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Assessment of genetic diversity and population structure of an endemic Moroccan tree (*Argania spinosa* L.) based in IRAP and ISSR markers and implications for conservation

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Abstract Argan Tree is well known for its precious oil extracted from its seeds particularly used for the nutritional and cosmetic benefits. Because of the high international demand, the argan tree suffers from overexploitation and its cultivation is rare. Thus, the assessment of the genetic variation of this endemic tree is critically important for designing conservation strategies. In the present study and for the first time, genetic diversity of the global natural distribution of argan tree (*Argania spinosa* L.) in Morocco was assessed. Four IRAP (inter-retrotransposon amplified polymorphism) primer combinations and seven ISSR (inter-simple sequence repeat) primers amplified 164 and 248 scorable polymorphic bands respectively. Polymorphic information content (PIC = 0.27), resolving power (Rp = 15) and marker index (MI = 10.81) generated by IRAP primer combinations were almost identical to those generated by ISSR primers (PIC = 0.27, Rp = 9.16 and MI = 12). AMOVA analysis showed that 49% of the genetic variation was partitioned within populations which is supported by Nei's genetic differentiation (Gst = 0.5391) and the overall estimate of gene flow (Nm) being 0.4274. The STRUCTURE analysis, PCoA (principal coordinate analysis) and UPGMA (unweighted pair-group method with arithmetic mean) based on the combined data matrices of IRAP and ISSR divided the 240 argan genotypes into two groups. The strong differentiation observed might be due to the geographical distribution of argan tree. Our results provide crucial insight for genetic conservation programs of this genetic resource.

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Introduction

The Argan (*Argania spinosa* L., Sapotaceae) is a thorny evergreen tree endemic of arid and semi-arid regions in Morocco. In 1998, the "UNESCO's Man and the Biosphere Program" declared it as a Biosphere Reserve. This tree covers currently an area of 900,000 ha (HCEFLCD 2012) and stretches on the southwest region in addition to three small populations in the North (Oued Grou) in Northeast (Benié snassen) and in the South (Goulmime) (Ehrig 1974; Prendergast and Walker 1992).

Argan trees protect the soil from erosion and help fighting desertification. The tree wood is very hard, it is used for heating and construction materials. The main product is the oil extracted from its seeds providing income for rural population. Argan oil is rich in unsaturated fatty acids (Chimi et al. 1994) and built a worldwide reputation for its cosmetic and nutritional uses. Despite its numerous properties, cultivation of argan is rare and suffers from overexploitation. The assessment of the genetic variation for species located in narrow geographic areas is critically important for designing conservation strategies (Vicente et al. 2011). Therefore, the accurate analysis of genetic structure and the understanding of distribution patterns of genetic diversity is necessary to preserve the evolutionary potential of species to cope with environmental changes which is one of the main conservation goals (Milligan et al. 1994; Reisch et al. 2003).

In recent years, different molecular techniques have been used to study genetic diversity of argan tree including RFLP of chloroplast DNA (El Mousadik and Petit 1996),

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SSR (Majourhat et al. 2008), ISSR (Yatrib et al. 2015) and AFLP (Pakhrou et al. 2016). However, all these studies have not taken into consideration global distribution of argan tree in Morocco and most studies have involved a limited number of samples. In this study, the set of samples selected provides global representation of the natural distribution area of argan tree. In order to obtain a reliable estimate of genetic diversity assessment, two molecular markers ISSR and IRAP were used. The inter-retrotransposon amplified polymorphism (IRAP) is a technique developed by Kalendar et al. (1999) for the amplification of intervening region between two retrotransposons sequences. This method requires a single polymerase chain reaction (PCR) and uses two LTR primers (Kalendar and Schulman 2006). Because of the high copy number of this molecular marker throughout the genome (Kumar and Bennetzen 1999) and the change caused by their insertion, this method has been applied in several genetic diversity studies of plant species including *Quercus brantii* (Alikhani et al. 2014), *Quercus libani* (Khadivi-Khub et al. 2015), *Pinus massoniana* (Fan et al. 2013) and *Pistacia* (Sorkheh et al. 2016). To our knowledge, no IRAP based study has been conducted on argan tree. The inter-simple sequence repeat (ISSR) is one of the most informative markers able to detect polymorphism with a limited budget and in a short time (Gupta et al. 1994; Wu et al. 1994; Zietkiewicz et al. 1994). Also, any previous knowledge of DNA sequence is not required for the primer design (Godwin et al. 1997). The present work was carried out to provide new molecular evidence and baseline information about the overall genetic variability of the natural areas of argan tree using two molecular markers ISSR and IRAP. In this context, we aim to investigate the distribution structure and genetic variability within and among the populations of argan tree considering its natural distribution in Moroccan areas. The obtained information will contribute to the foundation of the best conservation and management programs.

Materials and methods

Plant materials

A total of 240 individuals of *A. spinosa* L. were collected from 24 populations of different regions in Morocco. The sampled individuals were selected to provide global representation of the distribution area of argan. All the information and the geographical indicators of sampling sites are presented in Table 1. Fresh foliage samples were harvested from adult trees and stored at −80 °C for genomic DNA extraction.

DNA extraction

Isolation of genomic DNA (gDNA) was done according to the ISOLATE II Plant DNA Kit. The extracted total DNA was checked by electrophoresis on 1% agarose gel and the concentration was spectrophotometrically estimated (NanoDrop Technologies Inc., USA).

IRAP analysis

A total of 28 IRAP primer combinations were tested (Alavi-Kia et al. 2008). Initially, one individual from each population was used to perform a preliminary screening. Those producing a very low proportion of polymorphic markers were excluded. Finally, 4 primer combinations that provided reproducible and describable bands were chosen for final evaluation. The amplification reaction was performed according to the Alavi-Kia et al. (2008) protocol. The PCR conditions were optimized and the final PCR reaction mixture (15 µl) included 20 ng of template DNA, $1 \times$ PCR buffer, 2 mM MgCl$_2$, 0.2 mM dNTPs, 0.3 µM of primer and 1 U of Taq polymerase. The cycling program consisted of an initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 1 min, annealing temperature Ta (Table 2) for 1 min and 72 °C extension for 2 min with a final elongation step at 72 °C for 7 min. The PCR amplification products were separated by electrophoresis using 2% agarose gel (UltraPure^{TM} Agarose, Invitrogen) in $1 \times$ TAE. Gels were visualized under UV light after being stained with ethidium bromide.

ISSR analysis

The ISSR analysis was carried out according to the modified protocol described by Zietkiewicz et al. (1994). A total of 7 ISSR primers selected by Yatrib et al. (2015) were used in this study. The final PCR reaction was performed in 25 µl containing 1X PCR buffer, 1.5 mM of MgCl$_2$, 1 U/µl Taq DNA polymerase, 1.5 mM of dNTPs, 1.5 mM ISSR primer and 75 ng of total genomic DNA. PCR amplification was performed in GenAmp$^®$ thermal cycler (Applied Biosystem, CA, USA) following the reaction program: 94 °C for 30 s; 45 cycles of: 94 °C for 30 s; annealing temperature (Table 2) for 45 s; 72 °C for 2 min; and 72 °C for 7 min after the last cycle. The DNA amplification products of ISSR markers were separated by electrophoresis in 2.8% agarose gels.

Data analysis

The clear and visible amplified bands for ISSR and IRAP were considered as potential polymorphic markers and

Table 1 Locations and sample size of the 24 populations of *A. spinosa* L

Regions	Bioclimate	Populations	Code	Sample size	Longitude	Latitude	Altitude (m)
Region1	Semi-arid	Retmana	RT	10	9°19′	32°02′	72
	Semi-arid	Ouled Lhaj	OH	10	9°24′	31°56′	118
	Semi-arid	Jbel Kourati	JK	10	9°23′	31°47′	365
	Semi-arid	Mramer	MR	10	9°10′	31°38′	396
	Semi-arid	Rbai	RB	10	9°28′	31°32′	269
	Semi-arid	Tamesrart	TS	10	9°22′	31°21′	540
	Semi-arid	Neknafa	NK	10	9°33′	31°19′	242
	Semi-arid	Ait Issi	AI	10	9°22′	31°02′	980
	Semi-arid	Tmanar	TM	10	9°37′	31°00′	576
	Semi-arid	Timzgida Oufetass	TO	10	9°48′	30°00′	231
Region2	Sub-humid	TiziNt'est	TZ	10	8°23′	30°48′	1194
	Arid	Imouzzer	IZ	10	9°30′	30°39′	1100
	Arid	Admine	AD	10	9°21′	30°19′	83
	Arid	Tafraout	TA	10	9°03′	29°42′	900
	Arid	Ait Baha	AB	10	9°13′	30°06′	490
	Arid	Mnizla	MN	10	9°05′	30°33′	256
	Arid	Oulcadi	OL	10	8°28′	30°17′	1235
	Arid	Aoulouz	AO	10	8°06′	30°37′	788
	Arid	Doutana	DT	10	9°13′	30°43′	1194
Region3	Arid	Lakhssas	LA	10	9°43′	29°24′	954
	Saharan	Guelmim	GU	10	10°06′	29°06′	351
	Saharan	Assa Zag	AZ	10	9°25	28°25′	328
Region4	Sub-humid	Oued Grou	OG	10	6°22′	33°27′	412
	Sub-arid	Benié Snassen	BS	10	2°34′	34°35′	246

scored as 1 for presence and 0 for absence to generate a binary data matrix for each primer. The efficiency of each primer was evaluated by calculating the total number of polymorphic bands, the polymorphism information content (PIC) following Roldán-Ruiz et al. (2000) and the marker index (MI) according to Powell et al. (1996). The ability to distinguish ISSR and IRAP markers between the individuals was determined by calculating resolving power (Rp), using formula by Prevost and Wilkinson (1999). The different parameters of genetic diversity and the structure analysis were performed using the combined data of ISSR and IRAP. Nei's genetic diversity index (H), effective number of alleles (Ne) and Shannon diversity index (I) were calculated using GenALEx 6.5 (Peakall and Smouse 2006, 2012). The coefficient of genetic differentiation (Gst), gene flow (Nm = 0.5(1 − Gst)/Gst) and total genetic diversity (Ht) were estimated for total population using POPGENE software version 1.32 (Peakall and Smouse 2006; Yeh et al. 1999). The variation among the four regions, among and within populations were analyzed based on the molecular variation (AMOVA) using GenA-lEx version 6.5 (Peakall and Smouse 2006). In order, to test the correlation between geographical and genetic distances among populations, a Mantel test was carried out with 999

permutations. The genetic similarities according to Jaccard's coefficient of 240 individuals of *A. spinosa* L. were calculated using the SMIQUAL program of the analysis system NTSYS-pc (Numerical Taxonomy System, version 2.11) (Rohlf 2000). The resulting matrices were used to construct the dendrogram through unweighted pair-group method with arithmetic mean (UPGMA) using SAHN module as recommended by most studies (Alikhani et al. 2014; Lind and Gailing 2013). The principal coordinate analysis (PCoA) was conducted on a covariance matrix using GenALex6.5 to describe the relationship between populations. The population structure was determined for 240 genotypes of argan tree. The molecular data were processed using the STRUCTURE software based on the Bayesian estimate which assigns individuals to a number K of genetically homogeneous groups without a priori group designation. For the analysis with the program STRUCTURE, the length of burn-in and MCMC (Monte Carlo Markov Chain) were set to 10,000 for each K based on the work of Evanno et al. (2005), suggesting that this level is sufficient. Twenty independent replications (runs) were performed for each simulated value of K, varying from 2 to 20. Subsequently, the likely number of populations was calculated based on the highest log value of the likelihood

Table 2 Degree of polymorphism and information content for ISSR primers and IRAP primer combinations

Primers	Sequence of primers	Ta[a]	TF[b]	PP[c]	PIC[d]	MI[e]	Rp[f]
ISSR							
ISSR 1 (FL2)	$(AC)_8CG$	59.9	34	100	0.29	9.81	14
ISSR 2 (FL3)	$(AC)_8CT$	57.6	32	100	0.29	9.30	13
ISSR 3 (FL4)	$(AG)_8CC$	51.7	24	100	0.30	7.32	10
ISSR 4 (FL6)	$(CA)_8AG$	53.7	29	100	0.25	7.16	10
ISSR 5 (FL8)	$(GA)_8CC$	56.0	28	100	0.30	8.77	12
ISSR 6 (FL9)	$(GA)_8CG$	56.0	53	100	0.22	11.64	15
ISSR 7 (FL10)	$(GA)_8CT$	57.6	48	100	0.21	10.10	13
Total			248		1.87	64.10	86
Mean			35.43		0.27	9.16	12
IRAP Combinations							
Sukkula/Sukkula	GATAGGGTCGCATCTTGGGCGTGAC/ GATAGGGTCGCATCTTGGGCGTGAC	53.0	40	100	0.22	8.88	12
3′LTR/Sukkula	TGTTTCCCATGCGACGTTCCCCAACA/ GATAGGGTCGCATCTTGGGCGTGAC	50.0	33	100	0.31	10.23	14
5′LTR1/Sukkula	TTGCCTCTAGGGCATATTTCCAACA/ GATAGGGTCGCATCTTGGGCGTGAC	50.0	44	100	0.26	11.54	15
LTR6150/LTR614'9	CTGGTTCGGCCCATGTCTATGTATCCACACATGTA/ CTCGCTCGCCCACTACATCAACCGCGTTTATT	43.0	47	100	0.27	12.58	19
Total			164		1.06	43.23	59
Mean			41		0.27	10.81	15

[a] Annealing temperature

[b] Total number of fragments

[c] Percent polymorphism

[d] Polymorphic information content

[e] Marker Index

[f] Resolving power

(LnP(K)) using STRUCTURE Harvester (Earl and von-Holdt 2011) based on the method developed by Evanno et al. (2005).

Results

Level of polymorphism and informativeness obtained with ISSR and IRAP

Four IRAP primer combinations and seven ISSR primers were selected to amplify a total of 240 DNA samples of argan tree, the level of polymorphism and informativeness are presented in Table 2. The 4 IRAP primer combinations produced a total of 164 polymorphic amplicons with a range of 4 to 18 bands per primer combinations. The total number of fragment ranged from maximum 37 (LTR6150/LTR6149) to minimum 33 (5′LTR1/Sukkula). Also, the total number of amplified fragments by ISSR was 248 polymorphic bands that could be unambiguously scored. Primer ISSR 6 generated the largest number of fragment (53) while the primers ISSR 3 generated the smallest number of fragment (24). Furthermore, polymorphic information content (PIC) values of the four IRAP primer combinations ranged from maximum 0.31 (3′LTR/Sukkula) to minimum 0.22 (Sukkula/Sukkula) while marker index (MI) varied from 8.88 (Sukkula/Sukkula) to 12.58 (LTR6150/LTR6149) with an average of 10.81. The values of PIC of the 7 ISSR primers ranged between 0.21 (ISSR 7) and 0.30 (ISSR 3 and ISSR 5) with an average of 0.27 and the mean value for MI was 9.16 ranging from 11.64 (ISSR 6) to 7.16 (ISSR 4). Resolving power (Rp) of the IRAP primer combinations ranged from 12 (Sukkula/Sukkula) to 19 (LTR6150/LTR6149) with an average of 15 while Rp of the ISSR primers varied from 10 (ISSR 3 and ISSR 4) to 15 (ISSR 6). The primers ISSR 6 and LTR6150/LTR6149 were the most informative according to the Rp and MI values.

Genetic diversity and population structure analysis

The diversity parameters of all 240 individuals were calculated based on the combined data of ISSR and IRAP. The highest values of Shannon index (I), Nei's genetic diversity

(H), observed number of alleles (Na), effective number of alleles (Ne) and the percentage of polymorphic bands (PPB) was observed in Lakhsass population (I = 1.194, H = 0.125, Na = 0.864, Ne = 1.202, P = 42.48%) (Table 3). The total genetic diversity (Ht) of *A. spinosa* L. was 0.1879. Furthermore, the AMOVA showed that genetic variation within populations was 49% while 40% was among populations and 11% of molecular variation was partitioned among regions (Table 4). The proportion of total diversity residing among populations (Gst) was 0.5391 which indicates a high level of genetic differentiation among *A. spinosa* L. populations. The gene flow (Nm) based on the Gst was measured to be 0.4274. The results of this analysis suggest that the gene flow among populations throughout the entire distribution area is restricted with a high genetic differentiation. The Mantel

Table 3 Genetic variation and polymorphic features estimated using IRAP and ISSR markers among 24 populations of the *A. spinosa* L

Populations	Na[a]	Ne[b]	I[c]	H[d]	PPB[e] (%)
Retmana	0.633	1.107	0.114	0.071	27.91
Ouled Lhaj	0.590	1.109	0.110	0.069	25.49
Jbel Kourati	0.590	1.142	0.133	0.087	27.91
Mramer	0.633	1.109	0.113	0.071	27.18
Rbai	0.621	1.144	0.136	0.088	28.64
Tamesrart	0.675	1.106	0.115	0.071	29.37
Neknafa	0.580	1.095	0.099	0.062	24.51
Ait Issi	0.541	1.108	0.106	0.068	23.79
Tmanar	0.522	1.078	0.086	0.053	21.84
Timzgida Oufetass	0.631	1.116	0.117	0.074	27.43
TiziNt'est	0.779	1.141	0.145	0.091	34.47
Imouzzer	0.614	1.116	0.118	0.074	27.43
Admine	0.704	1.168	0.157	0.103	33.25
Tafraout	0.769	1.199	0.185	0.121	37.62
Ait Baha	0.782	1.197	0.183	0.119	37.62
Mnizla	0.617	1.114	0.114	0.072	26.94
Oulcadi	0.682	1.152	0.145	0.094	30.83
Aoulouz	0.614	1.152	0.139	0.091	27.91
Doutana	0.680	1.126	0.128	0.080	29.85
Lakhssas	0.864	1.202	0.194	0.125	42.48
Gulemim	0.828	1.198	0.189	0.122	40.53
Assa Zag	0.799	1.180	0.175	0.112	38.59
Oued Grou	0.624	1.151	0.141	0.092	28.64
Benié Snassen	0.583	1.104	0.107	0.067	25.24
Mean	0.665	1.138	0.135	0.087	

[a] Observed number of alleles

[b] Effective number of alleles

[c] Shannon's information index

[d] Nei's (1973) gene diversity

[e] Percentage of Polymorphic Loci

test revealed that there was no significant correlation between genetic and geographic distance (r = 0.0246; p < 0.05).

To elucidate the genetic relationships among the studied genotypes of argan tree, the dendrogram based on all of the molecular data (IRAP + ISSR) was constructed using Jaccard coefficient in NTSYS 2.11 (Applied Biostatistics, Setauket, New York, USA) (Rohlf 2000). The genetic similarity coefficient ranged from 0.14 to 0.74 (Fig. 1). The dendrogram grouped the studied populations into two major clusters and the individuals were separated according to their populations. Some populations of each region were placed into the same sub-cluster and showed high similarities with each other. In fact, populations of region 1 (MR, RB, JB, RT, OH, TM, AI, TS, NK, TO) were grouped in cluster I and the populations of region 3 (GU, AZ, LA) were grouped in cluster II while populations of region 2 and 4 were scattered in cluster I and II. These findings suggest that there is no important correlation between genetic relationships and geographical origins.

Besides, the principal coordinate analysis was performed to visualize the relationships among populations in more details and it explained 48.22% of total variation. The first PCo explained 8.01% of the total variability while the second PCo and the third PCo accounted for 15.05 and 21.16% of the variance respectively. The PCoA presents the relationship among populations and the results were comparable to the cluster analysis UPGMA dendrogram which showed the same distribution (Fig. 2).

In order, to provide further evidence of hierarchical population structure, powerful computational tools meant to deduce population structure were used. The STRUC-TURE software v2.1 (Pritchard et al. 2000) performs Bayesian assignments of individuals to a given number of genetically homogenous clusters (K) of populations. The most likely K for the given range of 2–20, was computed using STRUCTURE HARVESTER (Earl and vonHoldt 2011) based on both the log likelihood and maximum ΔK (Evanno et al. 2005). The maximum value of ΔK was detected at K = 7, followed by K = 2. Furthermore, the results of neighbour-joining tree analysis and PCoA indicated that 24 populations of argan tree were divided into two groups which is in agreement with the STRUCTURE results at K = 2. This result indicated that the genetic structure of *A. spinosa* L. could be grouped into two populations and seven subpopulations. The first cluster mainly consisted of 13 populations, these populations were split into 4 sub-cluster. The second cluster consisted of 11 populations. This group was further separated into 3 sub-clusters (Fig. 3). One of these clusters was containing the population of region 1 and the other genetic cluster comprised all of the populations of region 3 while populations of region 2 and 4 were placed into both clusters.

Table 4 Analysis of molecular variance (AMOVA) of 24 populations of *A. spinosa* L

Source	Df[a]	SS[b]	MS[c]	VC[d]	% VC[e]	Ht[f]	Gst[g]	Nm[h]
Among populations	20	4606.69	230.33	20.537	40	0.1879	0.5391	0.4274
Within populations	216	5391.50	24.96	24.961	49			
Among regions	3	1542.21	514.07	5.348	11			
Total	239	11,540.39		50.846	100			

[a] Degrees of freedom

[b] Sum of squares

[c] Mean of squares

[d] Variance component

[e] %Variance component

[f] Total genetic diversity

[g] Coefficient of genetic differentiation

[h] Estimate of gene flow

Discussion

This is the first detailed report of genetic diversity of argan tree that took into consideration the global natural distribution of argan tree in Morocco. In this study we combined the performance of two molecular markers IRAP and ISSR to evaluate conclusively the genetic variation of a set of samples collected from 24 populations. This species is the only one representative of the *Argania* genus and it's endemic to a restricted area in Morocco.

The IRAP and ISSR techniques were used to study the genetic diversity and structure of different forest tree such as *Quercus libani* G. Olivier (Khadivi-Khub et al. 2015) and *Quercus brantii* (Alikhani et al. 2014). Although, the mean of polymorphic bands of IRAP (41) was higher than ISSR (35.43) their percent of polymorphism bands and the diversity index consisted of PIC value were similar. Also, the two-marker techniques target different regions of the genome. In fact, IRAP marker amplifies genomic DNA of the region between inserted retrotransposons (Kalendar et al. 1999) while ISSR system detects the polymorphism of the region between simple sequence repeats (Zietkiewicz et al. 1994). This explains the importance of combining two markers in obtaining reliable estimates of genetic diversity (Souframanien and Gopalakrishna 2004). During the present investigation, we have demonstrated the effectiveness and power of IRAP and ISSR markers to detect polymorphism and estimate the genetic diversity by calculating the MI and Rp parameters. Our results are comparable to those found by Baraket et al. (2009) on *Ficus carica* and Pecina-Quintero et al. (2013) on *Ricinus communis*. It is considered to be informative in distinguishing the individuals and could be successfully applied to assess genetic variation among and within *A. spinosa* L. populations.

Our analysis of IRAP and ISSR molecular markers indicated that argan tree has maintained a moderate genetic

diversity (Ht = 0.1879) despite of its fragmented area. This genetic diversity reflects the historic genetic variability which is quite common in long lived plant species (Geert et al. 2007; Honnay et al. 2005).

The genetic analysis of populations based on combined data (ISSR and IRAP marker systems) showed divergence among the studied populations. The analysis of all clusters did not show clear clustering pattern of geographically closer populations. This result is consistent with those found by Yatrib et al. (2015) using ISSR markers on 17 populations suggesting that the geographic distribution is not the principal factor shaping the present population genetic structure. However, the dendrogram revealed that most of individuals were separated according to their populations. This result can be explained by the reproduction system of argan tree and its pollen scattering mechanism by wind and insects (Bani-Aameur et al. 1999; Hamrick and Godt 1996; Scotti-Saintagne et al. 2004).

The distribution of genetic differentiation among and within populations depends on gene flow rate between populations. The gene flow of a species is a function of the size of populations, the movement of seeds and pollen, the degree of isolations and the distribution of the habitats it occupies (Dumolin-Lapègue et al. 1997). The present analysis revealed a high genetic differentiation among the studied populations (Gst = 0.5391), this could be seen as evidence of low gene flow between the populations (Nm = 0.4). Similar results were found by Mouhaddab et al. (2015) using ISSR markers and by Pakhrou et al. (2016) using AFLP markers. The high differentiation among *A. spinosa* L. populations is consistent with studies of other endemic species like *Cupressus gigantean* (Gst = 0.36) (Xia et al. 2008) while it was higher than other forest species such as sessile oak (Gst = 0.026) (Kremer et al. 2002). Moreover, the high genetic differentiation and gene exchange between studied populations are expected for several reasons. Firstly, the studied
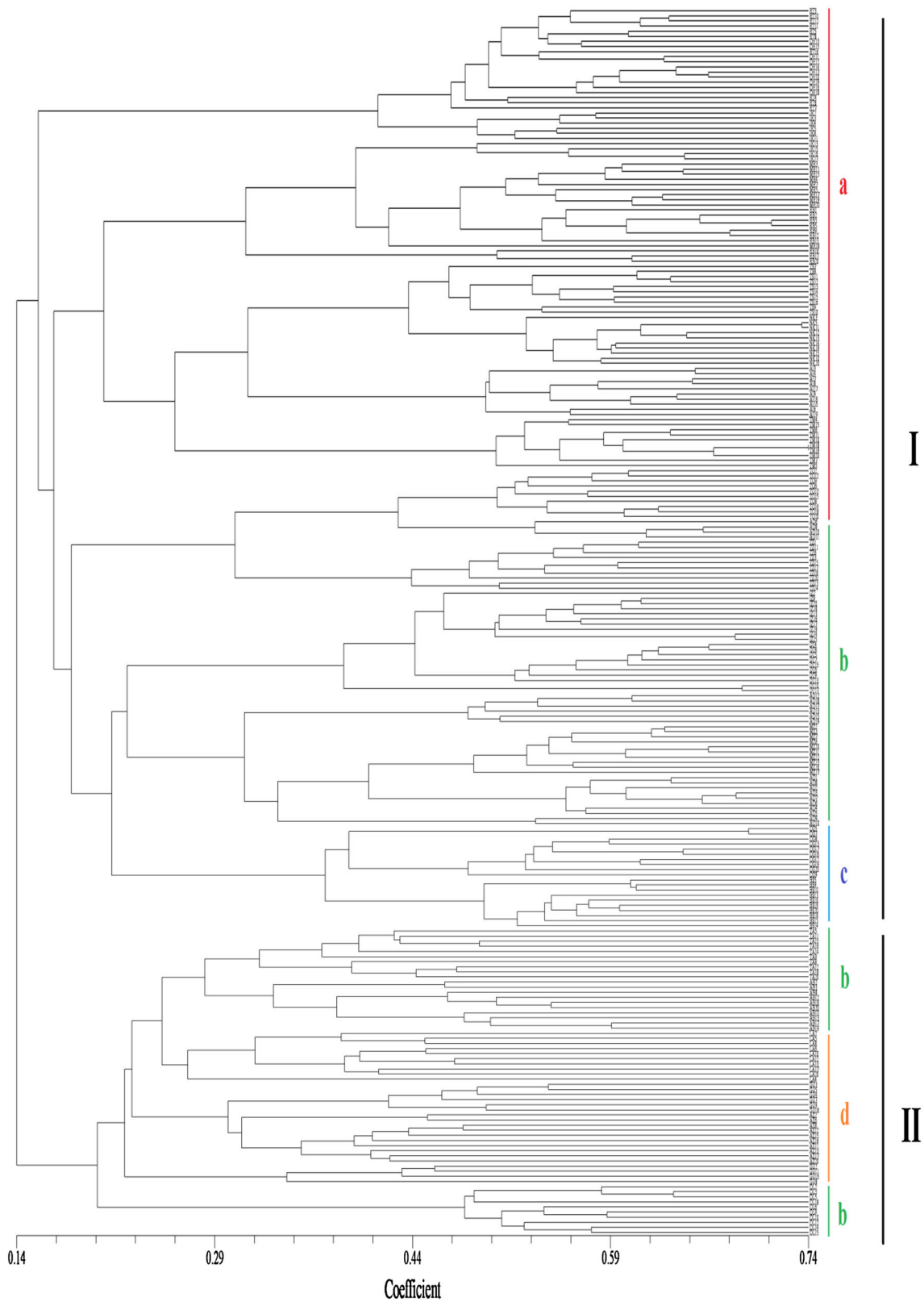
Fig. 1 UPGMA dendrogram produced using Jaccard's coefficient based on combined data of two molecular markers (IRAP and ISSR) in the studied 240 individuals of *A. spinosa* L. I: Cluster I grouped all populations of region 1 and region 4 and few populations of region 2;

II: Cluster II grouped all populations of region 3 and few populations of region 2. a: populations of regions 1; b: populations of region 2, c: populations of region 4; d: populations of region 3
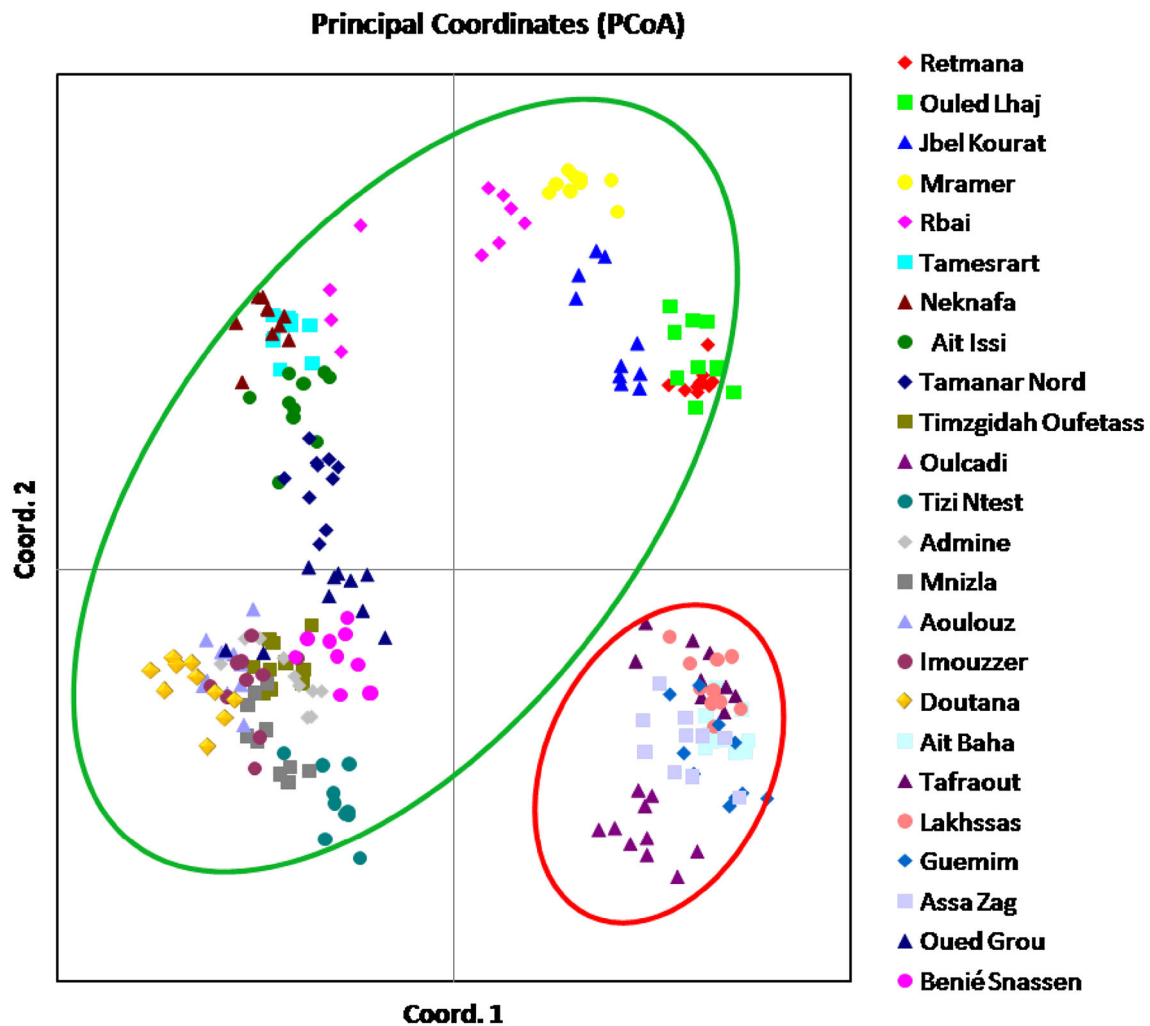
Principal Coordinates (PCoA)

Fig. 2 Principal coordinate analysis (PCoA) of 240 *A. spinosa* L. individuals from 24 populations, based on IRAP and ISSR analysis by GenAlEx 6.5. Percentage of total variance was 48.22% (axis1 = 8.01; axis2 = 15.05%; axis3 = 21.16%)

populations are located in different geographical areas under variable bioclimates (sub-humid, semi-arid, arid and saharan) and the distribution range of argan tree is limited to a specific area and naturally fragmented. This geographical distance indicates a barrier that can lead to difficulties in pollen and seeds scattering (Ding et al. 2008) and it's a probable cause of low gene flow in argan germplasm. Secondary, the strong differentiation observed could come from divergent adaptation of the species due to environmental factors causing a differentiated selection. Furthermore, the Mantel test indicated that there was no significant relationship between genetic and geographic distances. This result was confirmed by the UPGMA clustring, PCoA and STRUCTRE which indicates that the geographical distribution is not the main factor that shaped the current population genetic structure.

The main goal of current conservation plans is focused on maintaining species diversity (Margules and Pressey

2000; Rodrigues et al. 2013). The results revealed in this study enhance our understanding of population structure of argan tree and provide information which would have important management implications for this species.

Despite political and strategic actions implemented by the Moroccan government to conserve world heritage of the argan tree, the size of populations has decreased in recent times (de Waroux and Lambin 2012) due to a combination of loss of trees, lack of regrowth resulting from overgrazing, increasing aridity and human activities. Based on our results we recommend possible management strategies for the argan tree conservation. The first most important management practice is a periodic monitoring of natural distribution range and establishing a protection area where the exploitation of this tree is forbidden and especially in populations with low levels of genetic diversity such as Tmanar. This strategy can reduce the impact of human and animals activities and allow populations to
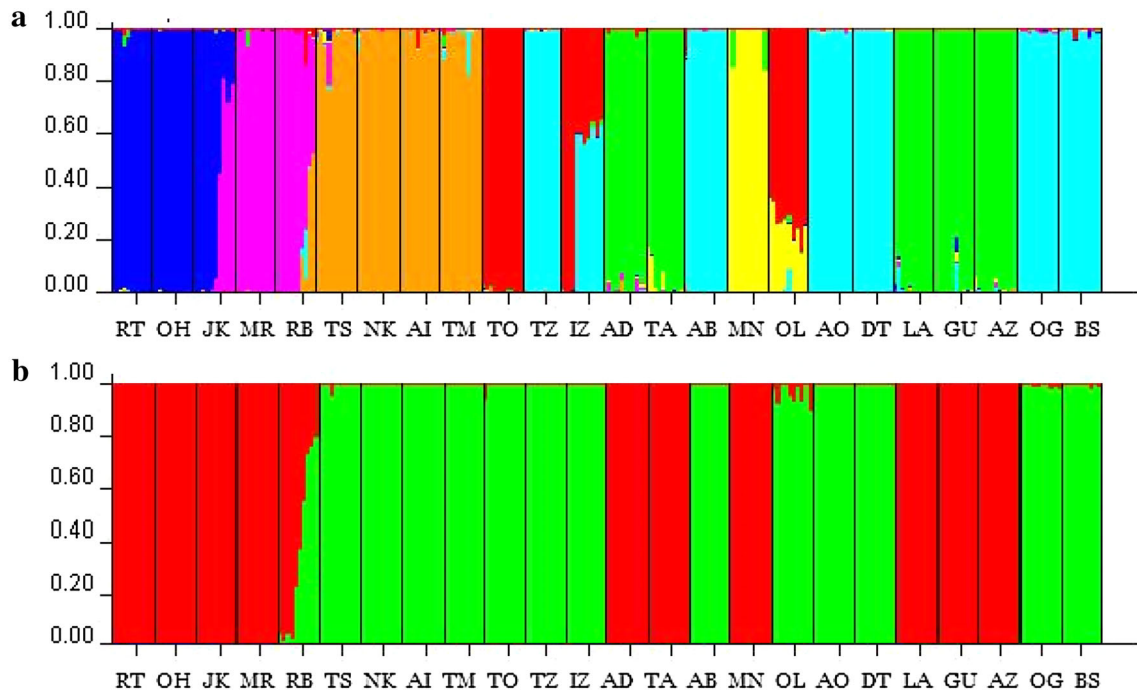
Fig. 3 Estimated populations structure of *A. spinosa* L. obtained with the STRUCTURE program. Results are based on IRAP and ISSR markers of the 240 samples. The maximum value of Delta K was detected at K = 7 (**a**), followed by K = 2 (**b**). In the barplots, each population is represented by a single *vertical line* divided into K *colors*, where K is the number of population assumed. Each *color* represents one cluster. Population's codes see Table 1

increase in size through natural regeneration. The second component of proposed strategy concerns the establishment of an ex situ conservation program by seed storage. Although the seeds from different population are needed to preserve most of the genetic variation and not to lose specific genes, it is suggested that the Lakhssas population will be the main source because of its higher levels of genetic diversity. In addition, considering the high genetic differentiation, a field gene bank should be built by a large scale plantation in a nature reserve. This strategy must take into account all existing populations and genetic groups in order to increase the chance of gene exchange and ensure a long-term conservation of most existing genetic variation. Finally, the safest way to preserve these genetic resources is probably through a combination of all these in situ and ex situ approaches but also increased the awareness of the local community about argan tree conservation. This last action can have a major influence on the efficiency of conservation strategy. Furthermore, owing the economic value of this tree, it is important to combine the studies on the distribution of genetic diversity with other information such as oil yield per population.

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