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Genetic diversity of Moroccan pomegranate (*Punica Granatum L.*) cultivars using AFLP markers

El Amine Ajal[1], Rania Jbir[2], Pilar Legua[3], Juan José Martínez[3], Rafael Martinez[3], Amel Salhi Hannachi[2], Abdelmajid Haddioui[1]*

[1]Team of Genetic and Plant Biotechnology, Laboratory of Management and Valorization of Natural Resources, Faculty of Sciences and Techniques, Morocco
University of Sultan Moulay Slimane, Beni Mellal, Morocco
[2]Laboratory of Molecular Genetics, Immunology & Biotechnology, Faculty of Sciences, El Manar University, Tunis, Tunisia
[3]Plant Science and Microbiology Department, EPSO, University Miguel Hernández, Ctra, Beniel, km. 3.2, 03312 Orihuela, Alicante, Spain

[*] **Corresponding author: ahaddioui@yahoo.fr**

Abstract

Pomegranate (*Punica granatum* L.) is one of the most important fruit crop cultivated in Morocco. However, little data is available on its genetic diversity. The genetic relationships among pomegranate cultivars were investigated using Amplified Fragment Length Polymorphism (AFLP) markers. Six pairs of primers were used to analyse 24 pomegranate cultivars obtained from the central regions of Morocco. A total of 519 scorable fragments were amplified, of which, 368 (71%) were polymorphic. Resolving power ranged from 13.16 to 28.75, and the average of polymorphism information content (PIC) per primer pair was 0.95. Coefficient of genetic differentiation between groups (G_{ST}) was 0.091, indicating that 9.10% of total genetic variability was among groups and 90.90% was within groups. The gene flow (Nm) was 4.992 among all of the groups. The UPGMA dendrogram and PCA analysis exhibited a genetic diversity structured independently from the geographical origin of cultivars and their denomination. These results proved that the tested primers were informative to discriminate among cultivars and to survey the genetic diversity in this fruit crop. The information may be useful to define conservation management program.

Keywords: *Punica granatum*; Molecular polymorphism; AFLP; Morocco.
Abbreviations: AFLP_ Amplified Fragment Length Polymorphism; CTAB_Cetyl-Trimethyl Ammonium Bromide; H_ Nei's gene diversity value; I_mean Shannon's information index; Rp_Resolving power.

Introduction

Pomegranate (*Punica granatum* L.), included in the Punicaceae family, is one of the earliest domesticated plant species, and believed to be a native to the southern Caspian belt (Iran) and northern Turkey (Janick, 2007), from where, thousands of years ago, they were dispersed to the Mediterranean Basin, East Asia, Europe, and North and South America (Teixeira da Silva et al., 2013). This specie has been regarded, for a long time, as a minor crop in various countries. However, during the last decade, *Punica granatum* L. has gained a tremendous worldwide popularity due to its nutritional values, specific organoleptic characteristics and numerous perceived health benefits (Filannino et al., 2013; Teixeira da Silva et al., 2013; Vázquez-Araújo et al., 2011). Indeed, when it is consumed as a fresh fruit or extracted beverages and juices or used as ingredients in herbal medicines and dietary supplements, pomegranate is known to be a natural source of bioactive compounds with a broad spectrum of bioactive properties, including anti-oxidant (Seeram et al., 2006; Borohov-Neori et al., 2009), anti-allergic (Damiani et al., 2009), anti-carcinogenic (Seeram et al., 2005; Khan, 2009), anti-diabetic (Julie, 2008; McFarlin et al., 2009), digestive protection (Wang et al., 2010), and anti-microbial (Al-Zoreky, 2009; Su et al., 2010, 2011) among others.

Thus, although there is no updated and accurate data available on pomegranate cultivation area and production in the world, due to the rapid increase in its expansion and production, it is estimated that around 1.5 million tons of pomegranate fruits are produced in the world annually (Holland and Bar-Yaakov, 2008). In this sense, Mediterranean countries are the main center for commercial cultivation of pomegranate (Verma et al., 2010). Nevertheless, despite this increasing in commercial importance of pomegranate and the expansion of its production area, the use of reduced number of pomegranate varieties cultivated for commercial purposes (including fruit size, color, shape, seed hardness, taste and flavor characteristics), has drastically affected the genetic diversity of this specie. Indeed, over 500 identified pomegranate varieties in the world, only 50 of which are known to be commercially cultivated (IPGRI, 2001). Consequently, there is a need to improve research on pomegranate genetic diversity characterization, conservation and sustainable management of local genetic resources. Identification and characterization of the collected genotypes constitutes an attractive task to examine level and distribution of genetic diversity in this crop. For a long period, the genetic diversity of *P. granatum* L. has been studied using morphological and

pomological traits (Zamani, 1990; Mars and Marrakchi, 1999). However, these analyses are less rewarding since they were based on parameters limited in number and/or highly influenced by the environmental conditions (Kumar, 1999; Ozgen et al., 2008; Kazemi alamuti et al., 2012). To overcome such inconvenience, PCR-based molecular markers such as Inter-Simple Sequence Repeat (ISSR) (Narzary et al., 2009; Ajal et al., 2014), Random Amplified Polymorphic DNA (RAPD) (Sarkhosh et al., 2006; Narzary et al., 2009; Hasnaoui et al., 2010), Simple Sequence Repeat (SSR) (Pirseyedi et al., 2010; Jbir et al., 2012) and AFLP (Yuan et al., 2007; Jbir et al., 2008; Moslemi et al., 2010; Ercisli et al., 2011) have been used to characterize cultivars and to establish genetic relationships between pomegranate varieties. In Morocco, during the last years, the cultivation of pomegranate has known a great expansion. The area reserved increased from 5820 ha in 2009 to 8218 ha in 2012 (MAPM, 2012). In 2012, the total pomegranate production is around 76 300 tons as reported (MAPM, 2012). Nevertheless, despite the increasing commercial importance of pomegranate in this country, relatively little is yet known regarding the genetic diversity of this species in Morocco. In this context, recent research focused on morphological, chemical characteristics, quality and bioactive compounds showed a wide variation among Moroccan pomegranate cultivars (Legua et al., 2012; Martínez et al., 2012; Hmid et al., 2013). The aim of this study was to examine the molecular polymorphism as well as the phylogenic relationships between 24 varieties of central regions of Morocco belonging to 2 geographical regions (provinces of Beni Mellal and Azilal) using AFLP technique. To the best of our knowledge, this is the first study of genetic diversity of Moroccan pomegranate cultivars.

Results

AFLP polymorphism

Six primers pairs were tested for their ability to generate AFLP banding patterns from DNA corresponding to the 24 cultivars studied. A total of 368 polymorphic bands out of total 519 bands were scored for the 24 pomegranate cultivars with a mean of 61 fragments per combination (Table 2). The largest number of polymorphic bands (79) was detected using primer combination E_{ACT}/M_{CAT} and the least number of polymorphic bands (30) were produced with primer combination $E_{ACA}/M_{CAG.}$ The percentage of polymorphic bands (PPB) ranged revealed for the 24 pomegranate cultivars ranged from 57% (E_{AAC}/M_{CAA}) to 85% (E_{AGC}/M_{CAA}), with an average of 71% per primer pair. Moreover, estimates of the resolving power (Rp) are used to determine the ability of primers to differentiate among cultivars. The (Rp) values varied from 13.16 to 28.75 for the E_{ACA}/M_{CAG} and E_{ACT}/M_{CAT} respectively, with a collective rate value of 149.03. Consequently, the E_{ACT}/M_{CAT} seems to be the most informative primer combination to distinguishing the cultivars. In addition, the polymorphism information content (PIC) ranged from 0.94 to 0.96 with an average of 0.95 per primer.

Intra groups genetic diversity

Estimates of genetic diversity in each group are summarized in Table 3. The percentage of polymorphic bands at the population level ranged from 71.47% in group II to 95.11% in group I. The Shannon information index (I) ranged from 0.409 in group II to 0.488 in group I with average of 0.508 at the group level. The total Nei's gene diversity was H= 0.337 varying from 0.278 (Group II) to 0.326 (Group I). The total diversity (H_T) was 0.33 and the mean genetic diversity within the groups (H_S) was 0.303 (Table 4). The coefficient of genetic differentiation between groups (G_{ST}) was 0.091, indicated that the mainly proportion of genetic variation (90.90%), was within groups and the remaining (9.10%) of the variation was among groups. The estimated gene flow (Nm) from one group to another over generation was averaged 4.992.

Genetic distances and cultivars clustering

The binary matrix data of polymorphism were computed to estimate the genetic distance among 24 Moroccan cultivars. The distance matrix exhibits a large average distance range from 0.17 to 0.69 (Table 5) suggesting that the genotypes studied are characterized by a great divergence. The lowest genetic distance of 0.17 was registered between Bouâdim Bzou (BM1) and Kharaji Bzou (KB); Ruby (RB1) and Papenschell (PP1) cultivars suggesting their great similarities at the DNA level. However, Grenade Jaune (GJ1) and Dwarf semi Evengreen (DE1), Sefri (SF2) and Zehri précoce (ZH2) cultivars seem to be the most divergent, since they presented the highest genetic distance of 0.69 and 0.66 respectively. The dendrogram constructed by UPGMA cluster analysis (Fig. 2) revealed three main clusters. The first cluster (I) is monophyletic branch consisted of Dwarf semi Evengreen (DE1) cultivar. The second cluster (II) consisted of Chioukhi (CK1) and Sefri (SF2) from Ahl Souss collection and Azilal region respectively. All the remaining cultivars are grouped in the third cluster (III). All this exhibited two sub-clusters. The first sub-cluster (III-1) was composed of one cultivar Grenade Jaune (GJ1) from Ahl Souss collection, while the second sub-cluster (III-2) was composed of the remaining cultivars. This result suggested that the cultivars studied are clustered independently from their geographical origin.

Principal component analysis

In this study, the data generated from AFLP markers were subjected to Principal Component Analysis (PCA). Results exhibited that the first two axes accounted for 24.45% of the total variability (Fig 3). Results showed that genotypes are randomly aggregated in two distinguished groups. In fact, significant divergence of Dwarf semi Evengreen cultivar (DE1) and Sefri (SF2) from all remaining cultivars was pointed out confirmed cluster analysis. This result suggested that a typically continuous genetic diversity characterising the cultivars studied.

Discussion

The present paper illustrates the AFLP markers to generate DNA fingerprints of 24 pomegranate cultivars. Using a set of primer combinations, a relatively large number of AFLPs have been permitted to survey genetic diversity and relationships among the cultivars studied. In fact, the percentage of polymorphic bands (61%), was higher than those reported for Tunisian pomegranate (57.5%) (Jbir et al., 2008) and Iran pomegranate (54.13%) (Moslemi et al., 2010), but it was lower than for Chinese pomegranate (73%) (Yuan et al., 2007). This result indicated that it was feasible to use AFLP for the study of pomegranate genetic diversity. In this work, the high percentage of polymorphism, level of genetic diversity and Shannon's information index were detected in group I (PPB = 95.11%; H= 0.326; I= 0.488). The total genetic diversity obtained in this study (H_T= 0.33) was higher

Table 1. Moroccan pomegranate studied and their geographical origin.

Cultivar	Label	Geographical origin	Group
Mollar Offin Hueso	MH1	Collection Ahl Souss	
Dwarf semi Evengreen	DE1	Collection Ahl Souss	
Negro Monstruoso	NM1	Collection Ahl Souss	
Djeibi	DJ1	Collection Ahl Souss	
Chelfi	CH1	Collection Ahl Souss	
Zehri d'automne	ZH1	Collection Ahl Souss	
Grenade rouge	GR1	Collection Ahl Souss	
Bzou	BZ1	Collection Ahl Souss	
Sefri	SF1	Collection Ahl Souss	I
Gordo de Jativa	GJ2	Collection Ahl Souss	
Wanderful	WF1	Collection Ahl Souss	
Papenschell	PP1	Collection Ahl Souss	
Djebali	DB1	Collection Ahl Souss	
Zehri précoce	ZH2	Collection Ahl Souss	
Ruby	RB1	Collection Ahl Souss	
Grenade jaune	GJ1	Collection Ahl Souss	
Chioukhi	CK1	Collection Ahl Souss	
Ounek Hmame	OH1	Collection Ahl Souss	
Kharaji Bzou	KB	Azilal	
Bouâdim Bzou	BM1	Azilal	
Hamde Bzou	HM1	Azilal	II
Sefri	SF2	Azilal	
Hamde Demnate	HM2	Azilal	
Bouâdim Demante	BM2	Azilal	

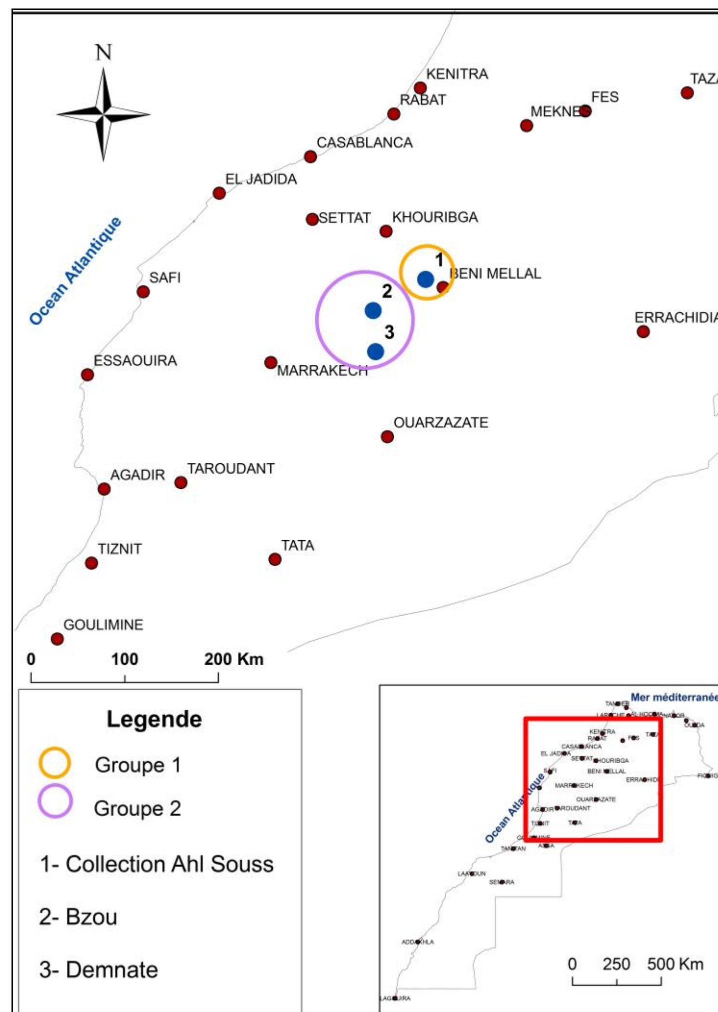

Fig 1. Map of Morocco which illustrates the geographical collection site of pomegranate and location of cultivars used in the study.

Table 2. Nucleotide sequence of AFLP adapters and primers.

	Primer Code	Sequence (5'-3')
Adaptors	E_N	CTCGTAGACTGCGTACG
		ATCTGACGCATGCTTAA
	M_N	GACGATGAGGTCCTGAG
		TACTCCAGGACTCAT
Pre-amplification	E_N	GACTGCGTACGAATTC**A**
	M_N	GATGAGTCCTGAGTAA**C**
Selective primers		
	E_{AGC}/M_{CAA}	CATCTGACGCATGGTTAAGN**AGC**
		TACTCAGGACTCATTN**CAA**
	E_{AAC}/M_{CAA}	CATCTGACGCATGGTTAAGN**AAC**
		TACTCAGGACTCATTN**CAA**
	E_{ACA}/M_{CAG}	CATCTGACGCATGGTTAAGN**ACA**
		TACTCAGGACTCATTN**CAG**
	E_{ACC}/M_{CTA}	CATCTGACGCATGGTTAAGN**ACC**
		TACTCAGGACTCATTN**CTA**
	E_{ACT}/M_{CAT}	CATCTGACGCATGGTTAAGN**ACT**
		TACTCAGGACTCATTN**CAT**
	E_{AAG}/M_{CTT}	CATCTGACGCATGGTTAAGN**AAG**
		TACTCAGGACTCATTN**CTT**

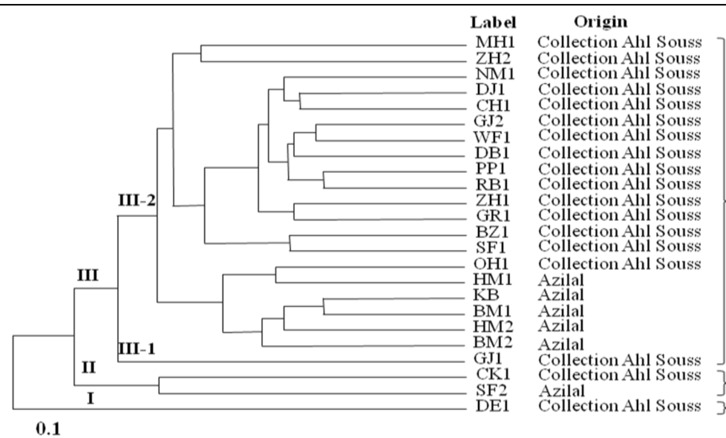

Fig 2. UPGMA dendrogram of 24 Moroccan pomegranate cultivars based on 368 AFLP markers. All the cultivars were divided into three main groups.

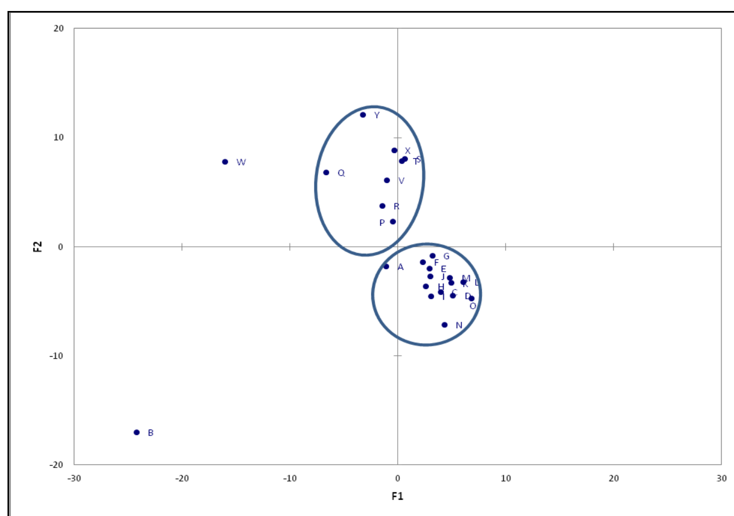

Fig 3. Dispersion of 24 Morocco pomegranate cultivars on the bi-plot (24.45% of the total inertia) of the Principal Component Analysis (PCA) based on 368 AFLPs markers. This shows the two grouped and divergence of two cultivars. (**A:**MH1; **B:**DE1; **C:**NM1; **D:**DJ1; **E:**CH1; **F:**ZH1; **G:**GR1; **H:**BZ1; **I:**SF1; **J:**GJ2; **K:**WF1; **L:**PP1; **M:**DB1; **N:**ZH2; **O:**RB1; **P:**GJ1; **Q:**CK1; **R:**OH1; **S:**KB; **T:**BM1; **V:**HM1; **W:**SF2; **X:**HM2; **Y:**BM2).

Table 3. Summary of AFLP data generated by six primer combinations for 24 Moroccan pomegranate cultivars.

Primer combination	TNB	NPB	PPB	Rp	PIC
E_{AGC}/M_{CAA}	79	67	85	26.13	0.94
E_{AAC}/M_{CAA}	104	59	57	25.33	0.96
E_{ACA}/M_{CAG}	45	30	67	13.16	0.95
E_{ACC}/M_{CTA}	86	60	70	27.16	0.96
E_{ACT}/M_{CAT}	111	79	71	28.75	0.95
E_{AAG}/M_{CTT}	94	73	78	28.50	0.94
Mean	86.5	61	71	24.84	0.95
Total	519	368		149.03	5.7

TNB: total number of bands; NPB: Number of polymorphic bands; PPB: Percentage of polymorphic bands; Rp: Resolving power; PIC: Polymorphism information content.

Table 4. Intra-group diversity of pomegranates.

Group	Mean H	Mean I	Polymorphic loci (%)
Group I	0.326	0.488	95.11
Group II	0.278	0.409	71.47
Total	0.337	0.508	

H: Nei's genetic diversity; I: Shannon's information index.

Table 5. Distribution of genetic diversity among pomegranate groups following Nei's method.

Group	Sample size	mean H_T	mean H_S	mean D_{ST}	mean G_{ST}	mean Nm
Group I	18	0.31	0.27	0.04	0.11	4.193
Group II	6	0.27	0.18	0.09	0.31	1.129
All groups	24	0.33	0.303	0.027	0.091	4.992

H_T: Total gene diversity; H_S: gene diversity within group; D_{ST}: gene diversity between groups; G_{ST}: genetic differentiation among pomegranate cultivars groups; Nm: gene flow.

than that obtained by Yuan et al. (2007) in China and lower than that reported by Moslemi et al. (2010) in Iran using AFLP marker. A relatively narrow genetic background of Tunisian pomegranate genetic resources has been demonstrated by the use of a set of 11 SSR loci that provided 25 alleles as reported by Hasnaoui et al. (2010). In fact, a low level of heterozygosity characterizes Tunisian pomegranate which can be explained in part by the vegetative propagation as a mode of reproduction of this fruit tree. The observed and the expected heterozygosity calculated for these resources varied from 0.037 to 0.592 and 0.036 to 0.491, respectively. Our results demonstrate the usefulness of AFLP markers to generate molecular polymorphism. The AFLP markers seem to be a powerful tool for fingerprinting and establishing genetic relationships among Moroccan pomegranate with high accuracy. Otherwise, the coefficient of gene differentiation (G_{ST}= 0.091) indicated that the proportion of genetic diversity among populations was low and a significant amount of genetic differentiation existed within populations. The gene flow (Nm = 4.992.) was higher than the one recorded in the Indian pomegranate (3.505) by Narzary et al. (2010). The high level of genetic diversity within groups and low level that among groups may be explained by the extensive gene flow between the different localities. The derived UPGMA dendrogram and the PCA analysis proved that the genotypes are clustered independently from their geographic origin**,** suggesting that a common genetic basis characterises these cultivars despite their phenotypic divergence as revealed by some recent studies on phenotypic diversity of these cultivars (Hmid et al., 2013). This agrees with the result obtained by Jbir et al. (2008) where Tunisian pomegranate cultivars clustering, based on AFLP makers, was not correlated with their geographic origin. The similar result was also reported in Chinese pomegranates where genetic distances between populations were not correlated to the geographical distance (Yuan et al., 2007). In fact, according to Mars (2001), cultivars are mainly selected by farmers with regards to agronomic traits related with the fruits parameters. Therefore, only a small part of the genome encoding these traits is involved in the farmers' empiric selection process. In addition, the two cultivars with the same name "Sefri" collected from the pomegranate collection and in farm fields are clustered into different groups. Hypothesis of problem of homonymy could be forwarded to explain this result since cultivars locally called by according to their origin and/or the fruit parameters such as size, colour, juice and seeds (Mars and Marrakchi, 1998).

Materials and Methods

Plant materials

The 24 Moroccan pomegranate cultivars used in this study (Table 1) were collected from the Pomegranate Collection of Ahl Souss situated in Beni Mellal region, and cultivated pomegranate trees in farm fields in Azilal region (Fig. 1). According to their geographical origin, considered cultivars were ranged into two groups: I and II. Approximately 30–50 young leaves of each cultivar were sampled from adult trees that were apparently free of pests and diseases. All the samples were washed with distilled water and stored at −80 °C until DNA extraction.

DNA extraction

Genomic DNA was extracted from young leaves following the modified Cetyl-Trimethyl Ammonium Bromide (CTAB) protocol (Doyle and Doyle, 1987). The quality and concentration of DNA were quantified spectrophoto-metrically and its integrity was checked by analytic agarose minigel electrophoresis (Sambrook et al., 1989).

AFLP analysis

About 500 ng of genomic DNA was used to genotype the 24 samples by the AFLP Regular Plant Mapping Protocol (Applied Biosystem, Foster City, CA, USA). The restriction-

Table 6. Genetic distance matrix for pomegranate cultivars based on AFLP data.

	MH1	DE1	NM1	DJ1	CH1	ZH1	GR1	BZ1	SF1	GJ2	WF1	PP1	DB1	ZH2	RB1	GJ1	CK1	OH1	KB	BM1	HM1	SF2	HM2	BM2
MH1	0.00																							
DE1	0.50	0.00																						
NM1	0.38	0.49	0.00																					
DJ1	0.33	0.51	0.20	0.00																				
CH1	0.33	0.48	0.22	0.20	0.00																			
ZH1	0.39	0.48	0.29	0.27	0.24	0.00																		
GR1	0.35	0.49	0.22	0.24	0.20	0.20	0.00																	
BZ1	0.36	0.53	0.36	0.29	0.29	0.31	0.30	0.00																
SF1	0.40	0.51	0.36	0.28	0.30	0.31	0.31	0.21	0.00															
GJ2	0.38	0.46	0.28	0.26	0.22	0.23	0.26	0.31	0.29	0.00														
WF1	0.39	0.51	0.20	0.23	0.21	0.25	0.22	0.33	0.23	**0.18**	0.00													
PP1	0.37	0.57	0.23	0.20	0.22	0.20	0.23	0.35	0.30	0.23	**0.18**	0.00												
DB1	0.36	0.53	0.26	0.21	0.24	0.28	0.24	0.34	0.30	0.21	0.20	0.19	0.00											
ZH2	0.31	0.60	0.36	0.26	0.33	0.37	0.39	0.36	0.41	0.34	0.29	0.30	0.29	0.00										
RB1	0.35	0.61	0.26	0.23	0.26	0.26	0.27	0.34	0.32	0.25	0.19	**0.17**	0.22	0.26	0.00									
GJ1	0.40	**0.69**	0.46	0.39	0.39	0.45	0.40	0.42	0.39	0.43	0.37	0.42	0.36	0.38	0.34	0.00								
CK1	0.35	0.62	0.48	0.42	0.37	0.43	0.44	0.44	0.44	0.44	0.43	0.45	0.46	0.46	0.44	0.39	0.00							
OH1	0.32	0.49	0.35	0.32	0.28	0.31	0.33	0.36	0.32	0.24	0.30	0.34	0.37	0.43	0.35	0.44	0.25	0.00						
KB	0.40	0.55	0.36	0.36	0.34	0.31	0.32	0.35	0.37	0.32	0.34	0.33	0.33	0.47	0.40	0.45	0.47	0.31	0.00					
BM1	0.38	0.55	0.33	0.36	0.31	0.29	0.32	0.37	0.39	0.32	0.32	0.34	0.36	0.45	0.40	0.52	0.43	0.28	**0.17**	0.00				
HM1	0.35	0.52	0.32	0.37	0.31	0.34	0.34	0.36	0.32	0.30	0.29	0.37	0.32	0.45	0.39	0.37	0.36	0.22	0.23	0.25	0.00			
SF2	0.51	0.53	0.56	0.53	0.45	0.54	0.50	0.57	0.56	0.51	0.52	0.52	0.51	0.66	0.58	0.45	0.36	0.42	0.51	0.51	0.44	0.00		
HM2	0.43	0.59	0.36	0.33	0.35	0.29	0.25	0.39	0.41	0.37	0.35	0.35	0.35	0.50	0.42	0.45	0.38	0.32	0.20	0.23	0.27	0.43	0.00	
BM2	0.49	0.60	0.45	0.45	0.41	0.35	0.36	0.51	0.53	0.40	0.42	0.45	0.42	0.54	0.47	0.47	0.47	0.37	0.24	0.25	0.28	0.49	0.24	0.00

ligation reactions and pre-selective amplifications were performed according to the protocol. The preselective amplification mixture was prepared by adding four µL of 20-fold diluted DNA from the restriction-ligation reaction, one µL AFLP preselective primer pairs (Applied Biosystem, Foster City, CA, USA), and 15µL AFLP core mix. The preselective amplification was carried out in a verity thermal cycles (Applied Biosystems) programmed at 72°C for 2 min, followed by 20 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 2 min, with an incubation step at 60°C for 30 min. The preselective amplification products were diluted ten-fold in TE0.1 buffer (20 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Three µL of pre-amplification product was re-amplified with 6 different primer combinations: $E_{AGC}/M_{CAA;}$ $E_{AAC}/M_{CAA;}$ $E_{ACA}/M_{CAG;}$ $E_{ACC}/M_{CTA;}$ E_{ACT}/M_{CAT} and E_{AAG}/M_{CTT}. One µL of each primer was used with 15µl AFLP core mix. Selective amplification was carried out in a verity thermal cycler (Applied Biosystem, Foster City, CA, USA) programmed at 94°C for 2 min, followed by 10 cycles of 94°C for 20 s, 66°C (-1°C/cycle) for 30 s and 72°C for 2 min, and 20 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 2 min with a subsequent hold for 30 min at 60°C. One µL of the selective amplification product was diluted in 25 µL of loading buffer mix (deionized formamide + GeneScan-500 ROX size standard; Applied Biosystems) and denatured at 95°C for 5 min. The PCR products were separated by capillary electrophoresis in an ABI 3130xl Genetic Analyzer (Applied Biosystem, Foster City, CA, USA).

Data analysis

Sequencing data were analyzed using the Peak Scanner™ software (versions 1.0; Applied Biosystems 2006). Peaks representing AFLP fragments were scored as binary matrix format with ‘‘1” for the presence of a band and ‘‘0” for its absence. For all primers combination, the total number of bands and the polymorphic ones were calculated. The ability of the most informative primer to differentiate between cultivars was assessed using the resolving power coefficient (Rp) (Prevost and Wilkinson, 1999) using the formula: $Rp = \sum Ib$, where $Ib = (2 \times |0.5 - p|)$ and p is the proportion of accessions containing the I band. The coefficient of gene differentiation among the groups within species was determined using Nei’s gene diversity method. The formula was $G_{ST} = D_{ST}/H_T$, $D_{ST} = H_T\text{-}H_S$, where, H_T is the total gene diversity, the H_S is the gene diversity within group, and the D_{ST} is the gene diversity between groups. The gene flow was determined as $Nm= 0.5*(1- G_{ST})/G_{ST}$. These parameters were determined using the PopGene 1.32 software (Francis and Yang, 2000). A genetic distance matrix was constructed from the data matrix by using the Genedist (version 3.572c) program based on the formula of Nei and Li (1979). A cluster analysis was made using the Unweighted Pair Group PHYLIP (Phylogeny Inference Package, version 3.5c) (Felsenstein, 1995) and TreeView (Win32, version1.5.2) (Page, 1996). The principal components analysis (PCA) was performed by measuring the binary matrix with XLSTAT program (Version 2006.5, Addinosoft) to confirm the similarity and diversity among the cultivars.

Conclusion

To the best of our knowledge, this is the first report on AFLP markers to characterize Moroccan pomegranate resources. The results of this study indicate that, in Morocco, the level of polymorphism in pomegranate is appreciably higher. AFLPs are also very promising genetic markers for cultivar identification. These markers are suitable and useful tool to characterize and identify the closely related clones obtained from local selection with regard to their high reproducibility and good discrimination power. It is imperative to increase the number of cultivars and the number of primers tested to access genetic diversity and elaborate a future improvement program.

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