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RESEARCH ARTICLE

Effect of habitat fragmentation on the genetic structure of the *Hypericum humifusum* L. (Hypericacae) in Tunisia

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Abstract

In Tunisia *Hypericum humifusum* occurs in small scattered populations decreasing progressively in size. However, no conservation programs are attempted to promote and preserve the potential value of this resource. In this work, we assessed the genetic diversity of 11 populations of *H. humifusum* collected from different geographical regions using nine isozyme systems. Fourteen polymorphic loci were detected and several ones were rare alleles according to population level. The species showed a high genetic diversity within populations (Ap = 1.72; P% = 54.54%; Ho = 0.214). A relatively high level of differentiation (F_{ST} = 0.154) associated with a restricted gene flow among populations (Nm = 1.37) was revealed. A significant population differentiation ($0.046 < F_{ST} < 0.260$) was observed. The relationship between F_{ST} and geographic distance matrices was not significant indicating that the genetic structure among populations is more linked to fragmentation of populations. Nei's genetic distances (D) among pairs of populations were low ($0.001 < D < 0.107$). The substantial differentiation between populations and their high genetic similarity suggest their recent divergence as a result of habitat fragmentation and isolation of populations. The PCA and UPGMA analysis, performed on all populations, showed three groups. Population groupings occur without evident relationship with bioclimates or geographic regions. The high differentiation between populations and their high genetic similarity suggest their recent divergence as a result of habitats fragmentation. The ex-situ conservation should be based on sampling seeds within rather than among populations.

Introduction

Plant species of the genus *Hypericum* are well known for their use in traditional medicine, due to the therapeutic efficacy of its many different species, some of which have been used as, bactericide, anti-inflammatory, diuretic and sedative properties (Dias et al., 2000). Extracts from aerial parts of *Hypericum* plants typically contain the phloroglucinol derivatives hyperforin and adhyperforin, the naphthodianthrones hypericin and pseudohypericin (Patocka 2003). The pharmacological activities of *Hypericum* extracts are mainly attributed to hypericins and hyperforin (Medina et al., 2006; Uzdensky et al., 2006). Flavonoids, the main components present in *Hypericum* plants, might play a role in preventing cardiovascular diseases and several kinds of cancer (Chu et al., 2000). Increased market demand for Hyperici herba has led to several investigations of secondary metabolite levels in plants from different areas of the world.

H. humifusum L., is the most widely distributed in the north and the center of the country mainly in crop fields, and mixed to cereal cultivation. The species is widely used in folk medicine for its antiseptic, astringent and antispasmodic properties (Le Floc’h, 1983).

The dynamic of *H. humifusum* populations was influenced by the dispersion of seeds and dry fragments or entire individuals by wind with unknown information about their dispersal distance (Béjaoui et al., 2010). The species tends to occur in scattered metapopulations, often with a low size and can become occasionally more abundant, forming extensive populations, within and at the edges of several crop fields with upper semi-arid zone (Béjaoui et al., 2010). The fragmentation of populations and their disturbance are main factors causing random genetic drift which enhances genetic erosion (Young et al., 1996; Van Rossum and Prentice, 2004), and reduces the adaptability of populations to future environmental changes (Ellstrand and Elam. 1993). Populations in fragmented habitats are expected to become differentiated due to founder effects, genetic drift and increased inbreeding, and a reduction in gene flow among populations (Templeton et al., 1990). A reduction in population size due to habitat fragmentation may result in loss of allelic richness or gene diversity (Lande, 1999). This can occur through population bottlenecks at the time of disturbance and genetic drift (Ellstrand and Elam, 1993). Habitat fragmentation in continuous, panmictic populations may create a metapopulation structure and limited gene flow. Metapopulations may experience frequent local extinctions and recolonization (Booy et al., 2000). Besides creating a metapopulation structure, habitat fragmentation may exacerbate local extinction, because it often alters demographic parameters and environmental factors. Thus the study of the genetic consequences of habitat fragmentation in *H. humifusum* populations has implications for species conservation.

Allozymes provide useful insights into the genetic variation of populations (Liu et al., 2006). They exhibit simple mendelian inheritance, codominant expression and are neutral or nearly neutral (Hamrick and Godt, 1989). However, the marker can lead for biased estimates of the genetic diversity, because the variation is detected only at coding loci, and reproducibility may vary according to both organs and development stage (Wendel and Weeden, 1989).

The aim of this study is to i) determine the pattern and extent of genetic diversity within and among Tunisian *H. humifusum* populations from different bioclimatic zones, using isozymes. ii) compare the level of genetic population structure revealed by data. iii) apply these informations for the development of appropriate conservation strategies.

2. Materiel and Methods

H. humifusum populations were collected from eleven sites belonging to the sub humid. upper semi-arid and lower semi-arid bioclimatic zones (Emberger, 1966). The populations were collected within *Triticum durum* and *T. aestivum* fields after harvesting, and in roadsides near these fields. The main ecological traits of sites were reported in Table 1. Ten plants from each population were sampled at distances exceeding 50 m to avoid the sampling of closely related individuals. Samples were placed on ice in plastic bags and transported to the laboratory for genetic analyses.

2.1. Isozyme electrophoresis

One hundred and fifty milligrams of leaves from each sample were ground with liquid nitrogen. After grinding 500 µL of Tris- HCl (0.1 M, pH 7.5) mixed with 1% (v/v) β-mercaptoethanol, 1.5% (w/v) bovine serum albumin (BSA), 10 µL EDTA and 50 mg polyvinylpyrrolidone 40000 were added. The homogenate was centrifuged for 25 min at 12000 rpm and at 4°C. The supernatant was adsorbed in Whatman no. 3 filter paper then introduced into gels.

2.2. Data analysis

Two gel-buffer systems using 13% starch gels were used to assay nine enzyme systems: Phosphoglucomutase (PGM, E.C.2.75.1), Phosphoglucoisomerase (PGI, E.C. 5.3.1.9), Malate dehydrogenase (MDH, E.C.1.1.1.37), 6-Phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44) and Isocitrate dehydrogenase (IDH, EC. 1.1.1.42) were assayed using a histidine-citrate gel and electrode buffer system at pH 6.5 and a gel buffer dilution of 1:3 (0.065 mol/L L-histidine and 0.02 mol/L citric acid). The histidine-citrate gel was run at 20 mA and at 4°C, for 8 hours. Staining protocols were carried out followed standard methods (Goodman et al. 1980; Weeden and Wendel, 1989). Zymograms were genetically interpreted according to standard principles (Wendel and Weeden, 1989). Loci were sequentially numbered (1, 2, 3…) in decreasing order of the anodal mobility. Alleles at a locus were coded alphabetically with the most anodally migration allozyme designated “a”. All enzymes were interpreted as dimeric enzymes except for PGM which is monomeric (Wendel and Weeden, 1989).

The genetic variation within populations or within ecological groups (each group includes populations from the same bioclimate) was estimated using allele frequencies, the percentage of polymorphic loci (P) at 95% criterium, the mean number of alleles per polymorphic locus (Ap), and averages of the observed (Ho) and expected (He) heterozygosities. Calculations were made using Biosys software package (Swofford and Selander, 1981). For each locus and each population, departure from Hardy-Weinberg equilibrium was estimated by Wright’s fixation index (F_{IS}) (Wright, 1965). The genetic divergence among populations was estimated by Nei's (1978) unbiased genetic distances. The genetic differentiation among populations or among ecological groups was estimated by Wright's (1965) F-statistics: F_{IT} (total inbreeding) and F_{ST} (subdivision among populations) and F_{IS} (inbreeding within populations), according to Weir and Cockerham (1984) estimates. Calculations were made using the program FSTAT versions 1.2 and 2.9.3 (Goudet, 1995; 2001). Means and standard errors over loci were obtained by jackknifing. The significance of indices was tested using permutations.

Gene flow (Nm) among populations was estimated by the average of the effective number of migrants exchanged between populations for each generation using Crow and Aoki's correction (Crow and Aoki, 1984), [$Nm = [(1/F_{ST})-1]/4\alpha$, where $\alpha = (n/(n -1))^2$, and n being the number of populations]. The relationship between Nm and geographic distances (km) was tested using Slatkin's method (Slatkin, 1993). The significance of the correlation between the two matrices was evaluated by Mantel's test (Mantel, 1967) using the program TFPGA 1.3 (Miller, 1997).

The genetic structure and the relationship among populations was determined using a Principal Component Analysis (PCA) generated from allelic frequencies for the polymorphic loci using the Multi-Variate Statistical Package MVSP 3.1 program (Kovach, 1999). Factorial Correspondence Analysis (FCA) generated from bands frequencies using a SAS program version 6 (SAS, 1990). A dendrogram using the Unweighted Pair Group Method with Arithmetic Averaging Algorithm (UPGMA) based on these distances was constructed.

3. RESULTS

3.1. Genetic diversity and population structure based on isozymes

Eight out of the five surveyed isozymes (MDH, PGM, IDH, PGI and 6PGD), were polymorphic. These systems led to the identification of ten loci, two out of them were monomorphic. The within population genetic diversity was high (Table 2). The allele IDH-1b is rare and characterized populations Slimene and Ain Errahma, respectively. The MDH-1b is present in all populations (data not shown). The number of alleles per polymorphic locus (Ap) varied from 1.4 (population El Ameime) to 2 (populations Aïn Younes and Aïn Errahma) with an average of 1.72. The average of polymorphism was 54.54%. The observed heterozygositiy (Ho) ranged from 0.061 (population Nebre) to 0.344 (population El Ameime). All Ho values were higher than those noted for He. However, the excess of heterozygosity was observed for all populations.

Table 3, yield Wright fixation indices (measuring the deviation from panmixia for loci detected) 52 tests have been performed, 41 out of them not present a deviation from the Hardy-Weinberg equilibrium (i.e. non-significant χ^2 test), 9 show a highly significant difference ($P < 0.01$) and three tests were significant ($P < 0.05$). At the loci level the only deficit of heterozygosity ($P < 0.001$) was observed for populations Daouar Chouarnia, Marjâa Aouem and Ain Errahma, respectively for loci PGM-2 and MDH-2. The loci PGI-1 and MDH-2 show an excess of heterozygosity for populations Ain Younes and Nebre 2, respectively.

F_{ST} values at the loci level varied from 0.046 (pgd-3) to 0.260 (pgm-2). A relatively high genetic structure among populations ($F_{ST} = 0.154$), and a low level of gene flow among them (Nm = 1.37) were observed (Table 4). Mantel's test did not show significant correlation between Nm and geographic distance matrices ($r = 0.01$, $P > 0.05$ after 1000 permutations).

The Principal Component Analysis (PCA), computed on allelic frequencies for the polymorphic loci for each population, showed that the first three principal axes represent 67.64% of the total variation. The first axis (30.27% of the total variation) is mainly correlated to loci MDH-2a, PGM-2a, PGM-2b, PGD-2a, PGD-2b, PGD-3a and PGD-3b. The second axis represents 20.47% of the total variation and corresponds to PGI-1a, PGI-1b, PGI-1c, MDH-1a, PGM-2c, PGD-1a and PGD-1b. The third principal component (16.71% of the variation) was more correlated to IDH-1a, IDH-1b, MDH-1b and MDH-2b.

The plot of the first two PCA axes (50.74% of the total variation) revealed a wide dispersion of populations (Fig 1). The group, situated at the negative side of the axis 1, includes populations Aïn Younes, Borj Massaoudi and Zagouhan. Populations El Ameime and Marjâa Aouem constituted, situated at the positive side of axis 1. Populations from the sub humid (Menzel Temime and Slimene) and lower semi-arid (Nebre 1, Nebre 2, Daouar Chouarnia and Aïn Errahma).

The PCA did not clearly show that, for the majority of populations, grouping had resulted from ecological factors and/or geographic location. The plot according to axes 1 and 3 (46.98% of the total inertia) showed a similar genetic population structure.

The FCA based on band frequencies matrix for all populations according to the axes 1 and 2 (53.56% of the total variation) revealed a large overlap points, indicating a strong electrophoretic similarity between individuals (Fig 2). A similar genetic population structure comparable to that observed by the PCA was observed.

Nei's (1978) genetic distance values between pairs of populations (data not shown) were low and varied from 0.001 (between populations Borj Messaoudi and Nebre1 despite their geographical distance to 0.107 (between populations El Ameime and Daouar Chouarnia from the upper semi-arid and lower semi-arid bioclimate, respectively). The average of Nei's distance over all populations was 0.035.

Populations neighboring have low genetic distances for populations Menzel Temime Zagouhan, Nebre 2 and Daouar Chouarnia. The genetic identity indices between populations are high, the highest index (I = 1.000) was observed between the pair of population Borj Messaoudi and Nebre1, respectively.

The UPGMA phenogram analysis showed three distinct groups of populations (Fig 3). The first one is constituted, respectively, by the populations Aïn Younes, Borj Messaoudi, Nebre1, Zagouhan and Daouar Chouarnia. The second includes the populations from upper semi-arid (Marjâa Aouem) and lower semi-arid (Aïn Errahma), respectively. Population El Ameime from the upper semi-arid bioclimate constituted the third group. Within each group, populations were not strictly clustered together according to bioclimate and/or geographic proximity.

Table 1. Location and main ecological traits for the eleven Tunisian of *H. humifusum* populations analysed.

Populations	**Bioclimatic zone** Q_2	**Latitude**	**Longitude**	**Climatic variation (°C)**	**Altitude (m)**	**Rainfall (mm/year)**
Aïn Younes	Usa $43<Q_2<64$	36°25' N	9°22' E	mild winter *4.5 <m<7*	100	300-400
Borj Massaoudi	Usa $43<Q_2<64$	35°70'N	9°13'E	mild winter *4.5 <m<7*	150	300-400
Zaghouan	Usa $43<Q_2<64$	36°26'N	10°46'E	mild winter *4.5 <m<7*	500	400-500
El Ameime	Usa $43<Q_2<64$	9°54'N	36°21'E	mild winter *4.5 <m<7*	600	400-500
Marjâa Aouem	Usa $43<Q_2<64$	36°28'N	9°50'E	mild winter *4.5 <m<7*	100	300-400
Nebre 1	Lsa $31<Q_2<37$	36°12'N	8°29'E	cool winter *1<m<3*	470	300-400
Nebre 2	Lsa $31<Q_2<37$	36°29'N	8°75'E	cool winter *1<m<3*	470	300-400
Daouar Chouarnia	Lsa $31<Q_2<37$	35°94'N	9°13'E	cool winter *1<m<3*	200	300-400
Aïn Errahma	Lsa $31<Q_2<37$	36°13'N	10°22'E	cool winter *1<m<3*	100	300-400
Menzel Temime	Sh $63<Q_2<97$	36°78'N	10°98'E	warm winter *m>7*	150	500-600
Slimene	Sh $63<Q_2<97$	36°71'N	10°51'E	warm winter *m>7*	18	500-600

a/ Bioclimatic zone: Sh: sub-humid; Usa: upper semi-arid; Lsa: lower semi-arid.
b/ Q_2: Emberger's pluviothermic coefficient (1966). $Q_2 = 2000P/ M^2 - m^2$ where *P* is the mean of annual rainfall (mm). *M* (K) is the mean of maximal temperatures for the warmest month (July) and *m* (K) is the mean of minimal temperatures for the coldest month (February). *P*. *M* and *m* values for each site were calculated for the period from 1953 to 2003 (Data provides by the Tunisian National Institute of Meteorology).

Table 2. Genetic diversity parameters according to the used marker for the 11 populations.

Populations	**N**	**Ap**	**P**	**H_o**	**He**	**F_{IS}**
Aïn Younes	19.9	2.0	75.0	0.278	0.267	-0.041
	(0.1)	(0.3)		(0.071)	(0.077)	
Borj Messaoudi	19.3	1.8	62.5	0.274	0.206	-0.33
	(0.5)	(0.3)		(0.103)	(0.081)	
Zaghouan	19.9	1.6	50.0	0.223	0.195	-0.143
	(0.1)	(0.3)		(0.094)	(0.084)	
El Ameime	20.4	1.4	50.0	0.344	0.218	-0.577
	(0.2)	(0.2)		(0.159)	(0.099)	
Marjâa Aouem	20.1	1.8	25.0	0.144	0.093	-0.548
	(0.1)	(0.3)		(0.111)	(0.063)	
Nebre 1	18.1	1.6	50.0	0.219	0.181	-0.209
	(0.3)	(0.3)		(0.107)	(0.085)	
Nebre2	20.3	1.8	37.5	0.061	0.104	0.414
	(0.2)	(0.3)		(0.038)	(0.046)	
Daouar Chouarnia	20.0	1.6	50.0	0.231	0.153	-0.509
	(0.0)	(0.3)		(0.129)	(0.074)	
Aïn Errahma	20.5	2.0	75.0	0.269	0.211	-0.274
	(0.2)	(0.2)		(0.103)	(0.063)	
Menzel Temime	20.6	1.8	62.5	0.229	0.181	-0.651
	(0.2)	(0.3)		(0.093)	(0.067)	
Slimene	20.3	1.8	62.5	0.199	0.171	-0.163
	(0.2)	(0.3)		(0.095)	(0.076)	
Mean		**1.72**	**54. 54**	**0.214**	**0.180**	**-0.298**

Standard deviations are in parentheses.

N: mean sample size per locus; Ap: mean number of alleles per polymorphic locus; P: percent of polymorphic loci; Ho: observed heterozygosity;

He. expected heterozygosity.

ns not significant at $p > 0.05$.

* Highly significant at $p < 0.01$ after 200 permutations.

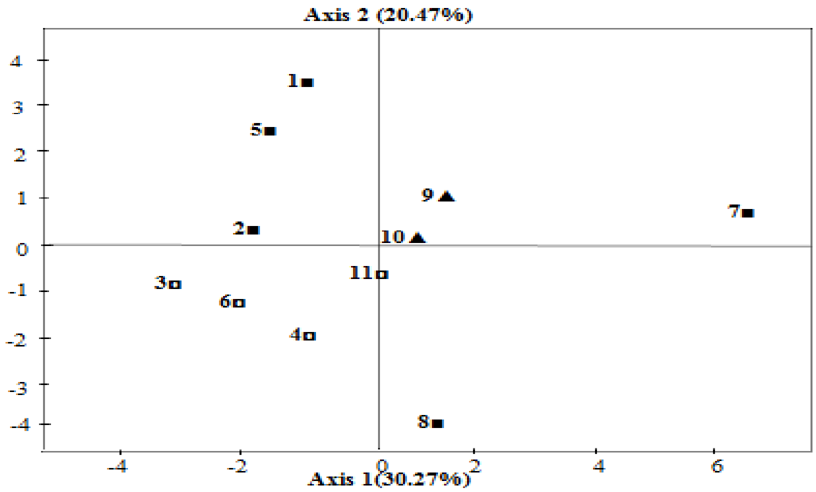

Figure 1. Principal Component Analysis (PCA) generated from allelic frequencies for the polymorphic loci for the 11 populations analysed of populations. Plot according to axes 1-2.

▲: Sub-humid. ■: Upper semi-arid. ◘: Lower semi-arid.

1 : Aïn Younes ; 2 : Borj Messaoudi ; 3 : Nebre1 ; 4 : Nebre2 ; 5 : Zagouhan ; 6 : Daouar Chouarnia;

7 : El Ameime ; 8 : Marjâa Aouem ; 9 : Menzel Temime ; 10 : Slimene ; 11 : Aïn Errahma.

Table 3. Fixation indices (Wright. 1978), deviation from panmixia and loci detected among populations of *H. humifusum* analyzed

Populations	**Locus**	**F**	**D**	**P**	
Aïn Younes	PGI-1	0.295	-0.313	0.000	**
	MDH-1	-0.290	0.258	0.528	*ns*
	MDH-2	-0.290	0.258	0.528	*ns*
	PGM-2	-0.192	0.161	0.538	*ns*
	PGD-2	-0.176	0.147	1.000	*ns*
	PGD-3	-0.081	0.054	1.000	*ns*
Borj Messaoudi	PGI-1	-0.015	-0.015	0.138	*ns*
	MDH-1	-0.125	0.094	1.000	*ns*
	MDH-2	-0.636	0.591	0.015	*
	PGM-2	-0.135	0.108	1.000	*ns*
	PGD-3	-0.081	0.054	1.000	*ns*
Zagouhan	PGI-1	-0.025	-0.002	0.062	*ns*
	MDH-2	-0.379	0.345	0.256	*ns*
	PGM-2	-0.026	0.000	1.000	*ns*
	PGD-2	-0.250	0.219	0.548	*ns*
	PGD-3	-0.081	0.054	1.000	*ns*
El Ameime	PGI-1	-0.499	0.462	0.000	**
	PGM-2	-0.667	0.667	0.005	**
	PGD-1	-0.053	0.026	1.000	*ns*
	PGD-2	-0.818	0.773	0.001	**
Marjâa Aouem	PGI-1	-0.026	0.000	1.000	*ns*
	PGM-2	-0.818	0.773	0.001	**
	PGD-2	-0.111	0.083	1.000	*ns*
Nebre1	PGI-1	-0.015	-0.015	0.138	*ns*
	MDH-1	-0.125	0.094	1.000	*ns*
	MDH-2	-0.636	0.591	0.015	*
	PGM-2	-0.091	0.061	1.000	*ns*
Nebre2	PGI-1	0.440	-0.453	0.000	**
	MDH-2	1.000	-1.000	0.000	**
	PGM-2	-0.024	0.000	1.000	*ns*
	PGD-3	-0.176	0.147	1.000	*ns*
Daouar Chouarnia	PGI-1	-0.306	0.273	0.256	*ns*
	MDH-2	-1.000	0.950	0.000	**
	PGM-2	-0.053	0.026	1.000	*ns*
	PGD-2	-0.111	0.083	1.000	*ns*
Aïn Errahma	IDH-1	-0.024	0.000	1.000	*ns*
	PGI-1	-0.036	0.011	0.578	*ns*
	MDH-1	-0.077	0.051	1.000	*ns*
	MDH-2	-0.200	0.171	1.000	*ns*
	PGM-2	-0.818	0.773	0.001	**
	PGD-2	-0.081	0.054	1.000	*ns*
	PGD-3	-0.081	0.054	1.000	*ns*
Menzel Temime	PGI-1	-0.462	0.427	0.158	*ns*
	MDH-2	-0.081	0.054	1.000	*ns*
	PGM-2	-0.212	0.182	1.000	*ns*
	PGD-2	-0.077	0.281	0.292	*ns*
	PGD-3	-0.313	0.051	1.000	*ns*

Table 3. (Cont.)

Slimene	IDH-1	-0.081	0.054	1.000	*ns*
	PGI-1	-0.282	0.251	0.675	*ns*
	MDH-1	-0.081	0.054	1.000	*ns*
	PGM-2	-0.167	0.139	1.000	*ns*
	PGD-2	-0.111	0.083	1.000	*ns*

F: Wright fixation index. P: probability of significance of the χ^2 test. D: deficit index (D < 0) or excess (D > 0) heterozygosity.
ns not significant at p > 0.05. * Highly significant at p < 0.01 after 200 permutations.

Table 4. Wright's F statistics (F_{IT}, F_{ST}, F_{IS}) for populations of *H. humifusum*.

Locus	F_{IS}	F_{IT}	F_{ST}	χ^2 cal F(ST)	
ICD-1	-0.067	-0.009	0.054	23.76	*ns*
PGI-1	-0.112	0.023	0.122	53.68	*ns*
MDH-1	-0.170	-0.057	0.096	42.04	***
MDH-2	-0.407	-0.160	0.176	77.08	***
PGM-2	-0.464	-0.083	0.260	226.72	***
PGD-1	-0.080	-0.028	0.048	19.2	*ns*
PGD-2	-0.308	-0.175	0.101	40.4	***
PGD-3	-0.053	-0.005	0.046	18.4	*ns*
Mean	**-0.267**	**-0.072**	**0.154**	**62.66**	

Standard errors in parentheses.
* Highly significant at p < 0.01 after 200 permutations.

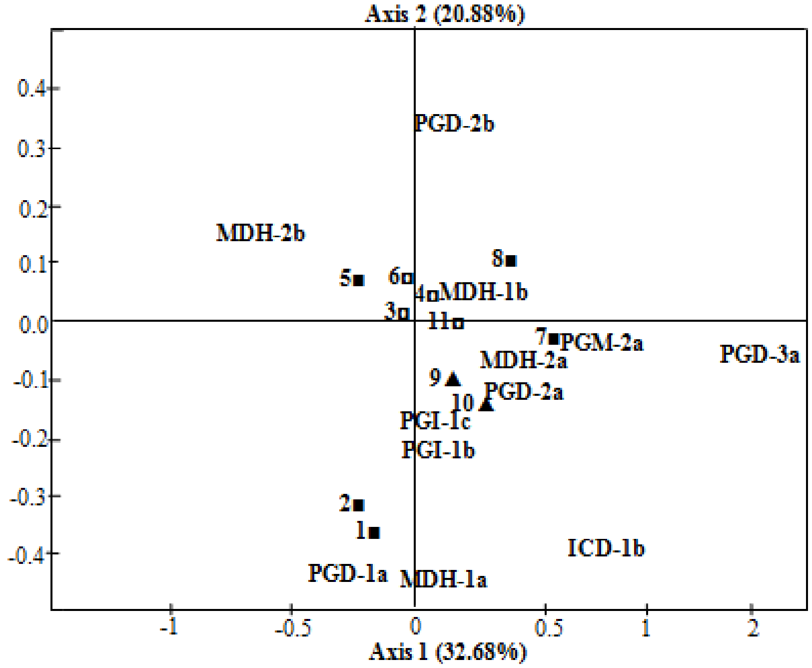

Figure 2. Factorial Correspondence Analysis (FCA) generated from variables and populations. Plot according to axes 1-2.

▲: Sub-humid. ■: Upper semi-arid. ◘: Lower semi-arid.
1 : Aïn Younes ; 2 : Borj Messaoudi ; 3 : Nebre1 ; 4 : Nebre2 ; 5 : Zagouhan ; 6 : Daouar Chouarnia ; 7 : El Ameime ; 8 : Marjâa Aouem ; 9 : Menzel Temime ; 10 : Slimene ; 11 : Aïn Errahma.

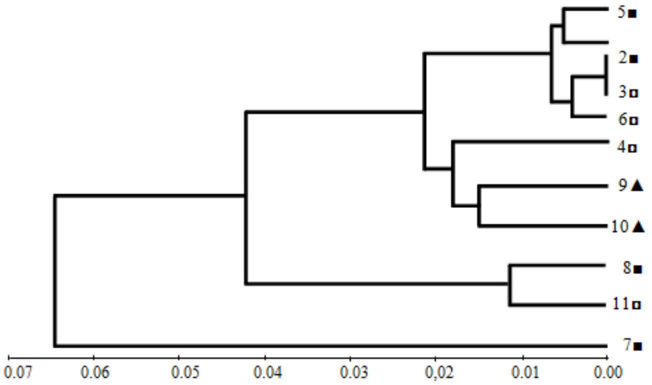

Figure 3. Dendogram of the eleven analysed *H. humifusum* based on Nei's (1978) genetic distances.
▲: Sub-humid. ■: Upper semi-arid. ◘: Lower semi-arid.
1 : Aïn Younes ; 2 : Borj Messaoudi ; 3 : Nebre1 ; 4 : Nebre2 ; 5 : Zagouhan ; 6 : Daouar Chouarnia ;
7 : El Ameime ; 8 : Marjâa Aouem ; 9 : Menzel Temime ; 10 : Slimene ; 11 : Aïn Errahma.

4. Discussion

The analysis of five isozymes polymorphism in Tunisian *Hypericum humifusum* showed that populations maintain high levels of genetic diversity within populations coupled with a significant excess of heterozygosity for most of them. The majority of total variation resided within populations supporting an outbreeding mating system as reported for the majority of *Hypericum* species (Matzk et al., 2001). Hamrick and Godt (1996) reported that isozyme variation within populations of outcrossing species was higher than that observed among populations. However, the mating system of *H. humifusum* has not been studied. Therefore, the assessment of the breeding system, via artificial auto-allo hybridizations, is necessary for further information about the dynamic of *H. humifusum* populations (Barcaccia et al., 2006; Bejaoui et al., 2010). The variation among populations could not be only explained by the influence of bioclimatic factors. In fact, the variations occur for both populations belonging to the same or different bioclimates. Other factors such as the level of site destruction, and the dispersal and reproductive potentials of populations might also explain the amount of variations (Coleman and Abott, 2003).

A relatively high genetic structure and a restricted level of gene flow among populations were detected. The observed F_{ST} value (0.154) is comparable to that reported for outcrosser and wind dispersal species (Nybom, 2004). In our study, the population genetic structure was not correlated with the geographical distances. Thus, an isolation by distance only could not explain the amount of the observed genetic structure. The substantial level of differentiation among populations seems to be more likely caused by habitat fragmentation and the limitation of gene flow through seed and pollen dispersal (Hamrick and Godt, 1989). Mean F_{ST} values for outcrossing species occurring in small scattered populations are known to be higher than those for species occurring in large and continuous populations (Crawford et al., 2001).

Population groupings in the PCA and UPGMA cluster are not always concordant with the geographic or bioclimatic zones. Our results were similar to those previously reported for *Hypericum brasiliense* populations which are shown to be little divergent genetically (Abreu et al. 2003). The low genetic distances and the substantial structure of *H. humifusum* populations indicate their recent fragmentation by the destruction of their habitats (Bejaoui ret al., 2010; Bejaoui et al., 2012).

Populations of *Hypericum humifusum* are severely affected by human activities causing a significant destruction of sites. The in-situ conservation is difficult to conceive because of regular intensive agricultural practices leading to disturbance of sites. Conservation of seeds in a gene bank should be the best way to ensure retention of allelic and genotypic diversity. Considering the significant differentiation among populations, preservation only of several populations would be insufficient to conserve all the genetic variation in the species. Therefore, to preserve the maximum of diversity in the species, all populations must be collected even at a small geographic range. Ex-situ conservation should aim to include firstly populations from the upper semi-arid bioclimate harbouring relatively high genetic diversity. Further studies including the variation of the chemical composition among populations and its relationship with the genetic diversity are necessary to elaborate efficient improvement program.

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