almond landraces (Figure 4). The cluster analysis based on 15 scoring traits placed the 3 almond landraces in 2 main groups. The 1st main group consisted of Hami Halo in one isolated group, while the other 5 landraces were clustered together. The 2nd main cluster included 2 sub clusters; Oga and Fark and 3 other landraces in a subgroup. Oga and Fark had unique traits such as no anthocyanin, white petal color, early flowering, flower buds most on one year old shoot, dense ramification, shell color intensity, marking of outer shell scribed, sweet kernel taste, intermediate softness of shell, high fruit drop, dark kernel color intensity, while other landraces showed in the sub-sub cluster that contained Hajari and Mukhmaly had similar traits in early flowering, light pink color of petals, flower buds were on 1-year-old shoot and spur, spreading tree habit and sweet kernel taste. In the 2nd sub-sub cluster Abu Dabos had unique traits such as intermediate duration of flowering, kernel pubescence and softness of shell, high fruit drop, and wrinkled kernel. The 1st main cluster consisted of Hami Halo which had unique traits such as late flowering, flower buds most on 1-year-old shoot, low anthocyanin coloration on shoot tip, intermediate ramification, spreading tree habit, light anthocyanin coloration on shoot tip, spreading tree habit, light shell color intensity, intermediate ramification, sweet kernel taste and softness of shell, high fruit drop, dark brown kernel color intensity, and wrinkled kernel. Clustering of landraces based on plant morphology was not consistent with genetic background based on RAPD. The mean value of genetic distance was 5.4. The highest genetic distance (7.52) was between Hami Hallo and Oga landrace, while the most similar landraces were reported in Fark and Oga (4.09). In this regard, 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 (Halasz et al., 2019).

Table 7. Euclidean distance based on 19 metric traits.

	Oga	Abu Dabos	Hajari	Hami Halo	Mukhmaly	Fark
Oga	0.00					
Abu Dabos	5.75	0.00				
Hajari	8.64	7.02	0.00			
Hami Halo	6.87	4.67	4.87	0.00		
Mukhmaly	6.97	5.08	3.60	2.31	0.00	
Fark	6.04	5.95	5.23	4.75	3.87	0.00

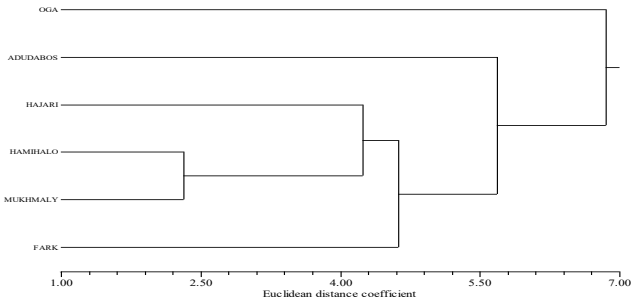


Figure 3.Dendogram using Euclidean distance coefficient for 6 almond landrace collected from Ajloun District based on 19 metric traits.

Table 8. Euclidean distance based on15 qualitative traits.

	Oga	Abu Dabos	Hajari	Hami Halo	Mukhmaly	Fark
Oga	0.00					
Abu Dabos	4.61	0.00				
Hajari	5.48	4.46	0.00			
Hami Halo	7.52	6.04	6.41	0.00		
Mukhmaly	6.56	4.37	4.29	5.07	0.00	
Fark	4.09	4.76	4.93	6.86	5.36	0

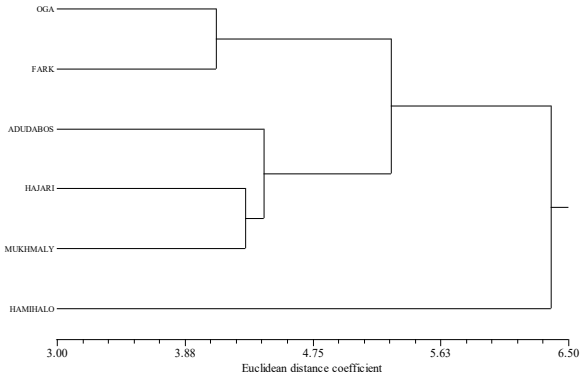


Figure 4. Dendogram using Euclidean distance coefficient for 6 almond landrace collected from Ajloun District based on 15 scoring traits.

RAPD Variation

Coefficients of genetic distance were calculated for paired comparison of the 6 almond landraces based on distance matrices generated from RAPD (Table 9). The mean value of genetic distance was 0.69. The highest genetic distance (0.85) was obtained between Abu Dabos and Fark, while the most similar landraces were obtained between Hajari and Oga with genetic distance of 0.5. The 6 landraces were grouped into 2 main clusters (Figure 5). The 1st cluster comprised from Oga, Hajari, Hami Halo and Abu Dabos, and the 2nd cluster included Fark and Mukhmaly. Two subgroups existed within the 1st main cluster. The 1st subgroup contained Oga and Hajari, while the 2nd subgroup contained Hami Halo and Abu Dabos. Principle coordinate analysis for almond genotypes is presented in Figure 5.

Table 9. Nei genetic distance based on RAPD between different almond landraces.

	Oga	Fark	Mukhmaly	Hami Halo	Hajari	Abu Dabos
Oga	0.00					
Fark	0.62	0.00				
Mukhmaly	0.71	0.68	0.00			
Hami Halo	0.65	0.68	0.71	0.00		
Hajari	0.50	0.80	0.68	0.73	0.00	
Abu Dabos	0.73	0.85	0.73	0.68	0.65	0.00

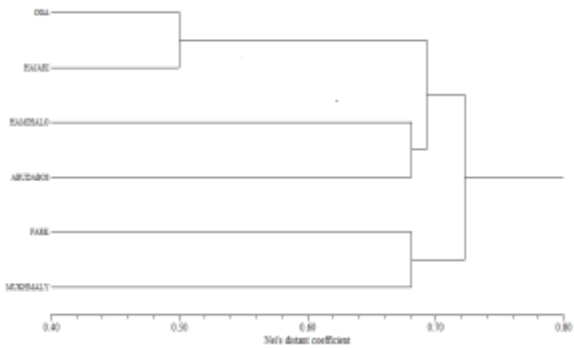


Figure 5. Dendogram using Nie distance coefficient for 6 almond landraces collected from Ajloun District based on RAPD.

Genetic distance estimate based on RAPD markers:

The data of the binary matrix was computed using the NTSY-pc program according to Nie genetic distance coefficient in order to estimate the genetic distance among 6 almond landraces. Nie genetic distance (N) identifies the distance between two genotypes on 0 to 1 scale. The obtained matrix exhibits genetic distances ranging from

0.5 to 0.85, with a mean of 0.697 (Table 10). These distances express high genetic diversity among local almond germplasm at the DNA level. The smallest genetic distance of 0.5 was observed between Mukhmaly landrace accessions and Oga landraces, while the largest genetic distance was 0.85 observed between Fark landraces and Abu Dabos landraces.

Table 10. Loading values of the 28 polymorphic markers on the first five principle components for the 6 almond landraces from Ajloun district.

		Function				
Primer	Size of the marker in Kb	PC1	PC2	PC3	PC4	PC5
P6-1	5.55	-.720	.533	.256	-.316	.179
P6-4	2.99	.211	-.931	-.266	-.087	-.106
P17-1	6.35	-.744	.112	-.106	.565	.322
P17-4	3.63	-.744	.112	-.106	.565	.322
P18-2	2.02	-.706	.161	.645	.222	.099
P21-4	2.33	.145	-.496	-.602	-.608	-.015
P22-4	2.93	-.706	.161	.645	.222	.099
P40-2	3.38	.671	-.081	.684	.090	.258
P40-5	1.78	.435	.438	.723	.198	-.239
P40-7	1.20	-.654	-.318	-.353	.462	-.364
P40-8	0.92	.088	-.809	.437	-.294	-.246
P41-2	4.68	.646	-.493	.457	.111	-.345
P41-3	4.25	-.706	.161	.645	.222	.099
P41-5	3.35	.830	-.158	-.003	.320	.429
P41-6	2.51	.350	-.663	.004	-.130	.649
P41-7	2.28	.159	-.452	.732	-.327	.358
P50-4	0.6	.356	.794	.330	-.339	-.140
P50-5	0.43	-.646	.493	-.457	-.111	.345
P50-6	0.39	.495	.770	-.379	-.135	.007
P62-2	3.27	-.150	.091	.922	-.284	-.196
P62-7	1.21	-.654	-.318	-.353	.462	-.364
P59-4	1.81	-.073	.511	-.472	-.705	.114
P59-5	1.47	.830	-.158	-.003	.320	.429
P54-4	0.59	-.176	.476	.356	-.782	-.065
P54-5	0.43	.495	.770	-.379	-.135	.007
P52-3	2.36	.699	.463	-.007	.534	-.106
P52-5	1.67	.699	.463	-.007	.534	-.106
P52-7	0.47	.699	.463	-.007	.534	-.106
% of variance		32.8	23.9	20.5	15.6	7.122
Cumulative variance (%)		32.833	56.716	77.253	92.878	100.000

Principle component analysis for 6 almond landrace accessions using RAPD data:

The result of the principle coordinate analysis for 6 almond genotypes is presented in figure 6. The distribution obtained by plotting the 1st 3 Eigenvectors calculated for the 6 almond accessions produced a separation that is similar to the UPGMA dendrogram based on Nie's genetic distance matrix. Oga and Mukhmaly, Hajari and Abu Dabos, and Hami Halo and Fark produced 3 separate groups. The 5 functions account

for 32.8, 23.9, 20.5, 15.6, and 7.12% of the total genetic variation. The principle component analysis showed that some alleles contributed more to total genetic variation. The most important alleles found to explain the variation for all polymorphic markers obtained in PC1 were P17-1(0.744), P41-5(0.830), and P59-5(0.830); in PC2 P6-4(0.931), P40-8(0.809) and P50-4(0.794), in PC3 were P18-2(0.654), P40-5(0.732) and P41-7(0.732), in PC4 were P59-4(0.705) and P54-4(0.782), and in PC5 was P41-6(0.649). The initial screening of RAPD primers was based on a group of RAPD primers consisting of 62 primers. The bands showed reproducible and scorable amplification that was used in the multivariate analysis. A total of 71 RAPD markers were amplified using the 12 pre-selected primers. All primers gave at least one polymorphic band. The amplification products of all tested landraces obtained by RAPD primers are shown in Figure (7). The number of total reproducible RAPD markers varied from 5 to 7, with fragment sizes ranging from 390 to 6350 bp. The 12 RAPD primers generated 426 data points, among which 113 data entries were for present bands (1), and 313 for absent bands (0). Bands with the same molecular weight and mobility were treated as identical fragments. One to five polymorphic bands were generated with a mean of 5.91 bands/primer ranging from 5 to 9 bands/primer. The percentage of polymorphic bands (PPB)/primer ranged from 20% in P18 and P22 to 71.4% in P41. The discriminating power (PIC value) of each RAPD primer used in the study varied from 0.28 in p17 and p52 to 0.5 in p21. The banding profile for each landrace is unique and could be used for varietal identification.

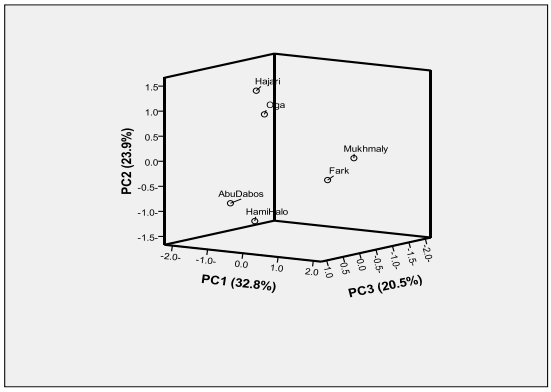
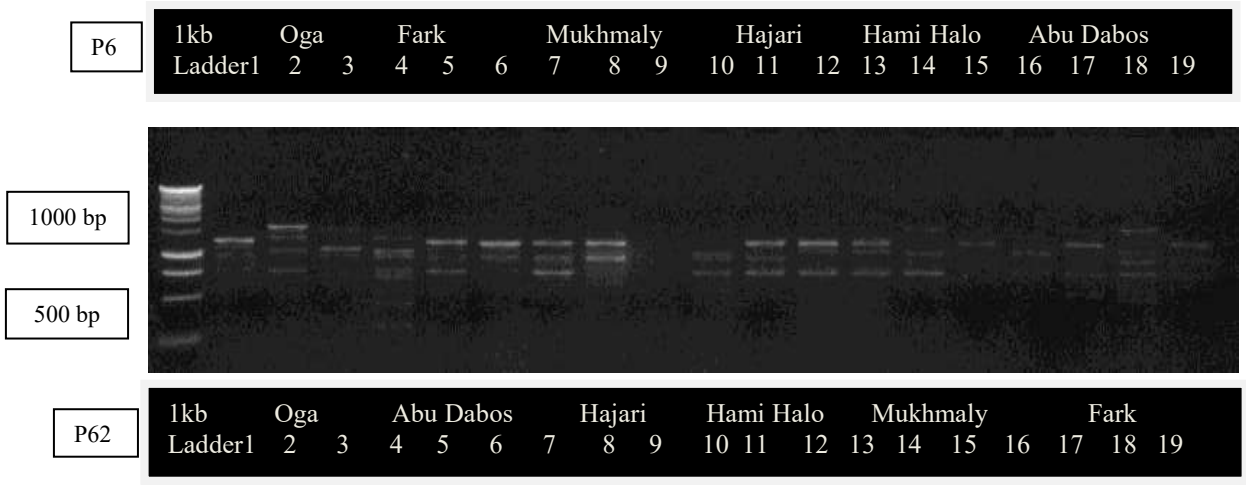


Figure 6. Principle component analysis plot for 6 almond landraces collected from Ajloun district base on polymorphic RAPD markers.



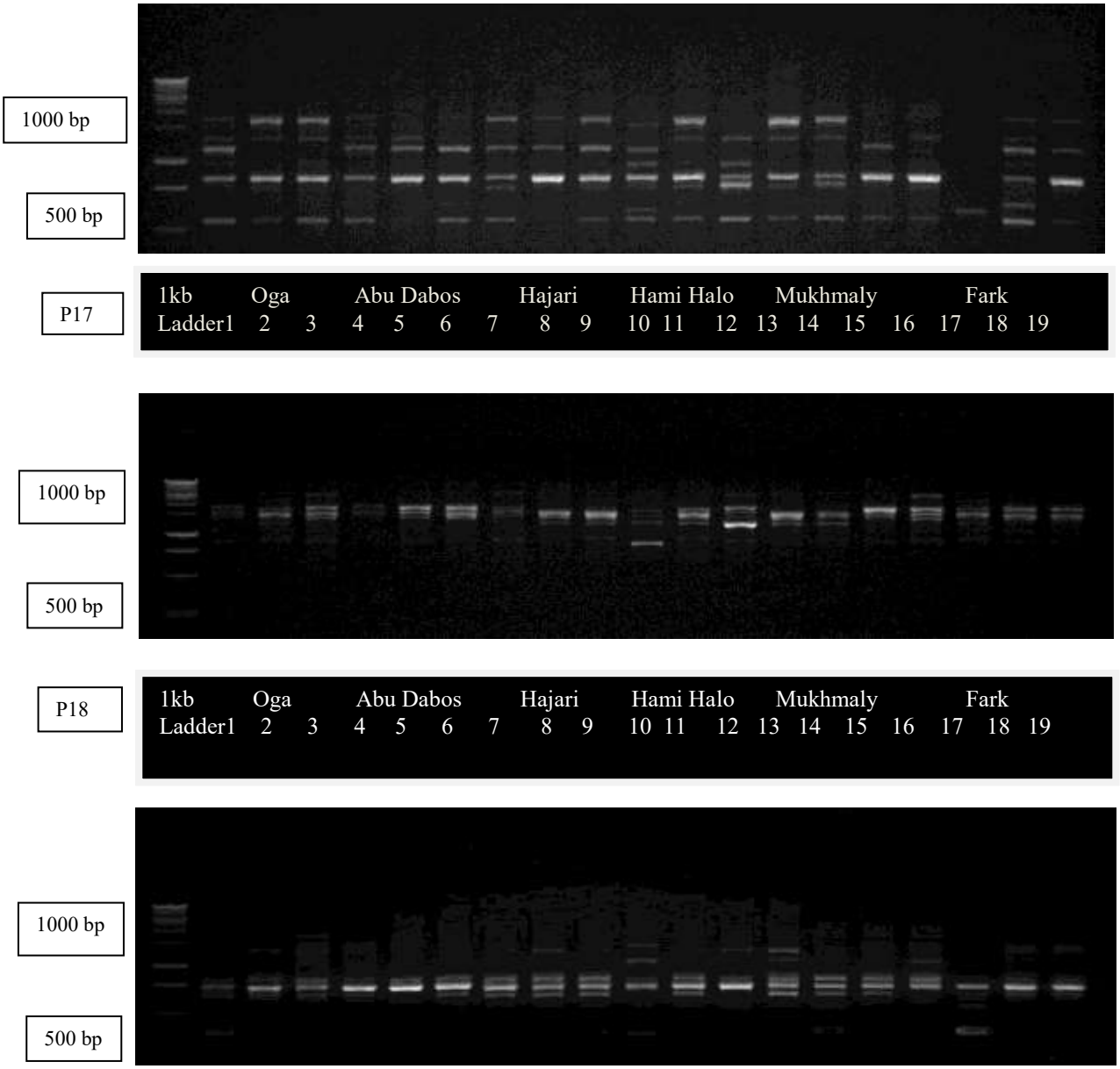


Figure 7. Photograph of ethidium bromide stained gel showing bands of RAPD amplification products of P6, P62, P17 and P18 primer for 6 almond landraces. Lanes 1-3, 4-6, 7-9, 10-12, 13-15 and 16-19 are respectively PCR products obtained from Oga, Fark, Mukhmaly, Hajari, Hami Halo, and Abu Dabos.

DISCUSSION

In the present study, a total of 34 traits were selected to characterize the available almond landraces from Jordan to find out the efficacy of morphological traits in genotype identification. According to the morphological descriptor of IPGRI-FAO (1985), these traits are well-defined traits for identifying almond genotypes. Moreover, many traits have economic importance, especially those related to nut and kernel traits, pest

resistance, and abiotic stress tolerance, and consequently serve as target traits for almond breeding programs. Our findings are in line with other studies of Khadivi-Khub et al. (2008) which support that morphological characterization of almonds is a reliable way to estimate the genetic diversity and the level of genetic distance among almond landraces. However, morphological traits are mostly influenced by external environmental factors such as temperature, rainfall and humidity, and agricultural practices (Sakar et al., 2019). Therefore, using some plant molecular genetic tools (i.e., DNA markers) is considered a solution to eliminating the effect of external environmental factors (Mirali and Nabulsi, 2003). Various methods were developed for the genetic characterization of almond germplasm including non-PCR (Aranzana et al., 2003) and PCR-based DNA markers (Colic et al., 2010; Mahood and Hama-Salih, 2020), and they have proved to be valuable genetic tools for varietal identification and estimation the level of genetic variation. RAPD is a widely used technique in almond genetic diversity studies (Mahood and Hama-Salih, 2020), and genetic mapping (Tanksley et al., 1999). The PCR-based markers including RAPD have the advantage of detecting the differences among genotypes using a small quantity of DNA (20-50 ng) without the need for any previous information on DNA sequences. RAPD markers have been reported to be as efficient as SSR (Xu et al., 2004) and RAPD was found to be more efficient in discriminating almond germplasm (Mirali and Nabulsi, 2003; Mahood and Hama-Salih, 2020). The use of RAPD markers in estimating the genetic diversity in almond germplasm is remarkable (Gregory, 2004).

Morphological Characterization of Jordanian Almond Landraces

A considerable magnitude of variation was found among almond landraces which could be utilized in plant breeding programs. The landraces significantly differed for all metric traits tested. Nut, kernel, and leaf-related traits showed medium to high CV values. Almond landraces were polymorphic for 15 out of 20 scoring traits, showing two or more phenotypic classes/traits. A high CV value indicates a high level of variability among these traits and a high possibility for selection for desirable traits. Since the landraces were grown at the same location, most variation recorded among landraces is mostly related to genetic differences. Traits recorded in this study were obtained from almond materials grown in the same site with minor microclimate variation. Therefore, the present phenotypic values do not include large differences due to environmental factors. Similarly, high phenotypic variability was reported in international almond collections from Iran (Sorkheh et al., 2009), Turkey (Askin et al., 2007), Spain (Vargas et al., 2001), France (Dicenta et al., 2003), and Lebanon (Talhouk et al., 1999). International almond collection exhibited a high level of variability in blooming-related traits, and nut and kernel-related traits. Such traits could be an efficient system to discriminate among almond landraces. Kernel and nut weights are the most important marketable traits. These traits are always a target for plant breeders and almond growers. In this study, a wide range of variation was recorded among almond landraces, i.e. kernel weight of the six landraces ranged from 0.80 to 1.60 g; Oga, Fark, and Abu Dabos had kernel weights larger than 1 g which were considered suitable for commercial purposes and within consumer preferences. Kernel size could be varied according to inter- and intra-seasonal variation in environmental conditions (Dicenta et al., 1993). Another factor that affect kernel size could be pollination. Pollen source was found to have a significant effect on nut and kernel weight, with fruit coming from cross-pollination being heavier than that from self-pollination (Oukabi et al., 2002). However, Ortega et al. (2006) found significant differences between open and self-pollination for nut weight but not for kernel weight.

The nut dimension is used by the industry for marketing nuts. It is important to mark almond nuts with desirable dimensions and shapes to receive the highest price from growers. High variability was recorded in kernel and nut dimensions (width and length) in almond germplasm. In our study, the longest kernel was recorded in Oga and Abu Dabos, while the widest kernel was recorded in Oga and Fark. The kernel shape of Jordanian

almond collections ranged between Narrow and extremely broad. The most desirable shape is oblong which is present in Mukhmaly and Fark. Kernel size was significantly large in Oga, and kernel size of Jordanian almond collections ranged between small and large. Nut shape was significantly larger in Abu Dabos and Fark. Based on the fruit index values, the nut shape of Jordanian almond collections ranged between round and extremely narrow. Nut size was high for Oga, the nut size of the Jordanian almond collection ranged between small and extremely large. Such variation in kernel size is required to meet different market needs, longer, more oblong nuts are often desirable for sliced or slivered products since longer nuts produce more uniform sliced products (Schirra, 1997). From this perspective, Mukhmaly and Fark with oblong shapes are the most desirable landraces for industrial uses. Kernel size is commercially important, and larger sizes generally confer greater value (Godini, 2002). The kernel taste was sweet in all tested landraces except Hami Halo with medium bitterness. The sweet character of the kernel has undoubtedly been of vital importance in the breeding of almonds, from the first selection processes carried out by farmers to the current research programs (Socias and Felipe, 1998). As a consequence of this constant selection, a progressive decrease in the frequency of alleles responsible for bitter flavor in this species has taken place (Socias, 1998). Nevertheless, some varieties, which have been grown for years and are commercially viable, carry these alleles and, when combined with each other can produce seedlings with bitter kernels. The nearest amygdalin precursor is not produced in the kernel but is transported from the mother plant, which is the one that has the bitter genotype (Ferhner et al., 1990), so that all the kernels of a tree will have either sweet or bitter kernels (Grasselly and Crossa-Raynaud, 1983). Among 38 almond local genotypes, 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 (Mahood and Hama-Salih, 2020). Furthermore, 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).

Almond is an early-blooming species, thus it is susceptible to spring frosts. From this perspective, Hami Halo and Abu Dabos bloomed later than other landraces, which could be utilized as a source of genes to escape frost stress. Although almond is resistant to low temperature in winter, certain degree and duration of low temperature in spring frost are lethal to most reproductive organs during the blooming period (Kodad et al., 2005). Therefore, when determining the almond variety's susceptibility to frost damage, blooming time is important. Moreover, almond genotypes are clustered together according to their bloom periods (Kodad et al., 2005). Duration of blooming is important in selecting satisfactory pollinizers. Usually, varieties within the same bloom group or from adjacent bloom groups will overlap and cross-pollinate satisfactorily, except in the case of incompatible combinations. However, the closer that varieties bloom together, the better the opportunity for cross-pollination and the setting of a crop. The reasons for this are better overlap of flower receptivity and the tendency of bees to fly between trees at a similar stage of bloom. Early- and late-blooming varieties will not have sufficient bloom overlap, and therefore will not provide adequate cross-pollination for each other (Kodad et al., 2005). There are hard-and soft-shell varieties; the soft shell varieties are the basis of the industry. Genotypes with soft shells are easier to be threshed compared with those with a hard shells. However, one major trait to select for almond varieties is shell hardiness. The shelled almonds should be free of shell debris and foreign materials and pest damage. Shell is a protective cover that prevents the kernel from infection. Fark and Hajari had the smallest leaf dimensions. Small leaves were found to be associated with the environment since almond populations are located in drier areas than those located in semi-humid or more humid regions (Talhouk et al., 1999).

Fark landrace had the smallest number of pores/nut while the other five landraces had high pores number. This trait could be used as morphological marker for varietal identification. This trait is genetically controlled and it seems not to be affected by water supply (Kester et al., 1991). All landraces showed spreading tree habit except Oga with weeping growth habit. Ramification was medium to extremely dense in all landraces. Dense ramification combined with spreading or weeping growth habit considered as desirable traits since they allow more light and air penetration inside the tree. As an undesirable trait interfering with an upright and strong trunk, also a negative correlation was observed among ramification, length and diameter of main trunk (Nikoumanesh et al., 2010). This relationship indicates that more ramification by the induction of lateral branches hamper an upright trunk growth and possibly could affect the time needed in reaching to a proper thickness of budding and/or grafting in nurseries (Nikoumanesh et al., 2010). Low anthocyanin accumulation was observed in Abu Dabos, Hami Hallo and Mukhmaly, while other landraces showed no anthocyanin accumulation. Anthocyanidins are an important group of phenolics in higher plants. Flavonoid composition of plants is generally variety dependent and affected by geographic region, environmental conditions, cultivation practices, and exposure to abiotic stresses, i.e. pests, weather, and UV light. Anthocyanin accumulation is influenced by the ripeness status, processing, and storage of plant-based materials (Lavedrine et al., 2000). Studies on the distribution of flavonoids (i.e., anthocyanin) in almonds revealed that most flavonoids were present exclusively in the skin, while nonflavonoids contributed to the majority of total phenolics in the kernel. The flavonoids in the almond skin layer act as phytoalexins protecting the seeds and nuts against pests and other environmental stress factors (Koes, 1994). Kernel pubescence was high in Fark and intermediate in Abu Dabos and Mukhmaly. Other landraces exhibited light kernel pubescence. Generally, light color and a rough surface texture of kernels are considered desirable traits in almonds. Fark had high pubescence and light brown seed coat color intensity which is desirable for processing and consumer preferences. Since the seed coat may represent from 5 to 10% of the kernel weight (Romjaro et al., 1977), a thin seed coat is desired for blanching to limit weight loss during processing. However, for other processes such as roasting, a thicker or rougher seed coat facilitates coating with salts and flavorings. Kernel surface texture is determined primarily by the seed coat and is affected by both macro and microscopic features. Kernel shriveling is often associated with differences in vascular bundle sizes within the same seed coat (Kester and Gradziel, 1996). Cultivars showing greater pubescence and associated darker kernel colors are less desirable for nuts consumed raw (Romjaro et al., 1977).

Genetic Polymorphism and RAPD Patterns

The RAPD reactions were repeated twice, and 12 RAPD primers were used to screen Jordanian almond landraces. Only reproducible bands were selected using varying annealing temperatures ranging from 30°C to 35°C. The mean level of the proportion of polymorphic loci ranged from 20.0% to 71.4%. With an average of 4.4 bands/primer, a total of 71 bands were generated, ranging in size from 246 to 6350 bp. The percentage of polymorphic bands ranged from 20% in P22 to 71.43% in P41. Previous studies on cultivated almonds (Nikoumanesh et al., 2010) have reported more or less similar higher numbers of polymorphic bands by a single primer. The low number of polymorphic alleles might be due to the narrow gene pool of the cultivated almond, which is usually propagated in a clonal manner by graphing and cutting. Despite the medium level of polymorphism, this set of RAPD markers had high potential in discriminating the 6 landraces. The PIC values for the 12 RAPD primers ranged from 0.28 to 0.50 indicating their low to intermediate discriminating power. Similarly, Mirali and Nabulsi (2003) reported a low number of polymorphic bands/primers ranging from 1 to 5, and those with 1 polymorphic band were more frequent than those having more than 3 polymorphic bands. However, other studies revealed a high level of polymorphism among international almond collections

grown at 2 gene banks in Southern Syria, which could be due to the wide genetic base of this material. According to Nikoumanesh et al. (2010), RAPD is a very powerful and attractive approach to exploring the level of genetic diversity among cultivated and wild almond germplasm.

Pair-wise genetic Nei's genetic distance among the 6 landraces ranged from 0.50 to 0.85 with an average value of 0.69. In an earlier RAPD study conducted by Al-Ghzawi et al. (2009), they observed genetic diversity values of 0.0 to 0.5 among different unknown cultivated almond genotypes and undefined wild almond germplasm. In a collection of cultivated almonds from 2 Syrian gene banks, they observed a genetic distance ranging from 0.04 to 0.30. Similarly, relatively wide genetic distances were observed using morphological traits which in agreement with results obtained by RAPD markers. The Euclidean distance among the 6 almond landraces ranged from 5.5 to 10.14 with mean values of 7.7. The non-significant correlation was obtained between genetic distance based on RAPD markers and those calculated based on morphological data indicating that no consistency between these 2 marker systems. Similarly, the correlation coefficients were found to be very weak between different marker systems. Nikoumanesh et al. (2010) and Kadkhodaei et al. (2011) found a low correlation between genetic distance based on SSR and RAPD and those created using morphological markers. Moreover, Shiran et al. (2007) reported no association between RAPD and SSR markers in a study on the relatedness among Iranian and internationally cultivated almond germplasm. Kadkhodaei et al. (2011) showed germplasm is primarily related to geographic origin. The clustering patterns obtained by morphological and RAPD markers were unrelated. Landraces such as Oga and Hajari that were closely clustered in RAPD dendrogram did not do so in morphology-based dendrograms. Other studies indicated a similar trend and inconsistency between RAPD and morphology-based dendrograms in almonds (Nikoumanesh et al., 2010). The 1st three principle components explained 44.79%, 24.6%, and 15.2%, respectively of the total variation based on morphological data. For RAPD markers, principle component analysis explained 32.8%, 23.9%, and 20.5% of the total variation. Our results revealed that nut traits such as shell-related parameters, nut weight, nut size, nut width, kernel size, shell hardiness as well as leaf-related traits are the most contributors to the total phenotypic variation in Jordanian almond landraces. For each factor, a loading value above 0.8 is considered significant. Sorkheh et al. (2009) found that nut weight, nut size, nut width, kernel size, and shell hardiness had the highest loading values in the first component in phenotypic study aimed at studying the variation among Amygdalus species from Lebanon. On the other hand, Nikoumanesh et al. (2010) found that leaf-traits are predominant in the first component and contributed the most to for the total genetic variation. Genetic diversity of 38 almond local genotypes was investigated using RADP and ISSR markers and it was found that 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 (Mahood and Hama-Salih, 2020). 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. Analysis of molecular variance (AMOVA) demonstrated a high-level genetic differentiation within a population of 88% for RAPD and 87% for ISSR (Mahood and Hama-Salih, 2020).

In conclusion, all metric and scoring traits recorded in this study show high variability, suggesting that they could be a valuable source in almond breeding programs. Although the number of landraces investigated in this study is limited, they revealed a high level of variability in different important agronomic parameters with commercial importance and target traits for selection by growers and breeders. Morphological and RAPD markers

are informative markers to explore the genetic diversity among almond landraces and for investigating the relatedness among almond germplasm. However, further studies might be required to study the intra-landrace variability. Although results showed high polymorphism in Jordanian almond landraces, a weak correlation between distances based on morphological markers and those obtained from RAPD markers indicates that clustering-based RAPD markers are independent of those obtained by morphological characterization. The traits mainly contributed to this variation were nut traits such as shell-related parameters, nut weight, nut size, nut width, kernel size, shell hardiness, and leaf-related traits.

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التنوع الجيني لأنواع اللوز **الاردني المختلفة المتأتية من خلال السمات المورفولوجية ومؤشرات تحليل الحمض النووي متعدد اشكال التضخيم العشوائي**

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ملخص

تم دراسة العلاقات ما بين ستة سلالات أردنية للوز باستخدام التحاليل المورفولوجية ومؤشرات تحليل الحمض النووي متعدد أشكال التضخيم العشوائي (RAPD). بينت النتائج انه من ضمن ستة سلالات من اللوز تمت دراستها، كان شكل اللوز، وحجم النواة ، وطول اللوز، وحجم اللوز، وطول القشرة قد اظهر مستوى عال من التباين (CV>30%)، في حين أظهر طول النواة، وعرض القشرة، وطول العقد الداخلية، وعرض اللوز قيما منخفضة نسبيا في التباين .(CV<20%) وأظهر تحليل المركب الرئيسي ان المركبات الخمس الاولى قد فسرت جميع التنوعات او التغايرات المورفولوجية خلال السلالات التي تمت دراستها. وكانت سمات النواة والجوزة هي الغالبة في المركبات الثلاث الأولى لتساهم بغالبية التنوع الكلي الموجود بين السلالات. وتم استخدام المسافة الاقليدية لبناء عناقيد من البيانات المورفولوجية والتي تم توزيعها بشكل فردي لمجموعتين رئيسيتين مع مسافة تراوحت من 5.5 إلى 10.14. وشكلت سلالات اللوز الحجري، الحامي الحلو والمكملي مجموعة رئيسية واحدة مع أحجام صغيرة للثمر، في حين شكلت السلالات الثلاث المتبقية (الاوغا، فارك، وأبو دبوس) السلسلة الثانية مع أحجام كبيرة للثمر. ومن بين 62 تضخيم عشوائي مسبق للحمض النووي، تم اختيار 12 وحدة منها لتحليل التنوع. وتم وضع الدرجات لـ 71 وحدة من بينها 28 وحدة متعددة الأشكال. وتراوحت قيمة المعدل لتعدد الأشكال أو التنوع لكل وحدة من 20% الى 74.2%. وتراوح معامل ارتباط مسافة نيز من 0.5 إلى 0.85 مع معدل 0.70. وكشف التحليل الجزيئي عن انفصال غير متسق ما بين السلالات مقارنة ما هو طبقا لسمات التنوع وتعدد الأشكال. وعلى الرغم من محدودية عدد السلالات المتواجدة في عجلون خلال المراقبة، إلا انه تمت ملاحظة تنوع كبير على مستويات كلا من تعدد الأشكال والحمض النووي ما يشير إلى أن سلالات اللوز الأردني غنية وان هناك مواد ومركبات جينية قيمة لتحسين اللوز.

الكلمات الدالة: اللوز، Prunus amygdalus، سلالات محلية، التنوع الجيني، السمات المورفولوجية، RAPD، الأردن.