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Full Length Research Paper

Genetic diversity and chemical polymorphism of Tunisian *Lavandula multifida* L. (Lamiaceae) populations

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Eleven Tunisian natural populations of *Lavandula multifida* L., from different geographic regions and bioclimates, were assessed for their variability using six polymorphic loci and 35 terpenoids. Isozymes were revealed by 13% gel electrophoresis. Volatiles were analysed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). A high genetic diversity within populations and within ecological groups was revealed by allozymes as a result of both of the outbreeding system of the species and the large size of populations before fragmentation. Cluster constructed using Nei's genetic distances showed high differentiation between populations. Those belonging to the same bioclimatic zone were not strictly grouped together. Essential oil composition varied among populations. Carvacrol (21.14 to 47.02%), acrylic acid dodecanyl ester (8.96 to 14.06%) and *β*-Bisabolene (12.96 to 19%) were the main components. Chemical population's structure, at the ecological group level, based on terpenoids was concordant with that of isozymes. Matrices of Wright's F_{ST} and Euclidean distances were also correlated. Population differentiation performed on combined data yielded similar to that shown using each marker separately. Conservation strategies should take into account the levels of genetic diversity and chemical variation in relation to population and bioclimate.

Key words: *Lavandula multifida,* Tunisia, natural populations, Isozymes, essential oil, bioclimate.

INTRODUCTION

Lavandula multifida L. (Lamiaceae), growing mainly in Mediterranean basin, is a perennial shrub (Emberger, 1966; Pottier-Alapetite, 1981; Miller, 1985). The species is exploited in traditional medicine to treat rheumatism and colds (El-Hilaly et al., 2003). It has hypoglycemic and anti-inflammatory properties (Gamez et al., 1987; Sosa et al., 2005). The demand for this plant is increasing for Industrial (pharmaceutic, perfumery and cosmetic industries) and ornamental purposes.

In Tunisia, the species grows wild in different bioclimatic zones extending from the upper semi-arid to the upper arid. *L. multifida* occurs in fragmented populations in the Central and the South of the country. It occurs in destroyed habitats such as rocky exposed ridges and slopes, and garrigues derived from the destruction of *Pinus halepensis* L. and *Juniperus phœnicea* L. forests (Nabli, 1995).

Tunisian populations are more disturbed as a result of an increasing clearing, overgrazing and overexploitation. Habitat fragmentation reduced genetic diversity of the species, enhanced inbreeding level and led to a rapid differentiation between populations (Chograni et al., 2008). The loss of genetic diversity affected population's evolution and reduced their future adaptation to environmental changes (Gilpin and Soulé, 1986; Chen et al., 2004). The maintenance of sufficient genetic diversity, both within and among populations, is one of the main goals in conservation planning as genetic diversity provides the template for adaptation and evolution of

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populations and species (Grant, 1998; Stuessy and Ono, 1998; Wang et al., 2005).

Chemical constituents have been widely used in systematic and evolution studies of aromatic and medicinal species (Adams, 2000; Slavbovska et al., 2001; Grayer et al., 2003; Khanuja et al., 2005; Trindade et al., 2009; Morone-Fortunato et al., 2010). At the intraspecific level, these compounds have been mainly used to assess variation among populations (Von Rudolff, 1975; Sagnard et al., 2002; Adams et al., 2003, 2006). This variation has been attributed to environment (climatic and edaphic) and/or genetic factors (Sagnard et al., 2002; Adams et al., 2006). Concordance between genetic and terpenoid variation among populations was previously reported (Adams et al., 2003; Khanuja et al., 2005; Morone-Fortunato et al., 2010). However, the relationship between the two variables remains inadequately defined (Vogel et al., 1996; Adams et al., 2003). The *in situ* maintenance of populations depends on the existence of a sufficient level of genetic diversity to face biotic and abiotic variations (Barret and Kohn, 1991; Hamrick and Godt, 1989). Thus, the analysis of genetic variation based both on genetic (morphology, isozyme and molecular) and chemical markers (terpenoids and flavonoids) is crucial in understanding population future maintenance to the development of conservation and improvement programs.

In this work, we investigated the population differentiation of *L. multifida* growing in different bioclimatic regions using both terpenoid and isozymic markers. This study constitutes a compilation of two previous works published separately on the genetic diversity and essential oil variation in Tunisian *L. multifida* (Chograni, 2009; Chograni et al., 2009). We address the following questions: (i) what is the pattern of variation within and among populations based on each marker? (ii) is there a relationship between chemical and isozymic data? (iii) what is the implication of the detect variation for conservation strategies?

MATERIALS AND METHODS

Surveyed populations and sampling

Eleven *L. multifida* populations were sampled from the upper semi-arid, lower semi-arid and upper arid bioclimatic zones according to Emberger's Q_2 pluviothermic coefficient (Emberger, 1966). The main ecological features and the geographic location of populations are reported in Figure 1 and Table 1. In each population, 20 individuals were sampled. Because of the vegetative reproduction of the species, samples were collected at a distance exceeding 10 m from each other to avoid collecting multiple plants from the same parent. For each population, isozyme analysis was performed on 20 individuals; volatiles were assessed on 10 plants taken at random from those used for the isozyme study.

Isozyme polymorphism analysis

Seven isozymes: alcohol dehydrogenase (Adh, EC 1.1.1.1), phosphoglucoisomerase (Pgi, EC 5.3.1.9), phosphoglucomutase (Pgm, EC 2.7.5.1), leucine aminopeptidase (Lap, EC 3.4.11.1), glutamate oxaloacetate transaminase (Got, EC 2.6.1.1), isocitrate dehydrogenase (Icd, EC 1.1.1.42) and 6-phosphogluconate dehydrogenase (6-Pgd, EC 1.1.1.44), were assessed using 13% starch horizontal gel electrophoresis. Enzyme extraction, staining procedures and zymogram genetic interpretation were carried out according to the methods of Chograni et al. (2008).

Essential oil analysis

The essential oil composition was determined by gas chromatography (GC) and gas chromatography- mass spectrometry (GC-MS) analysis of *n*-hexane extracts of aerial parts following the methods of Chograni et al. (2009). Compounds were identified by comparing their retention times with authentic samples injected under the same chromatographic conditions and in relation to their mass spectra with the HP chemstation database HP NBS 75K.L. Percentages of volatiles were calculated using the normalization method from the GC peak areas without correction factors.

Data analysis

Isozyme polymorphism and population structure

The genetic variation within populations or within ecological groups was assessed using the number of polymorphic loci (Ap), the percentage of polymorphic loci (P) and the average observed (Ho) and expected (He) heterozygosities. Departure from Hardy-Weinberg equilibrium was assessed by the F_{IS} inbreeding coefficient (Wright, 1951). Significance of deficit or excess of heterozygotes was tested using randomization procedures (Goudet, 2001). The divergence among populations was estimated by Nei's (1978) unbiased genetic distances. On the basis of these distances, a dendrogram using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) procedure was obtained. Calculations of gene diversity parameters and the construction of the phenogram were carried out using version 1 of the Biosys Program (Swofford and Selander, 1981).

Differentiation among populations or among eco-geographical groups was evaluated by Wright's (1951) F-statistics: F_{IT}(total inbreeding), F_{ST} (subdivision among populations) and F_{IS} (inbreeding coefficient within populations) according to Weir and Cockerham's (1984) indices using the program FSTAT versions 1.2 and 2.9.3 (Goudet, 1995, 2001). The significance of indices was tested after 1000 permutations.

Mantel's test (Mantel, 1967) was performed to evaluate isolation by distance of populations (Slatkin, 1993). For this purpose, pair wise log10 transformed values of Nm were regressed against geographical distances. Nm is the average of effective number of migrants exchanged between populations of each generation and calculated as $Nm = ¼ [(1/F_{ST}) - 1]/[4α]$, where $α = ¼ (n/(n - 1))^2$, n is the number of populations (Crow and Aoki, 1984).

Essential oil variation

Essential oil variation among ecological groups was assessed by a variance analysis (ANOVA procedure) (SAS, 1990) performed on constituent averages calculated for populations from the same group. The chemical population structure using all compounds was assessed by a principal component analysis (PCA) (SAS, 1990).

The relationship between oils variation and geographic distances among populations was estimated by a Mantel test (Mantel, 1967) performed on matrices of Euclidean distances and geographic

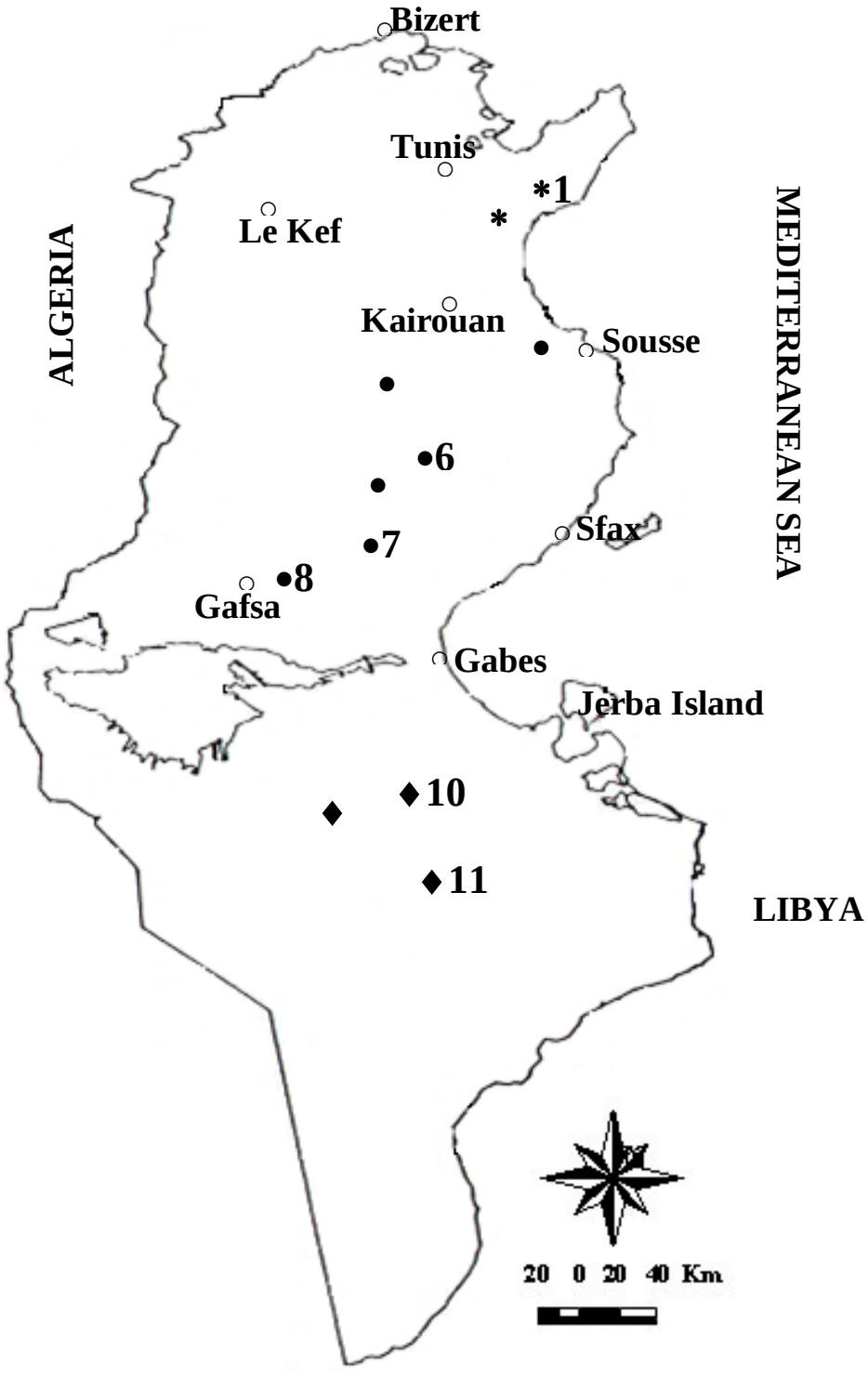

Figure 1. Map of Tunisia: Geographical distribution of the analysed *Lavandula multifida* populations. 1, 2,…: population code (Table 1). ∗,•,♦, populations belonging to the upper semi-arid, lower semi-arid and upper arid zones, respectively.

Combined analysis

A PCA was used to assess population structure based on the combined data of percentages of volatiles and frequencies of loci. The used loci and terpenoids were selected on the basis of the level of their correlation ($r > 0.3$) to axes 1 and 2 of previous PCAs performed on each set of data (Data not shown).

A Mantel's test (at $P < 0.05$ and after 1000 permutations) was performed to assess the correlation between Euclidean distances and Wright's F_{ST} matrices calculated on terpenoid and isozyme

Table 1. Main ecological traits for the 11 Tunisian *Lavandula multifida* populations analyzed.

Bioclimatic zone*	Population	Label	Q_2*	Altitude (m)	Latitude (N)	Longitude (E)	Rainfall (mm/year)
Upper semi-arid	Bou Argoub	1	67.70	300	36°25'	10°28'	450
	Jedidi Jeb. Mt	2	65.65	450	36°25' N	10°28' E	450
Lower semi-arid	Khniss	3	35.24	100	35°43' N	10°43' E	356
	Aïn Errahma	4	35.24	100	36°13' N	10°22' E	350
	Hammam Sousse	5	35.24	150	35°11' N	10°27' E	350
	El Fayedh	6	34.40	550	35°10' N	9°35' E	313
	Ouslett Jeb. Mt[a]	7	34.39	500	35°47' N	9°42' E	313
	Cherichera Jeb. Mt	8	34.39	450	35°59' N	9°81' E	350
Upper arid	Bouhedma Jeb. Mt	9	33.39	790	34°32' N	9°36' E	268
	Cherarda	10	28.83	400	35°9' N	10°2' E	313
	Goubrar Jeb. Mt	11	33.39	600	34°50' N	9°27' E	268

a, Djebel Mountain; * Bioclimatic zones were defined according to Emberger's (1966). (Q_2) pluviothermic quotient; $Q_2 = 2000P/(M^2 - m^2)$, P, the mean of annual rainfall (mm); M, average of maximal temperature (°K: Kelvin degree) for the warmest month (June); m, the average of minimal temperature (°K) for the coldest month (February). P, M and m values for each site were calculated for the period from 1953 to 2003. Data was provided by the Tunisian National Institute of Meteorology.

data, respectively.

RESULTS

Isozyme genetic diversity and population structure

A high genetic diversity within the populations was observed (Table 2). The mean number of alleles per polymorphic locus (Ap) ranged from 1.8 (populations 1 and 11) to 2.3 (population 2) (data not shown) with an average of 2.05. The percentage of polymorphic loci (P) for all populations was 74.24%. The averages of observed (Ho) and expected (He) heterozygosities were 0.230 and 0.231, respectively. A high genetic diversity was observed within the lower semi-arid (Ap = 2.1, P = 75%, He = 0.232) and upper arid populations (Ap = 1.93, P = 77.76%, He = 0.256). The populations belonging to the upper semi-arid showed the highest deficiency of heterozygotes (F_{IS} = 0.046) (Table 2).

A high differentiation among populations, estimated by Wright's F_{ST} index, was observed (F_{ST} = 0.283) (Table 3). The Mantel test (r = 0.106; P = 0.25 > 0.01) and the regression analysis showed no significant correlation between gene flow and geographic distance.

F_{ST} values among populations belonging to the same ecological group ranged from 0.036 (upper semi-arid) to 0.414 (lower semi-arid). The differentiation between ecological groups was significant and higher between discontinuous ones (Table 3).

The dendrogram based on Nei's (1978) genetic distances (Figure 2) showed two major population aggregates. The first one is represented by 10 populations belonging to different bioclimatic zones. The second group contained population 8 (Cherichera Jeb. Mt) belonging to the lower semi-arid zone.

Essential oil compounds variation

The analyses of the volatile oils isolated from the 11 populations showed that 35 components were always detected in every volatile oil sample, amounting to 89.90% of total oil (Table 4).

Carvacrol (21.14 to 47.02%), acrylic acid dodecanyl ester (8.96 to 14.06%) and *β*-bisabolene (12.96 to 19%) were the main components detected.

Euclidean distance values showed significant variation between all pairs of populations (Table 5). Low distance value (8.11) was observed between populations 3 and 5 from the same bioclimatic zones and 73.5 km geographically distant, whereas the highest value (51.8) was noted between populations 6 and 9 belonging to lower semi-arid and upper arid bioclimates, respectively (59.3 km distant each from another). The correlation between geographic and Euclidean distance matrices between population pairs, estimated by the Mantel test was not significant (r = 0.2; P = 0.08>0.01).

The principal component analysis performed on all populations using the amounts of all constituents according to axes 1 and 2 (89.63% of the inertia) showed three population aggregates (Figure 3). Populations 1, 2, 3, 4, and 5, belonging to the upper semi-arid and lower semi-arid zones, constituted the first group. In this group, the distribution of populations was according to several constituents such as germacrene D and camphene (populations 2 and 3). The second group, formed by the populations 7, 8, 9, 10 and 11, was characterized by high

Table 2. Summary of genetic diversity parameters for populations and ecological groups.

Population	Genetic diversity				
	Ap	P	Ho	He	F_{IS}
All populations	2.05 (0.263)	74.24	0.230 (0.086)	0.231 (0.068)	0.016 (0.098)^{ns}
Within ecological group					
Upper semi-arid	2.05 (0.25)	66.65	0.185 (0.086)	0.207 (0.064)	0.046 (0.171)^{ns}
Lower semi-arid	2.1 (0.25)	75	0.233 (0.086)	0.232 (0.064)	0.029 (0.141)^{ns}
Upper arid	1.93 (0.3)	77.76	0.256 (0.072)	0.247 (0.07)	-0.026 (0.055)^{ns}

Ap = mean number of alleles per polymorphic locus; P = percentage of polymorphic loci; Ho and He are the observed and the expected heterozygosities, respectively; standard errors are in parentheses; ns, not significant at P > 0.05.

Table 3. F-statistics (F_{IT}, F_{ST}, F_{IS}) calculated for all populations within and among groups

Population	F_{IT}	F_{ST}	F_{IS}
All populations (for all polymorphic loci)	0.300 (0.148)**	0.283 (0.094) **	0.016 (0.098)^{ns}
Within ecological group			
Upper semi-arid	0.081 (0.169)^{ns}	0.036 (0.029)*	0.046 (0.171)^{ns}
Lower semi-arid	0.441 (0.191)**	0.414 (0.134)**	0.029 (0.141)^{ns}
Upper arid	0.047 (0.076)^{ns}	0.070 (0.034)**	-0.026 (0.055)^{ns}
Among ecological groups			
Lower semi arid/upper semi-arid	0.389 (0.186)**	0.063 (0.022)**	0.345 (0.183)**
Lower semi arid/upper arid	0.302 (0.149)**	0.014 (0.014)*	0.292 (0.146)**
Upper semi-arid/upper arid	0.077 (0.065)*	0.038 (0.017)**	0.040 (0.057)^{ns}
Lower semi arid/upper semi-arid/upper arid	0.292 (0.148)**	0.034 (0.012)**	0.265 (0.143)**

** Highly significant at P < 0.001, * significant at P < 0.05 and ns not significant at P > 0.05 (after 1000 permutations).

amounts of acrylic acid dodecanyl ester and β-phellandrene. The third group was formed by population 6 (El Fayedh) from the lower semi-arid zone. It was distinguished by a high amount of caryophyllene oxide. All groups shared high contents for the major compounds.

Combined analysis

The first three axes of the PCA, performed on terpenoid percentages and allelic frequencies matrices, represented 93.39% of the total variation.

The plot obtained according to axes 1 and 2 (82.91% of the inertia) showed a high population's dispersal of each bioclimatic zone. However, populations from contiguous zones were less separated than those belonging to discontinuous ones (Figure 4).

Mantel's test and the regression analysis performed to assess correlation between F_{ST} and Euclidean distance matrices indicate a high correlation (r = 0.624, P < 0.01) between the two data sets.

DISCUSSION

The present study reveals that the genetic variation within population and within ecological group was high. These could be explained by the outcrossing system and the size of population before habitat fragmentation (Hamrick and Godt, 1989). A high differentiation among populations and among ecological groups due to low level of gene flow in relation to geographical distance was revealed. The increasing anthropic pressures may accentuate this differentiation.

Tunisian *L. multifida* essential oil includes carvacrol /acrylic acid dodecanyl and ester/*β*-bisabolene chemotype. These constituents have been also found to be the major compounds of *L. multifida* from different Mediterranean regions (that is, Spain and Morocco) (Bellakhdar et al., 1985; Garcia-Vallejo et al., 1989). The population structure based on terpenes is in accordance with that obtained using isozymic markers. The high chemical variability among populations could be mainly explained by genetic factors rather than by ecological factors. In fact, populations from the same bioclimatic

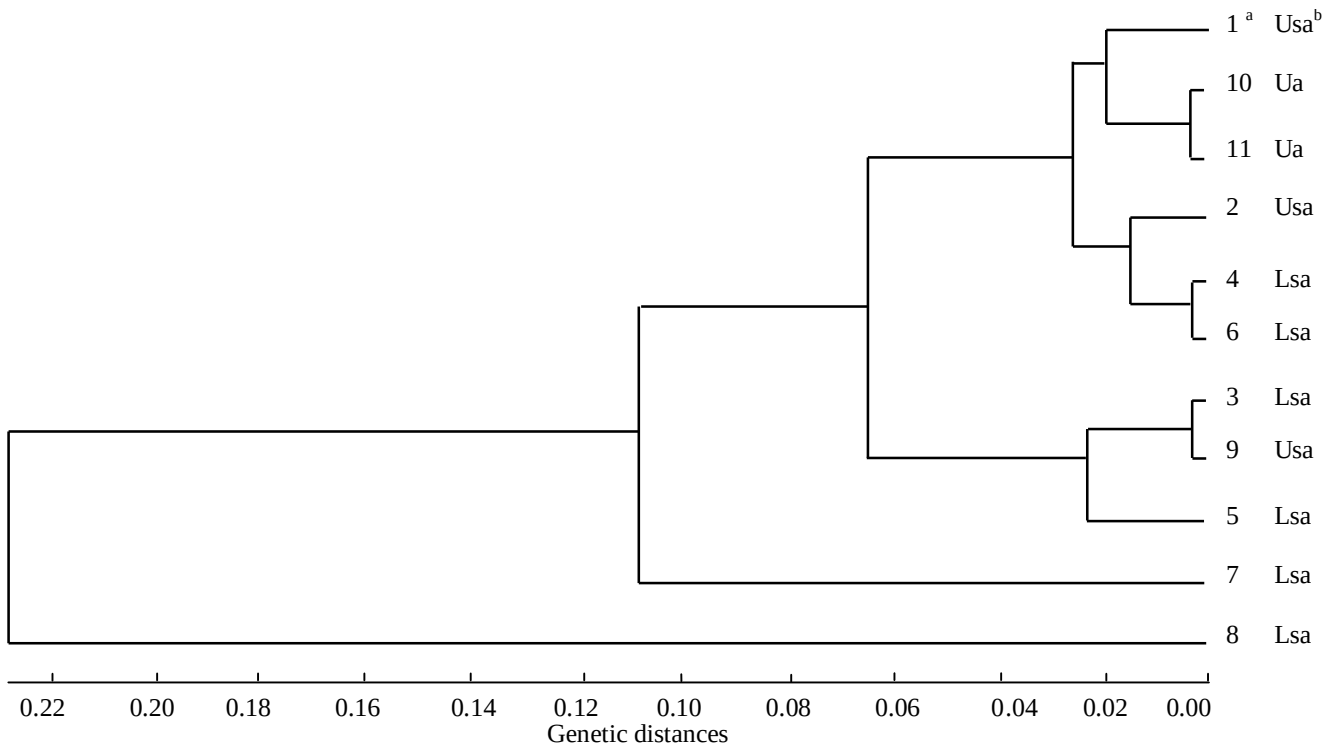

Figure 2. Dendrogram based on Nei's (1978) genetic distances for the 11 *L. multifida* analyzed populations. a, population code; b, bioclimatic zone; Usa, upper semi arid ; Lsa, lower semi arid ; Ua, upper arid.

Table 4. Mean percentage composition of essential oil in Tunisian *L. multifida* at species (As) and ecological group levels

Number	Constituent	RI[a)]	Upper semi-arid (1-2)	Lower semi-arid (3-8)	Upper arid (9-11)	As	F-test[b)]
1	α-Thujene	1030	tb	0.03b	1.46a	0.75	9.78**
2	α-Pinene	1032	0.23e	0.69e	0.80ed	0.57	5.14**
3	Camphene	1076	0.73a	0.12d	td	0.42	11.68**
4	β-Pinene	1118	0.06a	tb	tb	0.06	0.60^{ns}
5	Sabinene	1132	tc	0.13c	tc	0.13	4.10**
6	Car-3-ene	1159	tb	0.05b	tb	0.05	1.27*
7	Myrcene	1174	1.45b	1.57b	tb	1.51	2.39**
8	α-Phellandrene	1176	3.17bc	2.41c	5.66b	3.75	3.33**
9	Limonene	1203	tc	0.86ab	0.42c	0.64	3.91**
10	1,8-Cineole	1213	1.83b	0.46c	tc	1.14	19.81**
11	β-Phellandrene	1218	3.68bcd	5.68cd	12.85a	7.40	8.60**
12	γ-Terpinene	1255	0.61ab	0.21c	0.44c	0.42	5.32**
13	*p*-Cymene	1280	1.04cd	0.91bc	1.28b	1.08	20.78**
14	α-Terpinolene	1290	tb	0.10b	tb	0.10	4.20**
15	Camphor	1532	0.23b	0.10c	tc	0.12	21.10**
16	β-Bourbonene	1535	tb	0.24b	tb	0.24	24.10**
17	α-Gurjunene	1544	0.46a	tc	tc	0.46	9.41**
18	β-Cubebene	1549	0.42b	0.31b	tb	0.36	3.34**
19	α- Fenchol	1577	tc	tc	0.31b	0.31	11.60**
20	Terpinen-4-ol	1607	0.17d	0.61d	1.31c	0.70	23.71**
21	trans-β-Caryophyllene	1612	2.17a	0.35c	tc	1.26	20.34**
22	Allo-Aromadendrene	1661	tb	0.14b	0.05b	0.10	3.14**

Table 4. Contd.

23	Germacrene D	1726	ta	0.14a	ta	0.14	0.46^{ns}
24	*β*-Bisabolene	1741	19.00ab	14.94e	12.96cde	15.63	2.33**
25	*γ*-Cadinene	1776	tb	tb	0.29b	0.29	0.88^{ns}
26	Cis-α-Bisabolene	1785	ta	0.17a	0.19a	0.18	0.86^{ns}
27	Geraniol	1843	0.06c	tc	0.11c	0.08	14.33**
28	Caryophyllene oxide	2008	0.06b	7.19b	tb	3.62	32.32**
29	Nerolidol	2009	2.40a	0.22c	0.33c	0.99	3.92**
30	Spathulenol	2144	0.14c	0.19c	0.06c	0.13	6.92**
31	T-cadinol	2147	0.24c	tc	0.04c	0.14	19.99**
32	Carvacrol	2203	47.02b	32.50bc	21.14cd	33.55	15.27**
33	*α*-Bisabolol	2212	0.05b	tab	tb	0.03	0.39^{ns}
34	Caryophyllenol	2277	tb	1.05b	3.16b	2.11	6.26**
35	Acrylic acid dodecanyl ester	2840	8.96bc	11.48cde	14.06cd	11.50	11.42**
Total identified components			**94.14**	**82.82**	**76.93**	**89.90**	

Values followed by the same letter are not significantly different (Duncan's multiple range test at P<0.001). (a) Relative retention indices calculated against n-alkanes on the HP-INNOWAX capillary column; b, F-test of the variance analysis (F38/150 degrees of freedom) is highly significant (**) at P < 0.001 and significant (*) at P < 0.01; ns, not significant at P< 0.05%; t, traces (% < 0.05%).

Table 5. Euclidean distances (above diagonal) and geographic distances (km) (below diagonal) between all pairs of populations analyzed.

Population	Bioclimatic zone										
	Upper semi-arid		Lower semi-arid						Upper arid		
	1	2	3	4	5	6	7	8	9	10	11
1		16.20	22.67	17.64	8.11	48.43	16.73	12.20	35.28	17.73	27.16
2	24.00		36.39	20.87	12.10	50.98	29.24	16.97	47.97	27.16	34.99
3	93.75	105.00		25.49	26.79	50.08	12.22	22.28	19.21	18.80	23.09
4	34.50	46.50	67.50		15.47	43.96	19.35	13.03	32.75	12.92	16.00
5	73.50	85.50	26.25	43.50		47.67	20.20	11.79	38.17	19.68	28.07
6	179.25	191.25	127.50	146.25	121.50		47.47	46.44	51.78	45.96	45.68
7	109.50	118.50	100.50	78.00	78.75	82.50		15.33	20.13	15.42	20.92
8	118.50	130.50	92.25	88.00	75.75	64.50	21.75		33.48	15.87	23.26
9	236.25	247.50	169.50	201.00	168.00	59.25	141.00	121.50		27.93	25.73
10	162.00	175.50	97.50	129.00	96.00	35.25	83.25	61.50	84.00		11.63
11	184.50	197.25	123.75	150.75	122.25	15.00	90.75	72.75	60.75	27.75	

zone clustered separately from one another. However, the spatial chemical structure among populations may also result from local adaptation to micro ecological factors, acting to the selection of particular compounds dictated by the fragmentation of populations and their isolation. The cultivation of populations in the same experimental conditions allowed further information concerning the maintenance of oil profiles during cultivation and would help attempts to elucidate the diversification of populations.

At the ecological group level, the population structure based on isozymes was concordant with that observed using terpenoids. The concordance between the two sets of data is consistent with the findings of other studies using terpenoid and genetic markers (isozymes, RAPDs and ISSR) (Sagnard et al., 2002; Adams et al., 2003, 2006; Zaouali and Boussaid 2008). However, linkage between isozyme and genetic markers cannot be proved.

Isozymes are codominant and coded by a limited number of loci (Weeden and Wendel, 1989), whereas a single gene could code for several terpenes (Vogel et al., 1996). It is therefore necessary to analyze both Quantitative Trait Loci (QTL) and genetic markers to better explain the relationship between the two sets of variations.

Chemical and genetic *L. multifida* population structures were high. This may lead to increasing genetic drift and population extinction. So, preservation of populations is necessary to ensure their *in situ* maintenance. Limiting overgrazing and over collection is the best way of achieving conservation. The use of volatiles and isozymes could constitute a useful starting point for *in situ* conservation. The upper arid and upper semi-arid

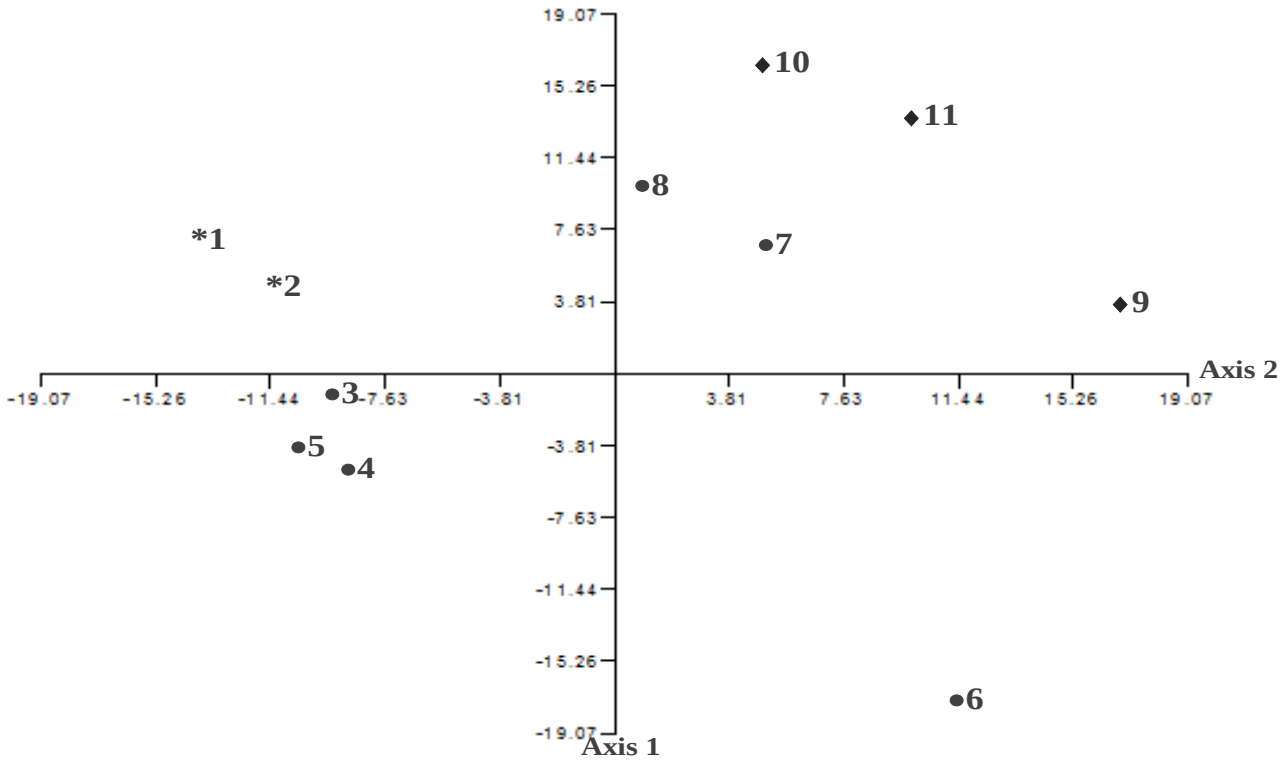

Figure 3. Principal component analysis performed on terpenes for the 11 analyzed populations. Plot according to axes 1-2. 1, 2,…: population code. ∗,●,♦, populations belonging to the upper semi-arid, lower semi-arid and upper arid zones, respectively.

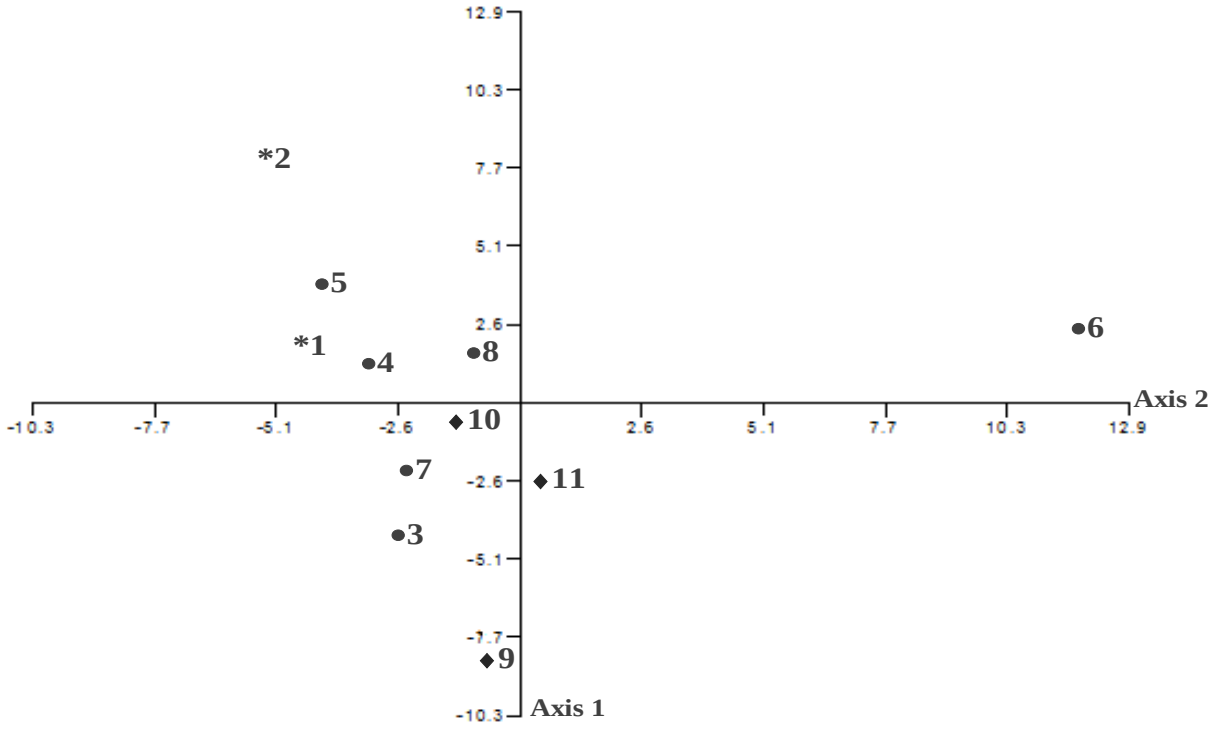

Figure 4. Principal component analysis performed on allelic frequencies and terpenoids for the 11 populations analyzed. Plot according to axes 1 to 2. 1, 2,…: population code. ∗,●,♦, populations belonging to the upper semi-arid, lower semi-arid and upper arid zones, respectively.

populations, with high genetic and essential oil variations, are the first to be protected. Because of the bioclimatic and geographic related variations, management should be made appropriate according to these factors. Ex situ population preservation should be based on the collection of maximum cuttings within population rather than between populations because of the high genetic variation within population. Cuttings should be transplanted in different bioclimatic zones to ensure efficient conservation.

In Tunisia, *Lavandula multifida*, occurs in small scattered populations as a result of habitat fragmentation. The low size of populations and their isolation led to high genetic drift, low gene flow and high differentiation among them. According to our genetic estimates, *L. multifida* populations suffer from genetic erosion. Their ability to respond to selective forces could decrease with the increasing anthropic pressures. Taking into account this consideration, as well as the small number and size of existing populations, efforts should be made to protect all populations. Limiting human activities (overgrazing, overharvesting, etc) may promote the expansion of populations. The variation of terpenoids within the species provides information for designing strategies for preserving the chemical diversity. Therefore, several constituents distinguish populations. Thus, populations exhibiting high amounts of these compounds should be protected. Their cultivation in the garden and the assessment of their genetic and chemical characteristics may allow the propagation of interesting genotypes.

REFERENCES

Adams RP (2000). Systematics of smooth leaf margin *Juniperus* of the western hemisphere based on leaf essential oils and RAPD DNA fingerprinting. Biochem. Syst. Ecol. 28:149-162.

Adams RP, Schwarzbach AE, Pandey RN (2003). The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: an example from *Juniperus*. Biochem. Syst. Ecol. 31:375-387.

Adams RP, Socorro M, Elizondo G, Elizondo MG, Slinkman E (2006). A new subalpine variety of *Juniperus blancoi* Martinez (Cupressaceae) from Durango, Mexico. Biochem. Syst. Ecol. 34:205-211.

Barret SCH, Kohn JR (1991). Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk DA, Holsinger KE (Eds), Genetics and Conservation of Rare Plants. Oxford University Press, New York. pp. 3-30

Bellakhdar J, Berrada M, Denier C, Holeman M, Ilidrissi A (1985). Comparative chemical study of the essential oils of ten populations of *Lavandula multifida* L. from Morocco. Biruiya. 1:95-106.

Chen JM, Wang JY, Liu X, Zhang YW, Wang QF (2004). RAPD analysis for genetic diversity of *Isoetes sinensis*. Biodivers. Sci. 12:348-353.

Chograni H (2009). *Lavandula multifida* L. (Lamiaceae) en Tunisie: Analyse de la structure génétique et de la variation de la composition des huiles essentielles des populations naturelles. Thesis, Faculté des sciences de Tunis.

Chograni H, Messaoud C, Boussaid M (2008). Genetic diversity and population structure in Tunisian *Lavandula stoechas* L. and *Lavandula multifida* L. (Lamiaceae). Biochem. Syst. Ecol. 36:349-359.

Chograni H, Zaouali Y, Rajeb C, Boussaid M (2009). Essential oil variation among natural populations of *Lavandula multifida* L. (Lamiaceae). Chem. Biodivers. 7:933-942.

Crow J, Aoki K (1984). Group selection for a phylogenetic behavioural trait: estimating the degree of population subdivision. Proc. Natl. Acad. Sci. U.S.A. 81:6073-6077.

El-Hilaly J, Hmammouchi M, Lyoussi B (2003). Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco). J. Ethnopharmacol. 86:149-158.

Emberger L (1966). Une classification biogéographique des climats. Recherches et travaux des Laboratoires de Géologie, Botanique et Zoologie. Faculté des Sciences, Montpellier. p. 43

Gamez MJ, JImenez J, Risco S, Zarzuelo A (1987). Hypoglycemic activity in various species of the genus *Lavandula*: *Lavandula stoechas* L. and *Lavandula multifida* L. Pharmazie 10(42):706-707.

Garcia-Vallejo MC, Garçia-Vallejo I, Velasco-Negueruela A (1989). Proceedings of the Eleventh International Congress of Essential Oils, Fragances and Flavors, Oxford & IBH Publ. Co, New Delhi.

Gilpin ME, Soulé ME (1986). Minimum viable populations: processes of species extinctions. In: Conservation Biology: The Science of Scarcity and Diversity (Ed ME Soulé). Sinauer, Sunderland, MA.

Goudet J (1995). FSTAT (version 1.2), a computer program to calculate F-statistics. J. Hered. 86: 485-486. Available from: http://www.Until.ch/izea/softwares/fstat.html.

Goudet J (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.2). http://www.Until.ch/izea/softwares/fstat.html.

Grant PR (1998). Evolution on islands. Oxford: Oxford University Press. p. 352.

Grayer RJ, Kite GC, Goldestone FJ, Bryan SE, Paton A, Putievsky E (2003). Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. Phytochemistry 43:1033–1039.

Hamrick JL, Godt MJW (1989). Allozyme diversity in plant species. In: Plant Population Genetics: Breeding and Genetic Resources (Eds ADH Brown, MT Clegg, AL Kahler, BS Weir). Sinauer, Sunderland, MA. pp. 43-63

Khanuja SPS, Shasany AK, Pawar A, Lal RK, Darokar MP, Naqvi AA, Rajkumar S, Sundaresan V, Lal N, Kumar S (2005). Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon Spreng* (Poaceae). Biochem. Syst. Ecol. 33:171-186.

Mantel N (1967). The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209-220.

Miller AG (1985). The Genus *Lavandula* in Arabia and Tropical N.E. Africa. Notes from the Royal Botanic Garden, Edinburgh. 42:503-528.

Morone-Fortunato I, Montemurro C, Ruta C, Perrini R, Sabetta W, Blanco A, Lorusso E, Avato P (2010). Essential oils, genetic relationships and in vitro establishment of *Helichrysum italicum* (Roth) G. Don ssp. italicum from wild Mediterranean germplasm. Ind. Crop Prod. 32:639-649.

Nabli MA (1995). Essai de synthèse sur la végétation et la phyto-écologie tunisiennes. II et III. Le milieu physique et la végétation. Ecologie végétale appliquée, I.O.R., Tunisia. 5-6:542.

Nei M (1978). Estimation of average heterozygosity and distance from a small number of individuals. Genetics 89:583-590.

Pottier-Alapetite G (1981). Flore de la Tunisie, Angiospermes dicotylédones. Gamopétales. I.O.R., Tunisie. p. 654.

Sagnard F, Barberot C, Fady B (2002). Structure of genetic diversity in *Abies alba* Mill. from southwestern Alps: multivariate analysis of adaptive and non-adaptive traits for conservation in France. For. Ecol. Manage. 157:175-189.

SAS (1990). SAS User's Guide: SAS STAT, SAS BASIC. Version 6, fourth ed. SAS Institute Inc., SAS incl, Box 8000. Cary, NC 27512-8000, Cary, NC.

Slatkin M (1993). Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

Slavbovska V, Jancic R, Bojovic S, Milosavljevic S, Djokovic D (2001). Variability of essential oil of *Satureja montana* L. and *Satureja kitaibelii* Wierzb. ex Heuff. from the central part of the Balkan peninsula. Phytochemistry 57:71–76.

Sosa S, Altinier G, Politi M, Braca A, Morelli I, Della Loggia R (2005). Extracts and constituents of *Lavandula multifida* with topical anti-inflammatory activity. Phytomedicine 12(4):271–277.

Stuessy TF, Ono M (1998). Evolution and speciation of island plants. Cambridge: Cambridge University Press. p. 358.

Swofford DL, Selander RB (1981). BIOSYS-1 a Fortran program for the

comprehensive analysis of electrophoretic data in population genetics and systematics. Heredity 72:281–283.
Trindade H, Costa MM, Lima SB, Pedro LG, Figueiredo AC, Barroso JG (2009). A combined approach using RAPD, ISSR and volatile analysis for the characterization of *Thymus caespititius* from Flores, Corvo and Graciosa islands (Azores, Portugal). Biochem. Syst. Ecol. 37:670–677.
Vogel BS, Wildung MR, Vogel G, Croteau R (1996). Abietadiene synthase from grand fir (*Abies grandis*). J. Biol. Chem. 38:23262–23268.
Von Rudolff E (1975). Volatile leaf oil analysis in chemosystematic studies of North American conifers. Biochem. Syst. Ecol. 2:137–167.
Wang ZS, An SQ, Liu H, Leng X, Zheng JW, Liu YH (2005). Genetic structure of the endangered plant *Neolitsea sericea* (Lauraceae) from the Zhoushan Archipelago using RAPD markers. Ann. Bot. 95:305-313.
Weeden NF, Wendel JF (1989). Genetics of plant isozymes. In: Soltis, DE, Soltis, PS (Eds), Isozymes in Plant Biology. Dioscorides Press, Oregon, USA. pp. 46-72.
Weir BS, Cockerham CC (1984). Estimating F-Statistics for the analysis of population structure. Evolution 38:1358-1370.
Wright S (1951). The genetical structure of populations. Ann. Eugen. 15:323-354.
Zaouali Y, Boussaid M (2008). Isozyme markers and volatiles in Tunisian *Rosmarinus officinalis* L. (Lamiaceae): A comparative analysis of population structure. Biochem. Syst. Ecol. 36:11-21.