ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2018.16.23

Research Article
Molecular Characterization of *Teucrium* L. (Lamiaceae) as a Prerequisite for its Conservation

Ream I. Marzouk and Ghada E. El-Badan

Department of Botany and Microbiology, Faculty of Science, Alexandria University, Alexandria, Egypt

Abstract

Background and Objective: *Teucrium* in Libya is represented by 11 species, five of which are endemic and among them problems at different taxonomic levels. Besides, Libya suffers from both human and environmental pressures and population fragmentation, which in turn lead to the loss of genetic diversity. There is an urgent need to assess this diversity therefor the aim of this study was to investigate the diversity among and within *Teucrium* as a prerequisite for conservation programming. **Materials and Methods:** The genetic diversity was assessed for 12 *Teucrium* taxa by RAPD and ISSR. Both the total and specific bands, polymorphism percentage, Shannon index (H) and Simpson index (D) were determined and the data was processed with cluster and principal component analyses. **Results:** *Teucrium* taxa were characterized with relatively low genetic diversity, particularly *T. polium* subsp. *flavovirens*. It was noted that the taxa of each section were not grouped together and the dendrogram resulted in a distinct sorting among the taxa at 0.545 similarity level. Both cluster and PCA analyses were clearly separated between *T. capitatum* and *T. polium* at the level of dissimilarity on par with other species. **Conclusion:** The study accepted *T. capitatum* and *T. polium* as two separate species and *T. polium* subsp. *flavovirens* as *T. luteum* subsp. *flavovirens* and for both Girian and Siert taxa, there was a doubt that they were assembled under *T. polium* which need to be confirmed by matching with other *Teucrium* species.

Key words: Biodiversity, conservation, endemic species, genetic diversity, ISSR, labiatae, libya, molecular markers, RAPD, *Teucrium*

Citation: Ream I. Marzouk and Ghada E. El-Badan, 2018. Molecular characterization of *Teucrium* L. (Lamiaceae) as a prerequisite for its conservation. Asian J. Biol. Sci., 11: 16-23.

Corresponding Author: Ream I. Marzouk, Department of Botany and Microbiology, Faculty of Science, Alexandria University, Alexandria, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Teucrium L. is the second considerable genus in Ajugoideae, which is often described as a contentious in demarcation at different taxonomic levels. It comprises more than 300 species of cosmopolitan distribution, especially in the Mediterranean region[1-5]. *Teucrium* species are rich in phenolic compounds and used for digestive and respiratory disorders and as diuretic, anti-septic, anti-rheumatic, anti-inflammatory, anti-oxidative, anti-microbial, anti-diabetic, hepatoprotective, hypolipidemic and anti-cancer[6,7].

Teucrium is represented in Libya by three sections-Chamaedrys (Mill.) Schreb., *Teucrium* L. and Polium (Mill.) Schreb. and eleven species. Five out of them were endemic; *T. apollinis* Maire and Weiller, *T. barbeyanum* Asch. and Taub. ex E.A. Durand and Barratte, *T. davaeanum* Coss., *T. lini-vaccarii* Pamp. and *T. zanonii* Pamp.[8,9].

The most problematic and heterogeneous section was Polium which contained more than 50% of the species and 82% of the endemicones[1,10]. It was classified into four subsections-Polium, Simplicipilosa, Pumilum and Rotundifolia-that were reduced to three by the eliminating of Rotundifolia[1,11]. The main taxonomic problem was under the subsection Polium, especially between *T. polium* and *T. capitatum* and among *T. polium* subspecies. The Euro+Med and WCPS[12,13] accepted five subspecies of *T. polium* in the Mediterranean region-aurasiacum (Maire) Greuter and Burdet, *clapae* S. Puech, *polium*, *purpurascens* (Benth.) S. Puech and *rupestricola* (Sennen) T. Navarro and Rosua. They also recognized the subspecies *capitatum* and *flavovirens* as *T. capitatum* and *T. luteum* subsp. *flavovirens*; respectively. Many authors interpreted this diversity as adaptive radiation and differentiation through polyploidy as a result of drought stress and regional establishment of the Mediterranean climate that facilitated the genus speciation[2,14-16].

Libya is a good example of representing both human and environmental pressures and population fragmentation that cause genetic bottle necks and promote genetic drift, which in turn leads to further loss of genetic diversity. Therefore there is an urgent need to assess the genetic diversity of these populations with particular attention to both endemic and polymorphic species. These assessments were crucial for planning effective conservation strategies[17,18]. Boulila *et al.*[2], Ding *et al.*[19], Saini *et al.*[20,21] and Sharma *et al.*[22] clarified that both RAPD and ISSR were valuable tools for these purposes. The RAPD featured many advantages such as low cost and speed, but with some disadvantages such as low reproducibility. Thus, mixing with other data from ISSR was very promising for distinguishing among species due to its high reproducibility[23].

The intention of the study is to assess the taxonomic relations and genetic diversity among Libyan *Teucrium* species-specially within the subsection Polium-based on both RAPD and ISSR, as a prerequisite for the conservation programming.

MATERIALS AND METHODS

Twelve *Teucrium* taxa were collected from its natural habitats in Libya in two flowering seasons; 2009 and 2010 (Table 1). The voucher specimens were deposited in the herbaria at Alexandria University, Egypt (ALEX) and Omar Al-Mukhtar University, Libya.

For both RAPD and ISSR analyses, genomic DNA was extracted from 10-20 mg dry seeds using GeneJET Genomic DNA purification kit (Thermo-Scientific). Ready-To-Go Analysis Beads (GE Healthcare Life Sciences, 27-9502-01 with primers) kit was performed for amplification reaction.

For RAPD analysis, six decamer oligonucleotide arbitrary primers were reproducible; RAPD-1 (GGTGCGGGAA), RAPD-2 (GTTTCGCTCC), RAPD-3 (GTAGACCCGT), RAPD-4 (AAGAGCCCGT), RAPD-5 (AACGCGCAAC) and RAPD-6

Table 1: Studied *Teucrium* species (*endemic), location, altitude, coordinates and habitats

Section	Species	Location, altitude and coordinates	Habitat
Chamaedrys	*Teucrium barbeyanum**	Shahhat Susah, 356 m, N32°50°30.42 E21°51°7.2	Rocky crevices, mountain hills and rocky places
Polium	*Teucrium apollinis**		Dry stony flat hills and maritime
	Teucrium capitatum	Tarhonah, 433 m, N32°29°48.78 E13°37°37.08	Open places
	*Teucrium davaeanum**	Wadi El Quttarh, 237 m, N32°01°35.82 E20°24°45.48	Mountain slopes
	*Teucrium lini-vaccarii**	Quasser-El Quaar, 338 m, N32°35°20.28 E13°50°18.18	Dry foot hills and banks of wadis
	Teucrium polium	Siert, 54 m, N31°08°56.1 E16°34°35.16	Gravelly places
		Girian, 743 m, N32°08°50.9 E13°00°50.9	Mountain hills
	Teucrium polium subsp. *flavovirens*	Al Hameida escarpment, 241 m, N32° 24°52.98 E20° 32°17.88	Rocky crevices, mountain hills and slopes
	*Teucrium zanonii**	Dryannah, 9 m, N32°19°42.12 E20°16°34.86	Maritime and gravelly sandy places
Teucrium	*Teucrium brevifolium*	Lathroun-Ras El Hellal, 23 m, N32°52 305'°0 E22°15°6.12	Mountain steppes and slopes
	Teucrium campanulatum	Wadi Errieg, 236 m, N32°32 230°0 E20°42°56.82	Mountains, calcarious and gravelly places
	Teucrium fruticans	El Rabtta-El Assbeh, 759 m, N32°07°12.6 E21°52°16.14	Mountain hills and slopes

(CCCGTCAGCA). Gene Amp Polymerase Chain Reaction (PCR) system cycler was achieved at 94°C for 7 min followed by 30 cycles at 94°C for 1 min, 35°C for 1 min and 72°C for 2 min and final extension step at 72°C for 6 min.

For ISSR analysis, six oligonucleotide arbitrary primers were reproducible; ISSR-1 (GAG[CAA]$_5$), ISSR-2 ([GA]$_8$T), ISSR-3 ([AG]$_8$T), ISSR-4 ([AC]$_8$C), ISSR-5 ([AGC]$_6$G) and ISSR-6 ([TG]$_8$A). Gene Amp Polymerase Chain Reaction (PCR) system cycler was achieved at 94°C for 5 min followed by 35 cycles at 94°C for 30 sec, 52°C for 45 sec and 72°C for 2 min and final extension step at 72°C for 5 min.

The amplified products from RAPD and ISSR was separated on 2% agarose gel, visualized under UV transilluminator and photographed via gel documentation system (Digi-doc, UVP Company, England). The discrete bands were scored as binary characters and UVP Doc-It® LS Image Analysis Software was used for molecular weights determination through 1000 base pair DNA ladder of Thermo scientific Co.

The genetic diversity among taxa was appraised using three estimators:

- The percentage of polymorphism = 100 (number of polymorphic bands/number of total bands)
- Shannon index (H) = $-\sum p_i \log_2 p_i$, where p_i is the frequency of a given band[24]
- Simpson index (D) = $1-\left\{\frac{\Sigma n_i(n_i-1)}{N(N-1)}\right\}$

where, n_i is the number of bands of species i and N is the total number of bands[25].

The Shannon index was in direct relation with both the richness (number of bands in a species) and the evenness (comparing the number of bands among species). On the other hand, Simpson index was approached to one where every species has a unique genotype. Due to the confusing of richness and evenness in the Shannon index, the combination between direct estimate of species richness (Shannon index) with some measure of dominance or evenness (Simpson index) were appropriate[26].

Both cluster analysis, using the unweighted pair group method with arithmetic mean (UPGMA) based on Jaccard distance and principal component analysis (PCA) were performed through PAST program[27].

RESULTS

The RAPD and ISSR patterns obtained from genomic DNA of *Teucrium* species using RAPD-1 and ISSR-1 were represented in Fig. 1.

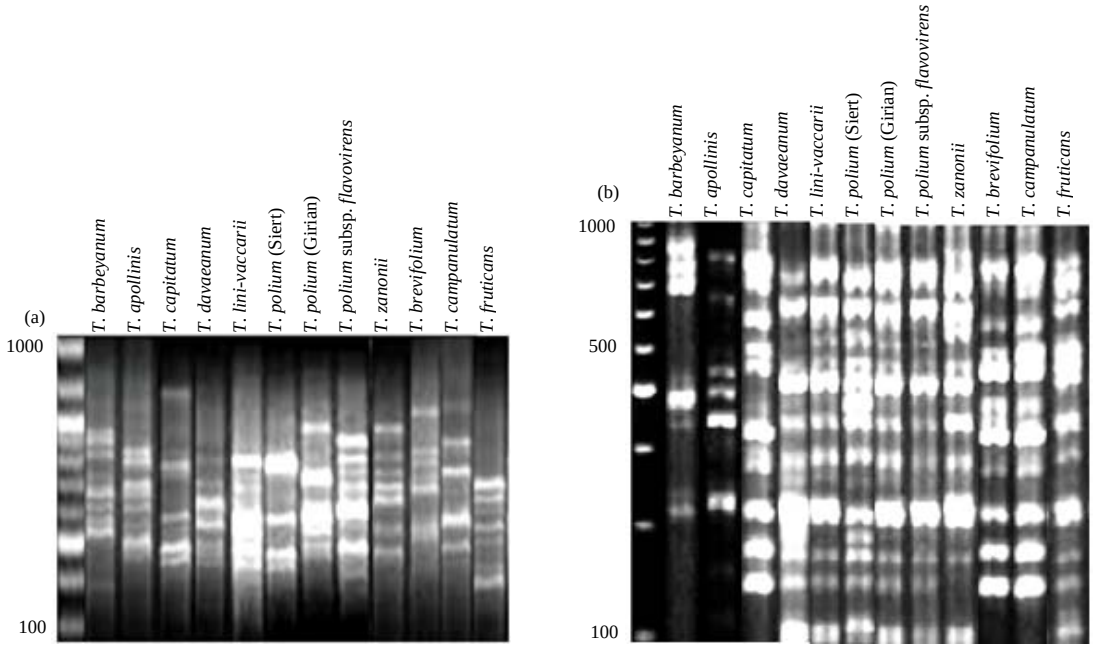

Fig. 1(a-b): RAPD and ISSR patterns obtained from genomic DNA of *Teucrium* species using RAPD-1 (GGTGCGGGAA) and ISSR-1; GAG(CAA)$_5$, (a) RAPD-1 and (b) ISSR-1

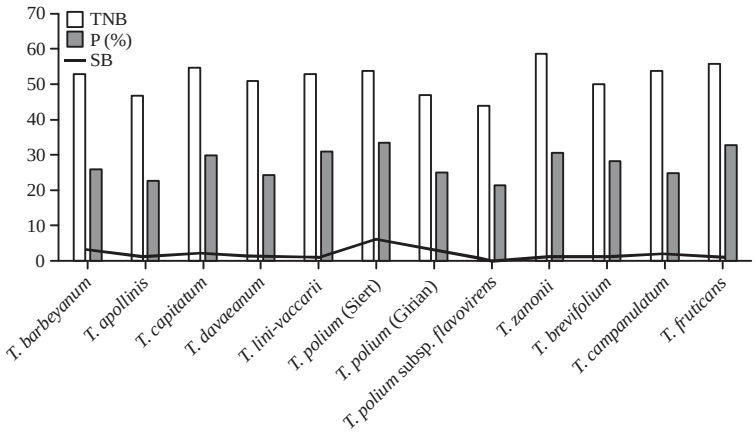

Fig. 2: Total number of bands (TNB), percentage of polymorphism (P %) and specific bands (SB) of *Teucrium* species generated from RAPD

Table 2: Shannon index (H) and simpson index (D) for RAPD and ISSR analyses

	RAPD		ISSR		RAPD and ISSR	
	H	D	H	D	H	D
T. apollinis	3.85	0.9787	4.127	0.9839	4.691	0.9908
T. barbeyanum	3.97	0.9811	4.19	0.9848	4.779	0.9916
T. brevifolium	3.912	0.98	4.143	0.9841	4.727	0.9912
T. campanulatum	4.007	0.9818	4.127	0.9839	4.762	0.9915
T. capitatum	3.989	0.9815	4.265	0.9859	4.828	0.992
T. davaeanum	3.932	0.9804	4.248	0.9857	4.796	0.9917
T. fruticans	4.025	0.9821	4.174	0.9846	4.796	0.9917
T. lini-vaccarii	3.97	0.9811	4.127	0.9839	4.745	0.9913
T. polium (Siert)	3.989	0.9815	4.043	0.9825	4.71	0.991
T. polium (Girian)	3.85	0.9787	4.174	0.9846	4.718	0.9911
T. polium subsp. *flavovirens*	3.784	0.9773	4.094	0.9833	4.644	0.9904
T. zanonii	4.078	0.9831	4.159	0.9844	4.812	0.9919

For RAPD analysis, 94 of 107 amplified fragments were polymorphic by 90.65%. The generated bands from each primer ranged from 73-171, with averages from 6.08-14.25. While the averages per species for the total number of bands, polymorphism (%) and specific bands were 51.92, 28.63 and 1.8, respectively. *Teucrium zanonii* attained the highest total produced bands and both Shannon's and Simpson's indices in RAPD, while *T. polium* subsp. *flavovirens* recorded the lowest values for all genetic diversity parameters (Fig. 2, Table 2). For ISSR analysis, the polymorphic bands were 210 from 226 generated fragments with 92.92%. The produced bands per primer varied from 102-152 with averages from 8.5-12.67. The averages of total number of bands, polymorphism (%) and specific bands per species were 63.91, 21.26 and 7.75, respectively. The highest total number of generated bands and polymorphism (%) was found in *T. campanulatum*, while *T. polium* of Siert achieved the lowest values in the same parameters along with the values of Shannon's and Simpson's indices. On the other hand, the specific bands varied from 3 in *T. brevifolium* to 16 in *T. zanonii* (Fig. 3, Table 2). For both RAPD and ISSR, the highest values of Shannon's and Simpson's indices were in *T. capitatum* and the lowest in *T. polium* subsp. *flavovirens* (Table 2). The discrimination among studied taxa in the non-hierarchical form by PCA accounted 41.604% of variance for the three first components, with 17.966, 12.839 and 10.799% for the 3 axes respectively. It was noted that the taxa of each section were not grouped together, especially those belonging to section Polium (Fig. 4). Within this section, both *T. capitatum* and *T. polium* were clearly separated and also *T. polium* from Siert was kept apart from other taxa of the same species. The dendrogram from UPGMA resulted in a distinct sorting among the taxa at 0.545 similarity level (Fig. 5). The *T. capitatum* and *T. polium* were distinguished at the lowest level of similarity (0.38). Both *T. polium* subsp. *flavovirens*

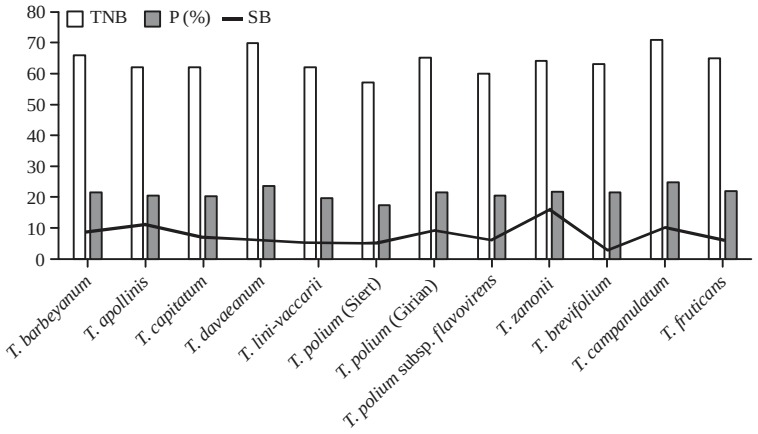

Fig. 3: Total number of bands (TNB), percentage of polymorphism (P %) and specific bands (SB) of *Teucrium* species generated from ISSR

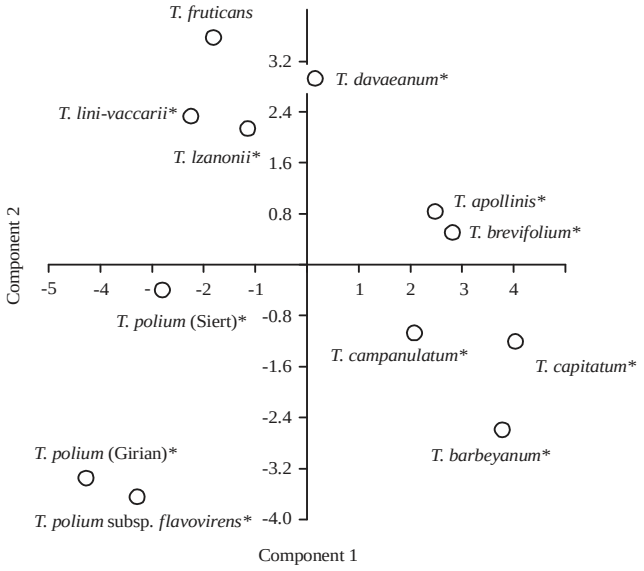

Fig. 4: PCO of *Teucrium* species based on both RAPD and ISSR analyses
*Species of section polium

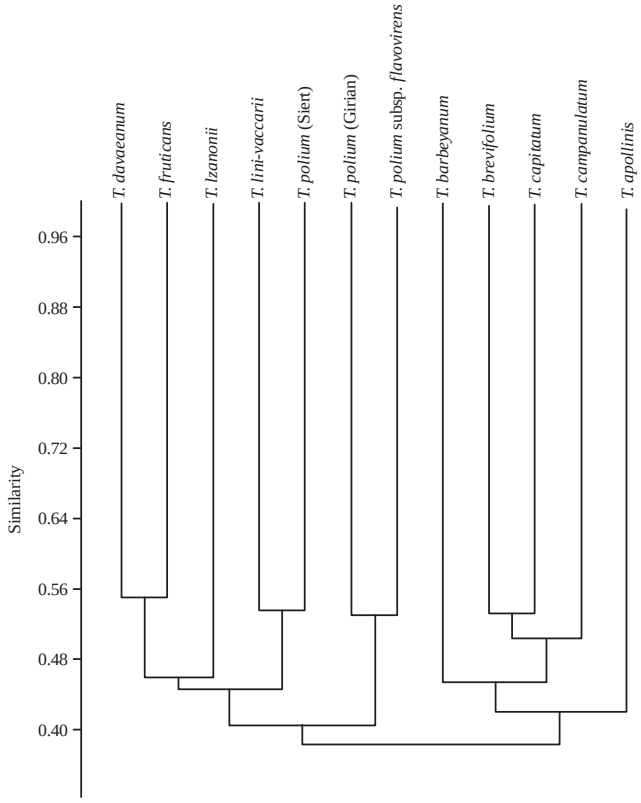

Fig. 5: Dendrogram of *Teucrium* species based on both RAPD and ISSR analyses

and the taxon of Girian were detached from that of Siert at 0.41similarity level, while *T. apollinis* and both *T. barbeyanum* and *T. zanonii* were segregated at 0.425 and 0.461 similarity levels, respectively.

DISCUSSION

Libya suffers from the destruction of biodiversity and the risk of genetic erosion due to desertification and several environmental and human crisis[28,29]. Therefore, the study evaluated the genetic diversity among Libyan *Teucrium* that is the second largest genus composed of endemic species which will assist in its conservation approach. *Teucrium* is well adapted to these pressures that has led to its speciation[16,30] and the present study confirms this idea by revealing relatively high Simpson's and Shannon's indices and stated why species were not grouped under the accepted sections. The genetic diversity assessment through different molecular markers and the combining of more than one

technique were important and both RAPD and ISSR are suitable tools for these evaluations particularly for endemic species[2,31-33]. The ISSR is more discriminative than RAPD which appeared through total number of bands, specific bands and both Simpson's and Shannon's indices for different species. The primers RAPD-4 and ISSR-4 record the highest number of common bands among taxa and the study recommends that they be genus specific. Luan *et al.*[34] pointed out that endemic species and those with small geographic ranges were characterized by low genetic diversity. These projections are evident in *T. apollinis*, which maintains low values for the total number of bands, the percentage of polymorphism, both Shannon's and Simpson's indices and relatively high specific bands as an average for both RAPD and ISSR-compared to other endemic species. These values may be related with the reduced population sizes and the geographical region of dry stony flat hills with relatively low humidity (38.9%) and high altitude (356 m). On the other hand, *T. zanonii* and *T. davaeanum* accomplish relatively high genetic diversity that can be interpreted through their distinctive habitats; loamy sand, relatively high humidity (72%) and low altitude (9 m) for the first and the presence of the second at the slope of Wadi El Quttarh[35]. Luan *et al.*[34], Maguire and Sedgley[36] and Zawko *et al.*[37] were pointed to a link between high genetic diversity in rare plants and the recent decline in population size, insufficient isolation time or wide gene flow. Therefore, the study recommends that priority be given to *in situ* and *ex situ* conservation for both species, particularly for vulnerable populations. Both *T. polium* and *T. capitatum* were among the most controversial species of taxonomic problems in *Teucrium*[1]. Many authors treated *T. capitatum* as a subspecies[38-40] and others referred to *T. polium* regardless of their subspecies[10,41-44]. The dendrogram and PCA are clearly separated between them and at the level of dissimilarity on par with other species, so the study accepts the level of species for both taxa. This is consistent with Marzouk *et al.*[8,9] for the distinctiveness of each species with specific nutlet and pollen micro-morphological attributes. In *T. capitatum*, the nutlet epidermal cells were specified with depressed anticlinal and convex external periclinal walls and the pollen with verrucate-scabrate sculpture. While *T. polium* was characterized by nutlet cells of raised, wavy anticlinal and concave external periclinal walls and pollen with verrucate-perforate sculpture. The three studied taxa of *T. polium* share 36 common bands of RAPD and ISSR, while 12, 11 and 6 of the finger-printing bands are distinctive in Girian, Siert and subsp. *flavovirens*, respectively. The taxon of Girian is closely related to the subsp. *flavovirens* through 29 common bands from RAPD and ISSR. Despite the segregation of Siert taxon, it shares 24 common bands with Girian and 8 bands with the subspecies. The great diversity in karyotyping pattern and ploidal levels within *T. polium* played an important role in its speciation[2,14-16,45]. Meanwhile, Askar[35] identified 12 essential oils in Libyan *Teucrium*; α-Cadinol, Camphene, β-Caryophyllene, α-Copaene, β-Elemene, Germacrene-B, Germacrene-D, Isoborneol, Limonene, Linalool, α-Pinene and Terpinen. He confirmed the absence of α-Cadinol and Isoborneol in Girian taxon, Linalool and Terpinen in Siert taxon and α-Cadinol, α-Pinene and Terpinen in the subsp. *flavovirens*. Thus, the study agrees with WCPS[13] to accept *T. polium* subsp. *flavovirens* as *T. luteum* subsp. *flavovirens*. For the other two taxa; Girian and Siert, there is a doubt that they are assembled under one species which need to be confirmed by matching with other *Teucrium* species.

CONCLUSION

Teucrium were characterized with relatively low genetic diversity and about 42% of its species were endemic that require conservation priority, especially for the populations of critical sizes or those subjected to human and climatic pressures. Also, the study highlights the validity of both RAPD and ISSR in the assessment of genetic diversity and the discrimination among and within *Teucrium*.

SIGNIFICANCE STATEMENT

This study evaluated the genetic diversity among Libyan *Teucrium*, which will assist in the conservation approach of these species suffering from environmental and human crisis. The taxa achieved a low level of genetic diversity. Both dendrogram and PCA were clearly separated between *T. capitatum* and *T. polium*, so the study accepted the species level for each. The results also confirmed the reallocation of *T. polium* subsp. *flavovirens* as *T. luteum* subsp. *flavovirens*. For the two taxa of *T. polium* from Girian and Siert, there was a doubt that they were gathered under one species.

REFERENCES

1. Navarro, T. and J. El Oualidi, 2000. Synopsis of *Teucrium* L. (Labiatae) in the Mediterranean region and surrounding areas. Flora Medit., 10: 349-363.

2. Boulila, A., A. Bejaoui, C. Messaoud and M. Boussaid, 2010. Genetic diversity and population structure of *Teucrium polium* (Lamiaceae) in Tunisia. Biochem. Genet., 48: 57-70.
3. Radulovic, N., M. Dekic, M. Joksovic and R. Vukicevic, 2012. Chemotaxonomy of Serbian *Teucrium* species inferred from essential oil chemical composition: The case of *Teucrium scordium* L. ssp. *scordioides*. Chem. Biodivers., 9: 106-122.
4. Ozcan, T., T. Dirmenci, E. Martin and F. Altınordu, 2015. Cytotaxonomical study in five taxa of the genus *Teucrium* L. (Lamiaceae). Caryologia, 68: 1-8.
5. Blanca, G., M. Cueto and J. Fuentes, 2017. *Teucrium teresianum* sp. nov. (Lamiaceae) from southern Spain. Nordic J. Bot., 35: 14-19.
6. Stankovic, M.S., M.G. Curcic, J.B. Zizic, M.D. Topuzovic, S.R. Solujic, S.D. Markovic, 2011. *Teucrium* Plant species as natural sources of novel anticancer compounds: Antiproliferative, proapoptotic and antioxidant properties. Int. J. Mol. Sci., 12: 4190-4205.
7. Jaradat, N.A., 2015. Review of the taxonomy, ethnobotany, phytochemistry, phytogeography and phytotoxicity of germander plant (*Teucrium polium* L.). Asian J. Pharm. Clin. Res., 8: 13-19.
8. Marzouk, R.I., S.M. El-Darier and A.M. Askar, 2016. Nutlet micromorphological characters of *Teucrium* taxa (*Lamiaceae*) in Libya. Phytotaxa, 263: 245-254.
9. Marzouk, R.I., M. Salama and A.B.M. Askar, 2017. Pollen morphology of *Teucrium* L. (Lamiaceae, Ajugoideae) in Libya. Bangladesh J. Plant Taxon., 24: 219-226.
10. Dinc, M., S. Dogu, A.D. Koca and B. Kaya, 2011. Anatomical and nutlet differentiation between *Teucrium montanum* and *T. polium* from Turkey. Biologia, 66: 448-453.
11. Navarro, T., 2010. Genus *Teucrium* L. Flora Iberica. (In Spanish).
12. Euro+Med, 2006. Euro+MedPlantBase-the information resource for Euro-Mediterranean plant diversity. http://ww2.bgbm.org/EuroPlusMed/PTaxonDetail.asp?NameCache=Teucrium%20polium and PTRefFk=8000000
13. WCPS., 2018. World checklist of selected plant families. Facilitated by the Royal Botanic Gardens, Kew. http://wcsp.science.kew.org/
14. Puech, S., 1990. Contribution a l'etude de biosystematique des Teucrium de la section polium (Labiatae) de Tunisie. III. Bull. Soc. Bot. France, Lett. Bot., 137: 63-767.
15. El Oualidi, J., S. Puech and T. Navarro, 2002. Geographical variation and successive adaptive radiations of yellow-flowered *Teucrium* (Labiatae) in the Mediterranean region. Bot. Rev., Vol. 68. 10.1663/0006-8101(2002)068[0209:GVASAR]2.0.CO;2
16. Salmaki, Y., S. Kattari, G. Heubl and C. Brauchler, 2016. Phylogeny of non-monophyletic *Teucrium* (Lamiaceae: Ajugoideae): Implications for character evolution and taxonomy. Taxon, 65: 805-822.
17. Laikre, L., F.W. Allendorf, L.C. Aroner, C.S. Baker and D.P. Gregovich *et al*., 2010. Neglect of genetic diversity in implementation of the convention on biological diversity. Conserv. Biol., 24: 86-88.
18. Yang, Q., Y. Fu, Y.Q. Wang, Y. Wang and W.H. Zhang *et al*., 2014. Genetic diversity and differentiation in the critically endangered orchid (*Amitostigma hemipilioides*): Implications for conservation. Plant Syst. Evol., 300: 871-879.
19. Ding, G., D. Zhang, Y. Yu, L. Zhao and B. Zhang, 2013. Population genetic diversity and divergence of the halobiotic herb *Limonium sinense* estimated by AFLP and ISSR and implications for conservation. Plant Syst. Evol., 299: 131-138.
20. Saini, R.K., K.R. Saad, G.A. Ravishankar, P. Giridhar and N.P. Shetty, 2013. Genetic diversity of commercially grown *Moringa oleifera* Lam. cultivars from India by RAPD, ISSR and cytochrome P_{450}-based markers. Plant Syst. Evol., 299: 1205-1213.
21. Sharma, S.S., K. Aadil, M.S. Negi and S.B. Tripathi, 2014. Efficacy of two dominant marker systems, ISSR and TE-AFLP for assessment of genetic diversity in biodiesel species *Pongamia pinnata*. Curr. Sci., 106: 1576-1580.
22. AlRawashdeh, I.M., 2015. Genetic variability among and within wild *Teucrium polium* L. populations at Wadi Shueib area in Jordan. J. Agric. Biol. Sci., 10: 267-273.
23. Boubaya, A., H. Hannachi, N. Marzougui, T. Triki, F. Guasmi and A. Ferchichi, 2013. Genetic diversity assessment of *Lawsonia inermis* germplasm in Tunisian coastal oases by ISSR and RAPD markers. Dendrobiology, 69: 31-39.
24. Lewontin, R.C., 1972. The apportionment of human diversity. Evol. Biol., 6: 381-398.
25. Pielou, E.C., 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol., 13: 131-144.
26. Magurran, A.E., 2004. Measuring Biological Diversity. Blackwell Publishing, Oxford, UK.
27. Hammer, O., D.A.T. Harper and P.D. Ryan, 2001. PAST: Paleontological statistics software package for education and data analysis. Paleontol. Electron., 4: 1-9.
28. Elshatshat, S. and A. Mansour, 2014. Disturbance of flora and vegetation composition of Libya by human impacts: Costal region of Al-Jabal Al-Akhdar area as model. J. Applied Sci. Res., 5: 286-292.
29. Agiel, N. and F. Mericli, 2017. A survey on the aromatic plants of Libya. Indian J. Pharm. Edu. Res., 51: S304-S308.
30. El-Mokasabi, F.M., 2017. Studies on the flora of Libya. ContROL., Vol. 2017.
31. Djabou, N., A. Muselli, H. Allali, M.E.A. Dib, B. Tabti, L. Varesi and J. Costa, 2012. Chemical and genetic diversity of two Mediterranean subspecies of *Teucrium polium* L. Phytochemistry, 83: 51-62.

32. Tabtib, B., 2014. Assessment of genetic differentiation of two Mediterranean subspecies of *Teucrium*. Bull. Georg. Nat. Acad. Sci., 8: 72-75.
33. Thilaga, S., R.R. Nair, M.R. Kannan and D. Ganesh, 2017. RAPD markers for screening shoot gall maker (*Betousa stylophora* Swinhoe) tolerant genotypes of amla (*Phyllanthus emblica* L.). J. Genet. Eng. Biotechnol., 15: 323-330.
34. Luan, S., T.Y. Chiang and X.U.N. Gong, 2006. High genetic diversity vs. low genetic differentiation in *Nouelia insignis* (Asteraceae), a narrowly distributed and endemic species in China, revealed by ISSR fingerprinting. Ann. Bot., 98: 583-589.
35. Askar, A.M., 2015. Chemoecological and palynological studies on *Teucrium* L. species in Libya. Ph.D. Thesis, Chemoecological and palynological studies on *Teucrium* L. species in Libya.
36. Maguire, T.L. and M. Sedgley, 1997. Genetic diversity in *Banksia* and *Dryandra* (Proteaceae) with emphasis on *Banksia cuneata*, a rare and endangered species. Heredity, 79: 394-401.
37. Zawko, G., S.L. Krauss, K.W. Dixon and K. Sivasithamparam, 2001. Conservation genetics of the rare and endangered *Leucopogon obtectus* (Ericaceae). Mol. Ecol., 10: 2389-2396.
38. Antunes, T., I. Sevinate-Pinto, J.G. Barroso, C. Cavaleiro and L.R. Salgueiro, 2004. Micromorphology of trichomes and composition of essential oil of *Teucrium capitatum*. Flavor Fragrance J., 19: 336-340.
39. De Martino, L., C. Formisano, E. Mancini, V. De Feo, F. Piozzi, D. Rigano and F. Senatore, 2010. Chemical composition and phytotoxic effects of essential oils from four *Teucrium* species. Natural Prod. Commun., 5: 1969-1976.
40. Fayed, A.A., M. Zareh, N. Hassan and A. Faried, 2015. A systematic revision of the genus *Teucrium* (Lamiaceae) in Egypt. Nordic J. Bot., 33: 389-400.
41. Savvidou, S., J. Goulis, I. Giavazis, K. Patsiaoura, P. Hytiroglou and C. Arvanitakis, 2007. Herb-induced hepatitis by Teucrium polium L.: report of two cases and review of the literature. Eur. J. Gastroenterol. Hepatol., 19: 507-511.
42. Niazmand, S., M. Esparham, T. Hassannia and M. Derakhshan, 2011. Cardiovascular effects of *Teucrium polium* L. extract in rabbit. Pharmacogn. Mag., 27: 260-264.
43. Pesaraklu, A., M. Mianabadi, M.B.B. Najjar, A. Sattarian and A. Baghizadeh, 2013. Genetic diversity of different populations of Iranian *Teucrium polium* L. using RAPD markers. Iran. J. Rangelands For. Plant Breed. Genet. Res., 21: 24-31.
44. Esfandyari, S., M. Farshadfar, H. Safari and H. Shirvani, 2017. Genetic variability of *Teucrium polium* ecotypes using ISSR molecular markers. Iran. J. Rangelands For. Plant Breed. Genet. Res., 25: 135-146.
45. Pavlova, D. and M. Vasileva, 2010. Variation in morphology of *Teucrium polium aggr*. populations in Bulgaria. Open Life Sci., 5: 880-887.