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Full Length Research Paper

Inter-specific relationships among two Tunisian *Thymus* taxa: *Thymus capitatus* Hoffm. et Link. and *Thymus algeriensis* Boiss. et Reut. using molecular markers

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Genetic relationships between two sympatric species *Thymus capitatus* Hoffm. et Link. and *Thymus algeriensis* Boiss. et Reut. (*Thymus hirtus* Willd. subsp. *algeriensis* Boiss. et Reut.) were assessed using random amplified polymorphic DNA (RAPD) markers. Eighteen natural populations from different geographical and bioclimatic zones were evaluated. The seven selected primers generated 121 RAPD markers for *T. capitatus* (103 polymorphic; P = 85.12%) and 154 for *T. algeriensis* (141 polymorphic; P = 91.56%). The genetic diversity within *T. capitatus* and *T. algeriensis* populations based on Shannon's index was high (H'_{pop} = 0.303 and 0.307, respectively). A high genetic differentiation was revealed (G_{ST} = 0.359 and Φ_{ST} = 0.284 for *T. capitatus*, G_{ST} = 0.335 and Φ_{ST} = 0.296 for *T. algeriensis*). The large proportions of the genetic variation were observed within populations for the two studied taxa. A high genetic population's structure was also estimated by a principal component analysis (PCA). Unweighted pair-group method using arithmetic average (UPGMA) cluster analysis based on Nei and Li's coefficient among populations identified that *T. algeriensis* and *T. capitatus* populations were clustered into two distinct groups. The high genetic divergence between the two species corroborates their taxonomic status.

Key words: Genetic diversity, inter-specific relationships, molecular markers, *Thymus capitatus*, *Thymus algeriensis*.

INTRODUCTION

Thymus is a large genus of Lamiaceae family comprising about 215 species and particularly prevalent in the Mediterranean area (Jalas, 1971). The systematics of species remains difficult because of the interspecific hybridization, polyploidy and morphological similarities among species (Morales, 1996; Tzakow and Constantinidis, 2005). The genus *Thymus* is known in several countries as a spice and food preservative, as well as a protective and curative remedy for many ailments. In Tunisian flora, the genus is represented by four species. Among them, *T. capitatus* and *T. algeriensis,* which can be sympatric in a wide part of their distribution area, are well represented (Pottier-Alapetite, 1981; Ben ElHadj Ali et al., 2010). Almost all species of this genus are currently used in popular medicine for stimulant, aromatic, antispasmodic, sedative, antioxidant, antibacterial and antiaflatoxigenic, and also in the treatment of diarrhoea, digestive and respiratory system disorders (Dob et al., 2006; Matta et al., 2007; Hazzit et al., 2009; Razzaghi-Abyaneh et al., 2009; Safaei-Ghomi et al., 2009).

In Tunisia, *T. capitatus* is known under the vernacular name of ''Zaater''. This shrub is about 20 to 60 cm height, the leaves (6 to 12 mm) are opposite and linear/lanceolate, the flowers are hermaphrodite large (7

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to 10 mm) and grouped in dense terminal heads with an uneven calyx (5 mm), with the upper three lobed and the lower cleft, and the corolla (5 to 8 mm) is deeper and pink or purple or white. The flowering period begins in June and continues until August. The species is a diploid (2n = 2x = 30) (Morales, 1996) and predominantly bee-pollinated outcrossing shrub, exhibiting both vegetative and sexual propagations (Petanidou and Vokou, 1993). Natural regeneration of genets from seeds is extremely rare because of the difficulty of seedling survival, mainly consequent to the low habitat quality (climatic factors, soil degradation, etc) (Eriksson, 1998; Pérez-Garcia et al., 2003). In Tunisia, *T. capitatus* populations are located in different bioclimatic zones (extending from the sub-humid to the upper arid) on sandy and often on rocky soils, under a rainfall varying from 300 to 1000 mm/year and at altitudes ranging from 150 to 500 m (Nabli, 1995).

T. algeriensis is an endemic plant of semi arid and arid areas of Tunisia and Algeria (Pottier-Alapetite, 1981; Le Floc'h and Boulos, 2008). It is a short lived, diploid (2n = 2x = 30) and gynodioecious shrub (Morales, 1996). It reproduces by seeds and can reach 20 to 50 cm in height. Leaves are opposite and linear/lanceolate (6 to 12 mm). Flowers with ovate bracts and pink purplish or whitish purple corolla are small (5 to 7 mm). Hermaphrodite (male fertile) and female (male sterile) plants can occur in the same populations. Flowering takes place between April and June. The species is believed to be an outcrosser as most thymes and often pollinated by bees (Tarayre and Thompson, 2002; Orellana et al., 2005). However, self pollination may occur in hermaphrodite plants. In Tunisia, *T. algeriensis* populations are distributed from the sub-humid to the lower arid bio-climates at altitudes ranging from 120 to 1100 m. The species grows on poor fertile calcareous soils and occurs in scattered small populations showing different level of destruction, mainly due to overharvesting and overgrazing.

In the present study, we used RAPD approaches to characterize two Tunisian species of *Thymus* and evaluate their genetic relationships. This method is broadly used in plant population genetic and differentiation studies (Monteleone et al., 2006; Solouki et al., 2008; Zheng et al., 2008; Trindade et al., 2009). Generally, RAPD has allowed the resolution of complex taxonomic relationships (Wolff and Richards, 1999; Casiva et al., 2002; Ruana et al., 2004) and phlogenetic studies (Dababneh, 2007; Mariette et al., 2007; Paolini et al., 2009).

MATERIALS AND METHODS

Surveyed populations and sampling

The plant materials used in this study were collected from nine populations across 6 bioclimatic zones according to Emberger's (1966) pluviothermic coefficient (Q_2): sub-humid, upper semi-arid, mean semi-arid, lower semi-arid upper arid and lower arid bioclimates (Table 1 and Figure 1). The random sample size in each population was ten individuals. Fresh leaves were sampled from individuals, at least 20 m apart, to reduce the chance of consanguinity.

Genomic DNA extraction and PCR conditions

Total genomic DNA was isolated from about 0.5 g young leaves using a modified CTAB protocol, as outlined by Lodhi et al. (1994). DNA quantity was estimated spectrophotometrically (absorbance at 260 nm). An agarose gel (0.8%) stained with ethidium bromide was used to verify the quality of DNA.

PCR was performed in a 25 µL reaction volume containing 50 ng DNA templates, 5 µL of 5 X reaction buffer, 40 pmoles of primer, 200 µM of each dNTP, 2.5 mM $MgCl_2$ and 1.5 U Taq polymerase (Promega). The mixture was overlaid with one drop of mineral oil. Reactions were performed in Stuart Thermal Cycler (Maxi-Gene) programmed for an initial denaturation step of 94°C for 2 min, followed by 45 cycles of 30 s at 94°C, 1 min at 36°C (annealing step), and 2 min at 72°C (elongation step). An additional 10 min period for elongation at 72°C followed this cycle. To test reproducibility between and within runs, DNA from same two plants was included in every PCR run. A negative control without DNA was also used in every PCR run. Eight primers were tested and only seven out of them were selected (OPJ-06, OPJ-08, OPJ-10, OPJ-12, OPJ-13, OPJ-14 and OPJ-16). Amplification products were separated by electrophoresis in 1.5% agarose gels in TAE buffer (pH 8), stained with ethidium bromide, and visualized under UV light using a DOC PRINT Photo Documentation System. Molecular weights were estimated using a 200 bp DNA Promega ladder.

Data analysis

RAPD bands were scored as presence (1) or absence (0) of bands and transformed into a binary matrix. Each marker band was assumed as being a single locus. For each primer, the percentage of polymorphic bands (P%) was estimated for the studied populations. The genetic diversity within population was estimated using P% and Shannon's index (H'). Shannon's index was also used to estimate the average diversity (H_{pop}) over all populations ($H_{pop} = -1/n\ \Sigma\ H'$; where n is the number of populations), and the diversity (H_{sp}) at species level ($Hsp = -\Sigma ps \log 2\ ps$; where ps is the frequency of presence or absence of the RAPD in the whole sample). The proportion of diversity within populations was estimated as H_{pop}/H_{sp}. Population differentiation was analyzed for polymorphism between populations by the gene differentiation coefficient (G_{ST}) [GST = (Hsp - Hpop)/Hsp]. The different indices were calculated by the POPGENE computer package (Yeh et al., 1999). The comparison among Shannon's diversity indices at the population levels was performed using a variance analysis (ANOVA procedure, SAS, 1990) and Duncan's test (Dagnelie, 1975).

The partitioning of the genetic variation within and among populations, besides H_{pop}/H_{sp} and G_{ST} estimates, was evaluated by AMOVA using WINAMOVA program, version 1.55 (Excoffier et al., 1992). Φ-statistics: Φ_{ST} (differentiation among populations), Φ_{CT} (differentiation among ecological groups) and Φ_{SC} (differentiation among populations within groups) were calculated. The significance of variance components and that of Φ-statistics were estimated using permutation procedures (NTSYS-pc, version 2.0, Rohlf, 1998). The genetic similarity between individuals was estimated using the Nei and Li's (1979) similarity coefficient S_{xy} [$S_{xy} = 2m_{xy}/(m_x + m_y)$, where, m_{xy} is the number of bands shared by samples x and y, and m_x and m_y are the number of bands in samples x and y, respectively]. The genetic distance (D_{xy}) between individuals was estimated using the complementary value S_{xy} [$D_{xy}=1-S_{xy}$]. In addition a PCA based on allelic frequencies was performed to

Table 1. Location and main ecological traits for *Thymus capitatus* and *Thymus algeriensis* populations analysed.

Species	Code	Population	Bioclimatic zone[B]	Latitude	Longitude	Q_2[C] coefficient	Altitude (m)	Rainfall (mm/year)
Thymus capitatus	C_1	El Hairech Jeb. Mt.[A]	Sh	36°10'N	8°4'E	64.52	400	500 - 600
	C_2	Abderrahmen Jeb. Mt.	Sh	36°40'N	10°41'E	65.64	420	500 - 600
	C_3	Bou Argoub	Usa	36°31'N	10°3'E	59.72	100	400 - 500
	C_4	Mansour Jeb. Mt.	Usa	36°17'N	9°36'E	45.72	600	400 - 500
	C_5	Essers	Msa	36°76'N	9°40'E	44.24	604	400 - 500
	C_6	Goubellat Jeb. Mt.	Msa	36°60'N	9°52'E	39.75	300	300 - 400
	C_7	Khenis	Lsa	35°45'N	10°49'E	34.05	300	300 - 400
	C_8	Aïn Errahma	Lsa	36°13'N	10°22'E	33.24	100	300 - 400
	C_9	Toujene	Ua	33°27'N	9°58'E	29.19	600	100 - 150
Thymus algeriensis	A_1	Sabbah Jeb. Mt.	Sh	36°46'N	9°1'E	83.5	460	600 - 700
	A_2	Bahra	Usa	36°14'N	8°36'E	49.77	450	400 - 500
	A_3	Mansour Jeb. Mt.	Usa	36°17'N	9°36'E	45.72	600	400 - 500
	A_4	Essers	Msa	36°76'N	9°40'E	43.24	610	400 - 500
	A_5	Chaambi Jeb. Mt.	Msa	35°11'N	8°45'E	44.9	1010	400 - 500
	A_6	Chrechira Jeb. Mt.	Lsa	35°8'N	9°29'E	35.3	560	300 - 400
	A_7	Toujene	Ua	33°27'N	9°58'E	29.19	600	100 - 150
	A_8	Ouled Bou Saad	La	34°27'N	8°35'E	18.43	320	150 - 200
	A_9	Douaou Jeb.Mt.	La	34°38'N	9'30'E	28.83	650	150 - 200

[A]Jeb. Mt: Jebel Mountain; [B]bioclimatic zone: Sh, sub-humid; Usa, upper semi-arid; Msa, mean semi-arid; Lsa, lower semi-arid; Ua, upper arid. [C]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 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 2006 (data provided by the Tunisian National Institute of Meteorology).

ordinate the relationship among all populations of the two species using the Multi-variate Statistical Package (MVSP) 3.1 program. The divergence between population's species was also estimated by Nei and Li's genetic similarity calculated among population pairs.

The unweighted pair-group method using arithmetic average (UPGMA) was used to construct a dendrogram representing all populations.

RESULTS

RAPD analysis

RAPD analysis of 90 individuals from nine *T. capitatus* populations using seven primers generated a total of 121 bands with fragments ranging in the size from 200 to 2000 bp (an average of 17.28 bands per primer). Of these bands, 103 were polymorphic and the P% for this species was 85.12%. The number of polymorphic bands amplified per primer varied from 10 to 22, with an average of 14.71 (Table 2). For *T. algeriensis* populations (90 individuals), a total of

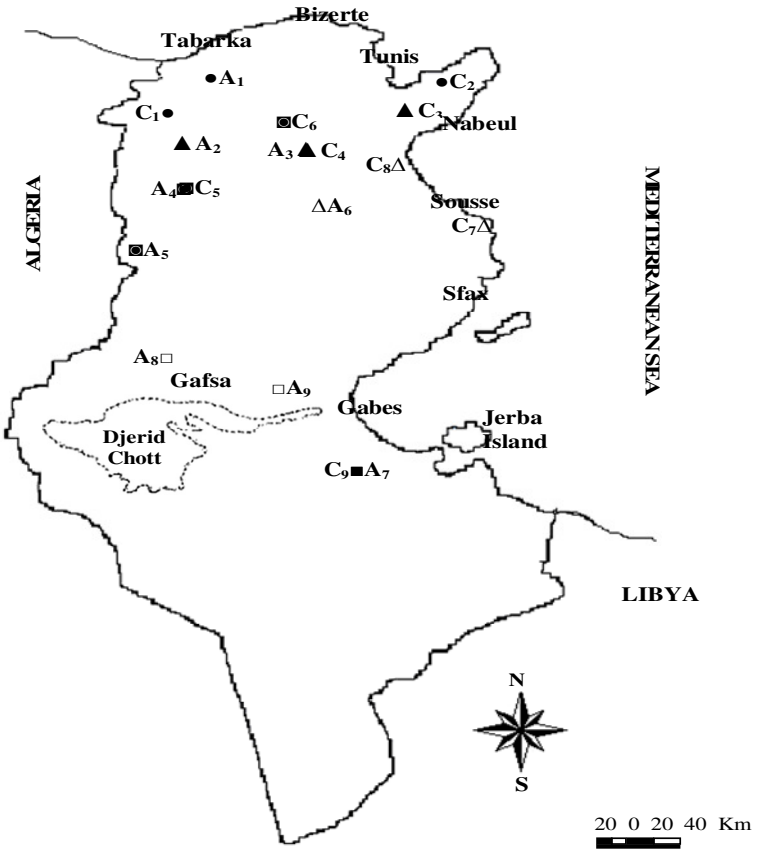

Figure 1. Map of Tunisia: Geographical distribution of *Thymus capitatus* and *Thymus algeriensis* populations analysed. C_1 to C_9: *T. capitatus* populations ; A_1 to A_9: *T. algeriensis* populations. Symbols indicate bioclimates: ●Sub-humid; ▲upper semi-arid; ◙ mean semi-arid; ∆ lower semi-arid; ■ upper arid, □ lower arid.

154 RAPD fragments were amplified in the size ranging from 200 to 2000 bp with 141 polymorphic bands (91.56%). The number of bands varied from 20 (OPJ-10) to 24 (OPJ-13 and OPJ-16) with an average of 22 at the species level (Table 2). Bands 1700, 820 and 1200 bp revealed respectively by OPJ-06, OPJ-10 and OPJ-14 were monomorphic for all populations of the two taxa. Exclusive bands according to primer were however found in *T. algeriensis* populations. For example, bands 460, 750 and 1200 bp (OPJ-06), bands 240 and 360 bp (OPJ-08), bands 300, 580 and 680 bp (OPJ-10), bands 780 bp (OPJ-12) and 1500 bp (OPJ-12), bands 200, 1000 and 1600 bp (OPJ-13), 320, 600 and 800 bp (OPJ-14), and bands 520, 850 and 900 bp (OPJ-16). Moreover, bands according to 340 and 550 bp (OPJ-06), 700 and 1500 bp (OPJ-08), 500 and 1500 bp (OPJ-10), 1000 bp (OPJ-12), 420 and 660 bp (OPJ-13), 220 and 500 bp (OPJ-14), 420 and 1100 bp (OPJ-16) were restricted only to *T. capitatus* populations.

For *T. capitatus*, the average percentage of the polymorphic loci detected was 69.26% (Table 3). Shannon's diversity index (H') values, estimating the within-population variability, did not show significant differences among populations (ANOVA test, $p > 0.05$). The average of H' value for all populations was 0.303, indicating a low level of the genetic diversity within *T. capitatus* populations. At the species level, the Shannon's index was moderate (H_{sp} = 0.473). The proportion of within-populations variation (H_{pop}/H_{sp} = 0.641) was high (Table 3), while in *T. algeriensis*, the percentage of polymorphic loci was 59.74%. The genetic diversity index (H') varied significantly among populations (ANOVA test, $P = 0.03 < 0.05$) with an average of 0.307. The averages within all populations (H_{pop}) and within the species (H_{sp}) were 0.307 and 0.461, respectively. The most of the variation occurred within populations (H_{pop}/H_{sp} = 66.5%) (Table 3).

Genetic structure and divergence among species

For *T. capitatus*, the G_{ST} value estimating the genetic structure among populations was 0.359 (Table 3). The within-population component of variance estimated

Table 2. RAPD primers and bands produced for 18 Tunisian populations of *Thymus capitatus* and *Thymus algeriensis*.

Primer	Sequence	*Thymus capitatus*			*Thymus algeriensis*		
		Total bands	Polymorphic bands	% Polymorphic loci	Total bands	Polymorphic bands	% Polymorphic loci
OPJ06	5'TCGTTCCGCA 3'	13	11	84.61	22	21	95.45
OPJ08	5'CATACCGTGG 3'	22	22	100	21	21	100
OPJ10	5'AAGCCCGAGG 3'	20	18	90	20	16	80
OPJ12	5'GTCCCGTGGT 3'	15	13	86.67	21	20	95.24
OPJ13	5'CCACACTACC 3'	13	10	76.92	24	22	91.67
OPJ14	5'CACCCGGATG 3'	17	11	64.71	22	19	86.36
OPJ16	5'CTGCTTAGGG 3'	21	18	85.71	24	22	91.67
Total over loci		121	103	85.12	154	141	91.56
Mean per primer		17.28	14.71	85.13	22	20.14	91.48

Table 3. Genetic diversity of *Thymus capitatus* and *Thymus algeriensis* populations, as estimated by Shannon's index.

Specie	*Thymus capitatus*	*Thymus algeriensis*
Polymorphism (%)	69.26	59.74
H_{pop} [A]	0.303[ns]	0.307*
H_{sp} [B]	0.473	0.461
H_{pop}/H_{sp} [C]	0.641	0.665
G_{ST} [D]	0.359	0.335

[A]Average within all populations; [B]total genetic diversity among all individuals; [C]proportion of diversity within all populations; [D]differentiation among populations. *Significant, $p < 0.05$; [ns]not significant (ANOVA test).

through AMOVA accounted for 71.61% of variation (Table 4). The mean Φ_{ST} value among all populations was 0.284 and was significantly different from zero. The differentiation among groups was low (Φ_{CT} = 0.019). For *T. algeriensis*, the level of the differentiation among populations estimated by G_{ST} (0.335) was high (Table 3). The within-population component of variance estimated through AMOVA accounted for 70.37% of overall variation (Table 4). The mean Φ_{ST} value among all populations was 0.296 ($p < 0.001$), indicating a high differentiation among them. The differentiation among ecological groups (Φ_{CT} = 0.116, $p < 0.001$) or among populations within ecological groups were also significant (Φ_{SC} = 0.212, $p < 0.05$). The PCA based on allelic frequencies matrix for all populations showed that the first three principal axes accounted for 60.33% of the total variation. The plot according to the first two PCA axes (54.75%) showed a clear distinction among the two species populations and revealed two major population groups (Figure 2). The first one projected at the negative side of axes 1 and 2, is represented by *T. capitatus* populations. The second group, situated at the positive side, includes populations of *T. algeriensis*. Squared Nei and Li's genetic similarity

Table 4. Nested analysis of molecular variance (AMOVA) at hierarchical levels of *Thymus capitatus* and *Thymus algeriensis* populations.

Specie	*Thymus capitatus*	*Thymus algeriensis*
Total variance (%)		
Within populations	71.61	70.37
Among populations	28.39	29.63
Φ-statistics		
Φ_{ST}	0.284**	0.296**
Φ_{CT}	0.019 ^{ns}	0.116**
Φ_{SC}	0.272**	0.212**

**Significant, $p < 0.001$ (after 1000 permutations), ^{ns}not significant.

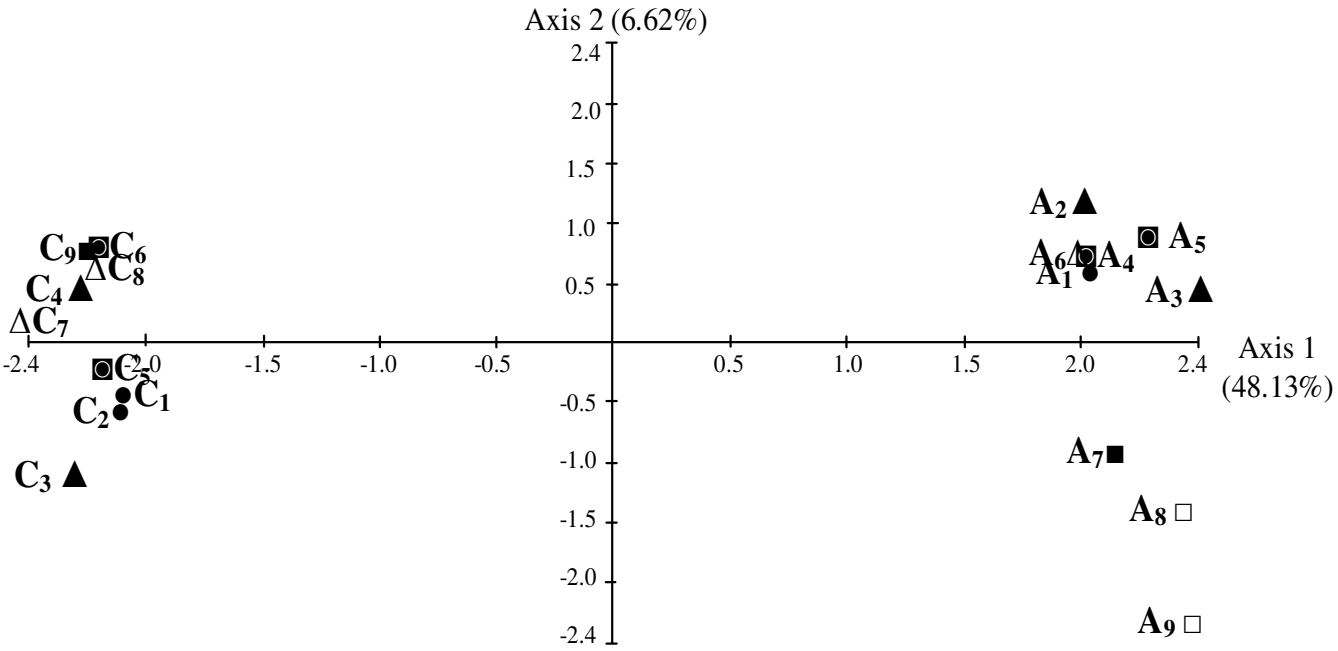

Figure 2. Principal coordinates analysis (PCoA) generated from Nei and Li's similarity coefficient for the 18 populations analysed. Plot according to axes 1-2. C_1 to C_9, *Thymus capitatus* populations; A_1 to A_9, *Thymus algeriensis* populations. ● Sub-humid; ▲ upper semi-arid; ◙ mean semi-arid; ∆ lower semi-arid; ■ upper arid; □ lower arid.

among individual pairs ranged from 0.259 to 0.909 (Data not shown). The lowest value (0.259) was observed between two individuals from Douaou Jebel Mountain population (*T. algeriensis*) and El Hairech Jebel Mountain population (*T. capitatus*). However, the highest value (0.909) was noted between two individuals (6 and 9) from the same population Toujene (*T. capitatus*; C_9). Table 5 shows the Nei and Li's coefficient genetic similarity between populations. This coefficient ranged between 0.477 and 0.954. The lowest value (0.477) was observed between population Douaou Jebel Mountain from *T. algeriensis* (Population A_9) and population Essers from *T. capitatus* (Population C_5), while the highest value (0.954) separated two populations of *T. capitatus*: Aïn Errahma (Population C_8) and Khenis (Population C_7). The UPGMA dendrogram generated from Nei and Li's similarity matrix for all populations showed two distinct groups of populations according to the taxa, which often coincides with the geographic distance between the populations (Figure 3). The first cluster (GI) consisted of *T. algeriensis* populations, while the second (GII) exclusively composed of *T. capitatus* populations.

DISCUSSION

Results herein represented the first use of molecular (DNA) markers to characterize genetic diversity and

Table 5. Similarity matrix among *Thymus capitatus* and *Thymus algeriensis* accessions by Nei and Li's coefficient based on RAPD bands.

Population	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8
C_2	0.927																
C_3	0.898	0.915															
C_4	0.904	0.921	0.883														
C_5	0.917	0.907	0.906	0.912													
C_6	0.946	0.936	0.908	0.950	0.945												
C_7	0.909	0.898	0.907	0.940	0.917	0.937											
C_8	0.908	0.897	0.887	0.949	0.897	0.936	0.954										
C_9	0.922	0.912	0.901	0.926	0.893	0.941	0.931	0.930									
A_1	0.542	0.534	0.511	0.559	0.552	0.564	0.520	0.534	0.523								
A_2	0.554	0.555	0.525	0.569	0.555	0.574	0.567	0.563	0.569	0.849							
A_3	0.538	0.548	0.518	0.571	0.548	0.568	0.543	0.565	0.545	0.844	0.882						
A_4	0.548	0.549	0.528	0.546	0.523	0.568	0.536	0.549	0.555	0.836	0.866	0.862					
A_5	0.552	0.562	0.541	0.568	0.536	0.581	0.557	0.579	0.568	0.835	0.888	0.845	0.899				
A_6	0.557	0.558	0.537	0.564	0.549	0.586	0.545	0.558	0.564	0.808	0.856	0.827	0.867	0.898			
A_7	0.558	0.568	0.527	0.574	0.559	0.596	0.563	0.559	0.565	0.812	0.789	0.790	0.833	0.840	0.830		
A_8	0.556	0.557	0.535	0.571	0.557	0.585	0.552	0.557	0.554	0.844	0.866	0.837	0.862	0.845	0.835	0.840	
A_9	0.487	0.486	0.491	0.502	0.477	0.518	0.491	0.486	0.493	0.830	0.813	0.832	0.841	0.807	0.813	0.852	0.874

relationships between two sympatric *Thymus* species (*T. capitatus* and *T. algeriensis*) in Tunisian flora. Our study on the spatial structure of the two species showed that the *T. capitatus* and *T. algeriensis* populations maintains high genetic diversity within populations (H_{pop}/H_{sp} = 0.614 and H_{pop}/H_{sp} = 0.665, respectively). Compared with other species analysed by RAPDs, the observed within-population diversity was lower than that reported for several Lamiaceae species with an outcrossing mating system (Verma et al., 2007; Boulila et al., 2009). From the result of this work, the high genetic diversity inside populations could be due to genetic bottlenecks associated probably with founder events (Thompson, 1999, 2002). Nevertheless, the mixed mating system (vegetative and sexual reproduction modes) could also contribute to decreasing the level of the genetic diversity within population. In our work, several RAPD loci appeared to be specific to populations according the taxa. Populations from the same bio-climate (populations Mansour Jeb. Mt. (C_4 and A_3), Essers (C_5 and A_4), and Toujene (C_9 and A_7)) do not reveal specific loci, and for this reason the detection of these loci might not reflect adaptability to ecological factors.

At interspecific level, *T. capitatus* and *T. algeriensis* populations were highly differentiated (G_{ST} = 0.359 and Φ_{ST} = 0.284; Φ_{ST} = 0.296 and G_{ST} = 0.335, respectively). The observed amount of differentiation was higher than the average for perennial outcrossing species (G_{ST} = 0.22, Φ_{ST} = 0.27) (Nybom and Bartish, 2000; Nybom, 2004) or species with a mixed mating system (G_{ST} = 21.2 - 24.0%) (Hamrick and Godt, 1996). This high differentiation could be explained by genetic drift due to limited gene flow via seed and/or pollen dispersal. It is well known that the most thymes species were qualified to be short distance dispersal species. Thompson (2002) reported that pollen and seed dispersals in thymes are highly localised, increasing the tendency for reproduction to occur within spatially localized groups. Furthermore, AMOVA analysis further revealed that most of the total variation was found within populations (71.61% for *T. capitatus* and 70.37% for *T. algeriensis*). This suggests that mating occurs mainly among individuals within a sub-population thus favouring the divergence among populations. Our RAPD-based AMOVA studies show that most genetic variation in the two *Thymus* species is distributed within populations rather than between them, indicating a relatively

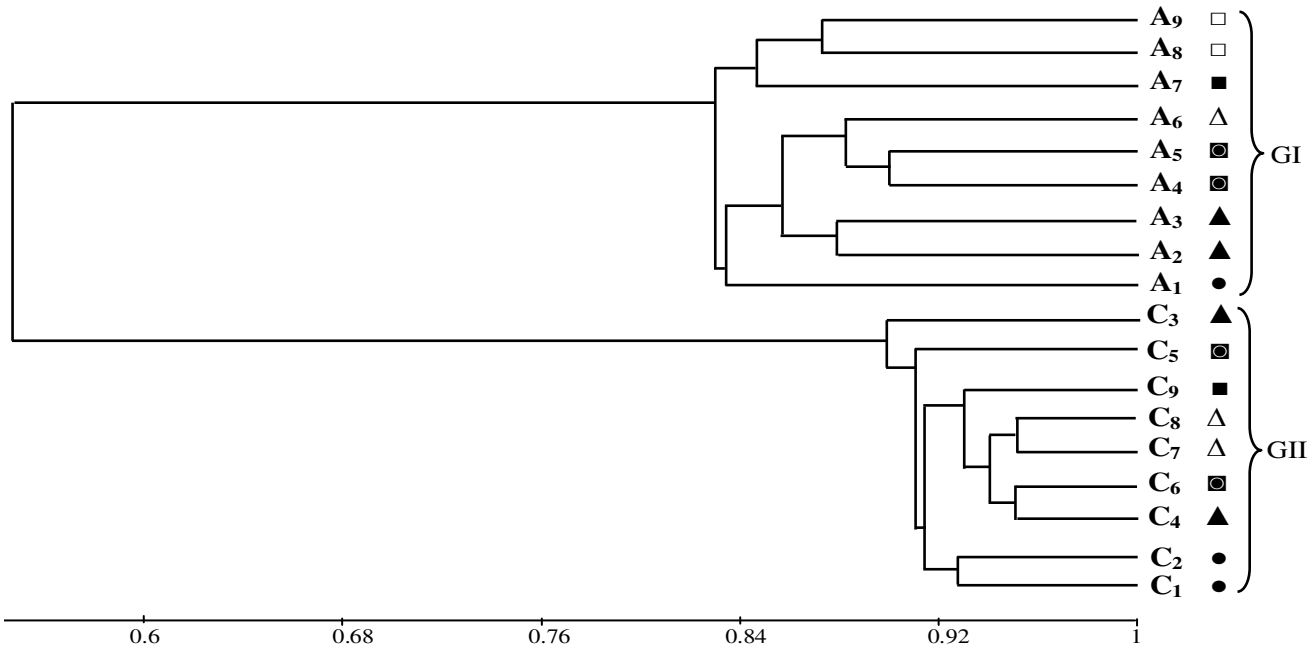

Figure 3. UPGMA dendrogram generated from Nei and Li's similarity coefficient among the two taxa of *Thymus* populations. C_1 to C_9, *Thymus capitatus* populations; A_1 to A_9, *T. algeriensis* populations. ● Subhumid ; ▲ upper semi-arid; ◙ mean semi-arid; Δ lower semi-arid ; ■ upper arid; □ lower arid.

restricted population differentiation as expected in outcrossing species. Such a pattern of population genetic structure has been previously reported for perennial species (Messaoud et al., 2007; Boulila et al., 2009).The most apparent difference between two studied *Thymus* species is the higher level of polymorphic markers in *T. algeriensis* suggesting a higher level of genetic variability. The higher level of this genetic diversity could be explained by the mating system of *T. capitatus* which attributed to be a hermaphrodite shrub, while *T. algeriensis* is a gynodioecious shrub with hermaphrodite (male fertile) and female (male sterile) plants occurring in the same population. A high distinction between populations and individuals (data not shown) of these closely related species was revealed indicating that genetic differentiation mainly occurs at local space scale due to limited gene flow and genetic drift. Thus, the gene flow via seed and pollen dispersion between adjacent populations, represent a critical determinant of the genetic structure of natural plant populations (Brown, 1978). This fact confirms again that RAPDs are appropriate to achieve classification at the species and intra-species levels.

In addition, multivariate analysis as showed by PCA analysis, performed on all populations, revealed that populations plotted as a broad scatter, and gathered often with relationship to the species. Two major population groups according to taxa were revealed. The UPGMA analysis divided samples into two distinct groups of species indicating a genetic separation between the two taxa due to interspecific reproductive barriers. The interspecific differentiation was also supported by the presence of specific alleles. Our findings, based on RAPDs, corroborate previous morphological studies which classified *T. capitatus* and *T. algeriensis* into distinct species belonging to different sections. However, according to morphological data, *T. capitatus* and *T. algeriensis* are closely related (Manicacci et al., 1998). Indeed, several authors have considered the position of *T. capitatus* in relation with the other *Thymus* species as a taxonomic problem. However, Jalas (1971) have considered that there is sufficient geobotanical, morphological, cytological and chemotaxonomic evidence to include *T. capitatus* in *Thymus,* but separated from other species by being placed in a particular subgenus: *Coridothymus* (Reichenb. ill.). Tunisian thyme occurs in small scattered populations showing high population genetic differentiation coupled with a low level of gene flow. The overgrazing, over-gathering and the increasing habitat destruction may jeopardize the maintenance of most populations. These processes may increase genetic drift.

Conclusion

RAPD were successful in revealing variations in *Thymus* at the species level. The combined analysis of UPGMA clustering and PCA plot revealed that *T. capitatus* and *T. algeriensis* were clustered more distantly. The

occurrence of species-specific RAPD markers allowed clear identification of each species, although these markers must be considered as putative species-specific markers due to the limited sampling of *T. capitatus* and *T. algeriensis* species.

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