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Genetic diversity and population structure of the endangered argan tree (A*rgania spinosa* L. Skeels) in morocco as revealed by SSR markers: Implication for conservation

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Abstract

Argania spinosa L. is an endangered tree of great socio-economic and ecological value in Morocco. In this study, thirteen nuclear SSR primer pairs were used to assess the genetic diversity and structure of 24 natural populations, including 240 individuals, representing 4 geographic regions. A total of 245 alleles were detected with an average of 18.5 per locus ranging from 6 to 35. The polymorphism information content (PIC) was in the range of (0.487-0.936) showing the good discriminating power of the SSR loci used. The observed and the expected heterozygosity across all populations and loci ranged from 0.372 to 0.777 and from 0.486 to 0.735, respectively. Analysis of molecular variance (AMOVA) revealed that the main variation existed within populations (78%) rather than among populations (12%). The Mantel test displayed no significant correlation between the geographic distance and the genetic distances for all populations. The STRUCTURE analysis and UPGMA clustering grouped 240 samples from 24 populations into two subgroups. Implications of the results for argan tree conservations are also discussed in this paper.

Keywords: Simple Sequences Repeats; Argan tree; genetic diversity; population structure; conservation strategy.

Introduction

The natural vegetation in the Mediterranean ecosystem is subjected to severe climatic conditions (Nogués and Baker, 2000). Some species are well adapted to these conditions, for example, *Argania spinosa* L. Skeels, also known as the argan tree. It is the only representative species of the tropical family Sapotaceae in Morocco (Majourhat et al., 2007). This slow-growing species may be shrubby in appearance or can grow as a tree reaching 7-10m in height (Díaz-Barradas et al., 2010). Abundant throughout the arid and semi-arid regions of south-west of Morocco, the argan forest is key to maintaining the ecological balance and helps safeguard biodiversity due to its hardy characteristics and its powerful role in protection against desertification. It has also of great socio-economical interest because it is a multipurpose tree (Prendergast and Walker, 1992). Each part of the tree is usable and it ensures the subsistence of around 3 million people, including 2.2 million people in rural area where sylvo-pastoral systems are based on the argan tree (Chaussod et al., 2005).

It is an endemic species to Morocco and grows in a harsh environment, surviving heat, drought and poor soils. Despite its importance, it remains a wild species vulnerable to extinction from ongoing fragmentation of its natural habitats (M'Hirit et al., 1998; Nouaim, 2005; Aabd et al., 2014). Research into the basic biology and ecology of the species is crucial for the establishment of effective conservations programs. Genetic diversity and population structure studies can afford a preliminary assessment of the conservation status of this important resource. This information may help guide the development of germplasm collections and thus the preservation (*in-situ* or *ex-situ*) of this species in the face of climate change and habitat fragmentation.

In this study, we used Simple Sequence Repeat (SSR or microsatellite) markers to evaluate the amount and partitioning of genetic diversity between populations of Argan tree throughout its distribution area. SSRs are known for their high degree of reproducibility and the ease of detection by PCR using pairs of flanking primers and the requirement of only a small amount of templateDNA. Simple Sequence Repeats markers have proved to be an efficient and practical tool in the study of genetic diversity and population structure of endemic and endangered plant species (Varshney et al., 2005). As well as their use in genome mapping, they are also ideal for genetic studies of population structure (Dayanandan et al., 1998). Due to their codominance, and

high reproducibility, these markers are greatly superior to the commonly used RAPD (Bani-Aameur and Benlahbil, 2004), ISSR (Mouhaddab et al., 2015; Yatrib et al., 2015) and AFLP (Pakhrou et al., 2016) that have previously been used on some populations of *A. spinosa* L. Skeels. Thus, in the present study, and for the first time, SSR markers are used to evaluate the genetic diversity of the argan forest across all its distribution area.

Here, thirteen highly polymorphic SSRs were used to analyze the genetic diversity and population structure of 24 argan populations (240 total individuals) representing all the distribution area of this species. The major objectives of this work aimed to address the following questions: (i) how is the genetic diversity partitioned within and between populations of this endangered species across all its distribution area. (ii) How closely are the genetic patterns linked to the geographical distributions? (iii) And based on the genetic structure, what measures should be adopted in the future for the conservation of this important species?

Results

Characteristics of microsatellite markers

A set of thirteen microsatellites combined in three multiplex panels were used to analyses 24 wild populations of the argan tree, covering all of its distribution area for the first time.

A total of 245 alleles were revealed across 240 individuals of *A. spinosa* L. Skeels. Low null alleles frequency (An) was detected ranging from -0.001 (MH07) to 0.329 (MH08). The number of observed alleles (*Na*) per locus ranged from 6 (ME05) to 35 ($ASMS_{2012\_04}$) with a mean value of 18.84 and a size ranging from 121 to 324bp. The effective number of alleles (*Ne*) varied from 2.374 to 16.613 per locus, with an average of 7.361. The observed and expected heterozygosities ranged from 0.242 to 0.832 and from 0.576 to 0.94, respectively (Table 2). Polymorphism information content (PIC) was between 0.488 (ME11) and 0.937 (ASMS01) with a mean number of 0.779, and 12 out of 13 loci were highly informative (PIC> 0.50). Mea Shannon index (I) was 2.075 ranging from 1.039 (ME11) to 2.980 (ASMS01), indicating the high polymorphism at the 13 microsatellite loci tested.

Genetic diversity

Intra-population microsatellite variation parameters based on allelic frequencies, are presented in Table 3, and showed that observed heterozygosity (*Ho*) varied from 0.392 in Guelmim to 0.777 in Mramer with an average of 0.645, whereas expected heterozygosity (*He*) was slightly higher than the observed heterozygosity, it ranged from 0.486 in Guelmim to 0.735 in the population of Lakhsass with a mean value of 0.674. Within the 24 populations, 58 unique alleles in the argan populations were detected at 10 loci. The highest allelic richness was observed within the Lakhssass population (*Ar*=7.8), while the lowest was found within the Guelmim population (*Ar*=3.538). A total of 25 private alleles were detected in 19 out of 24 populations distributed in the 4 geographic regions.

Fixation indice (Weir and Cockerham, 1984), which is a measure of inbreeding was calculated for each population. Estimated values of Fis for the wild 24 populations ranged from -0.123 (NK) to 0.278 (BS) with a mean value of 0.051 (Table 3).

Genetic differentiation

Pairwise F_{ST} values were examined in order to identify possible correlation between geographic distance and population differentiation. Estimates of pairwise F_{ST} among populations of *A. spinosa* identified the smallest genetic distance (0.0255) between the JK and MR populations, whilst the greatest distance (0.210) was observed between the DT and GU populations (Table 4**)**. There was no positive correlation between the geographical and genetic distances, as showed by the Mantel test (r= 0.241 P>0.05). However, when we performed the Mantel test for the geographic and genetic distances without taking in consideration the populations of the North (BS and OG), a positive correlation was obtained. Similarly, the AMOVA indicates that genetic variation was higher within populations (84%) rather than among populations (12%) and among regions (10%) of *A. spinosa* (Table 5, P<0.001).

Genetic relationship

The genetic relationship among the populations was examined by UPGMA cluster analysis based on Nei's pairwise genetic distance. The UPGMA clustering divided the 24 populations into two genetic groups **(**Fig. 2). Group I included 5 populations, Tafraout, Ait Baha, Guelmim, Lakhssas and Assa Zag, which were sampled from the Saharien region of the country. Group II comprised the rest of the populations. Within this latter group, Rbai, Mramer, Ait Issi, Tamesrat and Neknafa were grouped together (IIa) and were sampled from the same region which is the Argan region, whereas the other 14 populations were grouped together (IIb), including Beni Snassen and Oued Grou from the relics the North of the country. This typology is not in agreement with the geographical distribution of these populations; hence the populations from the North were genetically close to population in the Atlas Mountains region in the South West of the country.

The method of (Evanno et al., 2005) for the estimation of the most likely number of genetic clusters (K) revealed a maximum model of Δk with K=2, Δk =334.25. Confirming the groups obtained in SSR cluster analysis (Fig. 3). These results suggest that the most probable number of genetically distinct population groups was 2, based on our data of 240 individuals from 24 populations.

Discussion

Genetic diversity plays a very important role in a species evolution, allowing good adaptation to new environments. Populations with more variation tend to possess a variety of alleles that are suitable for tackling the changing environment (Hamrick and Godt, 1996a; Groom et al., 2005; Xing et al., 2014).

Populations of *Argania spinosa* L. Skeels have declined tremendously in size and are fragmented and degraded (le Polain de Waroux and Lambin, 2012). Thus, in the present study, we employed thirteen pairs of microsatellites markers in order to examine the genetic diversity and patterns of population differentiation in the argan forests of Morocco.

Our results suggest that the genetic diversity in this species is relatively high (Table 3, *He*=0.674 at population level) compared to corresponding genetic coefficients of other endangered species (Hamrick and Godt, 1996; Feyissa et al., 2007). Also the coefficient of differentiation among the 24 populations was estimated to be 0.170 using SSR marker, which is a slightly lower but comparable to previous finding

Table 1. Sampling location and size.

Regions	Code	Population	Abbreviation	Latitude	Longitude	Altitude (m)	Sample size
Region1	23	Oued Grou	OG	33°27'44.3"	6°22'94.5"	403	10
	24	Beni Snasssen	BS	34°51 095	2°34'983	195	10
Region2	1	Retmana	RT	32°02'05.4"	9°19'24.2"	58	10
	2	Ouled Lhaj	OH	31°56'13.7"	9°24'11.7"	120	10
	3	Jbel Kourati	JK	31°47'22.4"	9°24'03.00"	360	10
	4	Mramer	MR	31°38' 23.9"	9°10' 02.03 "	396	10
	5	Rbai	RB	31°32'07.8"	9°28' 34.4 "	269	10
	6	Tamsrart	TS	31°21'34.9"	9°22' 58.0 "	540	10
	7	Neknafa	NK	31°19'20.6"	9°33'56.1"	242	10
	8	Ait Issi	AI	31°02'13.5"	09°22'32.4"	980	10
	9	Tamanar Nord	TM	31°00'41,5"	9°37'20,3"	576	10
	10	Timzgidah Oufetass	TO	31°00'20,2"	9°48'09,02"	231	10
Region3	11	Oulcadi	OL	30° 17'19. 5"	8° 29'08. 5"	1247	10
	12	Tizinit Est	TZ	30° 48'50. 4"	8° 23'43. 9"	1178	10
	13	Admine	AD	30°19'53,1"	9°21'38"	83	10
	14	Mnizla	MN	30°33'44,8"	9°05'39,2"	256	10
	15	Aoulouz	AO	30° 37'11.2"	9° 06'47. 0"	782	10
	16	Imouzzer	IZ	30°39'00,9"	9°30'54"	1100	10
	17	Doutana	DT	30° 43'35. 4"	9° 14'01. 5"	796	10
	18	Ait Baha	AB	30°06'27,1"	9°13'32,6"	490	10
	19	Tafraout	TA	29°42'47,2"	9°03'21,5"	900	10
Region4	20	Lakhssas	LA	29°24'12,4"	9°43'50,3"	954	10
	21	Guelmim	GU	29° 06'51, 8"	10° 06'40, 5"	349	10
	22	Assa Zag	AZ	28° 25'53, 6"	9° 25'22, 5"	336	10
Mean						535.04	10

Table 2. Characteristics of 13 investigated SSR markers and the diversity detected in 240 argan individuals.

Locus	Primer Sequences	T°C	Alleles size (pb)	*Na*	*Ne*	*Ho*	*He*	*I*	*FIS*	F_{ST}	*PIC*	*An*
ASMS01	F:GTTTCTTGCAGTTTGAGAATTGAAAGGACAACG R: CCTCCCATCTAATATTTCTAGTTCCATCC	57	143-178	29	16.613	0.892	0.940	2.980	-0.037	0.085	0.937	0.025
ASMS20	F: GTTTCTTAATACTTCAATGCGAAGGTCGTG R: ATTACTCCCAACCTCAGTCAGC	53.8	121-250	34	11.541	0.796	0.913	2.874	0.016	0.115	0.908	0.065
ASMS2012_04	F: CCAATAATAGAAACACCCGGAA R: GTTTCTTACTTAGCCCACCTTCCTCTTCT	55	167-323	35	9.227	0.771	0.892	2.628	0.066	0.075	0.883	0.068
ASMS2012_34	F: CCCATTGTAGACTTCCGCTTAC R: GTTTCTTAACCACAGAGAGCAGCAACTTT	55	199-324	34	12.299	0.813	0.919	2.802	0.030	0.088	0.913	0.058
ASMS2012_37	F: CGGAAAGGAATTAGGATTTGG R: GTTTCTTCGGTTCGTCTCTTCTCCAGTAT	55	180-314	32	12.842	0.804	0.922	2.836	0.046	0.086	0.917	0.065
MH07	F:ATTGCAGCATATCCACACCA R: GCAAAGGGTGATGGGTTAGA	56	125-151	8	2.597	0.617	0.615	1.314	-0.210	0.171	0.581	-0.001
MH04	F:GCACTCTCCATGGTTCCAGT R:AAAGAGTCAATGGCGTGAGC	52	161-196	13	4.561	0.775	0.781	1.820	-0.124	0.117	0.753	0.003
MH06	F:ACACGCACAAAACAAACCAA R:TTCTTGAAGGAGGGTTGCTC	56	158-195	14	4.968	0.700	0.799	1.782	-0.137	0.229	0.769	0.058
ME11	F:TTGCTATTTGCCTGTT R:TTCATCACCTTCCTCTC	48	224-240	9	2.357	0.242	0.576	1.039	0.300	0.400	0.488	0.269
MH20	F:GAAGTTTTGACCATTTGGGAAT R:GACATAACACTAACCCTTCACGA	56	136-165	10	5.047	0.638	0.802	1.786	0.052	0.161	0.773	0.100
MH08	F:GTAATGGGAGCCGTTTGAGA R:CTGGGTAGCATTTGTTGCAT	56	173-200	12	6.234	0.383	0.840	1.988	0.280	0.366	0.820	0.329
ME05	F:GTTTGTATGGTTTCGGTT R:CTTCGTTTTCAGTAGGTCTC	52	245-258	6	4.1854	0.563	0.761	1.553	0.086	0.191	0.723	0.127
MH12	F:TGCGGAACTGTGGAAAGAGT R:ATCCACACAATGACTGACG	56	189-211	9	3.217	0.392	0.689	1.583	0.349	0.128	0.663	0.213

Note: F: Forward primer; R: reverse primer; T°C: PCR annealing temperature; Na: Observed number of Alleles; Ne: Effective number of alleles; *He*: Expected heterozygosity, *Ho*: Observed heterozygosity; I; Shannon information index, Fst = Genetic differentiation coefficient among populations, PIC: Polymorphism information content, An; the frequency of null alleles.

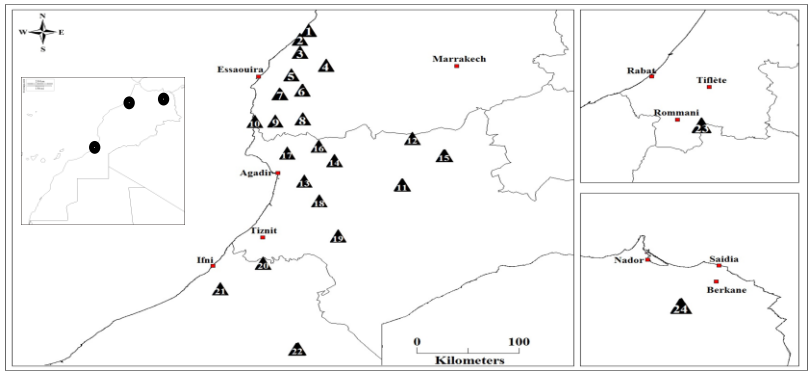

Fig 1. Map of the sampled regions of Morocco indicating the location of the Argan samples, See table 1 for codes

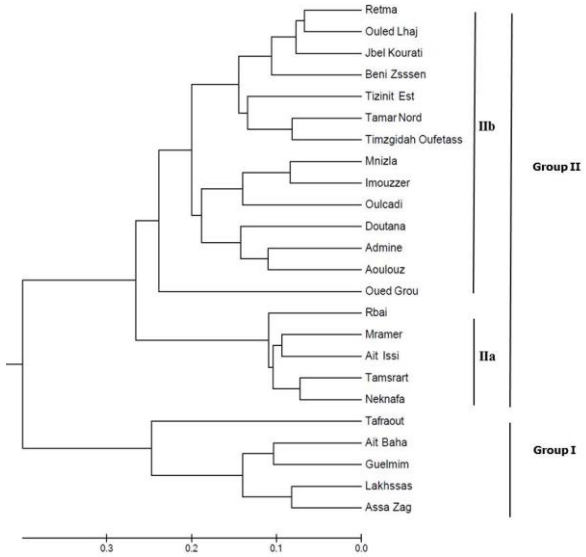

Fig 2. UPGMA dendrogram of genetic relationships among 24 collected populations of Argan based on Nei's (1972) genetic distance using SSR data.

Table 3. Genetic diversity, inbreeding coefficient in 24 argan populations based on 13 microsatellites loci.

Population	N	Na	Ne	Ar	Ho	He	F	Np
Oued Grou	10	5.077	3.624	5.1	0.631	0.640	0.040	3
Beni Snasssen	10	4.846	3.441	4.8	0.438	0.613	0.278	5
Retmana	10	6.769	5.109	6.8	0.738	0.679	-0.088	3
Ouled Lhaj	10	6.154	4.644	6.2	0.746	0.716	-0.044	1
Jbel Kourati	10	6.077	4.252	6.1	0.654	0.690	0.047	2
Mramer	10	6.692	5.256	6.7	0.777	0.727	-0.084	3
Rbai	10	6.077	4.974	6.1	0.677	0.698	0.035	0
Tamsrart	10	6.308	4.168	6.3	0.685	0.671	-0.043	3
Neknafa	10	6.154	4.607	6.2	0.754	0.686	-0.123	0
Ait Issi	10	6.308	4.915	6.3	0.677	0.664	-0.056	3
Tamanar Nord	10	5.769	4.373	5.8	0.654	0.646	-0.022	0
Timzgidah Oufetass	10	6.615	4.758	6.6	0.654	0.685	0.049	2
Oulcadi	10	6.000	4.366	6.0	0.638	0.689	0.053	0
Tizinit Est	10	6.308	4.467	6.3	0.669	0.715	0.075	2
Admine	10	6.077	3.430	6.1	0.508	0.598	0.180	5
Mnizla	10	6.077	4.249	6.1	0.646	0.706	0.125	3
Aoulouz	10	6.231	4.744	6.2	0.738	0.718	-0.017	3
Imouzzer	10	5.692	4.124	5.7	0.585	0.691	0.173	4
Doutana	10	5.846	3.968	5.8	0.631	0.667	0.088	2
Ait Baha	10	6.308	4.559	6.3	0.592	0.645	0.109	2
Tafraout	10	6.000	4.481	6.0	0.677	0.717	0.083	1
Lakhssas	10	7.846	5.600	7.8	0.723	0.735	0.050	7
Guelmim	10	3.538	2.417	3.5	0.392	0.486	0.159	0
Assa Zag	10	6.538	4.593	6.5	0.592	0.686	0.163	4
Mean	10	6.054	4.380	6.054	0.645	0.674	0.051	3.05

Note: N: sample number; *Ho*: observed heterozygosity; *He* = expected heterozygosity; F: fixation index; *Ar*: Allelic richness; Np= Number of private alleles.

Table 4. Estimates of pairewise F_{ST} among the 24 natural populations of Argan in Morocco. All estimates were significantly greater than zero (P<0.001).

	Oued Grou	Beni Zsssen	Retma	Ouled Lhaj	Jbel Kourati	Mramer	Rbai	Tamsrart	Neknafa	Ait Issi	Tamar Nord	Timzgidah Oufetass	Oulcadi	Tizinit Est	Admine	Mnizla	Aoulouz	Imouzzer	Douta	Ait Baha	Tafraout	Lakhssas	Guelmim	Assa Zag
Oued Grou	0																							
Beni Zsssen	0.070	0																						
Retma	0.094	0.132	0																					
Ouled Lhaj	0.097	0.131	0.058	0																				
Jbel Kourati	0.113	0.141	0.067	0.031	0																			
Mramer	0.104	0.126	0.060	0.031	0.026	0																		
Rbai	0.122	0.135	0.077	0.050	0.028	0.031	0																	
Tamsrart	0.126	0.127	0.090	0.078	0.087	0.084	0.083	0																
Nekfa	0.115	0.120	0.091	0.084	0.098	0.090	0.095	0.044	0															
Ait Issi	0.146	0.145	0.109	0.092	0.105	0.108	0.110	0.056	0.050	0														
Tamar Nord	0.137	0.138	0.094	0.077	0.093	0.093	0.094	0.034	0.041	0.034	0													
Timzgidah Oufetass	0.126	0.131	0.087	0.081	0.092	0.088	0.095	0.039	0.044	0.053	0.035	0												
Oulcadi	0.128	0.142	0.081	0.050	0.050	0.046	0.059	0.081	0.095	0.114	0.107	0.086	0											
Tizinit Est	0.101	0.112	0.066	0.040	0.048	0.038	0.057	0.066	0.077	0.096	0.078	0.072	0.031	0										
Admine	0.164	0.174	0.123	0.091	0.088	0.088	0.091	0.094	0.112	0.132	0.112	0.097	0.060	0.074	0									
Mnizla	0.118	0.138	0.081	0.051	0.058	0.043	0.059	0.089	0.084	0.113	0.095	0.077	0.047	0.047	0.073	0								
Aoulouz	0.105	0.129	0.073	0.038	0.063	0.050	0.070	0.087	0.085	0.105	0.091	0.074	0.057	0.044	0.083	0.047	0							
Imouzzer	0.137	0.133	0.093	0.073	0.076	0.067	0.079	0.063	0.054	0.080	0.065	0.054	0.067	0.054	0.070	0.060	0.053	0						
Douta	0.128	0.146	0.091	0.068	0.089	0.077	0.094	0.079	0.090	0.104	0.087	0.071	0.092	0.068	0.118	0.088	0.043	0.071	0					
Ait Baha	0.133	0.124	0.094	0.080	0.091	0.077	0.088	0.067	0.071	0.099	0.072	0.063	0.080	0.057	0.063	0.070	0.061	0.037	0.083	0				
Tafraout	0.122	0.134	0.082	0.043	0.057	0.061	0.067	0.076	0.084	0.087	0.075	0.067	0.056	0.051	0.068	0.055	0.039	0.051	0.060	0.060	0			
Lakhssas	0.043	0.060	0.077	0.063	0.079	0.072	0.080	0.084	0.076	0.097	0.092	0.084	0.083	0.065	0.118	0.071	0.067	0.087	0.083	0.086	0.070	0		
Guelmim	0.158	0.124	0.186	0.181	0.198	0.174	0.194	0.191	0.167	0.196	0.192	0.186	0.184	0.145	0.211	0.174	0.160	0.157	0.203	0.159	0.164	0.109	0	
Assa Zag	0.054	0.041	0.110	0.107	0.111	0.103	0.110	0.113	0.100	0.129	0.122	0.113	0.127	0.099	0.156	0.117	0.109	0.114	0.124	0.112	0.117	0.050	0.127	0

Table 5. Analyses of Molecular variance (AMOVA) among and within Argan populations.

Source	d.f	Sum of squares	Variance component	Total Variance (%)	P-value
Among Regions	3	283,318	1,282	10%	0<0.001
Among Pops	20	528,503	1,628	12%	0<0.001
Within Pops	216	2191,100	10,144	78%	0<0.001

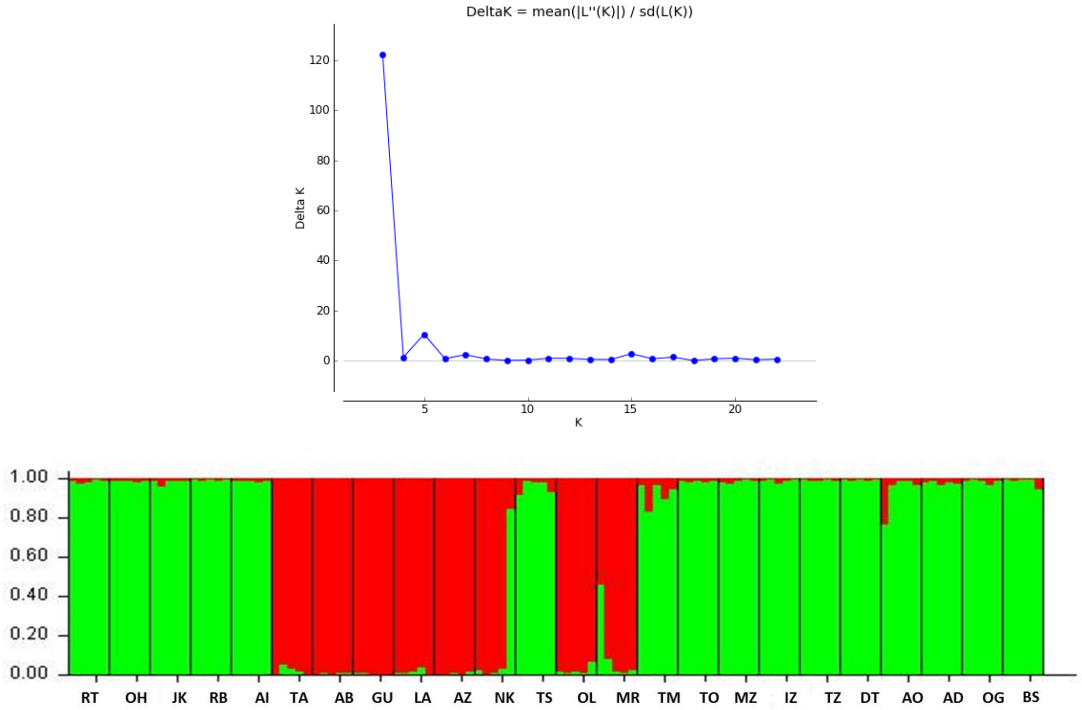

Fig 3. Estimation of L(K) and ΔK for each K value. A: ΔK values for different number of population assumed (K) in the STUCTURE analysis. B: Estimated population structure of the 24 populations with K=2 using STRUCTURE program.

for the argan forest based on alloenzymes (Fst=0.24) (El Mousadik and Petit, 1996) and based on ISSR markers (Gst= 0.40) (Mouhaddab et al., 2015) and (Gst= 0.39) (Yatrib et al., 2015). Compared to other deciduous woody plants with similar geographical distribution such as *Fraxinus mandshrurica* (He= 0.564; Fst= 0.010) (Hu et al., 2008), *Acer mono* (He= 0.737, Fst= 0.073) (Liu et al., 2014), the argan tree is considered as one of the most genetically differentiated forest resources.

Hamrick et al., (1992) has reviewed plant alloenzymes literature in plant species, summarizing parameters of genetic diversity, with special attention to trees. The results obtained in our study, comparing to what has been previously reported, showed that the argan tree, like most other woody species, maintains more variation within species and within populations but less variation among populations. Also, it appears that the argan tree, based on SSR data, has higher levels of total genetic variation (Fst=0.17) than many angiosperm where the average (Gst =0.10) .These results were also confirmed by the AMOVA, which showed that around 12% of the total molecular variance was attributable to diversity between populations (Table 5), rather than intra-population diversity.

Generally the genetic structure of plant populations reflects the interaction of multiple evolutionary processes including habitat fragmentation, population isolation, genetic drift, long-term evolutionary history and gene flow (Schaal et al., 1991). In the case of the argan tree, the low among-population genetic differentiation was mainly due to its breeding system (entomogam), its unique biological traits and the genetic isolation of its populations. It is also consistent with reports addressing other threatened or endemic species like *Moringo oleifer* Lam. (Muluvi et al., 1999) and *Elaeagnus mollis* Diels (Wang et al., 2012b).

These factors may also be associated with the amount of total genetic variation and its partition among and within populations. Also, the breeding system and the isolation of populations can increase genetic differentiation among plant species (Hogbin and Peakall, 1999). A migration rate of Nm=1 is hypothetically necessary to counter population divergence due to genetic drift (Wright, 1931). In our study, the estimated gene flow was 1.294; hence the abundant gene flow among populations might be caused by the pollen and seed movement in particular.

Moreover, *A. spinosa* L. Skeels is an important socio-economical plant, and the individuals and their habitat's size has been dramatically decreased, from an average of 27.4 trees/ha to 15.2 trees/ha between 1997 and 2007 (le Polain de Waroux and Lambin, 2012). Thus, the recent human intervention through overgrazing and over exploitation might be responsible for the endangered status of this species rather than low genetic diversity.

Also, the consistent fragmentation in its distribution and population size has increased genetic differentiation. Furthermore, the fact that the argan tree grows under different environmental conditions (Msanda et al., 2007) which means that each population is adapted to local environment could also promote genetic differentiation among populations (Hu et al., 2010). This explanation is supported by cluster and Bayesian analyses which showed that the germplasm collection of the argan tree can be divided into two main groups, which is consistent with the UPGMA tree. However, the Mantel test showed no significant correlation between genetic distance and geographic distance. Therefore, the present genetic patterns of the argan tree could be mainly due to an isolation-by-distance model (Wright, 1943), which predicts that the genetic distance between pairs of populations will increase as a function of the geographic

distance between them due to limited gene dispersal by pollen and seeds.

Conservation implications

Highs levels of genetic diversity are present in the sampled trees in *A. spinosa* L. Skeels population across its distribution area in Morocco.

Sampling for conservation and *in-situ* management of genetic resources must assure the existent diversity is conserved. Preserving the genetic variation among populations should focus on preserving the most genetically distinct populations although preserving the variation within populations must also conserve large core populations to ensure diversity is not lost because of genetic drift (Addisalem et al., 2016; Namkoong, 1988).

The maintenance of genetic variation is the major objective in conservations plans for endangered species (Hamrick and Godt, 1996b; Wang et al., 2012a). The information provided in this study give valuable baseline data on the genetic populations of *Argania spinosa* L. Skeels. These results showed that the argan populations is one of the most genetically differentiated forests, which has significant implications for the conservation on endangered species to improve the genetic basis for the argan breeding system.

First, private alleles are detected in 19 out of 24 populations. These populations should be prioritized for *in-situ* conservation. Also, *ex-situ* conservation can be critical to safeguarding endangered species (Heywood and Iriondo, 2003). Thus, we recommend the establishment of a core collection based from the different genetic cluster of this species. Finally, *in-situ* and *ex-situ* conservation must be combined to better conserve the valuable genetic diversity of the argan forest.

Materials and Methods

Sample Collection

A total of 240 individuals of *Argania spinosa* L. Skeels were sampled from 24 wild populations covering the whole distribution area of the argan forest (Table 1, Fig 1). From each population, fresh young leaves were collected from 10 randomly selected trees with a minimum of 50 m distance between them to avoid resampling from the same individuals. The locations of the studied populations were listed in Table 1 and presented in Fig 1. Plant material was then labeled and frozen at -80°C until further use.

DNA extraction

Genomic DNA was extracted from 50mg of dried leaf tissue using ISOLATE Plant DNA Mini kit (Bioline, USA) according to the manufacturer's instructions and quantified by spectrophotometry (ND-2000, Nanodrop, USA).

SSR Genotyping

After an initial screening of 24 SSR primers pairs, a total of 13 SSR pairs were used. Out of these, 5 SSR pairs are specific to *Argania spinosa* L. Skeels (El Bahloul et al., 2014) and the remaining 8 pairs were developed in 2 different species inside Sapotaceas family (*Manilkara huberi* (Azevedo et al., 2007); *Vitellaria paradoxa* (Cardi et al., 2005)) and were previously used on *Argania spinosa* L. Skeels to characterize the most common identified morphotypes of this species (Majourhat et al., 2008).

PCR essays were performed in 20µL reaction volumes including 1x reaction buffer, 50ng genomic DNA, 0.1 mmol L^{-1}dNTPs, 0.4 µmol L^{-1} of each primer, 2.5 mmol L^{-1} $MgCl_2$, and 1U Taq DNA polymerase (Promega, Madison WI, USA). The GenAmpl thermocycler (Applied Biosystems, CA, USA) was used with the program: 94°C for 2min, 1 cycle; 94°C for 30s, 48-57°C for 30s and 72°C for 30s, 35cycles; 72°C for 7min, 1 cycle and hold at 10°C.

After checking PCR amplification on a 1% agarose gel, the PCR products were pooled according to allelic length and fluorescent dye; Pool one : MH04, MH06, ME11, MH07; Pool two: ME05, MH12, MH20, MH08; Pool three: ASMS01, ASMS20, ASMS2012_04, ASMS2012_24, ASMS2012_37.

PCR products (0.25µL) were mixed with 9.5 µL of formamide and 0.5µL of the GeneScan^{TM}500 ROX ®Size Standard (Applied Biosystems). The DNA fragments were denatured and size fractioned using capillary electrophoresis on an ABI prism 3130 DNA sequencer (Applied Biosystms, Foster City, USA). Subsequently, the peaks were visually inspected and scored as alleles on the basis of the peak graph and then analyzed using GenMapper V 5.0 software (Applied Biosystems).

Genotyping and analyzing data

Based on the microsatellite data, and the obtained matrix, a set of parameters reflecting the intra and the inter genetic diversity levels, including the average number of alleles (*Na*), number of effective alleles (*Ne*), allelic richness (*Ar*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Shannon information index (*I*), Polymorphism information content (PIC) and fixation indices (F) were calculated using GenAlEx v. 6.5 (Peakall and Smouse, 2006). Analysis of molecular variance (AMOVA; (Excoffier et al., 1992)) and Mantel tests (Mantel, 1967) were also conducted using GenAlEx 6.501. Wright's *Fst* pair-wise genetic distances *(Fst)*, the frequency of null alleles (An) per locus with Brookfield method and the number of private alleles (Np) were calculated using the PopGenReport package (Adamack and Gruber, 2014) on R software.

The trustworthiness of these clusters was tested using Bayesian-based approach using the software STRUCTURE v.2.3.3 (Pritchard et al., 2000). The membership of each genotype was run with a genetic cluster value range of K=1-24 with the admixture model. Ten replicates for each values of K were run. Each run was implemented with a burn-in period of 100.000 steps, followed by 10^6 MCMC (Monte Cerlo Marcov Chain) replicates (Pritchard et al., 2000). Ln(PD) was derived for each K and then plotted to find the plateau of the ΔK values (Evanno et al., 2005). The optimal number of groups was determined using the second-order rate of change approach of Evanno et al., (2005).

In addition, relationships among the populations were constructed by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Nei's genetic distance between all pairs of populations (Nei, 1972) using Molecular Evolutionary genetics Analysis (MEGA) 6.06 software (Tamura et al., 2013).

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