MOLECULAR CHARACTERIZATION OF ENDANGERED SINAI THYME (*THYMUS DECUSSATUS* BENTH.) GROWING ON GEBEL MOUSA, SOUTH SINAI, EGYPT

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ABSTRACT

Gebel Mousa located in Saint Katherine Protectorate (SKP), South Sinai, Egypt. It harbors many endemic and endangered medicinal plants that are overexploited and facing serious threat of extinction. From these important medicinally plant species, we select *Thymus decussatus* (near-endemic and endangered plant) to investigate the genetic diversity level among eight populations using both of random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) molecular markers. Seven primers were chosen for RAPD and six primers for ISSR analysis. Thirteen RAPD and ISSR primers amplified 97 bands with an average of 7.46 fragments / primer and the total number of polymorphic fragments was 84, thus, representing a level of polymorphism of 86.70%. Jaccard's similarity coefficient values ranged from 0.37 to 0.64. The highest similarly value (0.64) was scored between Farsh Shoeib and Corridor between Farsh El-Hamar & Farsh Shoeib populations as the closet. On the other hand, the lowest similarity value (0.37) was recorded between Farsh Ellya in direction of G. Mousa and Corridor between Farsh El-Loza & Farsh Ellya populations as most distant. The dendrogram separated the eight *T. decussates* populations into two clusters. The first cluster included Farsh El-Sefsafa, Farsh El-Hamar; Corridor between Farsh El-Loza & Farsh Ellya. The second cluster included Farsh Shoeib, Corridor between Farsh El-Hamar & Farsh Shoeib, Farsh Ellya and Farsh Ellya in direction of G. Mousa and Farsh El-Loza. Assessing of genetic polymorphisms among the *Thymus* populations at varying microhabitats can help in their genetic improvement and their conserving programs.

Keywords: *Thymus decussatus,* Saint Katherine Protectorate, Gebel Mousa, Molecular markers

1. INTRODUCTION

Saint Katherine Protectorate (SKP) located at Southern Sinai is considered one of the largest protected areas in Egypt which included the highest mountains rich with about 44 percent of endemic plant species in Egypt. In Southern Sinai, Mountains flora varies from the other areas because of the higher variability in ecosystems, climatic conditions, water environment, and elevations [1-3]. Gebel Mousa harbors many rare endangered and native plants that are overexploited and facing severe threat of extinction.

Thymus genus, belonging to family *Lamiaceae*, integrates 928 species distributed all over the world and among them about 215 species were mainly distributed in Mediterranean area [4-7]. Owing to its aromatic nature, thyme plant has good antimicrobial, antiparasitic, antispasmodic and antioxidant properties [5, 8]. Therefore, their medicinal properties made it one of the most essential and common medicinal herbs [9]. Three species known as *T. capitatus, T. bovei*, and *T. decussatus* have been reported for this genus in Egypt, the later one was used in this analysis.

Genetic polymorphism refers to the degree of genetic variation that can occurs at different scales including populations, subpopulation or even among neighboring individuals [10] and it plays an important role in adaptation of a plant species to environmental stimuli [11], Ecogeographical patterns affect the distribution of genetic diversity in a species. Association of genetic diversity with variation in ecogeographical patterns has been detected in several species of aromatic plants [12-16].

Knowledge of genetic variation among *T. decussatus* populations is critical for their survival and also for better use of their genetic resources. Sinai thyme is distributed in scattered and patches microhabitats [17] in SCP, where it was restricted to mountain heads at altitudes above 1500 m [18] and it was growing well in the bases of cliffs and wades. Thyme will be regularly a significant species for corrupted ecosystems particularly arid environments for longer grazing pressure [19].

DNA markers are used in determining genetic variability in many plant species. Random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSRs), simple sequence repeats (SSRs), and amplified fragment length polymorphism (AFLP) are the most widely used DNA markers [20-23]. Genetic variability of aromatic and medicinal plant populations has been analyzed using either RAPD or ISSR or both [24-26]. The genetic diversity among the different accessions or populations of thyme plant using either RAPD or ISSR or both molecular markers was evaluated in different studies. Genetic variation within and among eight populations of the endemic Tunisian *Thymus algeriensis* plant species collected from variant geographical habitats was assessed using seven RAPD primers[27]. Their results recorded presence of high genetic variation within and among the studied populations.

RAPD markers were also used in genetic identification of five accessions of Palestinian *Thymus* for their ability to produce polymorphisms[28].Moreover, genetic diversity of ten *Thymus kotschyanus* populations obtained from various ecosystems in Iran was analyzed using RAPD markers[29].Their findings showed high level of genetic diversity among the ten studied populations of *T. kotschyanus*. Even, twenty ISSR primers were applied for evaluation of the genetic relationships among fourteen *Thymus*accessionsbelonging to three *T. daenensis*, *T. kotschyanus* and *T. vulgaris* species[30].

Understanding the patterns of genetic variation between populations is important for the establishment of efficient conservation strategies for growing plants [31].Thus, great efforts should be carried out on preserving the rare endangered species as well as the endemic species with restricted geographical distribution [32].From these near-endemic and endangered plant species, *T. decussates* plant species growing in Gebel Mousa was selected in this study to assess the degree of genetic variation among their populations using both of RAPD and ISSR molecular markers.

2. MATERIAS AND METHODS

2.1. Study area and population sampling

The Sinai Peninsula forms a land bridge between Africa and Asia and its flora and fauna have been influenced by both continental masses; and contains the Saint Katherine Protectorate which covers the mountainous region of Southern Sinai. St. Catherine Protectorate is characterized by the highest mountains in Egypt, a dense Wadi system and an arid climate, it was declared as a protected area in 1996 due to its biological and cultural interest [33]**.**St. Katherine Protectorate is located between 33º 30’ to 34º 30’ E, and 28° 50’ to 29° 50’N (Fig. 1).Gebel Mousa (2285 m above sea level), located in St. Catherine Protectorate, is the most active tourist area near the city of Saint Katherine. The presence of *main vegetation* in the Gebel Mousa occurs on an altitude between 1950 to 2031 m above sea level (asl), in 4 central areas respectively from south to north as; Farsh Kaniset Al-Hamar, Farsh Al-Shoeibi, Farsh Al-Loza and Farsh Eilya. Eight populations of *T. decussatus* were collected from Gebel Mousa, St. Catherine Protectorate, South Sinai, Egypt (Fig. 2)during vegetation season of summer 2015.

Young aerial parts of at least five individuals from each population were collected randomly, where the location name and coordination points of each studied populations were located by a GPS (Table 1). The aerial parts were air-dried and transported back to our laboratory for DNA extraction. The voucher specimens were deposited in the Botany and Microbiology Department Herbarium Faculty of Science, Al-Azhar- University.

Fig. 1: Location map of St. Katherine Protectorate (SKP) with a black line boundary.

Fig.2: Photo of *Thymus decussatus* Benth.

2.2. Isolation and quantification of genomic DNA

The experiments of genomic DNA extraction and PCR amplification reactions were carried out in Biotechnology Central Laboratory, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. The genomic DNA was isolated from approximately 100 mg air-dried aerial part. Five plant samples from each population were mixed together as bulk for DNA extraction. Extraction of the bulking DNA was carried out using the DNeasy ® Plant Mini Kit (Qiagen, Hilden, Germany)according to the manufacturer manual. Air-dried tissue was ground using liquid nitrogen to a fine powder, then, the powder was transferred to an appropriately sized tube. Then, 400 µl of buffer AP1 and 4 µl of RNase were added to a maximum of 100 mg of ground plant tissue and mixed well. The mixture was incubated for 10 min at 65ºC and mixed 2-3 times during incubation by inverting the tube. Then, 130 µl of buffer AP2 was added to the lysate, mixed and incubated for 5 min on ice. Lysate was applied to the QIA shredder spin

column sitting in a 2 ml collection tube and centrifuged for 2 min at14000 rpm.

The supernatant was transferred to a new tube without disturbing the cell-debris pellet. Typically, 450 µl of lysate was recovered. Then, 0.5 volume of buffer AP3 and 1 volume of ethanol (96-100%) were added to the cleared lysate and mixed by pipetting. Then, 650 µl of the mixture was applied through DNeasy Mini spin column setting in a 2 ml collection tube. Then, centrifuged for 1 min at 8000 rpm and flow-through was then discarded. DNeasy column was then placed in a new 2 ml collection tube and 500 µl of buffer A was added onto the DNeasy column and centrifuged for 1 min at 8000 rpm. Then, 500 µl buffer A was added to DNeasy column and centrifuged for 2 min at 14000 rpm to dry the column membrane. DNeasy column was then transferred to a 1.5 ml microfuge tube and 100 µl of preheated (65°C) buffer AE was pipetted directly onto the DNeasy column membrane. Then, incubated for 5 min at room temperature and centrifuged for 1 min at 8000 rpm to elute the purified DNA from the DNeasy spin column.

Quality of the extracted genomic DNA was tested by running on 1 percent agarose gel and their concentration was calculated by calculating the ratio of optical densities measured at 260-280 nm with a spectrophotometer (Thermo Scientific Type UV1, England). Reasonable dilutions of the extracted DNA were made for further amplification and polymerase chain reaction (PCR) analysis. Isolated DNA was stored at -20°C for further PCR amplification reactions.

2.3. Primers used in RAPD and ISSR analysis

Twenty RAPD and sixteen ISSR oligonucleotides synthesized by Operon technologies (Almeda, Calif.) were obtained for PCR reactions. Seven primers for RAPD and six primers for ISSR were chosen for PCR analysis based on the banding profiles and DNA polymorphic patterns that are produced (Table2). Other remaining primers were did not give a clear amplifications.

2.4. PCR amplification reactions and conditions

PCR reactions were performed in 25 µl per tube[20], containing 2 µl DNA (20 ng), 1 unit Taq DNA polymerase enzyme, 2 µl 10X buffer, 2 µl MgCl2 (25 mM), 2 µl dNTPs (2.5 mM each), 2 µl primers (10 pmol) and 14.8 µl H2O. Reactions to the RAPD and ISSR-PCR amplification were performed in thermocyclers (MJ Research, USA, PCT-200). RAPD – PCR reaction was accomplished by one period of 5 min at 94°C and 30 cycles, each cycle included a 60-second denaturation stage at 94°C, a 60-second annealing stage at 36°C and a 120-second extension step at 72°C; followed by a final extension step at 72°C for 5 min. After the final extension step, the samples were cooled at 4°C. ISSR – PCR amplification reactions involved an initial denaturation step of 94°C for 5 min, followed by 35 cycles of denaturation step at 94°C for 30 seconds, an annealing step at 52°C for 45 seconds and extension step at 72 °C for 120 seconds; then a final extension step at 72°C for 5 min. After the final extension step, the samples were cooled at 4°C.The annealing temperature varied according to the melting temperature of each primer.

Table 1: List of the eight populations representing *Thymus decussatus* studied species

No.	Population Names	Latitude (N)	Longitude (E)	Altitude (AL)
1	Farsh El-Sefsafa	28.55361	33.96443	1969 m a.s.l
2	Farsh Shoeib	28.55115	33.96625	1983 m a.s.l
3	Farsh Ellya	28.54150	33.97392	2070 m a.s.l
4	Farsh Ellya in direction of G. Mousa	28.54921	33.97128	1984 m a.s.l
5	Farsh El-Loza	28.54837	33.97038	1992 m a.s.l
6	Farsh El-Hamar	28.54663	33.96773	2020 m a.s.l
7	Corridor between Farsh El-Hamar and Farsh Shoeib	28.54855	33.96982	1996 m a.s.l
8	Corridor between Farsh El-Loza and Farsh Ellya	28.54580	33.97434	2032 m a.s.l

Table 2: List of RAPD and ISSR primers and their nucleotide sequences used for analysis of *T. decussatus* populations.

RAPD Primers	Sequences (5' - 3')	GC %	ISSR Primers	Sequence (5' - 3')	GC %
OPA-07	GAAACGGGTG	60	**14A**	CTCTCTCTCTCTCTCTTG	50
OPB-02	CATCCCCCTG	70	**44B**	CTCTCTCTCTCTCTCTTG	50
OPB-07	GGTGACGCAG	70	**HB-08**	GAGAGAGAGAGAGG	57
OPB-09	GTTGCCAGCC	70	**HB-10**	GAGAGAGAGAGACC	57
OPC-04	GATGATCGCC	60	**HB-11**	GTGTGTGTGTGTTGTCC	53
OPZ-03	GGCTGTGTGG	70	**HB-12**	CACCACCACGC	73

2.5. Agarose gel analysis

PCR amplification products were analyzed and detected on 1.2 percent agarose gels by electrophoresis. Gel photos were then captured by digital camera with 312 nm UV filter adaptor under UV transilluminator. DNA ladder (100 bp, Pharmacia) was used as marker for molecular band detection. The bands amplified by RAPD and ISSR were identified by the presence or absence of bands on the gel at a particular location. Banding profiles could be considered distinct if only one polymorphic band had been observed. Gel Analyzer 3 (Egygene) program has been applied to scoring RAPD and ISSR bands.

2.6. Data analysis

The pairwise genetic similarities of all sample pairs were calculated using the Jaccard coefficient method [34] and the matrix of similarity was constructed. This similarity matrix was subjected to the unweighted pair group method for arithmetic average analysis (UPGMA) to produce dendrogram[35]. All of these computations were performed using version 2.0 of the NTSYS-PC software[36].

3. RESULTS AND DISCUSSION

3.1. Polymorphism revealed by RAPD and ISSR primers

RAPD and ISSR markers have been successfully applied in assessing the extent of genetic diversity among eight *T.decussatus* populations collected in Gebel Mousa, SKP, Southern Sinai, Egypt from eight microhabitats. PCR amplification with RAPD primers has resulted in the production of reproducible fragment patterns (Fig. 3) for all the evaluated populations. Table (3) summarizes the polymorphism generated by seven RAPD primers, where they are produced a total of 58 amplified bands with an average of 8.29 bands per primer. The amplified bands had a size range of 150–1650 bp, and the total number of bands per primer ranged from seven in OPB-09 and OPZ-07 to ten in OPB-02. All primers with variable percentages of polymorphism ranged from 50 percent (OPC-04 primers) to 100 percent (OPA-07 and OPB-02) with an average of 86.21 percent were found to be polymorphic. The number of polymorphic bands per primordial ranged from four (OPC-04) to nine (OPB-02), with an average of 5.86. Our findings suggested more variability among the populations studied (86.21%). GC content has been reported to be a factor deciding a primer's efficiency[37] since GC content is correlated with annealing temperature and is related to the generation of more DNA fragments.

Our results were entirely compatible with results on five Palestinian *Thymus* species, *T. kotschyanus*, *T. caespititius*, [28, 29, 38]. In addition, numerous research studies on *Thymus* species from several countries showed that the rate of polymorphism ranges from 62% to 92% [39,40,41-43]. Nevertheless, the polymorphism level obtained in this study is rather lower than the recorded average polymorphism (94.31 percent) [30], which examined genetic polymorphism in 13 *Thymus* accessions collected from different habitats in Iran using 20 RAPD primers. Our data showed that the number of DNA fragments produced per primer ranged from seven to ten which are close to the

result obtained in their analysis on populations of *Thymus vulgaris* [42]. High level of polymorphism recorded in our study reflects the great degree of genetic variation among *Thymus* populations collected from various microhabitats in Gebel Mousa. Such heterogeneity may be due to the high degree of gene exchange between the studied populations [29].

Numerous studies have documented that the RAPD method is an effective tool for thyme germplasm research. In the present research, the analysis of a large number of DNA fragments can be considered as a good representation of the *Thymus* taxa genome and the ability to distinguish all the samples studied by RAPD banding profiles indicates that this technique may provide a faster and cheaper way for the identification of the studied *Thymus* taxa.

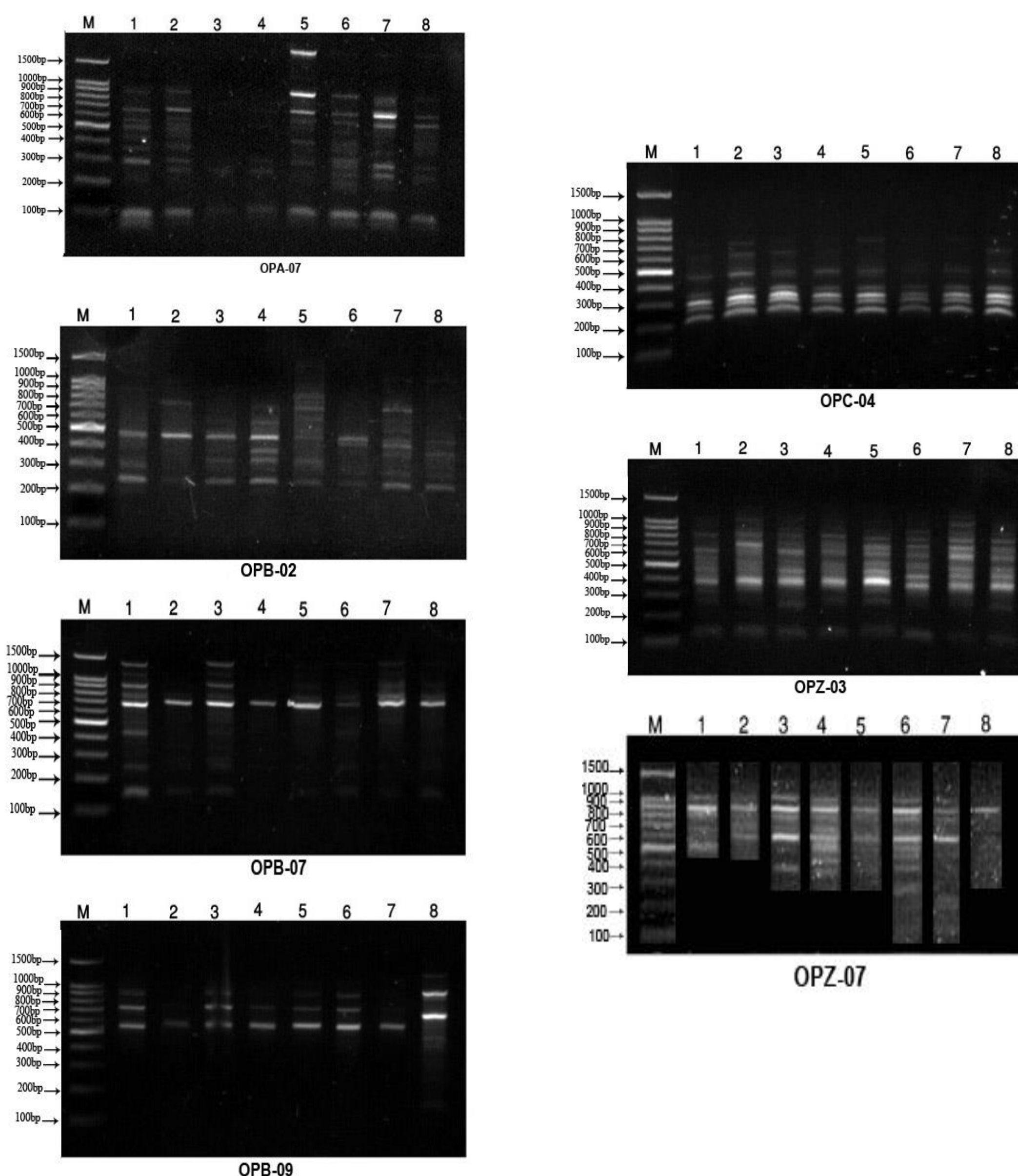

Fig.3: RAPD profiles of the eight *T. decussatus* populations produced by four primers OPA-07, OPB-02, OPB-07, OPB-09, OPC-04, OPZ-03 and OPZ-07. M: DNA ladder, lanes 1 to 8 represents the eight populations of *T. decussatus.* 1: Farsh El-Sefsafa, 2: Farsh Shoeib, 3: Farsh Ellya, 4: Farsh Ellya in direction of G. Mousa, 5: Farsh El-Loza, 6: Farsh El-Hamer, 7: Corridor between Farsh El-Hamar and Farsh Shoeib, 8: Corridor between Farsh El-Loza and Farsh Ellya.

The Polymorphism generated by six ISSR primers (Fig. 4) was summarized in Table 3. ISSR primers produced a total of 39 amplified bands, with an average of 6.5 bands per primer. The amplified bands had a size range of 200 – 1300 bp, and the total number of bands produced per primers ranged from five (HB-12) to eight (HB-10).All primers were found to be polymorphic with variable polymorphism percentages ranging from 71.43% (HB-08) to 100% (44B, HB-11 and HB-12) with an average of 87.18%. The number of polymorphic bands ranged from 3 (HB-08) to 7 (44B), averaging 5.33. Our results showed high variability among the populations surveyed (87.18 %).

At related study, it was observed that percentage of polymorphism ranged from 75 to 100 % with an average of 88.9% between 17 accessions of *Thymus daenensis*[44].In another study alsoit was recorded that the number of polymorphic bands ranged from 75% to 100%, with an average of 96.76% between fourteen *Thymus* accessions belonging to three species (*T. daenensis, T. kotschyanus, and T. vulgaris*) [30].The high polymorphic percentage reported in the above listed studies was completely in line with that obtained for *T.decassatus* populations in our analysis.

ISSR molecular marker performance is associated with motif sequences and its anchor series (Table 2). The motif sequences of the dinucleotide (GA) induce higher polymorphism than those replicates of the trinucleotides (CAC)[45]. This indicates that the ISSR markers can be a highly insightful, efficient, and reliable method for genetic diversity trials [46].

To characterize the degree of genetic variation among eight *T.decussatus* populations, the combination of the seven RAPD and the six ISSR primers were used (Table 3). As it was understood, the efficacy of the molecular marker techniques depends primarily on the degree of polymorphism produced by the primers used.

In the present analysis, 13 combined primers RAPD and ISSR produced 73 polymorphic bands out of 97 total amplified bands with an average of 86.70%, suggesting high genetic variation among the populations studied. RAPD and ISSR markers were used in similar studies to assess the genetic variation among *Nepeta septemcrenat, Thymus decussates* and *Phlomis aurea* plant species collected from Wadi Elfaraa, SCP, South Sinai, Egypt [47]. The primers of ISSR produced more polymorphic bands (23) than primers of RAPD (17), hence ISSRs were found to be more efficient than RAPD in estimating genetic diversity. Similar results also have been obtained for other plants including *Salvia* [48] and *Ocimum* [49].

3.2. Phylogenetic relationship based on amplified RAPD and ISSR bands

The similarity values among eight populations of *T. decussatus* according to the polymorphisms generated by seven RAPD primers as shown in Table 4. Results showed that the similarity coefficient values ranged from 0.35 to 0.60 with an average of 0.47 indicating the high degree of genetic variation between the studied populations. The highest similarly value (0.60) was observed between populations of Farsh El-Loza and Corridor between Farsh El-Hamar & Farsh Shoeib. The relatively high similarity value (0.58) was reported between Farsh El-Hamar and Corridor between Farsh El-Hamar & Farsh Shoeib on one side and corridor between Farsh El-Hamar & Farsh Shoeib and Corridor between Farsh El-Loza & Farsh Ellya on the other. Meanwhile, the lowest similarity value (0.35) was recorded between Farsh Ellya in direction of G. Mousa and Corridor between Farsh El-Loza and Farsh Ellya. Also, the quite low similarity value (0.36) was observed between populations of Farsh El-Sefsafa and Farsh Ellya in direction of G. Mousa. The moderate similarity level of 0.49 was recorded between Farsh Ellya and Farsh El-Hamar.

The results of Jaccard's similarity matrix (Table 4) and the UPGMA dendrogram (Fig. 5) revealed phylogenetic tree among the eight *T. decussatus* populations based on RAPD data. The eight *T. decussatus* populations were isolated by the phylogenetic tree into two clusters. The first cluster was split into two subclusters; the first subcluster included Farsh

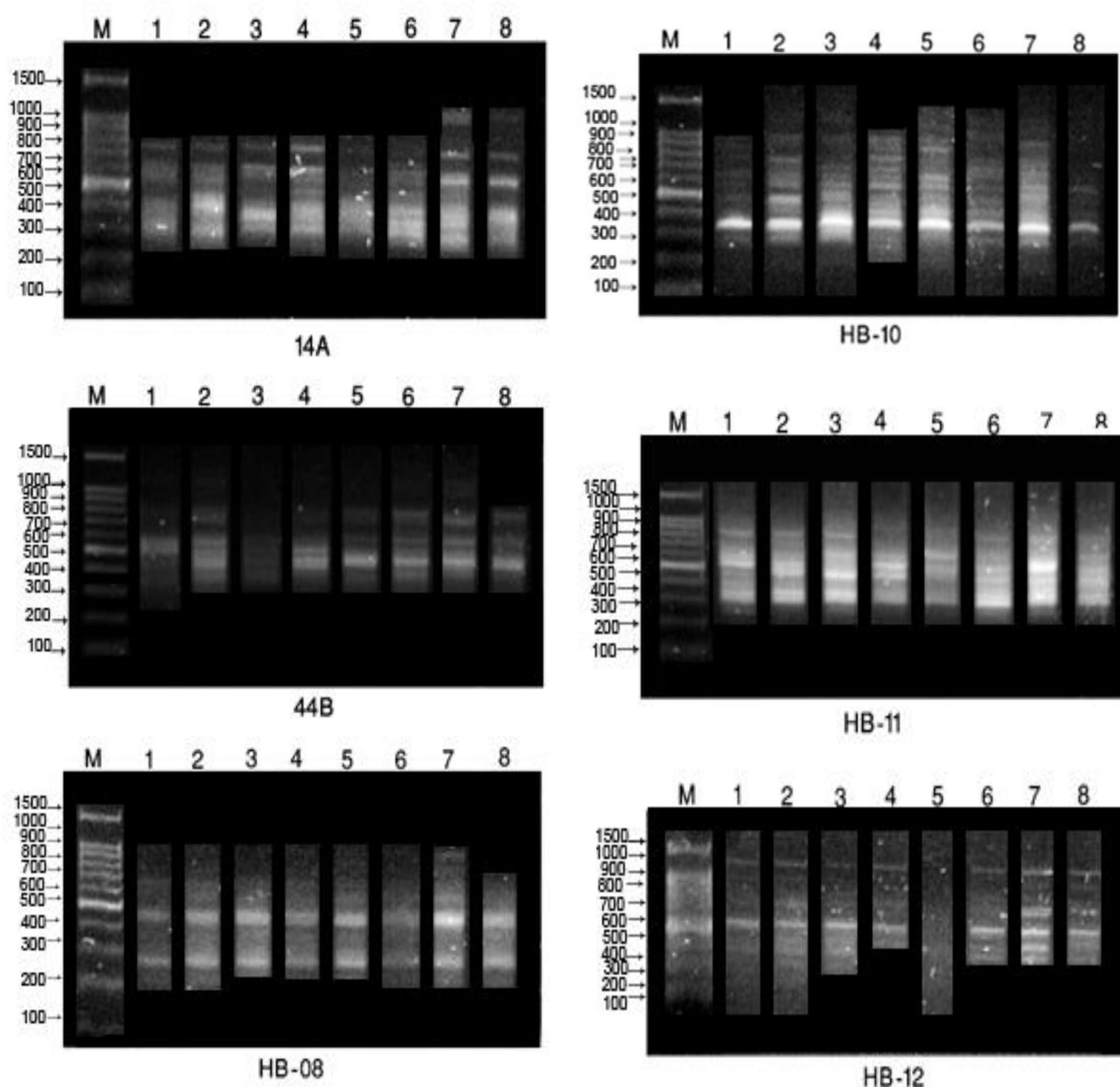

Fig. 4: ISSR profiles of the eight *T. decussatus* populations produced by four primers 14A, 44B, HB-08 and HB-10, HB-11 and HB-12. M: DNA ladder, lanes 1 to 8 represents the eight populations of *T. decussatus.* 1: Farsh El-Sefsafa, 2: Farsh Shoeib, 3: Farsh Ellya, 4: Farsh Ellya in direction of G. Mousa, 5: Farsh El-Loza, 6: Farsh El-Hamer, 7: Corridor between Farsh El-Hamar and Farsh Shoeib, 8: Corridor between Farsh El-Loza and Farsh Ellya.

El-Sefsafa and Farsh Ellya. While, Farsh El-Hamar and Corridor between Farsh El-Loza & Farsh Ellya populations formed the second subcluster. The second cluster also divided into two subclusters; the first su0bclusterinvolved Farsh Shoeib, Farsh El-Loza and Corridor between Farsh El-Hamar & Farsh Shoeib. The second subcluster included Farsh Ellya in direction of G. Mousa which was differentiated as separate identities.

Coefficient of similarity between eight populations of *T. decussatus* based on polymorphisms produced by six primers of the ISSR was shown in Table 5. The results showed that the coefficient values for Jaccard's similarity ranged from 0.30 to 0.74. The highest similarly level (0.74) was scored among Farsh Shoeib and Corridor between Farsh El-Hamar & Farsh Shoeib populations as the closet. A comparatively high similarity value (0.66) between Farsh Shoeib and Farsh Ellya in the

direction of G. Musa populations was also observed. On the other hand, the lowest similarity value (0.30) was reported as the most distant among populations of Farsh El-Sefsafa and Farsh El-Loza. The relatively low similarity value (0.33) between the Farsh El-Loza and Corridor populations between Farsh El-Loza & Farsh Ellya was also observed. The moderate level of similarity between the Farsh El-Sefsafa and Farsh Ellya populations was reported at 0.54.

The results of Jaccard's similarity matrix (Table 5) and the UPGMA dendrogram (Fig. 6) revealed phylogenetic tree among the eight *T. decussatus* populations based on ISSR data (Fig. 6). The phylogenetic tree separated the eight *T. decussates* populations into two clusters. The first cluster contained only one population of Farsh El-Loza as separate identity. The second cluster was split into two subclusters; the first subcluster included two clades: the first clade contained populations of Farsh El-Sefsafa and

Table 3: Total number of bands, monomorphic bands, polymorphic bands, unique bands and percentage of polymorphism obtained per each RAPD and ISSR primer for the eight populations of *T. decussatus***.**

Marker Type	Primer code	Total bands	Monomorphic bands	Polymorphic bands	Unique bands	Polymorphism (P%)
RAPD	**OPA-07**	8	-	6	2	**100**
	OPB-02	10	-	9	1	**100**
	OPB-07	9	1	8	-	**88.89**
	OPB-09	7	1	2	4	**85.71**
	OPC-04	8	4	4	-	**50**
	OPZ-03	9	1	8	-	**88.89**
	OPZ-07	7	1	4	2	**85.71**
	Average of P %					**86.21**
ISSR	**14A**	6	1	5	-	**83.33**
	44B	7	-	7	-	**100**
	HB-08	7	2	3	2	**71.43**
	HB-10	8	2	6	-	**75**
	HB-11	6	-	6	-	**100**
	HB-12	5	-	5	-	**100**
	Average of P%					**87.18**
Total		**97**	**13**	**73**	**11**	**-**
Mean		**7.46**	**1.00**	**5.62**	**0.85**	**86.70**

Table 4: The similarity coefficient values among eight populations of *T. decussatus* based on band polymorphisms generated by RAPD data. F: Farsh, G: Gebel, C: Corridor, D: Direction.

T.decussatus **Populations**	**F. El-Sefsafa**	**F. Shoeib**	**F. Ellya**	**F. Ellya in D. G. Mousa**	**F. El-Loza**	**F. El-Hamar**	**C. F. El-Hamar / F. Shoeib**	**C. F. El-Loza / F. Ellya**
F. El-Sefsafa	1.00							
F. Shoeib	0.41	1.00						
F. Ellya	0.57	0.43	1.00					
F. Ellya D. G. Mousa	0.36	0. 50	0.53	1.00				
F. El-Loza	0.56	0.52	0.55	0.51	1.00			
F. El-Hamar	0.54	0.43	0.49	0.45	0.44	1.00		
C. F. El-Hamar / F. Shoeib	0.51	0.55	0.50	0.54	**0.60**	0.58	1.00	
C. F. El-Loza / F. Ellya	0.53	0.40	0.39	**0.35**	0.45	0.53	0.58	

Farsh Ellya in the direction of G. Mousa, however the second clade included populations of Farsh Shoeib, Corridor between Farsh El-Hamar & Farsh Shoeib and Farsh Ellya. The second subcluster consisted of two populations (Farsh El-Hamar and Corridor between Farsh El-Loza & Farsh Ellya). The existence of such populations on the same cluster is in verifying their share of the same genetic pool.

Coefficient of similarity between eight populations of *T. decussatus* based on polymorphisms generated with seven RAPD primers and six ISSR primers was shown in Table 6. Ourdata appeared that the coefficient values for Jaccard's similarity ranged from 0.37 to 0.64. The highest similarly level of (0.64) was scored between FarshShoeib and Corridor between Farsh El-Hamar & Farsh Shoeib populations as the closet. A relatively high similarity value (0.59) between Farsh El-Hamar and Corridor among the Farsh El-Hamar & Farsh Shoeib populations was also observed. On the other hand, the lowest similarity value (0.37) was observed between Farsh Ellya in direction of G. Mousa and Corridor between Farsh El-Loza& Farsh Ellya populations as most distant. The relatively low similarity value (0.40) between the Farsh El-Loza and Corridor populations between Farsh El-Loza & Farsh Ellya was also observed.

In this study, the matrix generated by data of RAPD and ISSR polymorphisms allowed us

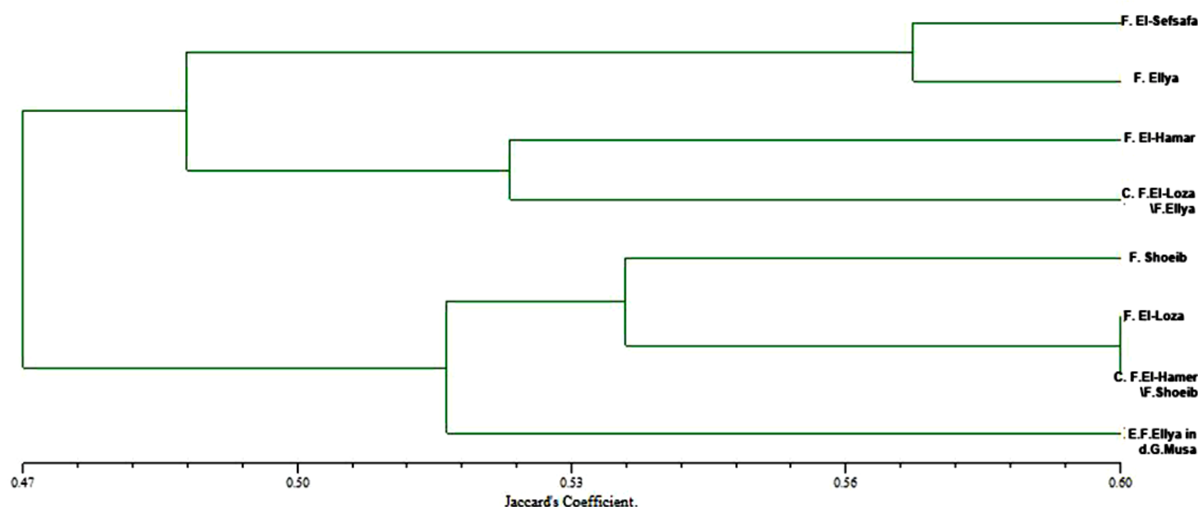

Figure 5: The dendrogram of genetic distance among the eight populations of *T. decussates* based onband polymorphisms generated by RAPD data.F: Farsh, G: Gebel, C: Corridor, D: Direction.

Table 5: The similarity coefficient values among eight populations of *T. decussatus* based on band polymorphisms generated by ISSR data. F: Farsh, G: Gebel, C: Corridor, D: Direction.

***T.decussatus* Populations**	**F. El-Sefsafa**	**F. Shoeib**	**F. Ellya**	**F. Ellya in D. G. Mousa**	**F. El-Loza**	**F. El-Hamar**	**C. F. El-Hamar / F. Shoeib**	**C. F. El-Loza / F. Ellya**
F. El-Sefsafa	1.00							
F. Shoeib	0.64	1.00						
F. Ellya	0.54	0.65	1.00					
F. Ellya D. G. Mousa	0.60	0.66	0.61	1.00				
F. El-Loza	**0.30**	0.43	0.48	0.48	1.00			
F. El-Hamar	0.48	0.55	0.55	0.41	0.38	1.00		
C. F. El-Hamar / F. Shoeib	0.50	**0.74**	0.60	0.51	0.45	0.60	1.00	
C. F. El-Loza / F. Ellya	0.48	0.50	0.55	0.41	0.33	0.55	0.60	1.00

to observe the relationship between the two types of markers evaluated here. RAPD was skewed towards lower similarity, while, those based on ISSR was skewed toward higher similarity. Likewise, it should have been noted that there was a broad correlation between RAPD and ISSR similarity matrixes, ISSR could be used in genetic variation assessment instead of RAPD. ISSR markers would be better methods for genetic studies as registered in other plants than RAPD. So, ISSR was concluded to be more insightful than RAPD [50, 51].

The results of Jaccard's similarity matrix (Table 6) and the UPGMA dendrogram (Fig. 7) revealed phylogenetic tree among the eight *T. decussatus* populations based on data of RAPD and ISSR (Fig. 7). The eight *T. decussatus* populations were divided by the dendrogram based on the RAPD and ISSR markers into two clusters. The first cluster was split into two subclusters; the first subcluster contained Farsh El-Sefsafa, while the second subcluster included two populations of Farsh El-Hamar and the corridor between Farsh El-Loza & Farsh Ellya. The second cluster was split into two subclusters; the first subcluster included two clades: the first clade included two populations of Farsh Shoeib and corridor between Farsh El-Hamar & Farsh Shoeib, while the second clade comprised Farsh Ellya and Farsh Ellya in the direction of G. Mousapopulations. The second subcluster contained Farsh El-Lozaas separate identity.

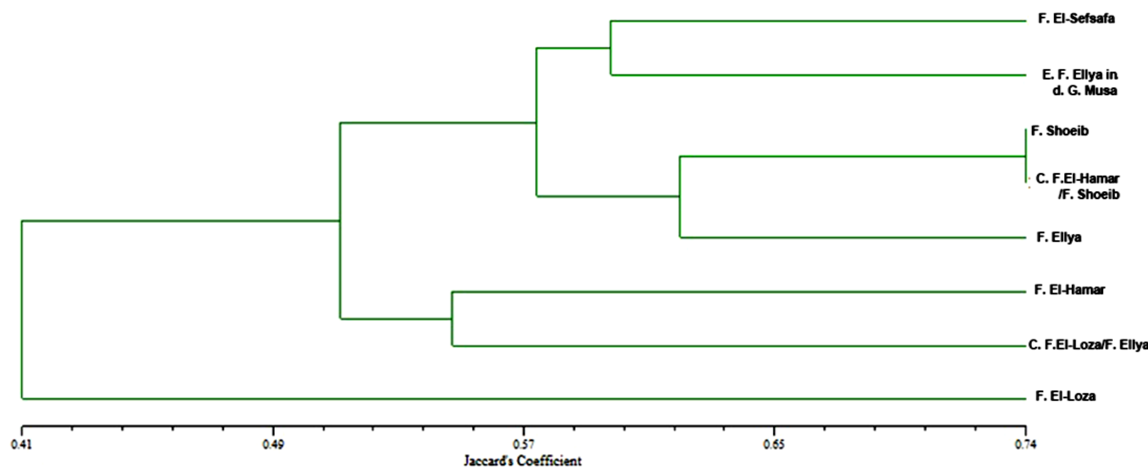

Fig. 6: The dendrogram of genetic distance among the eight populations of *T. decussates* based on band polymorphisms generated by ISSR data.F: Farsh, G: Gebel, C: Corridor, D: Direction.

Table 6: The similarity coefficient values among eight populations of *T. decussatus* based on band polymorphisms generated by both of RAPD and ISSR data. F: Farsh, G: Gebel, C: Corridor, D: Direction.

T.decussatus Populations	F. El-Sefsafa	F. Shoeib	F. Ellya	F. Ellya in D. G. Mousa	F. El-Loza	F. El-Hamar	C. F. El-Hamar / F. Shoeib	C. F. El-Loza / F. Ellya
F. El-Sefsafa	1.00							
F. Shoeib	0.50	1.00						
F. Ellya	0.56	0.52	1.00					
F. Ellya D. G. Mousa	0.45	0.57	0.56	1.00				
F. El-Loza	0.46	0.49	0.52	0.50	1.00			
F. El-Hamar	0.51	0.48	0.51	0.43	0.42	1.00		
C. F. El-Hamar / F. Shoeib	0.51	**0.64**	0.55	0.53	0.53	0.59	1.00	
C. F. El-Loza / F. Ellya	0.51	0.44	0.45	**0.37**	0.40	0.54	0.59	1.00

The phylogenetic tree suggested in the present study showed a very strong pattern of clustering for both DNA markers between the studied populations. The genetic closeness between the Farsh Shoeib and the corridor between populations of Farsh El-Hamar & Farsh Shoeib can be explained by the high degree of commonness among their individuals. The differences between the dendrograms produced by RAPDs and ISSRs could be explained separately by the different number of PCR products analyzed (58 for RAPD and 39 for ISSR).

The pattern of clustering referred primarily to the regional distribution of the *T. decussatus* populations at Gebel Mousa. Such findings support the fact that there are region-specific variations, which can be clarified by a long-term soil and climatic adaptation cycle. These findings are comparable to those of [52] in their study on endemic plant species (*Draba dorneri Heuff*), [16] in their study on three medicinal plant species belongs to the *Lamiaceae* family (*Nepeta septemcrenata*, *Ballota undulate* and *Teucrium polium*) which grows naturally in Saint Catherine Mountain at three different altitudes (1800, 2200 and 2600 m above sea level), and [53] in their study on *Commiphora wightii.* All of these result indicated that there are relationship between geographical distributions and the genetic diversity.

High genetic variation occurs between *T. decussatus* populations as recorded in our study contrasts with that often defined for mountain plants. For endemic plants from Tibet [54] and Central Asian desert plants[55] and several other studies of outcrossing endemic species, it was found that there was high genetic variability in colonies than in habitats[56, 57]. Differences in polymorphism among restricted species may be linked to the degree to which their populations, in accordance with [58, 59], occur in heterogeneous habitats. In order to maximize the use of genetic resources as interesting materials in plant breeding programmes, better knowledge of areas of high genetic diversity is also important.

Despite the short geographical distances which separate the current *T. decussatus* populations, high genetic variation was observed between the populations studied using both RAPD and ISSR markers. Strong genetic diversity for the endangered *Caesalpinia echinata* has also been reported [60]; the endangered pine[61], the uncommon Mexican pine[62], the native *Agave victoriae-reginae* [63], three Florida endemics (*Eryngium cuneifolium*, *Hypericum cumulicola* and *Liatris ohlingerae*; [64], the annual Florida *Warea carteri* [65] and the endemics *Iris cristata* and *I. lacustris* [66]).

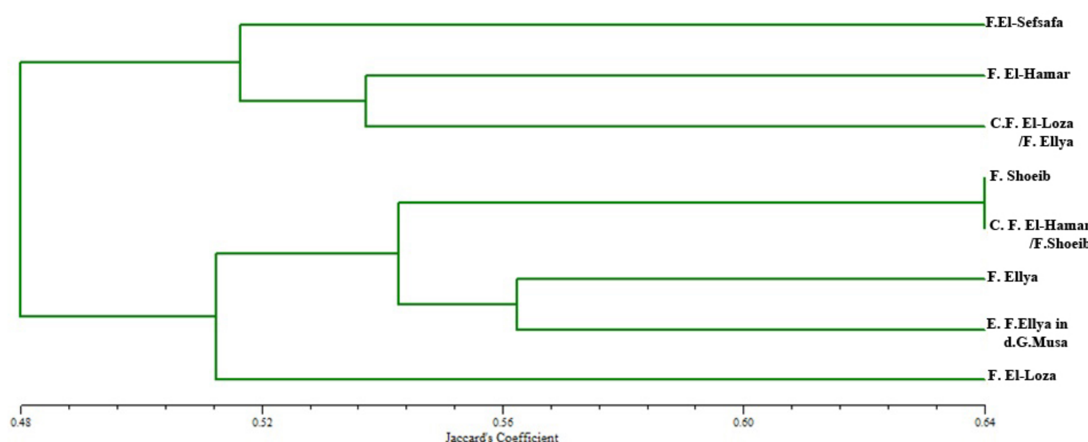

Fig. 7: The dendrogram of genetic distance among the eight populations of *T. decussatus* based on band polymorphisms generated by RAPD and ISSR data.F: Farsh, G: Gebel, C: Corridor, D: Direction.

It is known that extent and distribution of genetic diversity is significantly influenced by the mating mechanism and reproduction mode. Higher homozygosity results in low levels of genetic variation in self-compatible species, while species with primarily outcrossing mating systems experience higher rates of genetic variation[41].The findings of this analysis showed that there is a high level of genetic variation between the studied *Thymus* populations which is expected due to the high level of heterozygosity due to the cross-pollinating existence of the plant developed during the evolution processes.

4. CONCLUSION

Both RAPD and ISSR analyses showed the same percentage polymorphism results, as 86.21 percent for RAPD markers and 87.18 percent for ISSR markers and 86.70 percent for combined markers (RAPD+ISSR). It can be concluded that amongst the *Thymus* populations rising in the Gebel Mousa region there is a high genetic diversity. Specific geographical and ecological conditions permit certain potential genetic modifications or DNA changes such as translocation, deletion, point mutation and so on. The molecular analysis using markers RAPD and ISSR showed that both markers were able to differentiate between the different populations of *T. decassatus* and can thus be used to research the degree of genetic variation between T. Populations decussatus.

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الملخص العربى

التوصيف الجزيئى لنبات الزعتران السيناوى المهدد بالانقراض النامى على جبل موسى، جنوب سيناء، مصر

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تواجه العديد من النباتات الطبية الهامة المتوطنة والنادرة والتى تنمو طبيعيا على جبل موسى بمحمية سانت كاثرين بجنوب سيناء مصر خطر الانقراض نتيجة الاستخدام الجائر لتلك النباتات. من بين تلك النباتات الطبية تم أختيار ثمانية عشائر تمثل ثمانية مواقع من النوع النباتى *Thymus decussatus* المعروف باسم الزعتران أو زعيتر (القريب من المتوطن والنادر) لدراسة مستوى التنوع الوراثى بين تلك العشائر الثمانية باستخدام كلا من واسمات تقنية التضاعف العشوائى متعدد الاشكال للحمض النووى DNA (RAPD) واسمات تقنية تكرار التسلسل البسيط المتداخل (ISSR). ولقد أظهرت ثلاث عشرة بادئة من خمس وعشرون بادئة من كلتا التقنيتين تم استخدامها كفاءة عالية فى اعطاء تباينات بين العشائر النباتية الثمانية محل الدراسة حيث أعطت هذه البدئات 97 حزمة كلية من بينها 84 حزمة غير متشابهة بنسبة تباين تتراوح من 50 الى 100 % بمعدل تباين وصل الى 86.70 %. ولقد بلغ متوسط عدد الحزم مع البادئة الواحدة 7.46 حزمة. ولقد تراوحت قيم معامل تشابه جاكارد من 0.37 % الى 0.64 % وأن اعلى قيمة تشابه (0.64 %) سجلت بين عشيرة فرش شعيب وعشيرة الممر بين فرش الحمار وفرش شعيب عاكسا قربهم الوراثى. فى حين كانت اقل قيمة تشابه (0.37 %) سجلت بين عشيرة فرش ايليا فى اتجاه جبل موسى و عشيرة الممر بين فرش اللوزة وفرش ايليا عاكسا البعد الوراثى بينهم. وتم تحديد درجة القرابة الوراثية بإستخدام نظام ال UPGMA على أساس معامل تشابه جاكارد بين عشائر الزعتران الثمانية محل الدراسة على أساس تحليل ناتج التباين ومثلت بشجرة القرابة. بناءا على هذه الشجرة تم تمييز عشائر الزعيتر الثمانية الى مجموعتين أساسيتين تضم المجموعة الاولى ثلاث عشائر هى فرش الصفصافة و فرش الحمار والممر بين فرش اللوزة وفرش ايليا بينما تضم المجموعة الثانية خمسة عشائر هى فرش شعيب والممر بين كنيسة الحمار وفرش شعيب وفرش ايليا وفرش ايليا فى اتجاه جبل موسى وفرش اللوزة.أظهرت تلك النتائج أن واسمات RAPD و ISSR الجزيئية يمكن استخدامها فى تقييم التباين الوراثي بين عشائر نبات الزعتران الطبية الموزعة فى بيئات دقيقة متنوعة فى جبل موسى والتى قد تساعد فى برامج تحسينها الوراثى و صيانتها.