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Patterns of Genetic Diversity and Structure at Fine Scale of an Endangered Moroccan Endemic Tree (*Argania spinosa* L. Skeels) Based on ISSR Polymorphism

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Abstract

The preservation of the diversity of endangered populations of argan trees, in their natural habitat, is a crucial step toward their conservation. The aim of the present study was to evaluate the genetic diversity of the argan trees in the wild, and to establish a phylogenetic map using DNA fingerprints. The ultimate goal was to develop a core set that would represent the existing diversity in the whole germplasm. In regard to this, 200 samples of *Argania spinosa* individual trees were collected from 10 different provenances in the region of Essaouira (Morocco). The genetic variation between and within these argan trees was investigated using previously described Inter-Simple Sequence Repeat markers. These markers generated a total of 149 fragments, in which 148 (99.33%) were polymorphic. The samples collected in the 'Ouled Lhaj' provenance showed the lowest diversity (% of polymorphic locus *P*=48.32%; genetic diversity Nei *h*=0.153; allelic richness *A*=1.483), compared to those collected in the 'Mramer' provenance (%*P*=68.46%; *h*=0.233; *A*=1.685). Also, the results showed a high level of genetic differentiation among provenances (AMOVA=44%, *Gst*=0.40), and a limited gene flow (*Nm*=0.73) between the provenances. In addition, these data suggested a low correlation between the genetic diversity of the tree and their respective geographical location in relation to the proximity to the littoral. Finally, a core collection of 13 genotypes that represent the essential of the detected diversity was established. The distribution pattern of this genetic diversity provides an important baseline data for the conservation strategies of argan tree species in the wild.

Keywords: argan tree, genetic diversity, ISSR, core collection, conservation

Introduction

The argan tree (*Argania spinosa* L. Skeels) is an endemic forest species in Morocco (Northern West of Africa), covering approximately 828000 hectares (Mhirit *et al*., 1998). This species belongs to *Sapotaceae* family and it is a diploid species (2n=24) (Majourhat *et al*., 2007), monoecious and allogamous (Msanda *et al*., 2005) which is believed to be pollinated by insects (El Mousadik and Petit, 1996a). *Argania spinosa* is a multi-purpose tree (oil-agriculture-forestry-pastoral) with a great genetic diversity (El Mousadik and Petit, 1996a; 1996b). Due to its diversity, the argan tree has a remarkable resilience to withstand climate change adverse, especially in geographical area with increasingly accentuated dryness. In Morocco, the argan tree plays an important role in preserving the local environment, and ensuring the socio-economical equilibrium in the area where it grows (Nouaïm *et al*., 1991). The area of Essaouira produces between 1000 to 2000 tonnes of oil per year, corresponding very roughly to a local population of 60000-120000 trees, producing 142 million to 286 million fruits per year (Moussouris and Pierce, 2000).

Knowledge of genetic diversity within and among populations is particularly important for conservation management (Jian *et al*., 2006). Indeed, species that lack an appropriate amount of genetic diversity are generally unable to survive in a changing environment (Qian *et al*., 2001). The levels

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Table 1. Geographic coordinates of selected argan trees

Origin sites	Code	Latitude (N)	Longitude (W)	Altitude (m)
'Jbel Jourati'	JK	31°47'	09°23'-09°24'	360-369
'Mramer'	MR	31°38'	09°09'-09°10'	374-397
'Retmana'	RT	32°02'	09°19'	58-80
'Tamsrourt'	TS	31°21'	09°21'-09°22'	536-637
'Ait Issi'	AI	31°02'	09°22'	966-989
'Timzgida Ouftass'	TO	31°00'	09°84'	189-231
'Ouled Lhaj'	OH	31°56'	09°23'-09°24'	116-125
'Rbai'	RB	31°31'-31°32'	09°28'	264-290
'Meknafa'	MK	31°19'	09°33'-09°34	242-266
'Tamanar'	TM	31°00'	09°37'	278-587

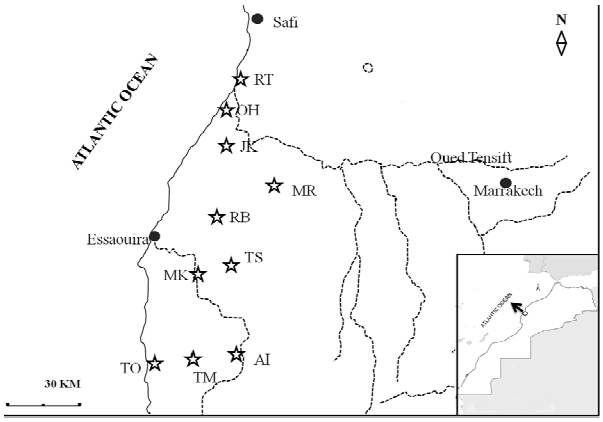

Fig. 1. The geographical distribution of the provenances of argan tree samples (see Table 1 for abbreviation)

of genetic variation between different populations and within the same population are the result of a dynamic interplay between several factors that include the gene flow, the selection effect, the inbreeding, the genetic drift and mutations (Hartl and Clark, 1994). Recently, molecular methods have provided accurate and reproducible tools to analyze the genetic variation of species, which is proven to be of great importance in planning efficient strategies for their conservation (Hamrick and Godt, 1996).

In Morocco, the argan ecosystem is subjected to a continuous degradation due to the anthropozoïc pressure, the dry climate, and the lack of natural regeneration. By consequence, the density of the argan tree forest suffered a regression from an average of 200 trees/ha to less than 30 trees/ha (Dupin, 1949; Nouaïm *et al*., 1991). As a result, the conservation of the remaining genetic resources of argan forest became a priority, especially after the UNESCO declared the argan ecosystem as a Biosphere Reserve in 1998.

Most of the studies regarding the genetic variability of the argan tree used agromorphological approaches (Ferradous, 1995; Aameur and Ferradous, 2001; Benlahbil, 2003; Bani-Aameur, 2004; Ait Aabd *et al*., 2011). These studies strongly highlighted the existence of a high phenotypic diversity. However, morphological characteristics are usually not sufficient to give a clear picture of the existing genetic diversity. By contrast, the assessment of genetic diversity using DNA markers was proven to be more accurate and reliable.

The level of the genetic diversity of the argan trees in the wild remains poorly investigated. In fact, only few studies that focused on the genetic diversity of *Argania spiniosa* were published to date. The earliest investigations studied the isozymes markers and the DNA of chloroplast using PCR-RFLP (El Mousadik and Petit, 1996a; 1996b), followed by studying the Random Amplified Polymorphic DNA (RAPD) (Bani-Aameur and Benlahbil, 2004; Majourhat *et al*., 2008), and more recently studies on the Single Sequence Repeats (SSR) or microsatellites (Majourhat *et al*., 2008). Among these various molecular tools, the Inter-Simple Sequence Repeats (ISSR) using the PCR, remains the most reproducible, and gives a larger number of polymorphic fragments per primer. Indeed, it allows the identification of a very high number of loci, using only a few primers (Zietkiewicz *et al*., 1994). This technique also does not require prior knowledge of DNA sequence in order to design primers, straightforward to perform and cost effective compared to SSR and SNP. ISSR markers have been often successfully used to evaluate the genetic diversity of many species, such as *Morus* spp. (Kar *et al*., 2008), Barby fig (*Opuntia ficus indica*) (Ganopoulos *et al*., 2015), *Quercus infectoria* (Rahmani *et al*., 2015).

The aim of the present study is to examine the genetic diversity of naturel populations of *Argania spinosa* in the wild. The ultimate goal of this work is to establish a core collection representing the diversity that still remains conserved in the argan germplasm located in the region of Essaouira.

Materials and Methods

The geographical area of the study

The samples used in this study, were collected from 10 different selected area (provenances) in the region of Essaouira (Fig. 1) (i.e. 'JbelKourati', 'Mramer', 'Retmana', 'Tamsrourt', 'Ait Issi', 'Timzgida Ouftass', 'Ouled Lhaj', 'Rbai', 'Meknafa' and 'Tamanar'). These provenances represent different eco-geographical conditions in regards to the altitude and the proximity to the littoral. The geographical data for each sample (latitude, longitude, and altitude) shown in Table 1 were collected using the global positioning system (GPS).

Biological materials

Fresh leaves were collected from 20 individual trees per each provenance, and then stored at -20 °C. The trees were selected randomly and their locations were individually registered using the Global Positioning System (GPS).

DNA extraction

The genomic DNA was extracted from 40 mg of grounded leaves, using a modified CTAB (Cethyltrimethyl Ammonium Bromide) procedure, initially described by Doyle and Doyle (1987). Briefly, 1 ml of CTAB extraction buffer (1 M Tris-HCl pH 8.0, 1.36 M NaCl; 0.5 M EDTA pH 8.0, 0.5 M Poly-Vinyl Pyrrolidone (PVP) and CTAB 2%) and 3 µl of β-mercaptoéthanol were added to the leaves and the mixture was incubated at 65 °C for 30 min. Genomic DNA was extracted using chloroform: iso-amyl alcohol (24:1) and centrifugation at 10000 rpm for 10 min, at 4 °C was performed. The DNA was precipitated, from the supernatant, using isopropanol (2/3 volume of supernatant) and incubate overnight at -20 °C. The precipitated DNA was collected by centrifugation at 14000 rpm for 15 min, and the pellet was washed successively in 76% ethanol containing 10 mM ammonium acetate and in 75% ethanol. Finally, the dried pellets of DNA were dissolved in 100

Table 2. List of ISSR primers used in this study

Code	Sequence (5'- 3')	T (°C)	[$MgCl_2$] mM	TNB	Size (bp)
ISSR1-8	CAGAGAGAGAGAGAGC	56.0	2.0	21	200-1500
ISSR3-8	YGGAGAGAGAGAGAGAGY	56.8	1.5	22	200-1000
ISSR4-8	YGACACACACACACACYG	53.5	2.0	28	200-2500
ISSR5-8	GTGTGTGTGTGTGTT	55.3	1.5	33	200-2000
ISSR6-8	YTACACACACACACACYT	56.8	1.5	19	400-1500
ISSR7/8	AGCAGCAGCAGCAGC	60.5	2.0	6	400-1500
ISSR8/8	CTCCTCCTCCTCCTCCTC	59.8	2.0	7	200-900
ISSR807	GAGAGAGAGAGAGAGAC	52.0	1.5	30	200-2000
ISSR808	CTCTCTCTCTCTCTCTA	50.7	2.0	24	200-2000
ISSR857	ACACACACACACACACG	52.0	2.0	26	200-2500

T°C: Temperature of annealing; TNB: Total number of band.

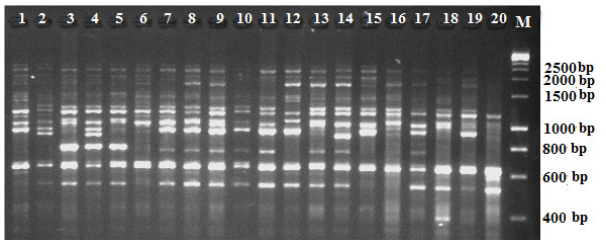

Fig. 2. DNA profile of ISSR amplification using the primer 4/8 with samples from the 'Mramer' provenance (M=size marker, bp=base pair)

µl of sterile ultrapure water. The quantity and the quality of DNA obtained were controlled by spectrophotometer and by electrophoresis in agarose gel (0.8%) containing ethidium bromide. Aliquots of DNA samples were prepared at a concentration of 50 ng/µl, and stored at -20 °C before use.

ISSR PCR amplification

In this study, ten ISSR primers that produced a clear, reproducible and relatively high polymorphism were used to estimate the genetic diversity of 200 samples (Table 2). These primers, previously described by Souto Alves *et al.* (2009), were successfully used to study a wide range of forest species (e.g., *Apterosperma oblata* (Su *et al.*, 2008), *Murraya koenigii* (Verma and Rana, 2011).

The DNA amplification by PCR was performed in a 10 µl reaction mixture containing 15 ng of genomic DNA, Taq polymerase buffer (1×), 0.2 mM of dNTP, 0.2 mM of primers (Eurofins) and 1U of Taq polymerase (Promega). The reaction mixture was supplemented with 1.5 to 2 mM of $MgCl_2$. The concentration of $MgCl_2$ varied according to the nature of the primers (Table 2). The PCR was performed in a Thermal Cycler (Applied Biosystems 2720), using a program that start with an initial denaturation cycle at 94 °C for 3 min; followed by 30 cycles of 45 s denaturation at 94 °C, annealing at 50-60 °C for 30 s (depending on the type of primers) and 1 min extension at 72 °C; and ending with final extension cycle at 72 °C, for 7 min. The PCR products were visualized by separation for 2 h at a constant voltage (90 V), on agarose gel (2%) (containing ethidium bromide).

Statistical analysis

The amplified fragments with the same mobility, on the agarose gel, according to their molecular weight (bp) were scored using a binary code [present (1) or absent (0)]. Each of these fragments represents a locus. The parameters related to the genetic diversity within and between different provenances [i.e. the percentage of polymorphic bands (*PPB*), Nei's gene diversity (*h*), Nei's genetic differentiation index among populations (G_{st}), allelic richness (*A*) and gene flow (*Nm*)] were calculated using POPGENE 1.32 (Yeh *et al.*, 2000). An estimate of *Nm* among populations was calculated using the formula of $Nm=0.5\ (1-G_{st})/G_{st}$ (McDermott and McDonald, 1993). The genetic distance matrix was employed to construct UPGMA tree (unweighted pair group method using arithmetic mean) using MEGA version 5 (Tamura *et al.*, 2011). The molecular variance (AMOVA), using GenALEX version 6.5b3 (Peakall and Smouse, 2012) was used to estimate the distribution of the genetic variation between different provenances and within the same provenance. The correlation between the genetic and geographic distances was estimated using the Mantel test. A Bayesian analysis of ISSR population structure on the entire data set, using STRUCTURE 3.2 (Pritchard *et al.*, 2000), was conducted to test for genetic admixture across provenances. STRUCTURE algorithm was run using the basic model with admixture and correlated allele frequencies, with the assumed number of genetic K clusters varying from 1 to 10 (the total number of provenances), ten replicate runs per K value(number of populations), and the burn-in period and Markov Chain Monte Carlo (MCMC) were set to 500,000 and 5000,000 iterations, respectively. To identify the number of K clusters explaining the observed genetic structure, statistical parameters defined by Evanno *et al.* (2005) based on the rate of change in the log probability of data between successive K values were used. Finally, it assembled a core collection that should represent the entire genetic diversity explored in the study using Powercore software (Kim *et al.*, 2007).

Results

Genetic diversity

The results showed (i.e. Fig. 2) a total of 149 bands detected by using 10 screened primers across 200 individuals, from the 10 provenances studied. Among them, 148 (99.33%) were polymorphic.

The 149 locus generated by 10 ISSR primers were sufficient to genetically characterize the studied provenances. To define the ISSR variation within the provenances, the POPGENE software was used to calculate five parameters that represent estimation for each locus, and the average represent the diversity in the loci. The analysis of the diversity within provenances (Table 3) showed that all the parameters followed the same direction (*A*; *h*; *NPB*; *%P*). The

Table 3. Genetic diversity within the argan provenances

Provenances	*A*	*h*	*NPB*	*%P*
'Retmana' (RT)	1.5302	0.2055	79	53.02
'Ouled Lhaj' (OH)	1.4832	0.1526	72	48.32
'Jbel Kourati' (JK)	1.6376	0.244	95	63.76
'Mramer' (MR)	1.6846	0.2332	102	68.46
'Rbaï' (RB)	1.6242	0.2032	93	62.42
'Tamsrourt' (TS)	1.557	0.209	83	62.42
'Meknafa' (MK)	1.6376	0.2053	95	63.76
'Ait Issi' (AI)	1.5772	0.2161	86	57.72
'Tamanar' (TM)	1.5638	0.1901	84	56.38
'Timzgida Ouftass' (TO)	1.6779	0.2398	101	67.79
Average	1.59733	0.20988	89	60.405
200 genotypes	1.9933	0.3530	148	99.33

A: Allelic richness; *h*: Nei's genetic diversity; *NPB*: Number of polymorphic bands; *%P*: percentage of polymorphism.

Table 4. Analysis of the molecular variance (AMOVA) among the argan trees provenances

Source of variation	DF	SS	MS	Est.var	%	*p*-value
Within provenances	9	2285,010	253,890	11,939	44%	0,010
Among provenances	190	2869,100	15,101	15,101	56%	0,010

DF: degree of freedom; SS: sum of squares; MS: Mean Square; Est.var: estimation variance; %: Percentage variance.

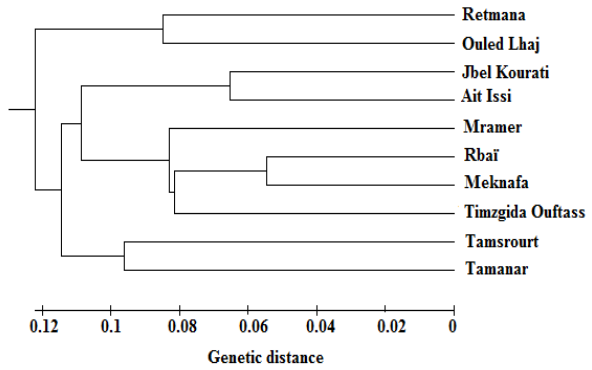

Fig. 3. UPGMA dendrogram based on Nei's genetic distances among the populations of the argan trees

highest and the lowest genetic diversity were observed in the 'Mramer' (MR) provenance (allelic richness, *A*=1.6846; Nei's genetic diversity, *h*=0.2332; % of polymorphic loci, *%P*=68.46) and 'Ouled Lhaj' (OH) (*A*=1.4832; *h*=0.1526; *%P*=48.32), respectively. On the other hand, considering all provenances as a single group, a great genetic diversity is noted (*A*=1.9933; *h*=0.353).

Genetic differentiation and gene flow

The AMOVA analysis showed that 56% of the total genetic variability resided between provenances (Table 4). This result was confirmed by the estimate of gene differentiation coefficient (*Gst*=0.4053), which indicates that 40.53% of the total genetic variability exist between different groups of trees and 59.47% was within the groups. The level of gene flow (*Nm*) was estimated to 0.7336, indicating a limited rate of gene exchange among provenances.

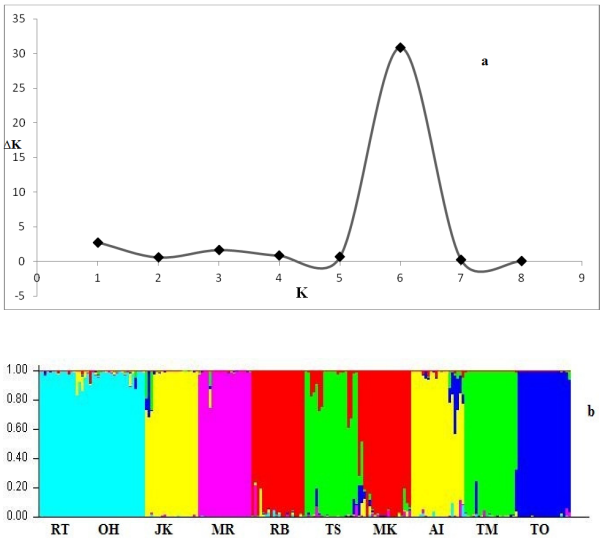

Fig. 4. The genetic relationships among the 10 provenances of argan tree (200 individuals) estimated using STRUCTURE program based on ISSR data; (a) The *ΔK* (Evanno *et al*., 2005) was plotted against various values of *K* suggesting *K=6* as the most likely number of clusters, (b) assignation of individuals to the genetic clusters at *K=6*. The y-axis shows the likelihood distance and the x-axis the accession and subgroup indicated by color. Each color represents one gene pool

Genetic relationships

The analysis with POPGENE indicated that the Nei's unbiased genetic distances is comprised between 0.1093 ('Rbaï' vs 'Meknafa') and 0.2942 ('Retmana' vs 'Mramer'). Based on this, the UPGMA dendrogram showed that the 10 provenances formed six major distinct groups of clusters (Fig. 3). The 'Retmana' and 'Ouled Lhaj' formed a single group, as well as, the 'Jbel Kourati' and 'Ait Issi' provenances. The same was observed in the 'Rbaï' and 'Meknafa' and the populations of 'Tamsrourt' with 'Tamanar', respectively. By contrast, the 'Mramer' and 'Timzgida Ouftass' formed two distinct groups of their own.

To measure the genetic divergence between these populations, it has been used the $G_{st\ pairwise}$ index (Palsbøll *et al*., 2007). The index values showed that all the populations studied were clearly differentiated. The highest genetic differentiation (0.3435) was particularly observed between 'Retmana' (RT) and 'Tamanar' (TM) (Table 5).

In the ISSR admixture analysis using STRUCTURE, the real K value with the highest value of ΔK for the 200 individuals was *K*=6 (Fig. 4a). The proportions of each provenance assigned into six clusters (cluster I and cluster II) (Fig. 4b) which result is in agreement with UPGMA dendrogram based on Nei's genetic identities. The Mantel's test (Mantel, 1967) results showed a positive but non-significant correlation between genetic structure and geographical distance (r=0.181, *P*=0.120, 999 permutations).

The construction of the core collection

Core collection is a subset of accessions from the entire collection that capture, with minimum redundancy, most of the available genetic diversity of a crop, a wild species or a group of

Table 5. The determination of the genetic differentiation between the different argan trees provenances using $G_{st\ pairwise}$ index (see Table 1 for abbreviation

Provenances	RT	OH	JK	MR	RB	TS	MK	AI	TM	TO
RT	*****									
OH	0.2701	*****								
JK	0.2932	0.3012	*****							
MR	0.3166	0.2874	0.2381	*****						
RB	0.2674	0.3217	0.2889	0.2392	*****					
TS	0.3318	0.3343	0.2929	0.2456	0.296	*****				
MK	0.2979	0.3346	0.2547	0.1973	0.1757	0.2622	*****			
AI	0.2912	0.3122	0.1778	0.2414	0.2846	0.2994	0.2584	*****		
TM	0.3435	0.3325	0.2721	0.2841	0.3187	0.2654	0.2905	0.302	*****	
TO	0.2707	0.3175	0.2364	0.2093	0.2256	0.265	0.2052	0.2564	0.2424	*****

Table 6. List of 13 trees out of 200 trees of entire collection included in core set formed by PowerCore

Individuals	Region	Latitude (N)	Longitude (W)	Altitude (m)	Number of loci
RT19	'Retmana'	32°02'28.5"	9°19'17.1"	77	100
TM19	'Tamanar'	31°00'45.7"	9°37'11.8"	565	70
MK11	'Meknafa'	31°19'16.5"	9°34'02.8"	261	85
TO4	'Timzgida Ouftass'	31°00'19"	9°48'11.3"	213	74
RT5	'Retmana'	32°02'18.7"	9°19'25.6"	70	86
AI19	'Ait Issi'	31°02'03.9"	09°22'34.3"	973	85
MR12	'Mramer'	31°38' 34.4	9°10'01.90"	378	85
JK5	'Jbel Kourati'	31°47'26.2"	9°23'59.90"	364	92
AI6	'Ait Issi'	31°02'10.7"	09°22'27.4"	983	98
TS8	'Tamsrourt'	31°21' 34,2"	9°22' 52,9"	550	83
TM17	'Tamanar'	31°00'44,7"	9°37'13,8"	562	74
MR17	'Mramer'	31°38' 40,4"	9°09' 55,50"	375	77
MR9	'Mramer'	31°38' 32,6	9°10' 06,40"	386	76

Table 7. Comparison of genetic diversity indices among the entire set and the core established

Genotypes	*A*	*h*	*NPB*	*%P*
200 genotypes	1.993	0.3530	148	99.33
13 genotypes of the core collection	1.9396	0.3630	140	93.96

A: Allelic richness; *h*: Nei's (1987) genetic diversity; *NPB*: Number of polymorphic bands; *%P*: percentage of polymorphism.

species (Brown, 1989a, b; Van Hintum *et al*., 2000, Belaj *et al*., 2012). Generally, information on geographic data, genetic data, or phenotypic data were used for grouping. To achieve this objective, an approach of maximization was adopted, using a heuristic algorithm implemented in the Power Core 1.0 software (Kim *et al*., 2007). This program allowed the selection of the most diverse genotypes covering all alleles existing in the original collection. The content of the core collection obtained using this software is shown in the Table 6. The results showed a Core collection representing existing genetic diversity that includes 13 genotypes from different regions selected by this program. This collection represents 6.5% of the total collection. The constructed core collection capture about 95% of the total diversity (*A*=1.9396; *h*=0.3630; *%P*=93.96). Indeed, the comparison of the genetic diversity indices between the genotypes included in the collection (13 genotypes) and the entire collection (200 genotypes) did not show any significant differences (Table 7).

Discussion

Genetic diversity

Genetic diversity is critical for the adaptation and the resistance to environmental changes and consequently for the long-term survival of a species. In this study, the high level of genetic diversity has reported in 'Mramer' provenance *A*=1.6846; *h*=0.2332; *%P*=68.46). By contrast, the argan tree from the provenance of 'Ouled Lhaj' (OH), located in the North and closest to the ocean (less than 3 km), were the less genetically diverse (*A*=1.4832; *h*=0.1526; *%P*=48.32). However, if considering all the provenances that were studied as a single source, a high genetic diversity is noted (*A*=1.9933, *h*=0.353) (table 3). In this work, the ISSR analysis using ten primers generated a higher polymorphism compared to other molecular markers such as isozymes (El Mousadik and Petit, 1996) and RAPD (Bani-Aameur and Benlahbil, 2004; Majourhat *et al*., 2008). In addition, the *Argania spinosa* showed a relatively higher mean of gene diversity using these ISSR markers compared to other species of trees (Zhao *et al*., 2006; JunMin *et al*., 2007; Sun *et al*., 2013; Vaishali *et al*., 2014, Rahmani *et al*., 2015). However, there was no significant difference between genetic diversity and altitude; high genetic diversity can be found in mountains and plains. Low and high genetic diversity between provenances could be attributed to many factors including, the geographical distribution combined with the level of isolation of the populations, the heavy deforestation and the extensive inhabitation.

Genetic differentiation and gene flow

The analysis of multi locus profiles has shown a very high level of differentiation inside the 10 provenances studied (G_{st}=0.4053; AMOVA=0, 44; *Nm*=0.7336), which is consistent with our previous reports that focused on isoenzymes and chloroplast DNA of the argan tree (G_{stn}=0.25; G_{stc}=0.60) (El Mousadik and Petit, 1996a; 1996b). Compared to other forest species, for instance the sessile oak that covers most of Europe

and that is characterized by a low differentiation index (G_{st}=0.026) (Kremer *et al*., 2002), it has been found that the argan tree is among the most genetically differentiated forest resources. This is consistent with reports in relation to other threatened or endemic species, like: *Pinus sylvestris* (G_{st}=0.3965) (Hui-yu *et al*., 2005); *Cupressus gigantea* (G_{st}=0.36) (Xia *et al*., 2008); *Machilus thunbergii* (G_{st}=0.4118) (Liu *et al*., 2013) and *Rheum palmatum* and *Rheum tanguticum* ($G_{st\ palmatum}$=0.537; $G_{st\ tanguticum}$=497) (Wang *et al*., 2012). The high genetic differentiation of the argan trees could be explained by several factors, including their geographical distribution, their breeding system (entomogame) and the genetic isolation of their populations. Indeed, the scattered distribution of species added to the topographic barriers can lead to difficulties in the dispersal of pollen and seeds, and consequently limit gene flow among populations (Lu *et al*., 2008). Also, it was suggested that endemism and limited distributions of populations within a species favor a high genetic differentiation (Hamrick and Godt, 1990).

Genetic relationships

The knowledge of the genetic relationships among provenances of *Argania spinosa* is important information for the efficient use and *ex situ* conservation. According to the present data analysis, using UPGMA dendrogram, the argan trees included in this study could be divided into 6 distinct groups or clusters. Four of them were formed by trees from two different provenances, (i.e. 'Retmana' and 'Ouled Lhaj', 'Jbel Kourati' and 'Ait Issi', 'Rbai' and 'Meknafa', 'Tamsrourt' and 'Tamanar'*).* By contrast, the remaining two groups were formed by trees coming from a single provenance (i.e. 'Mramer' and 'Timzgida Ouftass'*).* Bayesian analysis showed that the germplasm collection of argan tree of Essaouira could be represented by six large groups, which is consistent with UPGMA cluster analysis. Further analysis like $G_{st\ pairwise}$ confirmed this genetic structure. However, there was no correlation between the genetic structures of these populations and their respective geographic distance (r=0.181, *P*=0.120, 999 permutations), indicating that geographic isolation was not the main factors inducing genetic difference.

The genetic structure of plant populations reflects the interactions of various evolutionary processes including the long-term evolutionary history of the species (e.g., shifts in distribution, habitat fragmentation, and population isolation), genetic drift, mutation, breeding system, and selection (Slatkin, 1987; Hamrick and Godt, 1996; Schaal *et al*., 1998). Therefore, the development of genetic structure within and between the populations of the argan tree in the region of Essaouira is probably strongly associated with the gene flow caused by the movement of pollen and seeds.

Construction of nested core collections maximizing diversity

The purpose of core collections is to facilitate the use of germplasm by providing a set of accessions displaying the genetic diversity available in the larger collection (Brown, 1989a), and this core collection facilitate experimental trials to assess germplasm under contrasting environmental conditions (El Bakkali *et al*., 2013). The current data analysis provides 13 individuals (6.5% of the origin collection) defined by 10 ISSR markers (Table 6), which represent 100% of the genetic diversity of the entire collection (Table 7). The core collection established with heuristic analysis method could maintain specific alleles present in the entire collection (Zhang *et al*., 2011; Khaing *et al*., 2013). In this sense, core collections exist for some fruit tree species (Volk *et al*., 2005; Escribano *et al*., 2008; Santesteban *et al*., 2009; El Bakkali *et al*., 2013; Miyamoto *et al*., 2014). In a comparative study, Belaj *et al*. (2012) used Mstrat and PowerCore to construct a core collection of *Olea europaea* L. their results showed the high efficiency of PowerCore to capture all alleles present in the source collection. This is consistent with the results obtained in multiple previous studies that have also suggested the efficiency of PowerCore (Agrama *et al*., 2009; Zhao *et al*., 2011; Kaga *et al*., 2012; Zhang *et al*., 2012; Khaing *et al*., 2013). Considering the present results, this collection might be an appropriate choice for applications involving the conservation of genetic resources of the argan trees. Field investigations must be taken as complementary criteria to ensure optimal management of the argan forest, in particular, criteria of phenotypic and agronomic aspect, but also of socioeconomic and environmental nature as this will help to utilize germplasm in breeding programmes more effectively.

Conclusions

In the current study, the ISSR analysis was a key tool to demonstrate a high level of genetic diversity between the argan trees from twelve different areas, which provides a potential resource of germplasm that might be used in the future conservation programs. Indeed, the determination of the genetic relationships between argan tree will further improve the efficiency of sampling and using of germplasm resources.

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