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Molecular Characterization of Pomegranate (*Punica granatum* L.) Landraces Grown in Jordan using Amplified Fragment Length Polymorphism Markers

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Abstract: Amplified Fragment Length Polymorphism (AFLP) technique was used to asses the genetic diversity among twelve pomegranate landraces collected from three locations in Jordan. Eight AFLP primer combinations detected a total of 1433 bands with an average of 14.9 bands per landrace. *Mse*I+CTG and *Eco*RI+ATG primer combinations have the highest ability to discrimination the landraces; they revealed 265 bands with an average of 22.1 band/landrace. The polymorphism that detected by individual primer combinations ranged from 56.7 to 100%. The average genetic similarity ranged from 0.46 to 0.87 among the twelve tested landraces. The highest similarity was recorded between the landraces Qrati and Khdari1, Táeafi and Helow Khashabi. Landrace Zeglabi showed broad diversity comparing to the other landraces. However, all landraces were discriminated with the tested primer combinations. This study has emphasized the ability of AFLP in determining the genetic diversity among pomegranate landraces.

Key words: Pomegranate (*Punica granatum* L.), genetic diversity, landraces, amplified fragment length polymorphism markers

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the family Punicaceae which has a single genus *Punica* and two species *P. protopunica* Balf. and *P. granatum* L. According to Smith (1976), *P. granatum* L. has 2n = 2x = 16, 18 chromosomes. Pomegranate thought to be indigenous to the region of Iran where it is native (Stover and Mercure, 2007) and it is also thought to be a native in Turkey (Ercisli *et al*., 2007); It was spread to the Mediterranean countries at a very early date. Ozgen *et al*. (2008) mentioned the importance of evaluating and conserving the local genetic materials of pomegranate in the Mediterranean region. Pomegranate is grown commercially in Tunisia, Turkey, Egypt, Spain, Morocco, Iran, Afghanistan, India and to some extent, in the United States (California), China, Japan and Russia (Hayes, 1957; Kumar, 1990). In Jordan, Pomegranate is grown under irrigated and rain fed areas; the total planted area was 3,761 ha with a total production of 4,094 tons, in the average of 6 years (2000-2005).

The genetic diversity among and within genotypes plays a significant role in crops improvement through any breeding program. Genetic diversity at morphological as well as biochemical levels has a limitations due to the influence of environment on the performance of genotypes. Therefore, the genetic materials is considered the main component for studying or comparing between genotypes due to its stability and less affected by environment features. Many techniques have been used for these purposes, for instance; Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphism (AFLP).

Amplified fragment length polymorphism is based on the detection of DNA restriction fragments by PCR amplification. Caetano-Anolle's and Gresshoff (1997) reported that the AFLP technique allows production of very high density DNA marker maps for application in genome research, positional cloning of gene and for application in genetic analysis including cultivar identification and genetic diversity.

Sarkhosh *et al*. (2006) used one hundred RAPD primers to determine the diversity level among 24 Iranian pomegranate genotypes. Sixteen primers showed reliable polymorphic patterns and the genotypes produced similarities that ranged from 0.29 to 0.89. Other studies indicated that RAPD analysis was useful for revealing genetic diversity and for grouping pomegranate cultivars (Durgac *et al*., 2008; Zamani *et al*., 2007; Ercisli *et al*., 2007).

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De la Rosa *et al.* (2002) used AFLP and RAPD as dominant markers to locate suitable markers in the map of olive. A total of 166 AFLP markers were obtained from 15 different primer combinations. Resta *et al.* (2002) mentioned that AFLP analysis is an effective tool for the identification of olives cultivars and for the estimation of genetic distance. A selected group of 13 genotypes diffused in Apulia (Southern Italy) were compared using 18 primer combinations; about 792 AFLP loci and 241 polymorphism were found. Jubrael *et al.* (2005) used AFLP markers to discriminate 18 Iraqi date palm varieties. A total of 122 polymorphic AFLP loci were scored with an average of 17.4 polymorphic loci/primer combinations. They reported that AFLP technique is an efficient method for varietals identification and for estimating genetic relationship in date palm. Amplified fragment length polymorphism was used for comparing date palm varieties with florescence labeled primers. A total of 310 AFLP fragments derived from five primer combinations were yielded. The obtained AFLP markers were successfully used for identifying date palm plants (Diaz *et al.*, 2003). AFLP technique was used to detect polymorphism between California Almond cultivars, which were not distinguishable with other techniques (Boritzki *et al.*, 2000; Arnau *et al.*, 2001).

The genetic identification of genotypes is important for certified plant materials, but requires fast and reliable techniques (Bianchi *et al.*, 2002). Pomegranate has not taken attention in researches and few characterizations and genetic diversity-studies are available; it never been characterized at the molecular level in Jordan. Therefore, the aim of this study was to characterize some of the pomegranate landraces grown in different locations of Jordan using AFLP markers technique.

MATERIALS AND METHODS

This study was conducted during 2006-2008 at the research station of Al- Balqà Applied University.

Plant materials: Leaves of twelve pomegranate landraces were collected from three locations in Jordan that practice cultivation of pomegranate. These locations were Al-Rayan and Arjan in the North of Jordan and Al-Walah in the middle part of Jordan; they belong to the Ministry of Agriculture (Table 1). Fully-grown fresh leaves free of apparent pest and diseases were washed with distilled water, dried and then tightly wrapped in polyethylene film. All the samples were labeled and kept at (-80°C) until using.

Isolation of genomic DNA: Genomic DNA was isolated from the collected leaves using the cetytrimethyl ammonium bromide (CTAB) method described by Doyle and Doyle (1987). Three gram of frozen leaves were grounded to a fine powder in liquid nitrogen and then transferred into (50 mL) polypropylene tube containing (20 mL) of pre-warmed CTAB buffer {(2% w/v) CTAB, (1.4 M) NaCl (sodium chloride), (20 mM) EDTA (ethylenediaminetetraacetic acid), (100 mM) Tris-HCl pH 8.0, (1%) PVP (polyvinyl pyrrolidone), (0.2%) β-mercaptoethanol and (0.1%) sodium hydrogen sulfite ($NaHSO_3$)}. The mixture was incubated in a water bath with shaker for 1 h at (65°C). After cooling to room temperature, an equal volume of chloroform-isoamylalcohol (24:1) was added to each sample and kept on a shaker for 10 min. The samples were then centrifuged at (12000 rpm) for 15 min. The supernatant was transferred into new tubes and two volumes of cold absolute ethanol were added to each sample to precipitate the DNA. The tubes were placed at (-20°C) for 15 min to improve DNA precipitation; after that, they centrifuged at (12000 rpm) for 10 min. The supernatant was decanted carefully and the DNA was washed with (70%) ethanol. The tubes were placed on the laboratory bench for over night to ensure complete drying of the pellet. Four hundred microliter of TE buffer {(10 mM) Tris-HCl pH 8.0, (0.1 mM) EDTA} was added to each sample to dissolve the DNA. Five microliter of RNas was added to each sample and kept at (37°C) for 30 min to get rid of RNA. The DNA samples were then kept at (-80°C) until using.

Table 1: Names and sites of collection of pomegranate landraces used in the study

Landraces	Sites of collection
Sharabi	Arjan orchards (North of Jordan)
Qrati	
Emlasi	
Khdari 1	
Khdari 2	
Zeglabi	
Ta'eafi	Al-Walah orchards (Middle of Jordan)
Sleahi	
Helow Khshabi	Al-Rayan orchards (North of Jordan)
Helow Tari	
Belanawa	
Hamedi	

Detection of genomic DNA: Agarose gel (0.7%) electrophoresis was utilized to detect the DNA. The concentration was determined by using the spectrophotometer (Bio Wave, S2100 Diode Array) and the DNA was then diluted to (50 ng μL^{-1}). The quality of DNA was estimated by calculating the ratio of absorbance at (260 and 280 nm) according to Johnson (1994).

Digestion and ligation of DNA: Digestion and ligation of DNA were achieved following the procedure of Vos *et al.* (1995), which described in AFLP Core Reagent Kit (Invetrogene, life technologies, Cat. Number: 10482-016). The (25 μL) total volume of the digestion mixture for each sample was composed of: (13 μL) deionized sterile water,

(5 μL) reaction buffer {(50 mM) Tris-HCl pH 7.5, (50 mM) Mg-acetate and (250 mM) K-acetate}, (2 μL) of *Eco*RI/*Mse*I restriction enzymes {(1.25 unit μL^{-1}) of enzyme in (10 mM) Tris-HCl pH 7.4, (50 mM) NaCl, (0.1 mM) EDTA, (1 mM) Dithiothreitol (DDT), (0.1 mg mL^{-1}) BSA, (50%) glycerol (v/v)} and (5 μL) of template DNA. The samples were incubated at (37°C) for 3 h utilizing the thermocycler (MJ-Research, Model PTC 200). The digested samples were collected by brief centrifugation, incubated for 15 min at (70°C) and then placed on ice.

The digested samples were then ligated with corresponding adaptors by adding (25 μL) total volume of ligation mixture that consisted of: (24 μL) of the adapter/ligation solution {(5 pmol μL^{-1}) *EcoRI* adapters, (50 pmol μL^{-1}) *Mse*I adapters, (0.4 mM) ATP, (10 mM) Tris-HCl pH 7.5, (10 mM) Mg-acetate} and (1 μL) T4 DNA-ligase {(1 unit μL^{-1}) in (10 mM) Tris-HCl pH 7.5, (1 mM) DTT, (50 mM) KCl, (50%) glycerol (v/v)}. The samples were placed into the thermocycler at (20°C) for 2 h. After ligation step, the samples were diluted tenfold with TE buffer {(10 mM) Tris-HCl pH 8.0, (0.1 mM) EDTA} to be used in the preamplification step.

AFLP procedure: The preamplification reaction was performed with two oligonucleotide primers, one corresponding to the *Eco*RI-ends and the other corresponding to the *Mse*I-ends. One microliter of the ligated DNA was mixed with (9 μL) of preamplification mixture {(6.26 μL) deionized sterile water, (1 μL) of (10X) polymerase buffer that consist of [(100 mM) Tris-HCl pH 8.0, (15 mM) MgCl2, (500 mM) KCl and (0.1%) difco gelatin], (1 μL) of (2 mM) dNTPs, (0.3 μL) of (100 μM) of each primer (*Eco*RI+A and *Mse*I+C) and (0.14 μL) of (1unit) ampliTaq polymerase (Promega, Madison, USA)]. The PCR reaction was set as follow: (94°C) for 5 min followed by 21 cycles of 30 sec at (94°C), 40 sec at (56°C) and 1 min at (72°C). The final extension was 5 min at (72°C). After the preamplification step, samples were diluted tenfold with sterile deionized water to be used in selective amplification.

Selective amplification was achieved using primers possess three nucleotides at their 3' end. Eight primer combinations (Table 2) were used in the analyses. The selective amplification mixture was composed of (2.28 μL) sterile deionized water, (1 μL) of (10X) polymerase buffer, (1 μL) of (2 mM) dNTPs, (0.4 μL) 0f (100 μM) of each primer (*Eco*RI + 3 nucleotides and *Mse*I + 3 nucleotides), (0.2 μL) of (15 mM) MgCl2 and (0.22 μL) of (1unit) ampliTaq polymerase. Then, (3.5 μL) of the preamplifier templates DNA was added to each mixture. The AFLP selective amplification was carried out with the following cycle profile: cycle (1): 30 sec at (94°C), 30 sec at (68°C) and 60 sec at (72°C). Cycle (2-13): same PCR profiles as cycle one except for a sequential decreasing of the annealing temperature in each subsequent cycle by (0.7°C). Cycle (14-35): 30 sec at (94°C), 30 sec at (59°C), 60 sec at (72°C) and then 5 min at (72°C) for final extension. After that, the samples were kept at (-20°C) until using.

Acrylamide gel electrophoresis: Apelex sequencing unit (Vertical electrophoresis, Apelex, MT 1002, France) was used for gel generation. The short plate was treated with the binding solution {(3 μL) of bind silane (Promega, Madison, USA) mixed with (1 mL) of absolute ethanol and (0.5%) glacial acetic acid}. The long plate was cleaned and the surface was treated with the silicon grease (Loxeal engineering adhesives grasso silicone, Italy). The gel solution {(16 mL) of acrylamide/bisacrylamide 19:1 solution (Promega, Madison, USA), (16 mL) of (5X) TBE buffer [(0.1 M) Tris-base, (0.1 M) Boric acid, (2 mM) EDTA)], (33.6 g) of Urea (Promega, Madison, USA) were mixed, warmed, filtered and then the volume of the mixture was completed to (70 mL) by using sterile deionized

Table 2: Sequence of AFLP primer combinations used in this study

Name of primer	Sequence		
*Eco*RI-adaptor	5 '-CTCGTAGACTGCGTACC CATCTGACGCATGGTTAA-5'		
*Mse*I-adaptor	5 '-GACGATGAGTCCTGAG TACTCAGGACTCAT-5'		
Primer used in preamplification			
	CORE	ENZ	EXT
*Eco*RI	5 '- GACTGCGTACC	AATTC	+ NNN-3'
*Mse*I	5 '- GATGAGTCCTGAG	TAA	+ NNN-3 '
Primer combinations used in selective AFLP amplification			
*Eco*RI + 3 ' extra nucleotide*	5 '- GACTGCGTACCAATTC + A-2 -3 '		
*Mse*I + 3 ' extra nucleotide*	5 '- GATGAGTCCTGAGTAA + C-2 -3 '		

*The selective *EcoRI* primer with three selective nucleotides, 3 ' extra nucleotide ATG / ATT / AAT / ACA / ACC / ACT / AGA / AGG. *The selective *Mse*I primer with three selective nucleotides, 3 ' extra nucleotide CTG / CTT / CAT / CCA / CCC / CCT / CGA / CGG

water} was mixed with (48 μL) of Tetra Ethyl Methyl Ethylene Diamine (TEMED) and (480 μL) of Ammonium per Sulfate (APS). The solution was then poured on the vertical plates. After 1.30 h, the gel plates stand on the sequencing units and pre-heated for 30 min using Apelex power supply (800 V).

Samples loading and gel silver staining: Each of the selective amplification samples was mixed with (4 μL) of formamide dye {(98%) formamide, (10 mM) EDTA pH 8.0 and bromo phenol blue and xylene cyanol as tracking dyes}. The samples were then heated for 3 min at (94°C) and directly inserted in ice. Four microliter from each sample was loaded into the comb-teeth wells and run at constant current (800 V) in (1X) TBE buffer for 3.5 h. After electrophoresis was completed, the two plates were separated from each other and the long plate was prepared for staining.

The gel was fixed with 1 L of (10%) glacial acetic acid and agitated well for 20 min and then washed three times with tap water. The gel was transferred to a tray filled with 2 L of silver staining solution {(2 g) of silver nitrate and (3 mL) of (37%) formaldehyde dissolved in 2 L of double distilled water} and kept with agitation for 30 min. Then, the gel was washed with distilled water and set in a tray field with 1 liter of the developing solution {(30 g) of sodium carbonate, (1.5 mL) of (37%) formaldehyde, (200 μL) sodium thiosulfate mixed with 1 L of double distilled water} until the first band was appeared. The developing solution was changed once and the gel was set in it until all bands became visualized. Then, (500 mL) of fix-stop solution was added to the developing solution and kept for 3 min. After that, the gel was washed with tape water and kept on the refrigerator until got dry. Gel was visually scored from the photo taken by a scanner with high resolution.

Data analysis: For each primer combination, the number of polymorphic and monomorphic bands was determined. The bands were analyzed using Nei similarity index (Nei and Li, 1979), which excludes common negative data. Bands were scored as (1) for present and (0) for absent and entered into a data matrix. Unweighted Pair Group Method with Arithmetic mean (UPGMA) cluster analysis was used to identify genetic variation patterns among the landraces using the NTSYSpc program version 2.1 by Rohlf, 2000 (Exeter, Software, New York). The similarity equation was as follow:

$$\text{Similarity} = 2N_{ab}/(N_a + N_b)$$

Where:

N_{ab} = No. of scored amplification fragments with the same molecular weight shared between landraces a and b

N_a = No. of scored amplified fragments in landrace a

N_b = No. of scored amplification fragments in landrace

The markers size for each gel was estimated according to standard curves and regression equations that constructed based on the migration distance (mm) of the (50 bp) step DNA ladder and the log size of the DNA markers.

RESULTS

Genomic DNA that was isolated from pomegranate landraces revealed high quantity and quality. The DNA was detected by (0.7%) agarose gel electrophoresis. The concentrations of DNA samples ranged from 225 to 525 ng μL^{-1}. Amplified fragment length polymorphism bands and markers produced by the eight primer combinations with the three selective nucleotides and their distributions across the landraces are presented in Table 3. The total number of bands scored for the eight primer combinations were 1433. The primer combinations (*Mse*I+CTG/*Eco*RI +ATG) showed highest number (265) of bands and the primer combinations (*Mse*I+CCC/*Eco*RI+ACC) showed the lowest number (102) of bands. The primer combinations *Mse*I+CAT/ *Eco*RI+AAT and the primer combinations *Mse*I+CGA/ *Eco*RI+AGA gave the highest percentage of polymorphism (100%), while the lowest percentage of polymorphism (56.7%) was obtained from the primer combinations *Mse*I+CTT/*Eco*RI+ATT. The eight primer combinations also generated a total of 257 markers; 235 of

Table 3: Primer combinations, total number of markers, number of polymorphic markers, polymorphic percent, total number of bands and average number of markers/landrace that produced by eight primer combinations used in this study

Primer combinations	Total No. of markers[1]	No. of polymorphic markers[2]	Polymorphic (%)	Total No. of bands[3]	Average No. of bands/landrace[4]
*Mse*I+CTG / *Eco*RI+ATG	41	39	95.12	265	22.1
*Mse*I+CTT / *Eco*RI+ATT	30	17	56.70	236	19.7
*Mse*I+CAT / *Eco*RI+AAT	28	28	100.00	155	12.9
*Mse*I+CCA / *Eco*RI+ACA	41	39	95.12	214	17.8
*Mse*I+CCC / *Eco*RI+ACC	24	22	91.70	102	8.5
*Mse*I+CCT / *Eco*RI+ACT	31	30	96.80	164	13.7
*Mse*I+CGA / *Eco*RI+AGA	31	31	100.00	139	11.6
*Mse*I+CGG / *Eco*RI+AGG	31	29	93.50	158	13.2
Total	257	235	91.40	1433	14.9

[1]Total No. of differently sized AFLP markers amplified across all 12 landraces. [2]No. of AFLP markers found to be polymorphic across all 12 landraces. [3]Total No. of AFLP bands scored for all 12 landraces. [4]Average No. of AFLP markers scored per landrace

Table 4: Similarity coefficient values for pairwise comparison of 12 pomegranate landraces based on eight primer combinations

Landraces	Sharabi	Emlasi	Qrati	Khdari 1	Khdari 2	Zeglabi	Ta'eafi	Sleahi	Helow Khshabi	Helow Tari	Balanawa
Sharabi											
Emlasi	0.78										
Qrati	0.77	0.82									
Khdari 1	0.78	0.87	0.80								
Khdari 2	0.56	0.50	0.51	0.50							
Zeglabi	0.54	0.55	0.55	0.54	0.50						
Ta'eafi	0.77	0.81	0.82	0.83	0.48	0.56					
Sleahi	0.73	0.83	0.77	0.81	0.48	0.60	0.83				
Helow Khshabi	0.75	0.83	0.78	0.83	0.47	0.58	0.87	0.85			
Helow Tari	0.69	0.76	0.73	0.76	0.48	0.57	0.75	0.81	0.81		
Balanawa	0.61	0.65	0.66	0.67	0.51	0.63	0.66	0.70	0.68	0.69	
Hamedi	0.51	0.48	0.51	0.52	0.54	0.50	0.47	0.50	0.46	0.47	0.51

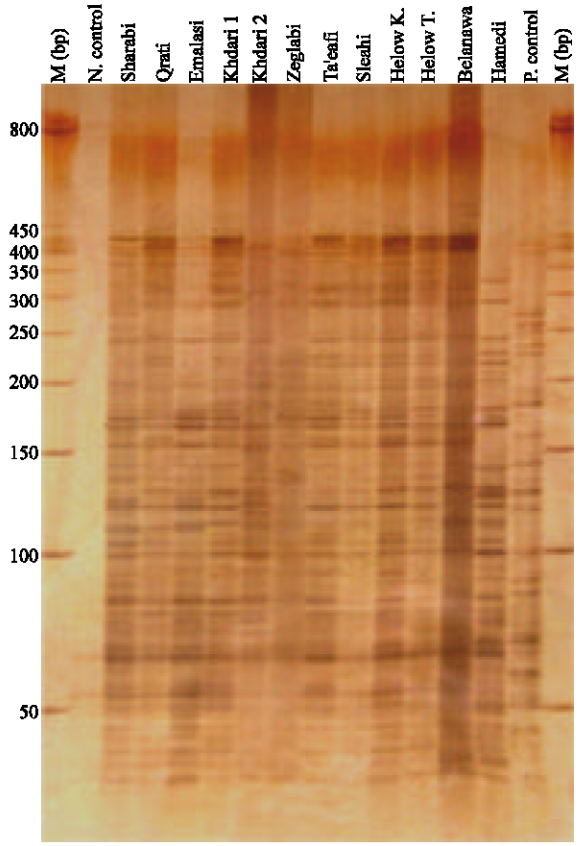

Fig. 1: Amplified fragment length polymorphism pattern of 12 pomegranate landraces obtained from silver-stained polyacrylamide gel for the primer combinations *Mse*I+CTG and *Eco*RI+ATG. M (bp): 50 bp step DNA ladder. N. control: Negative control. P. control: Positive control

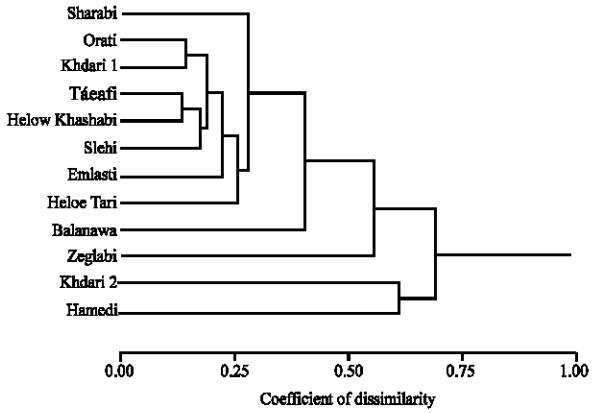

Fig. 2: Dendrogram of 12 pomegranate landraces generated by UPGMA cluster analysis of the similarity values on the basis of eight primer combinations

them were polymorphic which accounted 91.4% and the remaining 8.6 % were monomorphic. As an example, AFLP banding pattern for the primer combinations *Mse*I+CTG and *Eco*RI+ATG is shown in Fig. 1.

The similarity coefficients values between all possible pairs of landraces ranged from 0.46 to 0.87 (Table 4). The dendrograme constructed by UPGMA cluster analysis (Fig. 2) revealed two main clusters. The first one consisted of two landraces Khdari 2 and Hamedi. The second cluster was subdivided into three sub-clusters. In the first sub cluster, landrace Zeglabi was grouped with the other nine landraces. In the second sub cluster, landrace Balanawa was grouped the other remaining eight landraces. In the third sub cluster, landrace Sharabi was grouped with sub-clusters included the other seven landraces; in which landraces Qrati and Khdari1 grouped together with similarity equal to 85%, landraces Táeafi and Helow Khashabi grouped together with similarity equal to 87% and were in turn grouped with landrace Slehi. The landrace Emlasi was grouped with sub-groups included (Slehi, Helow Khashabi, Táeafi, Khdari 1, Qrati and Sharabi).

DISCUSSION

The success of any genetic conservation or breeding program is depending on understanding the amount and distribution of the genetic variation presented in the genetic pool. Morphological traits were used to describe

such genetic variation in pomegranate varieties. These traits are mainly related to the fruit and morphology characteristic and are complex and greatly influenced by environmental factures (Sarkhosh *et al*., 2006). Identification and characterization of varieties based on these morphological traits alone are difficult; the use of other biochemical and molecular markers could supply complementary useful information (Mars and Marrakchi, 1999).

In this study, eight primer combinations were used to characterize pomegranate at molecular level. The number of reproducible polymorphic markers for these primer combinations ranged from 6 markers (landrace Qrati with primer combination *Mse*I+CCC/*Eco*RI+ACC) to twenty eight markers (landrace Helow Khshabi with primer combination *Mse*I+CTG/*Eco*RI+ATG). The markers size ranged from 47 bp for primer combinations *Mse*I+CAT/*Eco*RI+AAT with all landraces except landraces Qrati and Helow Tari to 450 bp for primer combination *Mse*I+CTG/*Eco*RI+ATG with all landraces except Khdari 2 and Hamadi. The large number of polymorphic markers produced with the eight primer combinations indicated that pomegranate landraces are highly polymorphic and this agree with the findings of Sarkhosh *et al.* (2006), who mentioned that polymorphism detection efficiency among pomegranate varieties by RAPD technique was very high (90.96%).

The PCR amplification products allowed to distinguish each landrace and to study the genetic relationship among them. The amplification products obtained with the eight primer combinations were analyzed to compare the genetic relationships between landraces. Primer combinations *Mse*I+CAT/*Eco*RI+AAT and *Mse*I+CGA/*Eco*RI+AGA produced 100% polymorphic markers. These two primer combinations are recommended to be used for the identification of pomegranate landraces with less time and cost.

The similarity values ranged from 0.46 to 0.87 among landraces. High similarity was recorded between landraces Helow Khashabi and Ta'eafi. Regarding the cluster analysis, AFLP technique reliably distinguished all the twelve pomegranate landraces and showed that some of them have less genetic differences like (Qrati and Khdari 1) and (Táeafi and Hellow Khashabi) which indicated that these landraces may have similar genetic makeup. The analysis also showed that there was broad genetic diversity among landraces Khdari 2, Zeglabi, Bala-nawa and Hello Tari; these landraces produced the highest numbers of unique markers with the primer combinations 7, 6, 4, 4, respectively.

The results revealed that AFLP technique is one of the most powerful tools for evaluating genetic diversity among pomegranate landraces and this agree with other reports which mentioned that AFLP has been showed to be useful for studying genetic variation in many fruit trees such as date palm (Cao and Chao, 2002; Devanand and Chao, 2003; Jubrael *et al*., 2005), olives (Resta *et al*., 2002; De la Rosa *et al*., 2002) and other cultivars (Boritzki, 2000; Arnau *et al*., 2001). The results of this study also revealed that the diversity of the pomegranate displayed by the twelve landraces could be attributed to genetic causes.

In conclusion, the high percentage of polymorphic markers obtained from the primer combinations indicated that pomegranate is highly polymorphic fruit tree. Some landraces showed wide divergence and their favorable characters should be taken into consideration in future breeding programs. The results of this study support the need of establishing a gene bank for pomegranate to conserve the local landraces and to develop different projects to investigate the possibility of reserving these landraces *in vitro*. Amplified fragment length polymorphism markers can be used for pomegranate fingerprinting and for determining genetic relation among landraces grown in Jordan. Further researches are needed to study the morphological and agronomic traits of pomegranate landraces to support the findings of the present study.

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