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Assessing genetic diversity and constructing a core collection of an endangered Moroccan endemic tree [*Argania spinosa* (L.) Skeels]

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Abstract

Utilizing inter-simple sequence repeat (ISSR) markers, 240 genotypes of argan tree [*Argania spinosa* (L.) Skeels, *Sapotaceae*] were studied for genetic variability, phylogenetic relationship, and construction of core collection. ISSR markers chosen amplified a total of 147 bands, of which 141 (95.92%) were polymorphic. Nei's genetic diversity ranged from 0.15 to 0.29 with a mean of 0.20. Allelic richness was similar between all provenances with an average of 1.56. Our results showed a high level of genetic differentiation among provenances (AMOVA = 46%, Gst = 0.42) and a limited gene flow (Nm = 0.70). Unweighted Pair Group Method by Arithmetic, Principal Coordinates Analysis and a Bayesian-based approach clearly showed a high genetic structuring of the provenances while respecting the geographical proximity between the plains and high altitudes. There was a significant correlation between genetic distance and geographic distance by Mantel test (r = 0.52, p = 0.01). Thus, we generated a core collection with 14 entries that represent 100% of genetic diversity of the original collection, which will be useful for the future conservation programs.

Keywords: *Argania spinosa*, Conservation, Core collection, genetic diversity, ISSR markers.

Introduction

Knowledge of genetic diversity within and among populations is particularly important for conservation management (Jian *et al.*, 2006). Generally, it is investigated by pedigree studies, phenotype traits, biochemical and genetic characteristics, and molecular markers. However, the DNA markers show the variation at a genetic level, and are not affected by environmental conditions. They are distributed over the whole genome, and are more reliable than morphological markers. The Inter Simple Sequence Repeats (ISSR) have been used more frequently due to their ability to detect high levels of polymorphism. This technique is cost effective, straightforward to perform, and inexpensive (Zietkiewicz *et al.*, 1994) and does not require prior knowledge of DNA sequence for primer design.

Argan tree is an endemic forest species. It is widespread in arid and semi arid areas of south-west Morocco

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(Emberger, 1925), where distributes rich genetic resources covering approximately 948 200 hectares (FAO, 2010) mostly in the dry lowlands of the Souss valley and on the sunny mountain spurs of the Anti-Atlas and the High-Atlas. It has a major socio-economical role since it contributes to the livelihood and subsistence of rural population. The high-quality oil extracted from its seeds represents a significant contribution of fatty acids to the diet of the local population in the Souss valley. However, this species is subject to a continuous degradation due to the anthropozoïc pressure, the dry climate, and a lack of natural regeneration. Nevertheless, Dupin *et al.* (1949) mentioned a mean densities ranging from 150 to 250 trees per hectare in the Souss valley. Now, argan suffered a regression from an average of 200 trees / ha to less than 30 trees / ha in the plain (Nouaim *et al.*, 1993). For this reason, the argan forest has been declared a Biosphere Reserve by UNESCO in 1998. Therefore, the management and the conservation of the remaining genetic resources of argan forest is an urgent priority.

For endangered plant species, information on the level of genetic diversity and the population genetic structure is practically important for the management and development of conservation strategies (Hamrick, 1983; Hamrick & Godt, 1996). Currently, the genetic diversity of the argan tree is not well characterized. Few studies assessing polymorphism in *A. spinosa* have been described using isozymes markers and PCR-RFLP of chloroplast DNA (El Mousadik & Petit, 1996a; 1996b), Random Amplified Polymorphic DNA (RAPD) (Bani-Aameur & Benlahbil, 2004; Majourhat *et al.*, 2008), Single Sequence Repeats (SSR) or microsatellites (Majourhat *et al*., 2008) and recently Inter Simple Sequence Repeat applied to assess genetic diversity of the argan tree of Essaouira (Mouhaddab *et al*., 2015). In the present study, we used ISSR primers to examine the genetic structure of *A. spinosa* of the Souss valley and the Saharan area, in their natural habitat. Because these markers do not require prior knowledge of DNA sequence in order to design primers, they are highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature. In addition, ISSR markers provide highly polymorphic fingerprints and give a very high number of loci using a few primers (ensuring a good representation of the diversity of the genome) (Zietkiewicz *et al*., 1994; Kojima *et al*., 1998; Bornet & Branchard, 2001). Moreover, the ISSR markers are cheap and cost effective compared to SSR or SNP markers. ISSRs have been successfully used to estimate the extent of genetic diversity in a wide range of species which include *Morus* spp. (Kar *et al.*, 2008), Olive (*Olea eurepaea*) (Ergun, 2015), Barby fig (*Opuntia ficus-indica*) (Ganopoulos *et al.*, 2015) and *Quercus infectoria* (Rahmani *et al.*, 2015).

The aim of this study was to: i) reveal patterns of molecular polymorphism in argan trees, ii) explore the potential of ISSR markers in order to investigate the structure of genetic diversity of argan tree, and iii) establish a core collection in order to define conservation and management strategies for this species.

Materials and methods

The geographical area of the study

Twelve natural provenances of *A. spinosa* were sampled from southwest of Morocco at altitudes ranging from 84.15 to 1235 m, representing large areas of the plains and mountains and covering a wide range of ecological environments (Figure 1). Twenty trees were sampled per provenance. The geographical data for each sample (latitude, longitude, and altitude) are shown in Table 1 [data collected using the Global Positioning System (GPS)].

DNA isolation and PCR amplification

Total genomic DNA was extracted from young leaves of 240 trees using the CTAB method of Doyle (Doyle, 1987) with minor modifications. Ten polymorphic ISSR primers with high successes were used for this study (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 & Rana, 2011). Polymerase chain reactions were performed in a 10 µl volume containing 15 ng of genomic DNA, Taq polymerase buffer (1×), 0.2 mM of dNTP, 0.2 mM of primers

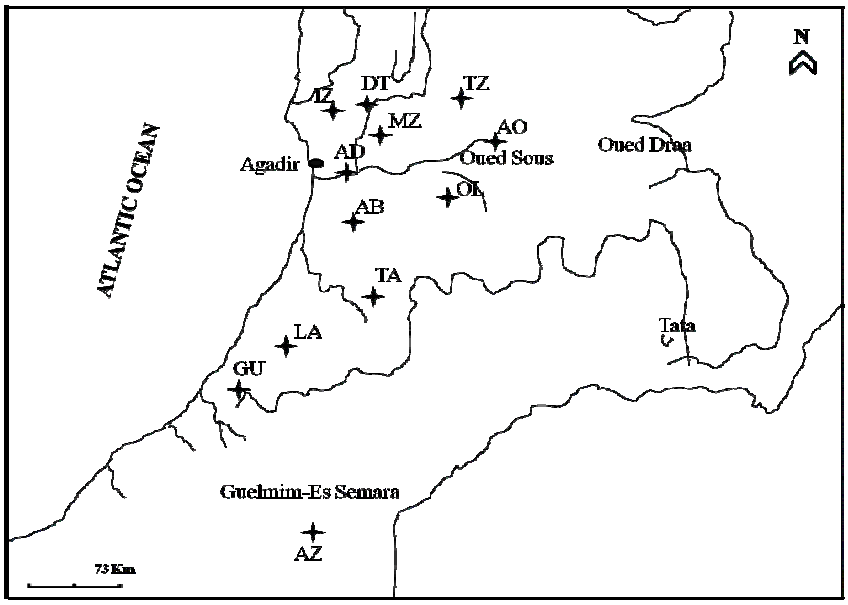

Figure 1. The geographical distribution of the provenances of argan tree samples (see Table 1 for abbreviation).

Table 1. Eco-geographical information about collection sites.

Provence	Provenance	Code name	Sample size	Longitude	Latitude	Altitude (m)
Taroudant	Oulcadi	OL	20	8°28'	30°17'	1235
Taroudant	Tizi Nteset	TZ	20	8°23'	30°48'	1193,95
Agadir	Admine	AD	20	9°21'	30°19'	84,15
Taroudant	Menizla	MZ	20	9°05'	30°33'	253,55
Taroudant	Aoulouz	AO	20	08°06'	30°37'	788,8
Agadir	Doutama	DT	20	9°13'	30°43'	791
Agadir	Immouzar	IZ	20	9°30'	30°39'	1093,6
Chtouka Ait Baha	Ait Baha	AB	20	9°13'	30°06'	511
Tiznit	Tafraout	TA	20	9°03'	29°42'	854
Tiznit	Lakhessas	LA	20	9°43'	29°24'	974
Guelmim-Es Smara	Guelmim	Gu	20	10°06'	29°06'	351,25
Guelmim-Es Smara	Assa Zag	AZ	20	9°24'	28°26'	328,95

(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, see Table 2. The PCR was performed in a Thermal Cycler (Applied Biosystems 2720), using a program that start with an initial denaturation cycle at 94ºC for 3 min; followed by 30 cycles of 45 s denaturation at 94ºC, annealing at 50-60ºC for 30 s (depending on the type of primers) and 1 min extension at 72ºC; and ending with final extension cycle at 72ºC, for 7 min. Amplification products were visualized by separation for 2 h at a constant voltage (90 V), on Agarose gel (2%).

Table 2. List of ISSR primers used in this study.

Code	Motif	Sequence (5'- 3')	T (°C)	[$MgCl_2$] mM	TNB	Size (bp)
ISSR1/8	(AG)8C	CAGAGAGAGAGAGAGAGC	56.0	2.0	21	200-1500
ISSR3/8	(GA)8YG	YGGAGAGAGAGAGAGAGAGY	56.8	1.5	22	200-1000
ISSR4/8	(AC)8YG	YGACACACACACACACACYG	53.5	2.0	28	200-2500
ISSR5/8	(GT)8T	GTGTGTGTGTGTGTGTT	55.3	1.5	33	200-2000
ISSR6/8	(AC)8YT	YTACACACACACACACACYT	56.8	1.5	19	400-1500
ISSR7/8	(AGC)6	AGCAGCAGCAGCAGCAGC	60.5	2.0	6	400-1500
ISSR8/8	(CTC)8	CTCCTCCTCCTCCTCCTC	59.8	2.0	7	200-900
ISSR807	(GA)8C	GAGAGAGAGAGAGAGAC	52.0	1.5	30	200-2000
ISSR808	(CT)8A	CTCTCTCTCTCTCTCTA	50.7	2.0	24	200-2000
ISSR857	(AC)8G	ACACACACACACACACG	52.0	2.0	26	200-2500

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

Data analysis

ISSR bands were scored as present (1) or absent (0) and entered into a binary matrix representing the ISSR profile of each provenance. The data matrix was analyzed using POPGENE version 1.32 software package (Yeh *et al.*, 2000) to calculate various genetic diversity parameters: percentage of polymorphic bands (PPB), percentage of polymorphic loci (% P), number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (h) (Nei, 1973), Nei's genetic differentiation index among populations (Gst) and gene flow (Nm).

The GenAlEx version 6.5b3 (Peakall & Smouse, 2012) was used to calculate estimates of population differentiation using a Principle Coordinate Analysis (PCA) and Analysis of Molecular Variance (AMOVA). The GenAlEx was also used to perform Mantel test (Mantel, 1967) in order to check relationships among genetic and geographical distances. The genotypes were then clustered by UPGMA (Unweighted Pair Group Method by Arithmetic averages) on the basis of Nei's (1978) genetic distance unbiased using MEGA version 5 (Tamura *et al.*, 2011). The population structure was examined with a Bayesian Markov Chain Monte Carlo method, which was implemented in the Structure 3.2 software (Pritchard *et al.*, 2000). The program was run by a number of clusters (K) from 1 to 12 with a burn-in period of 500,000 iterations followed by 5000,000 iterations. The most likely number of populations (K) was estimated using Evanno method (Evanno *et al.*, 2005) based on the rate of change in the log probability of data between successive K values. At the end, we estimated the minimum size necessary to capture all the observed alleles using maximization approach by a heuristic algorithm implemented in the Power Core 1.0 software (Kim *et al.*, 2007) and the procedure was employed in developing core subset from ISSR data.

Results and Discussion

Genetic diversity

A total 240 of individual trees *A. spinosa* from 12 provenances surveyed generated a total of 147 fragments using 10 selected ISSR primers, of which 141 (95.92%) were polymorphic (Table 3). The observed numbers of alleles (Na) ranged from 1.48 to 1.65 with an average of 1.56. The effective numbers of alleles (Ne) ranged from 1.25 to 1.42 with an average of 1.57. The highest and the lowest genetic diversities were observed in provenances Menizla (h = 0.29, NPB = 95, %P = 64.63) and Doutama (h = 0.15, NPB = 72 %P = 48.98) respectively. However, considering all provenances as a single group, a very important genetic diversity is noted (Na = 1.96, Ne = 1.57, h = 0.34, NPB = 141, %P = 95.92). In this study, ISSR analysis using ten primers generated a higherpolymorphism compared to other molecular markers such as isozymes (El

Mousadik & Petit, 1996b) and RAPD (Bani-Aameur & Benlahbil, 2004; Majourhat *et al*., 2008). Thus, *A. spinosa* showed relatively higher mean gene diversity as compared with other tree species using ISSR markers (Zhao *et al.*, 2006; JunMin *et al.*, 2007; Sun *et al.*, 2013; Vaishali *et al.*, 2014; Ergun, 2015). On the other hand, the provenances of mountainous argan with altitudes between 788.8 to 1235 m have similar levels of diversity to valley argan with altitudes ranging between 84.15 to 511 m. Therefore, there was no significant difference between genetic diversity and altitude. Low and high genetic diversity at provenances level could be attributed to many factors including restrictive geographical distribution, isolated populations, heavy deforestation, and extensive habitat.

Table 3. Genetic diversity within the argan provenances.

Provenances	Code	Na	Ne	h	NPB	%P
Oulcadi	OL	1.59	1.39	0.22	87	59.18
Tizi Nteset	TZ	1.52	1.33	0.19	76	51.70
Admine	AD	1.55	1.36	0.20	81	55.10
Menizla	MZ	1.65	1.42	0.29	95	64.63
Aoulouz	AO	1.52	1.31	0.18	77	52.38
Doutama	DT	1.48	1.25	0.15	72	48.98
Immouzar	IZ	1.63	1.40	0.23	93	63.27
Ait Baha	AB	1.58	1.34	0.20	86	58.50
Tafraout	TA	1.58	1.36	0.21	86	58.50
Lakhessas	LA	1.53	1.32	0.19	78	53.06
Guelmim	GU	1.53	1.30	0.18	78	53.06
Assa Zag	AZ	1.48	1.28	0.16	71	48.30
Average	-	1.56	1.34	0.20	8167	55.56
240 Trees	-	1.96	1.57	0.34	141	95.92

Na: number of alleles; **Ne**: effective number of alleles; **h**: Nei's (1987) genetic diversity; **NPB**: Number of polymorphic bands; **%P**: percentage of polymorphism.

Genetic differentiation among populations

Genetic differentiation between the different provenances using $Gst_{Pairwise}$ index varied from 0.18 (Tizi Nteset vs Ademine) to 0.38 (Guelmim vs Doutama) (Table 4). The coefficient of genetic differentiation (Gst) was 0.42, suggesting a high genetic differentiation among populations. Similarly, the hierarchical AMOVA for all ISSR data set showed that 54% of total variation was within provenances, 46% resided between provenances. The analysis of multilocus profiles showed a very high level of differentiation inside the twelve provenances studied, which is consistent with our previous reports using isozyme markers (Gst_n = 0.25), chloroplast DNA (Gst_c = 0.60) and ISSR markers (Gst = 0.41) of the argan tree (El Mousadik & Petit, 1996a; 1996b; Mouhaddab *et al*., 2015). The argan tree is among the most genetically differentiated forest resources, which is similar to the high genetic differentiation in endangered and endemic forester species like *Torreya jackii* (Gst = 0.63) (Li & Jin, 2007), *Cycas guizhouensis* (Gst = 0.43) (Xiao *et al.*, 2004), and *Populus cathayana* (Gst = 0.477) (Lu *et al.*, 2006). The high value of differentiation reflects the interactions of various factors including: a reduced geographic range, the saharan and arid interior climates, their breeding system (entomogame), and genetic drift or genetic isolation of populations.

The estimated number of migrants per generation (Nm) was 0.70, which suggested that the gene flow in *A. spinosa* was restricted. Gene flow is generally considered as the main factor that could homogenize the genetic structure of populations in their distribution area. According to Wright (1931), Nm¼ 1 is sufficient to overcome the effects of genetic drift. Also, species with low gene flow have higher genetic differentiation than species with high gene flow. These results can be explained by topographic barriers preventing the dispersal of seeds and pollen. Also, insect pollination is often associated with a reduced gene flow among populations. On the other hand, it

was suggested that endemism and limited distributions of populations within a species favor high genetic differentiation (Hamrick *et al.*, 1990).

Genetic structure

Population pairwise relationships showed the smallest genetic distance between the provenances Tizi Nteset and Ademine (0.1194) and the highest between provenances Doutama and Ademine (0.2809). UPGMA cluster analysis was performed to explore the relationship among provenances based on Nei's genetic distance. Twelve provenances of *A. spinosa* were clustered into two major distinct groups. The nine provenances (Tizi Nteset, Ademine, Menizla, Ait Baha, Tafraout, Assa Zag, Aoulouz, Lakhessas and Guelmim) were clustered into one group that were further delineated into two sub-clusters, while the provenances Oulcadi, Immouzar and Doutama were clustered into the other group (Figure 2). The pattern of genetic diversity and population structure was further analyzed with a Bayesian-based approach implemented in Structure software. We investigated the range from K = 1 to K = 12 and determined the posterior probability for each value of K using the estimated log likelihood of K. The real gene pools (K) were determined using the statistics proposed by Evanno *et al*. (2005). In the ISSR admixture analysis using Structure software, the highest ΔK value K = 4 appeared to be the perfect model for argan genetic structure in the plains and mountains of southwestern Morocco (Figure 3a). Bayesian approach structured all provenances into four groups (Figure 3b). The first one regrouped three provenances (OL, AD and IZ). The TZ, AO, MZ and AB constituted the second cluster. The third group included TA, AZ, LA and GU provenances. Doutama provenance constituted an isolated population. Principal coordinate analysis (PCoA) data based on Nei's genetic distance explained 37.68% of the total variation (Figure 4). The second axis (19.21%) separated the majority of provenances from the plains and mountains

Table 4. Genetic differentiation between the different provenances using $Gst_{pairwise}$ index.

	Oulcadi	Tizi Nteset	Admine	Menizla	Aoulouz	Doutama	Immouzar	Ait Baha	Tafraout	Lakhessas	Guelmim	Assa Zag
Oulcadi	****											
Tizi Nteset	0.278	****										
Admine	0.246	0.180	****									
Menizla	0,271	0.221	0 .235	****								
Aoulouz	0,267	0.218	0.302	0.238	****							
Doutama	0,314	0,344	0.367	0.280	0.359	****						
Immouzar	0,238	0,288	0.282	0.239	0.306	0.299	****					
Ait Baha	0,280	0,262	0.277	0.228	0.254	0.302	0.316	****				
Tafraout	0,266	0,229	0.276	0.203	0.277	0.304	0.279	0.219	****			
Lakhessas	0,329	0,278	0.294	0.279	0.335	0.238	0.313	0.260	0.248	****		
Guelmim	0,324	0,273	0.311	0.290	0.342	0.380	0.313	0.309	0.286	0.323	****	
Assa Zag	0,313	0.241	0.247	0.248	0.313	0.365	0.297	0.252	0.236	0.278	0.283	****

of high Atlas to those of the Anti-Atlas. These revealed similar groupings to UPGMA were in agreement with Bayesian analysis and confirmed the genetic distinctiveness of 240 genotypes. The Mantel test showed that there was significant correlation between geographical distance and pairwise genetic distance ($r = 0.52$, $P < 0.01$, 999 permutations) (Figure 5). These different analyses showed a similar genetic structure of all provenances studied. Thus, the provenance of Doutama is well separated from the other populations and represents a unique gene pool; this can be explained by its geographical isolation. These results indicate that the genetic relationships among provenances were consistent with their geographical proximity.

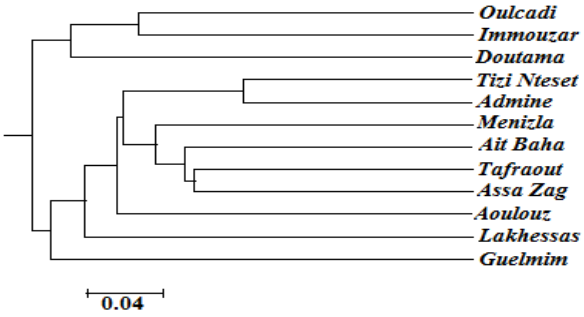

Figure 2. UPGMA cluster analysis of twelve provenances of *A. Spinosa* with ISSR markers. The dendrogram was made using the Nei's (1978) genetic distances.

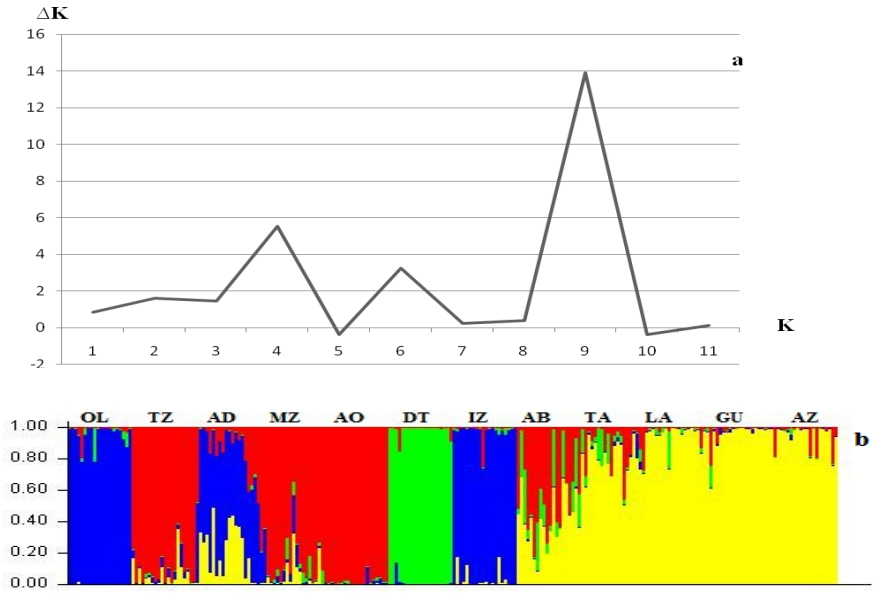

Figure 3. The genetic relationships among the 12 provenances of argan tree (240 individuals) estimated using STRUCTURE program based on ISSR data; (**a**) The ΔK (Evanno *et al.*, 2005) was plotted against various values of K suggesting K = 6 as the most likely number of clusters, (**b**) assignation of individuals to the genetic clusters at K = 4. The y-axis shows the likelihood distance and the x-axis show the accession and subgroup indicated by color. Each color represents one gene pool.

Figure 4. Relationships among 12 provenances of *A. spinosa* visualized by Principal Coordinate Analysis (PCoA) of ISSR data (see Table 1 for abbreviation).

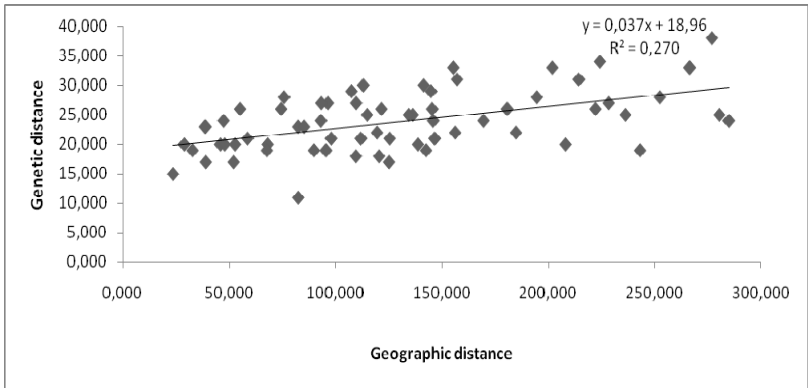

Figure 5. Mantel test between genetic distance and geographical distances (km) among argan provenances.

Developing a Core collection of argan tree

Despite the high genetic diversity found in the whole collection, the development of a core collection capable of maximizing variation while minimizing sample number could be desirable for argan tree breeding strategy, management conservation and scientific research. In this study, Powercore software (Kim *et al*., 2007) allowed the selection of the most diverse genotypes covering all alleles existing in the whole collection. The content of the core collection revealed by this software is shown in the Table 5. The number of entire set eventually retained on core collection was 14 trees of the original collection. The genetic diversity of this collection was analyzed by calculating the total number of alleles (N_a) and effective number of alleles (N_e), genetic diversity of Nei's (h); Number of polymorphic bands NPB; percentage of polymorphism %P. However, when compared to the whole collection, the core subsets had a similar genetic diversity of all the calculated parameters (Table 6). Therefore, this selection belongs to all provenances except Admine and Assa Zag which does not present a high level of genetic diversity to maintain. In a comparative study, Belaj *et al.* (2012) used Mstrat and PowerCore establish a core collection of *Olea europaea* L. their results demonstrated the high efficiency of PowerCore to capture all alleles present in the source collection. Additionally, Kim *et al.* (2007) reported that the core collections constructed by PowerCore preserve the set of alleles and/or observations existing in the core collection while maintaining 100% coverage, less redundancy and reproducibility of the selection using the same dataset. This is consistent with the results obtained in multiple previous studies that have also suggested the efficiency of PowerCore (Agrama *et al.*, 2009; Kaga *et al.*, 2012; Zhang *et al.*, 2012; Khaing *et al.*, 2013).

The core subsets developed in this study are representative of the genetic diversity of the collection and the core with 14 individuals successfully captured all of the alleles (100%) existing in the whole collection. It was selected with a relatively low sampling intensity (6% of the whole collection) but was highly efficient at maintaining genetic diversity, making it potentially appropriate choice for applications involving genetic conservation of the argan tree on the one hand. Moreover, by maximizing the genetic diversity in a smaller number of genotypes, this core could facilitate the study of the variability and correlation of morphologic and agronomic traits. Future studies may also afford interesting insights about currently unknown argan diversity (morphological traits, argan oil quality and productivity parameters, resistance to desertification, etc.) into the selected core subsets in comparison to the whole collection.

Table 5. The genetic basis of the collection of 14 individuals built by PowerCore.

Individuals	Region	Latitude (N)	Longitude (W)	Altitude (m)	Number loci
IZ7	Immouzar	9°30'51"	30°39'00,8"	1107	87
IZ15	Immouzar	9°30'46,2"	30°38'59,6"	1093	88
OL2	Oulcadi	8°28'54,4"	30°17'20"	1230	90
OL18	Oulcadi	8°29'24,4"	30°17'18,5"	1217	83
GU7	Guelmim	10°06'41,4"	29°06'53,6"	358	81
GU20	Guelmim	10°06'38,5"	29°06'43,3"	342	82
AO3	Aoulouz	08°06'37,6"	30°37'12,7"	803	87
AO13	Aoulouz	08°06'52,8"	30°37'11"	783	83
AB17	Ait Baha	9°13'38,2"	30°06'25,5"	507	82
TA12	Tafraout	9°03'33,9"	29°42'45,1"	847	84
MZ4	Menizla	9°05'38,5"	30°33'42,1"	258	87
DT18	Doutama	9°13'57"	30°43'30,8"	798	82
TZ2	Tizi Nteset	8°23'42,3"	30°48'52,2"	1180	93
LA7	Lakhssas	9°43'44,9"	29°24'05,7"	991	82

Implications for conservation of wild resources

The preservation of genetic variability is one of the main objectives in conserving endangered species (Hamrick & Godt, 1996). Previous studies showed that the most relevant criteria for measuring diversity should be allelic richness, in particular in the context of genetic conservation (Schoen & Brown, 1993; Bataillon *et al.*, 1996; El Mousadik & Petit, 1996b). Allelic richness certainly provides the best indicator to identify populations that warrant special conservation effort. The results of this study showed that *A. spinosa* has high genetic diversity, with the existence of a high differentiation among populations. Therefore, great efforts should be made to preserve all the extant populations and their habitats in the field, especially for those populations with higher genetic diversity, e.g., Menizla (Na = 1.65, Ne = 1.42, h = 0.29, %P = 64. 63), Immouzar (Na = 1.63, Ne = 1.40, h = 0.23, %P = 63. 27) and Oulcadi (Na = 1.59, Ne = 1.36, h = 0.22, %P = 59.18). These should be given priority for *in situ* conservation, and to be the source for the *ex situ* conservation. A core collection is a subset of large germplasm collection that contains individuals chosen to represent the genetic variability of the germplasm collection.

Table 6. Comparison of genetic diversity indices among the entire set and the core established.

Genotypes	Na	Ne	h	NPB	%P
Whole collection	1.96	1.57	0.34	141	95.92
Core collection	1.96	1.63	0.36	141	95.92

Conclusion

In summary, our study shows a genetic diversity of a core collection of an endangered Moroccan endemic tree (*A. spinosa*). Using genetic variability, phylogenetic relationship, and construction of core collection, we showed a high genetic structuring of the plant provenances and the geographical proximity between the plains and high altitudes. There still remains a general lack of data for understanding the dynamics *in situ* of genetic diversity in relation to environmental and anthropogenic factors. Potential improvements could be made by the integration of evolutionary factors over time and space that could be necessary for the translation of research results into descriptive tools to aid decision for conservation programs.

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