Genetic diversity of the endangered argan tree (*Argania spinosa* L.) (sapotaceae) revealed by ISSR analysis

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Abstract

Genetic variation in 150 trees of *A. spinosa* was estimated using inter-simple sequence repeat (ISSR). The plant material used in this study were originated from five provenances belonging to the principal provinces of argan ecosystem in southwest of Morocco. Based on the nine ISSR primers, 86 loci were scored. At the population level, percentage of polymorphic bands (PPB) was ranged from 75.58 to 82.56 %, Nei’s genetic diversity index (H) ranged from 0.30 to 0.34, Shannon’s information index (I) ranged from 0.44 to 0.49. Analyses of spatial genetic structure using UPGMA, the principal coordinate analysis and Bayesian-based approach from Structure program, showed that the entire provenances were successfully assigned to three groups. There was no significant correlation between genetic distance and geographic distance by Mantel test (r = 0.026, P = 0.942). The genetic differentiation coefficient (Gst = 0.22) was high, it was similar to the result of the analysis of molecular variance (28 % of the total variation among provenances). According to the genetic diversity results, the conservation and improvement strategies should be taking in consideration the provenance factor. So this study should be made appropriately the large sampling for the better representing of all diversity existing on the scale of the natural distribution of the argan.

Keywords: *Argania spinosa*, ISSR marker, Genetic diversity, Conservation

INTRODUCTION

Genetic diversity is necessary for adaptation to environmental changes and for long-term survival of a species. Although, the database of genetic diversity in natural populations of forest species is the initial step in the management of genetic resources. Various strategies have been employed to evaluate the degree of genetic diversity based on morphological, chemical, biochemical, and molecular markers. Evolutionary of genetic variation influenced by several forester improvements, including harvesting, refore Provenance, and other silvicultural practices. So, for endangered plant species knowledge the level of genetic diversity and the population genetic structure is practically important for the management and development of conservation strategies (Hamrick, 1983; Hamrick and Godt, 1996).

Argan tree is an important and popular forest tree. It is one of the endangered species in south west of Morocco, where distributes rich genetic resources and covers approximately 828 000 ha (M’Hirit et al., 1998). It is known for the quality of its oils. Used mainly for food,
MATERIALS AND METHODS

Plant material

Five natural provenances of *A. spinosa* were sampled from the south-west of Morocco at altitudes ranging from 109 to 916 m and covering a wide range of ecological environments. Thirty trees were sampled per provenance. The sampling locations and their origins were listed in Table 1 and Figure 1.

DNA extraction

Total genomic DNA was isolated from all the 150 trees using a Doyle and Doyle (1987) method with minor modifications. The 40 mg of leaf material was grinded with 1 ml of Cetyltrimethyl Ammonium Bromide (CTAB) extraction buffer containing 2% CTAB, 1 M Tris-HCl pH 8.0, 2% EDTA (0.5 M; pH 8.0), 2 M NaCl, and 2% Poly-Vinyl Pyrrolidone (PVP), with 3 µl of B-mercaptoethanol putted directly into Eppendorf tubes. Following incubation at 64 °C for 1 h, 1 ml of Chloroform: Iso-amyl Alcohol (24:1) was added, mixed for 15 min manually and centrifuged at 12000 rpm at 4 °C for 12 min, the supernatant was transferred to a new tube and then repeated two times. DNA was precipitated with iso-propanol (2/3 volume of supernatant), then incubated for night and centrifuged at 14000 rpm under 4 °C for 10 min. the supernatant discarded and the pellet washed with 70% ethanol containing 10mM ammonium acetate. The pellet was air dried for 20 min and suspended in 100 µl of sterile ultrapure water. The quality and concentration of the extracted DNA samples were determined with UV spectrophotometer and checked using 0.8 % agarose gel electrophoresis with TBE 1 % buffer containing ethidium bromide. After the determination of the initial concentration for each sample, the concentration of DNA was calculated and diluted to 100 ng/ul in sterile ultrapure water and stored at -20 °C for further PCR analyses.

ISSR analysis

Nine ISSR primers (Eurofins) were used to estimate the
Table 1. Eco-geographical information about collection sites

<table>
<thead>
<tr>
<th>Province</th>
<th>Provenance</th>
<th>Code name</th>
<th>Sample size</th>
<th>Climate</th>
<th>Altitude (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taroudant</td>
<td>Aoulouz</td>
<td>Ao</td>
<td>30</td>
<td>Arid</td>
<td>737 - 850</td>
</tr>
<tr>
<td>Essaouira</td>
<td>Had dra</td>
<td>Hd</td>
<td>30</td>
<td>Semi-arid</td>
<td>181 - 226</td>
</tr>
<tr>
<td>Agadir</td>
<td>Alma</td>
<td>Al</td>
<td>30</td>
<td>Arid</td>
<td>275 - 430</td>
</tr>
<tr>
<td>Chtouka Ait Baha</td>
<td>Biougra</td>
<td>Bi</td>
<td>30</td>
<td>Arid</td>
<td>109 - 137</td>
</tr>
<tr>
<td>Tiznit</td>
<td>Lakhsas</td>
<td>La</td>
<td>30</td>
<td>Arid</td>
<td>916 - 988</td>
</tr>
</tbody>
</table>

Figure 1. Map showing geographic distribution of the five sampled provenances of argan trees.

genetic diversity, representing di and tri repeats. Three of these contained the AC repeat motif with three possessed one to two nucleotides anchor sequence at the 3’ end and the 5’ end. Two contained the GA repeat motif, and two are tri-nucleotides repeat motif. PCR reactions were performed with a thermal cycler (Mastercycler gradient eppendorf) to determine the optimal annealing temperature (Ta). So, different temperature (Tm) was tested and MgCl₂ concentration. Temperatures leading to clear patterns were then repeated until the best Ta was selected for routine procedure. The reproducibility of the amplification was confirmed by carrying out two independent PCR with one negative control. The polymorphic ISSR markers are given in Table 2. So, the MgCl₂ concentration and annealing temperature varied according to the primer.
Table 2. List of nine ISSR-PCR primers, annealing temperatures (Ta), MgCl2 concentration optimized for PCR amplification and length of the amplification products

<table>
<thead>
<tr>
<th>Primer code</th>
<th>Motif</th>
<th>Sequence (5′–3′)</th>
<th>[MgCl2] (mM)</th>
<th>Ta (°C)</th>
<th>Size (pb)</th>
<th>TNB</th>
<th>NPB</th>
<th>PPB %</th>
<th>No. of bands in each population</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISSR 807</td>
<td>(GA)BC</td>
<td>5′GAGAGAGAGAGAGAGAC 3′</td>
<td>1.5</td>
<td>52.0</td>
<td>200 - 1000</td>
<td>12</td>
<td>11</td>
<td>91.67</td>
<td>12/12 12/12 12/12 12/12</td>
</tr>
<tr>
<td>ISSR 808</td>
<td>(CT)BA</td>
<td>3′CTCTCTCTCTCTCTCTCTA 5′</td>
<td>2.0</td>
<td>50.7</td>
<td>200 - 1400</td>
<td>14</td>
<td>10</td>
<td>71.43</td>
<td>14/14 7/14 9/14 10/14</td>
</tr>
<tr>
<td>ISSR 857</td>
<td>(AC)BG</td>
<td>3′ACACACACACACACACG 5′</td>
<td>1.5</td>
<td>52.0</td>
<td>250 - 1500</td>
<td>13</td>
<td>13</td>
<td>100.00</td>
<td>11/13 11/13 11/13 11/13</td>
</tr>
<tr>
<td>ISSR 1/8</td>
<td>(AG)BC</td>
<td>5′CAAGAGAGAGAGAGAGAC 3′</td>
<td>2.0</td>
<td>56.0</td>
<td>200 - 1000</td>
<td>11</td>
<td>11</td>
<td>91.67</td>
<td>12/12 12/12 12/12 12/12</td>
</tr>
<tr>
<td>ISSR 3/8</td>
<td>(GA)BG</td>
<td>5′YGAGAGAGAGAGAGAGAC 3′</td>
<td>1.5</td>
<td>56.8</td>
<td>300 - 1500</td>
<td>13</td>
<td>9</td>
<td>69.23</td>
<td>11/13 11/13 12/13 13/13</td>
</tr>
<tr>
<td>ISSR 4/8</td>
<td>(AC)BG</td>
<td>5′YGACACACACACACACYG 3′</td>
<td>2.0</td>
<td>53.5</td>
<td>200 - 2000</td>
<td>9</td>
<td>8</td>
<td>88.89</td>
<td>9/9 7/9 9/9 9/9</td>
</tr>
<tr>
<td>ISSR 5/8</td>
<td>(AC)BY</td>
<td>5′YTACACACACACACACYT 5′</td>
<td>1.5</td>
<td>56.8</td>
<td>400 - 1500</td>
<td>4</td>
<td>3</td>
<td>75.00</td>
<td>4/4 4/4 4/4 4/4</td>
</tr>
<tr>
<td>ISSR 7/8</td>
<td>(AGC)E</td>
<td>5′AGCACAGCAGCAGCAGCAGC 3′</td>
<td>2.0</td>
<td>60.5</td>
<td>400 - 1800</td>
<td>4</td>
<td>3</td>
<td>75.00</td>
<td>4/4 4/4 4/4 4/4</td>
</tr>
<tr>
<td>ISSR 8/8</td>
<td>(CTC)E</td>
<td>5′CTCCTCTCTCTCTCTCTCT 3′</td>
<td>2.0</td>
<td>59.8</td>
<td>400 - 2000</td>
<td>5</td>
<td>3</td>
<td>60.00</td>
<td>5/5 5/5 5/5 5/5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>86</td>
<td>71</td>
<td>82.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>9.56</td>
<td>7.89</td>
<td>80.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Y: stands for pyrimidine (C or G); Ta: Temperature of annealing; TNB: Total number of band; NPB: Number of polymorphic bands; PPB %: Percentage of polymorphism bands (%). Y: stands for pyrimidine (C or G)

Data analysis

Genetic determinism of ISSR primers is dominant. The amplified fragments of DNA were scored for presence (1) or absence (0) of bands, with each of these presented as an allele. Data was compiled in a 0/1 binary data matrix of 86 loci and 150 individuals using MS Excel. The data matrix was analyzed using POPGENE version 1.32 software package (Yeh et al., 2000) to calculate various genetic diversity parameters: the number of polymorphic loci, percentage of polymorphic loci, Nei’s (1973) gene diversity index, Shannon’s information index of genetic diversity. The genetic diversity for each population was measured by the number of alleles (Na), effective number of alleles (Ne); Nei’s (1973) gene diversity (H), Shannon’s information index (I), number of polymorphic bands (NPB) and percentage of polymorphic bands (PPB). Genetic differentiation among populations was estimated by pairwise Gst values.

So, at the interprovenances level, total genetic diversity (Ht), genetic diversity within populations (Hs) and Nei’s (1973) coefficient of genetic differentiation among populations (Gst) were calculated. The average level of gene flow (Nm) among populations was indirectly estimated by a traditional method based on Gst value [Nm = (1-Gst)/4Gst] (Slatkin and Barton, 1989). Analysis of molecular variance (AMOVA) was conducted to estimate the distribution of genetic variation among and within populations, using GenALEX version 6.5 (Excoffier et al., 2005). The variance components were tested statistically by non-parametric randomization tests using 999 permutations. A UPGMA (Unweighted Pair Group Method by Arithmetic averages) dendrogram was constructed on the basis of Nei’s (1978) genetic distances unbiased. Principle Coordinate analysis based on Nei’s (1978) genetic distances was constructed to obtain a graphical representation of the relationship between the 150 test individuals. Bayesian clustering approach was implemented.
ISSR amplification profiles using primer 808 (a) and 807 (b) for two provenances, Lanes 200.

for all ISSR markers using Structure 2.3.3 software (Falush et al., 2007; Pritchard et al., 2000), to evaluate the population structure and to assign individuals into subpopulations. The admixture model with correlated allele frequencies was selected as an appropriate option for the analysis. The burn-in period and Markov Chain Monte Carlo (MCMC) were set to 500,000 and 5000,000 iterations, respectively, and 10 replicates per K were run. As recommended by the software authors in the instructions manual successive K value (number of populations) from 1 to 10 was used to obtain the distinct cluster and to estimate number of subpopulation. So, the best K that resulted in the greatest variation among groups was selected based in data likelihood and DK modal value (Evanno et al., 2005). In order to check relationships among genetic and geographical distances, a Mantel test was performed (Mantel, 1967).

RESULTS

Extraction and purification of DNA

Total Genomic DNA was successfully extracted and purified from 150 accessions with three repetitions, using the modified CTAB protocol (Doyle and Doyle 1987). There were no difficulties to isolate DNA free from contaminating proteins and polysaccharides. In the preference, after precipitation of Nucleic acids into isopropanol solution, the homogenate was extracted twice with chloroform: iso-amyl alcohol (24:1) mixed for 10 min manually and centrifuged at 10,000 rpm. Different quantity of fresh leaves was tested 40 mg, 100 mg and 200 mg, so the genomic DNA was isolated from 40 mg fresh was retained from following extraction. Quantity of DNA extracted in 40 mg was measured by spectrophotometer and ranged between 100 ng/ul and 4000 ng/ul. The DNA purity ranged from 1.60 to 1.90 measured by ultraviolet spectrophotometer.

ISSR polymorphism

Selection of primers

The present study is the first to use inter-simple sequence repeat (ISSR) data to assess genetic diversity in A. spinosa provenances. So, in the preliminary study, we examined the microsatellite-based ISSR markers used in all study of genetic diversity with ISSR markers cited in (SoutoAlves and Vanusa da Silva, 2009) and were successfully used in a wide range of forest species, e.g., Apterospema oblata (Su et al., 2008), Murraya koenigii (Verma and Rana 2011), Populus cathayana (Lu et al., 2006), etc. that used a great number of primers with (AG), (GA), (CT), (AC). We first assayed the reproductively of these primers in order to select suitable polymorphic ISSR markers for the present study.

ISSR-PCR reaction system optimizing and ISSR profile analysis

The effects of magnesium concentration and annealing temperature were tested for each ISSR markers. Nine primers that produced clear and reproducible fragments were selected for further analysis. So, the annealing temperatures and magnesium concentrations used in the present study are summarized in Table 2. Using the data from all PCR amplification bands from 150 individuals, the nine ISSR primers amplified a total of 86 bands, of which 71 bands were polymorphic. The percentage of polymorphism of the amplified products was 82.56% (Table 2). The size of amplified bands ranged from approximately 200 to 2000 bp. Primer 8/8 possessed the lowest polymorphism (60.00%), while primers 857 showed the highest polymorphism (100%). The ISSR pattern of same genotypes of Lakhsas and Biougra provenances amplified by primer 808 and 807 is presented in Figure 2. In the present study, tri-ISSR
Table 3. Intra-population diversity measures of wild A. spinosa based on ISSR data. Na = number of alleles; Ne = effective number of alleles; H = Nei’s (1973) gene diversity; I = Shannon’s information index; NPB = Number of polymorphic bands; % PPB = Percentage of polymorphic bands.

<table>
<thead>
<tr>
<th>Provenance size</th>
<th>Na ± Standard deviation</th>
<th>Polymorphic bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aoulouz</td>
<td>30</td>
<td>1.81 ± 0.39</td>
</tr>
<tr>
<td>Had Dra</td>
<td>30</td>
<td>1.80 ± 0.40</td>
</tr>
<tr>
<td>Alma</td>
<td>30</td>
<td>1.77 ± 0.41</td>
</tr>
<tr>
<td>Biougra</td>
<td>30</td>
<td>1.83 ± 0.38</td>
</tr>
<tr>
<td>Lakhsas</td>
<td>30</td>
<td>1.76 ± 0.43</td>
</tr>
<tr>
<td>Mean</td>
<td>30</td>
<td>1.78 ± 0.34</td>
</tr>
</tbody>
</table>

Table 4. Genetic distance of Nei (1978) between provenances of A. spinosa.

<table>
<thead>
<tr>
<th>Aoulouz</th>
<th>Had Dra</th>
<th>Alma</th>
<th>Biougra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Had Dra</td>
<td>0.17***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alma</td>
<td>0.20***</td>
<td>0.19***</td>
<td></td>
</tr>
<tr>
<td>Biougra</td>
<td>0.19***</td>
<td>0.20***</td>
<td>0.07***</td>
</tr>
<tr>
<td>Lakhsas</td>
<td>0.19***</td>
<td>0.18***</td>
<td>0.19***</td>
</tr>
</tbody>
</table>

Table 5. Analysis of molecular variance (AMOVA) within and between provenances of A. spinosa.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>VC</th>
<th>% of variation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among provenances</td>
<td>4</td>
<td>666.77</td>
<td>166.69</td>
<td>5.13</td>
<td>28 %</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Within provenances</td>
<td>145</td>
<td>1878.43</td>
<td>12.96</td>
<td>12.96</td>
<td>72 %</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>2545.21</td>
<td>18.08</td>
<td>100 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df: Degree of freedom ; SS: Sum of squares; MS: Mean of squares; VC: Variance components.

(AGC)₆ and (CTC)₆ primer generated the lowest percentage of polymorphic markers. Previously, the dinucleotide repetitive sequence primers were found to be more amenable to ISSR analysis, namely such as microsatellite DNA sequences including (AG)n, (GA)n, (AC)n than (CT)n.

Genetic structure

The genetic diversity among the five argan provenances is showed in Table 3. The numbers of polymorphic loci ranged from 65 to 71, and the percentages of polymorphism (P) ranged from 75.58 % to 82.56 % with an average of 79.54 %. The observed numbers of alleles (Na) ranged from 1.76 ± 0.43 to 1.83 ± 0.38 with an average of 1.79 ± 0.40. The effective numbers of alleles (Ne) ranged from 1.53 ± 0.37 to 1.61 ± 0.38 with an average of 1.57 ± 0.38. The range of Nei’s genetic diversity (H) and Shannon’s information indexes (I) were 0.30 to 0.34 and 0.44 to 0.49, with an average of 0.32 ± 0.16 and 0.46 ± 0.22, respectively. Among these provenances investigated, Had Dra exhibited the highest genetic variability (H = 0.34; I = 0.49; PPB = 80.23 %), whereas the population Al exhibited the lowest variability (H = 0.30; I = 0.44; PPB = 77.91 %) as shown in Table 3.

Based on pairwise Nei’s genetic distances (Nei, 1978), the results showed higher significant differentiation among the five provenances, that ranged from 0.07 (between Biougra and Alma) to 0.23 (between Biougra and Lakhsas) Table 4.

Genetic differentiation was obtained using AMOVA analysis, and it was highly significant (P < 0.001). The results showed that 28 % of the genetic variation was portioned existed among the provenances and 72 % within the provenances (Table 5). According to AMOVA analysis, the Gst coefficient of genetic differentiation was high (0.22). The level of gene flow (Nm) was 1.78, indicating a low or limited rate of gene exchange among provenances.

Clustering and genetic relationships among argan provenances

The dendrogram obtained from UPGMA cluster analysis of genetic distance based on nine ISSR markers are presented in Figure 3. UPGMA cluster analysis showed
clear genetic distinct among the five provenances. So, the dendrogram consisted of three main clusters (Figure 3). Thus, the cluster I regrouped Ao and Hd provenance, cluster II clustered La provenance and cluster II regrouped Al and Bi provenances, with the most distinct distance from cluster I.

Relationships between individual populations were illustrated by principal coordinate analysis (PCO) for ISSR data and also support their UPGMA clustering (Figure 4). Three main clusters could be distinguished in PCO (Figure 4). The first cluster contained 60 individuals from Aoulouz and Had Dra provenance, whereas clusters II contained Alma and Biougra individuals respectively, the third principal coordinates isolate genotypes from Lakhsas provenance.

In general, genetic clustering obtained with Structure program were in good agreement with the UPGMA phenogram based on Nei’s genetic distance and PCO analyses. This program assessed by means of the Bayesian algorithm to estimates the likelihood of the individuals being structured in a given number of genetic clusters (or genetic populations, K). According to the results, the analyses of assignment probabilities for 150 individuals in each cluster reveals increased resolution of clusters supposed K= 3 (Figure 5). So, the genotypes were grouped into three main distinct clusters. And this program clustering showed that Lakhsas provenance is genetically distinct from two main clusters (Figure 5) and generate two homogeneity groups Alma with Biougra and Aoulouz with Had Dra provenances.

A Mantel test (Figure 6) was used to test the correlation between the genetic and the geographical distances. So, the result of this test showed a positive but non-significant correlation ($r = 0.026$), indicating that isolation by distance is not sufficient to explain the present genetic structure.
Figure 5. Bayesian analysis. The y-axis shows the likelihood distance and the x-axis show the accession and subgroup indicated by color. A total of 150 trees showing assignment to three subgroups; sub-group one (blue) accessions from Aoulouz and Had Dra; sub-group two (red) including Alma and Biougra accessions; sub-group three (green) containing predominately Lakhsas accessions.

Figure 6. Mantel test between genetic distance and geographical distances (km) among five argan populations.

DISCUSSION

Genetic variation of ISSR in *A. spinosa*

ISSR proved to be useful markers to assess the level of polymorphism and diversity of many forest plant species (Huang et al., 2008; Verma and Rana, 2011; He et al., 2012; Khan et al., 2012; Maquia et al., 2013). The present work is the first application of the ISSR markers to assess the genetic variability among argan provenances. The polymorphism level varies with the nature (unanchored, 3'-anchored, or 5'-anchored) and sequence of the repeats (motif) in the primer employed (Reddy et al., 2002). ISSR with these repeat motifs (AG), (GA), (CT), (TC), (AC), (CA) show higher polymorphism than primers with tri- or tetra-nucleotide repeats and are most abundant in ISSR genetics study (Reddy et al., 2002). Four of the nine primers used in this study generated the highest percentage of polymorphic markers (75 to 60 %). The dinucleotide repetitive sequence primers (GA)n, (AG)n, (AC)n, (CT)n, (CA)n were found to be more including in to analyze of genetic variability of several endangered, rare and endemic forest species (Qiu et al., 2004; Cao et al., 2006; Lu et al., 2006; Su et al., 2008; Kumar et al., 2010; Verma and Rana, 2011; Jin et al., 2013). In this study, ISSR analysis using nine primers generated more than 80 fragments for all the five populations and are generally 200-2000 pb long, and tend to generate higher polymorphism compared to other molecular markers such as isozymes (El Mousadik et al., 1996a) and RAPD (Bani Aameur and Benlahbil, 2004, Majourhat et al., 2008). So, genetic diversity of *A. spinosa* at the population level was considerably high when compared with various endangered and endemic forester species using various using ISSR markers (the percentage of polymorphic loci PPB = 79.54 %, Nei’s genetic diversity $H = 0.29$, Shannon’s information index $I = 0.43$). So, for *Torreya*
Population genetic diversity and genetic structure

As evident from the present study, the levels of genetic diversity determined among five populations of *A. spinosa* were high with respect to the various genetic diversity parameters used. Furthermore in populations of rare and endangered species, high levels of genetic diversity are expected, especially for those with strictly limited distribution and small population size (Hedrick, 1985; Ellstrand and Elam 1993). The genetic differentiation and gene flow are important indices to estimate the population genetic structure of species. The mean genetic differentiation coefficient (St) estimated from 86 loci was 0.22, indicating that our results were consistent with the finding found for isozyme markers (El Mousadik et al., 1996). The high genetic differentiation within populations of argan accessions was confirmed by the AMOVA analysis, which indicated that around 28% of the total variance was attributable to among population diversity and 72% was attributable to within population diversity. The high within population genetic estimated might be significant to indicating existing the several hypotheses which could explain this level of diversity in argan population.

There is a relative genetic approach between Alma and Biougra (Souss plain) on the one hand and between Had Dra and Aoulouz (Chialedma plateau and mountain western High Atlas) on the other hand, and isolation from Lakhsas (Mountain: Anti Atlas West). In other words, gene flow is limited between Lakhsas and other provenances. Geographical barriers and dominate wind direction from south to north may explain the importance of genetic divergences (St = 0.22). However, the mode of reproduction entomogame could contribute to the differences obtained, as found by previous work in the argan tree using allozyme marker (St = 0.25, El Mousadik and Petit 1996a), the PCR-RFLP and the DNAcp markers (St = 0.60, El Mousadik and Petit 1996b).

The genetic structure of plant populations reflects the interactions of many different processes including the long-term evolutionary history of the species (e.g., shifts in distribution, habitat fragmentation, and/or population isolation), mutation, genetic drift, breeding system, gene flow, selection and life form (Slatkin 1987; Hamrick and Godt 1996; Gabrielsen et al., 1997; Schaal et al., 1998). All of these factors may be associated with the amount of total genetic variation and its partitioning among and within populations. According to Hogbin and Peakall (1999), breeding system, genetic drift or genetic isolation of populations can cause high level of genetic differentiation among plant species populations. In this study, the gene flow (Nm) or the movement of gene within and between populations estimated was 1.78. Gene flow plays an important role in determining the genetic structure. Hence, Nm value greater than 1.0 is considered necessary to prevent divergence resulting from genetic drift (Wright 1951). Therefore, ample gene flow among populations might be attributed primarily to pollen movement.

Based on the Nei’s genetic distance, the largest genetic difference was occurred among Biougra and Lakhsas populations and a lower level was noted among Biougra and Alma population. As a possible, the low differentiation between the Biougra and Alma populations explain by the smooth topography dominate that may have facility gene flow, via both pollen and seeds. However, gene flow via seed dispersal show important that pollen dissemination in argan tree, when comparing the coefficients genetic nuclear differentiation (Gst) and cytoplasmic (Gstcp) (El Mousadik and Petit 1996b), dissemination via animals (ruminants) and some rodents would be also determining the space genetic structuring of the argan.

In contrast, there was no clear relationship between genetic diversity and altitude, high genetic diversity can be found in mountain and also in flat topography localization. Instead of altitude, topography seems to be the main factor affecting the genetic structure. Hence, dominated of rocky mountain massifs in Lakhsas played a dual role in isolate refuge and in differentiation with others populations. Analyses of spatial genetic structure showed the entire populations were successfully assigned to three subgroups, as indicated by PCoA, UPGMA and Bayesian-based approach from structure program indicating that ecological differentiation is the factor that promotes and maintains the divergence among different species. The genetic variation of argan tree is not only caused by environmental factors, but also by individual differences. This implication was supported by the result of the UPGMA dendrogram, and the PCoA also showed that there was no clear geographical trend.
between natural populations of A. spinosa. However, the correlation determined using the Mantel test between the diversity matrix based on genetic and geographic revealed a non-significant relationship (r = 0.063; p > 0.05), indicating that geographic isolation was not the main factors inducing genetic difference.

Implications for conservation

During 1970-2007, the geographical history of argan population size has been rapidly reduced (forest density declined by 44.5% during this period) (Waroux and population size has been rapidly reduced (forest density During 1970-2007, the geographical history of argan genetic diversity within and among populations of argan trees gives informations to guide conservation strategy. All used marker in others argan search provided similar pattern of genetic differentiation among populations. Thus, across our sample (five provenances), three gene pool was considered. So, considering the importance of genetic diversity within populations and high genetic differentiation among populations warrant urgent need for conservation of this natural population, so, we should take necessary measures to constitute a germplasm collection representing the essential of genetic diversity in natural area of Argan tree. So, this study oriented towards sampling strategies considering a maximum of eco-geographical variations, since over 20% of diversity is shared between populations. As a result, in situ conservation should be considered firstly.

Conclusion and perspective.

In conclusion, the results in this study indicated a great level of genetic variation among ISSR marker and ISSR was an effective method to evaluate the genetic diversity in argan tree.

AMOVA analysis provides high levels of genetic diversity within argan populations, indicating that each population of A. spinosa is very important for this endangered and endemic forested gene pool. The UPGMA, PCoA and Structure analyses were clustered the argan species into three groups. A Mantel test indicating that isolation by distance geographic is not sufficient to explain the present genetic structure. The ultimate goals of conservation should be designed to preserve high genetic variation within population and to prevent potential extinction for this endangered species. This study should be continued to further evaluate the genetic diversity across the natural distribution of the argan, in order to conceive the selection of stands to preserve and protect. The results show the importance of the genetic structure based on geographical distribution, which makes the goal of conservation and preservation of the genetic diversity of argan more pertinent.

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