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POLLEN MORPHOLOGY OF *TEUCRIUM* L. (LAMIACEAE, AJUGOIDEAE) IN LIBYA

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Abstract

Pollen grains of 11 taxa of *Teucrium* from Libya were examined using light microscopy (LM) and scanning electron microscopy (SEM) in order to provide better insight on the evaluation of palynological attributes for species characterization of *Teucrium* with special emphasis on the five Libyan endemic. Two main pollen shapes were documented; subprolate and prolate or perprolate in *T. fruticans.* The exine sculpture inspected at surface, operculum and pole were mostly verrucate, perforate or scabrate. *Teucrium fruticans* attained the phenomenon of pollen dimorphism with two distinct shapes and specifics sculpture for each form. The results validated the taxonomic significance of pollen grains for the discrimination among *Teucrium* species in Libya.

Introduction

Teucrium L. is the second-largest genus of subfamily Ajugoideae with cosmopolitan distribution over represented in the Mediterranean area (Navarro and EL Oualidi, 2000a; Radulović *et al.*, 2012; Yasaman *et al.*, 2016). The Mediterranean region is its main center of diversity, being represented by around 90% of the total *Teucrium* species in the world (Blanca *et al.*, 2017).

The pollen attributes were potentially useful for both species identification and phylogenetic implication (Abdel Khalik, 2016). These characters endorsed to be stable and of taxonomic significance for generic and specific delimitation of *Teucrium*. The size, exine sculpture and density of supratectal elements seemed to be of systematic value (Abu-Assab and Cantino, 1992; Dinç and Ozturk, 2008; Oybak-Dönmez and Inceoğlu, 1988; Oybak-Dönmez *et al.*, 1999; Navarro *et al.*, 2004).

In Libya, *Teucrium* is represented by eleven taxa of which *T. apollinis* Maire & Weiller, *T. barbeyanum* Asch. & Taub. *ex* E.A. Durand & Barratte, *T. davaeanum* Coss., *T. lini-vaccarii* Pam. and *T. zanonii* Pam. are believed to be endemic to Libya (Marzouk *et al.*, 2016).

The present study was performed to assess the taxonomic value of pollen grains for species characterization of *Teucrium*. It was also achieved to appraise these characters to the Libyan endemic species for the opportunity of their genetic resources conservation.

Material and Methods

Eleven taxa of *Teucrium* were collected from eleven locations in Libya, starting from Al-Gabal Al-Akhdar in the east to Gabal Naffusah (Garian) in the west, along 4500 sq. km. during two flowering seasons: 2009 and 2010. The studied species were sorted under three sections: Chamaedrys, Polium and Teucrium (Table 1). The voucher specimens are kept at the herbaria of Alexandria University (ALEX) and Omar EL-Mukhtar University (Libya).

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The samples were acetolyzed following Erdtman's technique (Erdtman, 1952). 1-3 specimens for each taxon were subjected in this work; at least 30 pollen grains per taxon were examined by using Zeiss light microscope with a micrometer eye-piece. For SEM, the anthers were transferred directly on a stub with double-sided tape, coated for 5 minutes with gold in a polaron JEC-1100E coating unit, and then photographed with JEOL JSM-5300 SEM (Faculty of Science, Alexandria University). The applied terminology based on Punt *et al.* (2007).

Table 1. *Teucrium* specimens used in the present study (sections after Siddiqi, 1985).

Section	Specimen number	Species	Coordinates
Chamaedrys	1-3	**T. barbeyanum* Asch. & Taub. ex E. A. Durand & Barratte	Shahhat Susah Coordinate pair: N32° 50°30.42 E21° 51°7.2
Polium	4-6	**T. apollinis* Maire & Weiller	
	7-8	*T. capitatum* L.	Tarhonah Coordinate pair: N32° 29°48.78 E13° 37°37.08
	9-11	**T. davaeanum* Coss.	Wadi El Quttarh Coordinate pair: N32° 01°35.82 E20° 24°45.48
	12-14	**T. lini-vaccarii* Pamp.	Quasser–El Quaar Coordinate pair: N32° 35°20.28 E13° 50°18.18
	15-17	*T. polium* L.	Sirut Coordinate pair: N31° 08°56.1 E16° 34°35.16
	18-19	*T. polium* subsp. *flavovirens* Batt.	Al Hameida escarpment Coordinate pair: N32° 24°52.98 E20° 32°17.88
	20-22	**T. zanonii* Pamp.	Dryannah Coordinate pair: N32° 19°42.12 E20° 16°34.86
Teucrium	23-25	*T. brevifolium* Schreb.	Lathroun–Ras El Hellal Coordinate pair: N32° 52 305'°0 E22° 15°6.12
	26-28	*T. campanulatum* L.	Wadi Errieg Coordinate pair: N32° 32 230°0 E20° 42°56.82
	29-30	*T. fruticans* L.	El Rabtta–El Assbeh Coordinate pair: N32° 07°12.6 E21° 52°16.14

*Endemic species.

Results and Discussion

The taxonomic complexity of *Teucrium* is reflected in the changes in its classification using different characteristics as pollen morphology (Díez *et al.*, 1993), karyology (Valdés-Bermejo and Sánchez-Crespo, 1978), indumentum characteristics (Manzanares *et al.*, 1983; El Oualidi and Puech, 1993; Navarro and EL Oualidi, 2000b), phytochemistry (Harborne *et al.*, 1986; Velasco-Negueruela and Pérez-Alonso, 1990; Bukhari *et al.*, 2014) and amino acid composition (Juani *et al.*, 2004). Meanwhile, there is an imminent danger for genetic erosion of Libyan wild species due to the increment in drought and anthropogenic activities, that has resulted in habitat loss and fragmentation leading to the diminishing of the germplasm reservoir (Al-Idrissi *et al.*, 1996). The

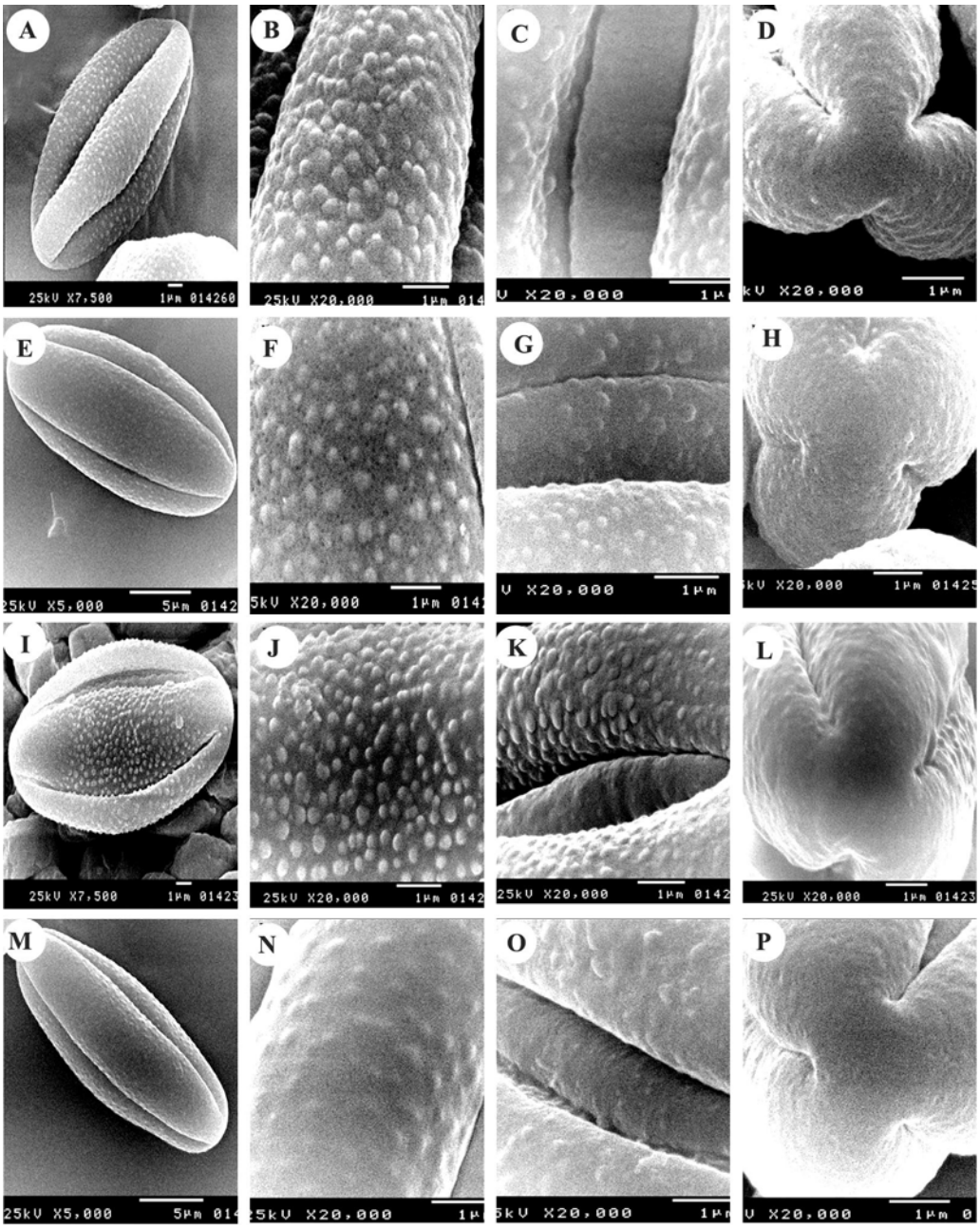

Fig. 1. Pollen grains of *Teucrium*: A–D, *T. barbeyanum*; E–H, *T. apollinis*; I–L, *T. capitatum*; M–P, *T. davaeanum*.

IUCN Red List of Threatened Plants (1998) recorded three endemic species; *T. apollinis, T. barbeyanum* and *T. zanonii* in addition *T. davaeanum* and *T. lini-vaccari* recorded by Marzouk *et al.* (2016). Consequently, the efforts must be intensified giving priority to study both endangered and endemic species through various characters.

In the present study, *Teucrium* pollen grains are monads, isopolar, radio symmetric, and tricolpate with opercula. The pollen shape is subprolate in *T. barbeyanum* (Fig. 1A), *T. campanulatum* (Fig. 3A) and *T. polium* subsp. *flavovirens* (Fig. 2L) and prolate in the rest of species. However, two shapes were recorded in *T. fruticans*; perprolate (form a) and prolate (form b) (Fig. 3I, O). That is in congruent with Ojeda and Díez (1992) for the recognition of the dimorphism phenomenon in this species. The polar axis length ranges from 30.02-31.2 µm in section Chamaedrys, from 30.41-37.01 µm in section Polium, and from 38.74-60.96 µm in section

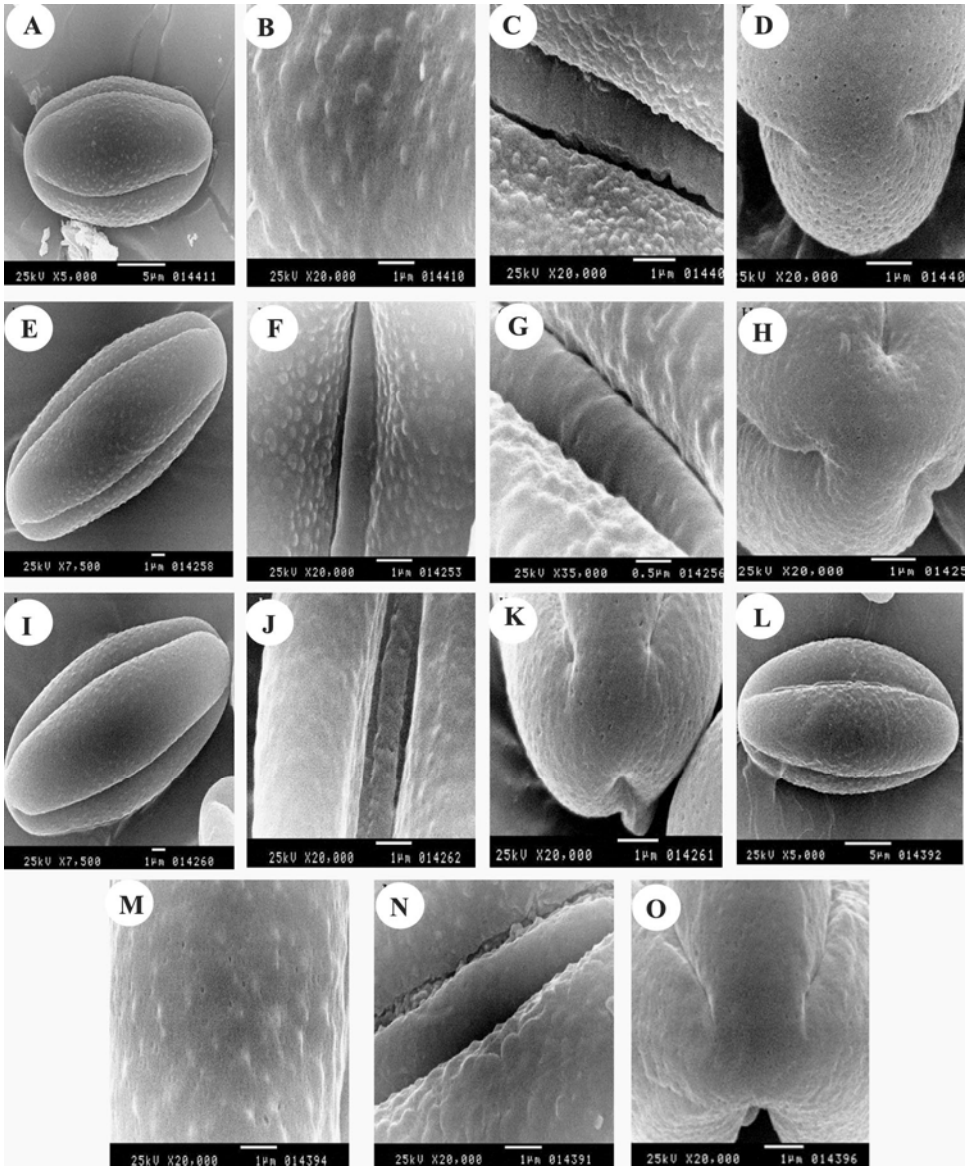

Fig. 2. Pollen grains of *Teucrium*: A–D, *T. lini-vaccarii*; E–H, *T. polium*; I–K, *T. polium* subsp. *flavovirens*; L–O, *T. zanonii*.

Teucrium (Table 2). The equatorial diameter varies from 23.47-28.28 µm in both sections Chamaedrys and Polium, and 19.63-39.23 µm in section Teucrium. Polar axis and equatorial diameter were found useful in separating two closely related taxa where it is large in *T. polium* than *T. polium* subsp. *flavovirens.* The largest colpus dimensions and mesocolpium diameter attain in section Teucrium while the smallest in section Polium (Table 2). The exine thickness assorts from 1.5-1.65 µm in *T. campanulatum* and the taxa of both sections Chamaedrys and Polium, while from 2.33-3.19 µm in the rest of section Teucrium (Table 2). Oybak-Dönmez and Inceoğlu (1988) and Dinç *et al*. (2008) specified *Teucrium* with either verrucate or verrucate-granulate exine sculpture, meanwhile Navarro *et al*. (2004) declared that the basal groups of *Teucrium* with verrucate sculpturing. The current study discriminates among the exine sculpture at the surface,

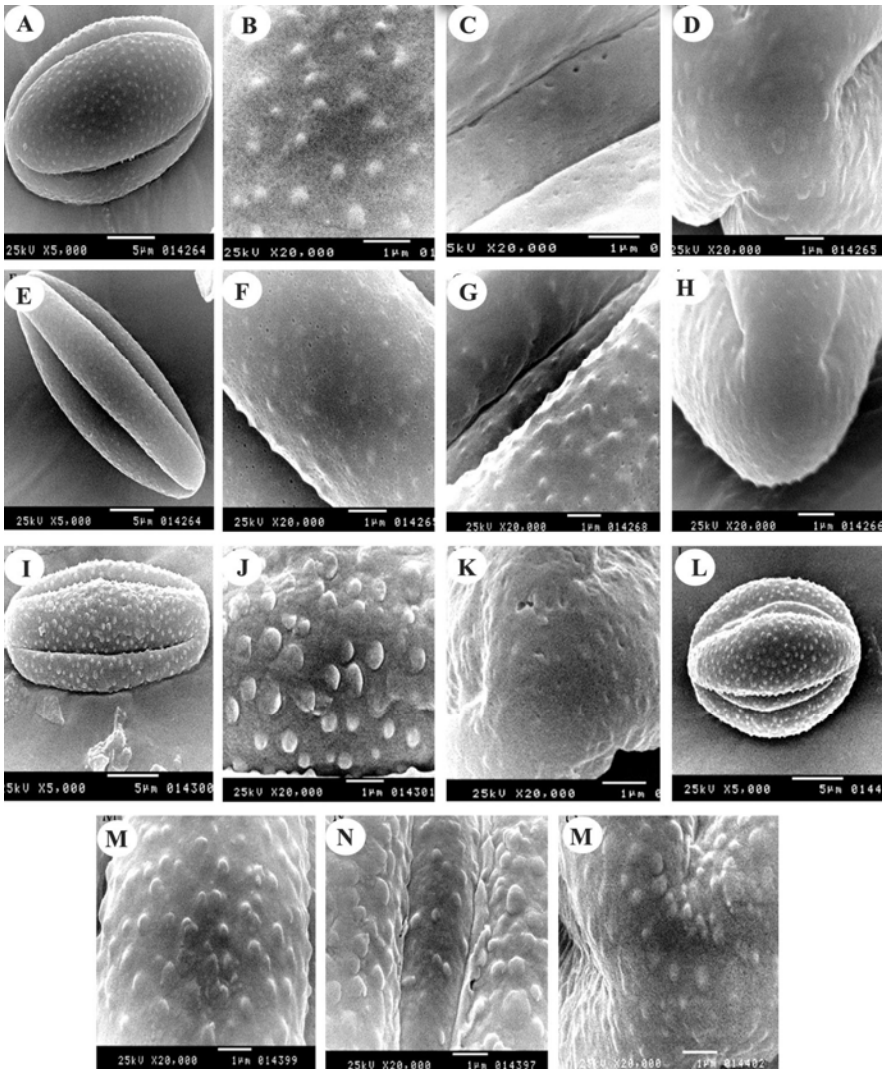

Fig. 3. Pollen grains of *Teucrium*: A–D, *T. brevifolium*; E–H, *T. campanulatum*; I–K, *T. fruticans* form a; L–O, *T. fruticans* form b.

Table 2. Pollen morphological characters for the examined taxa of *Teucrium*.

Section	Species	Polar axis "P" (µ m)	Equatorial diameter "E"(µ m)	P/E ratio	Shape	Colpus length (µ m)	Colpus width (µ m)	Meso-colpium (µ m)	Exine thick-ness (µ m)	Ornamentation		
										Surface	Operculum	Pole
Chamaedrys	*T. barbeyanum*	30.67[b]	24.75[a]	1.24[a]	Subprolate	23.11	3.80	21.85[a]	1.58	Verrucate-perforate	Perforate	Perforate
Polium	*T. apollinis*	34.87[a]	22.97[a]	1.52[e]	Prolate	23.65	3.16	20.14[a]	1.57	Verrucate-perforate	Verrucate- perforate	Perforate
	T. capitatum	34.11[a]	25.13[a]	1.36[a]	Prolate	24.45	3.87	21.46[a]	1.62	Verrucate decreased to scabrate towards the pole	Faint perforate	Faint perforate
	T. davaeanum	34.50[a]	24.06[a]	1.44[d]	Prolate	25.90	3.80	21.14[a]	1.58	Scabrate-verrucate and faint perforate	Faint perforate	Faint perforate
	T. lini-vaccarii	33.11[a]	24.35[a]	1.36[c]	Prolate	24.42	4.81	19.48[a]	1.60	Verrucate- perforate	Perforate	Perforate
	T. polium	36.30[a]	28.43[a]	1.28[b]	Subprolate	23.60	3.80	23.26[a]	1.58	Verrucate- perforate	Perforate	Perforate
	T. polium subsp. *flavovirens*	30.45[a]	24.83[b]	1.23[a]	Subprolate	22.5	3.99	16.84[a]	1.62	Scabrate-verrucate and faint perforate	Perforate	Perforate
	T. zanonii	36.98[c]	27.26[a]	1.36[a]	Prolate	24.93	3.92	23.17[a]	1.58	Scabrate-verrucate and perforate	Perforate	Perforate
Teucrium	*T. brevifolium*	43.17[e]	29.54[a]	1.49[a]	Prolate	31.50	3.88	23.75[a]	2.38	Verrucate- perforate	Perforate	Verrucate
	T. campanulatum	39.40[d]	32.20[d]	1.22[a]	Subprolate	27.54	4.81	24.35[a]	1.55	Scabrate-verrucate and perforate	Scabrate-verrucate and perforate	Faint perforate
	T. fruticans form a	42.77	19.63	2.18	Perprolate	30.13	4.85	20.11	2.40	Verrucate	Obscure	Scabrate-perforate
	T. fruticans form b	60.96	39.23	1.55	Prolate	41.73	7.14	25.09	3.19	Verrucate and faint perforate	Verrucate	Verrucate
	$LSD_{0.05}$	1.48	1.79	4.37	0.537	-----	-----	1.34	-----			

operculum and pole. In section Chamaedrys, the exine sculpture is verrucate-perforate at the surface and perforate at both operculum and pole (Fig. 1B, D). In section Polium, the verrucate-perforate surface sculpture accomplishes with *T. apollinis* (Fig. 1F), *T. lini-vaccarii* (Fig. 2B) and *T. polium* (Fig. 2F). While in *T. capitatum* (Fig. 1J, K) and *T. polium* subsp. *flavovirens* (Fig. 2J), the sculpture is verrucae to scabrate. Both *T. davaeanum* and *T. zanonii* specify with sculpture of scabrate-verrucate and faint perforate (Fig. 1N) and scabrate-verrucate and perforate (Fig. 2M), respectively. The faint perforate sculpture at both operculum and pole realizes in both *T. capitatum* (Fig. 1K, L) and *T. davaeanum* (Fig. 1O, P), while *T. apollinis* with verrucate-perforate at the operculum (Fig. 1K) and perforate in the rest of species. In section Teucrium, each species attains certain sculpturing, *T. brevifolium* reveals verrucate-perforate, perforate, and verrucate at the surface, operculum and pole, respectively (Fig. 3B, D). In *T. campanulatum*, the sculpture is scabrate-verrucate and perforate at both surfaces and operculum and faint perforate at the pole (Fig. 3F, H). *Teucrium fruticans* accomplishes sculpture dimorphism, the verrucate or verrucate and faint perforate at the surface (Fig. 3J, M), obscure or verrucate at the operculum (Fig. 3I, N), and scabrate-perforate or verrucate at the pole (Fig. 3K, O). The results indicated the validity of pollen characters for taxonomic implications and in the discrimination among *Teucrium* at both section and species levels.

References

Abdel Khalik, K. 2016. A systematic revision of the genus *Plectranthus* L. (Lamiaceae) in Saudi Arabia based on morphological, palynological, and micromorphological characters of trichomes. American Journal of Plant Sciences **7**: 1429–1444.

Abu-Assab, M.S. and Cantino, P.D. 1992. Pollen morphology in subfamily Lamioideae (Lamiaceae) and its phylogenetic implications. *In*: Harley, R.M. and Reynolds, T.(Eds), Advances in Lamiaceae Science. Royal Botanic Gardens, Kew, pp. 97–122.

Al-Idrissi, M., Sbeita, A., Jebriel, A., Zintani, A., Shreidi, A., Ghawawi, H. and Tazi, M. 1996. Libya: Country report to the FAO international technical conference on plant genetic resources. Leipzig, Germany.

Blanca, G., Cueto, M. and Fuentes, J. 2017. *Teucrium teresianum* sp. nov. (Lamiaceae) from southern Spain. Nordic J. Bot. **35**(1): 14–19.

Bukhari, N.A., Al-Otaibi, R.A. and Ibrahim, M.M. 2014. Biodiversity characteristics of *Teucrium polium* species in Saudi Arabia. Saudia J. Biol. Sci. **22**(2): 181–185.

Díez, M.J., Ojeda, F. and Colomer, M. 1993. Contribución a la palinologı a del genero *Teucrium* L. en la Penınsula Iberica e Islas Baleares y suinteretaxonmico. Lagascalia **17**: 119–134.

Dinç, M. and Ozturk, M. 2008. Comparative morphological, anatomical, and palynological studies on the genus *Stachys* L. sect. Ambleia Bentham (Lamiaceae) species in Turkey. Turkish J. Bot. **32**:113–121.

Dinç, M., Duran, A., Pinar, M. and Ozturk, M.2008. Anatomy, palynology and nutlet micromorphology of Turkish endemic *Teucrium sandrasicum* (Lamiaceae). Biologia **63**: 637–641.

El Oualidi, J. and Puech, S. 1993. Quelques marqueurs morphologiques des *Teucrium* section Polium (Lamiaceae) du Maroc: valeurs diagnostiques à différents niveaux d'integration. Acta Botanica Malacitana **18**:163–171.

Erdtman, G. 1952. Pollen Morphology and Plant Taxonomy. Angiosperms. Almqvist and Wiksell, Stockholm, pp. 539.

Harborne, J.B., Tomas-Barberan, F.A., Williams, C.A. and Gil, M.I. 1986. A chemotaxonomic study of flavonoids from European *Teucrium* species. Phytochemistry **25**: 2811–2816.

IUCN Red List of Threatened Plants. 1998. Walter, K.S. and Gillett, H.J. (Eds),1997. IUCN Compiled by the World Conservation Monitoring Centre. IUCN-The World Conservation Union, Gland, Switzerland and Cambridge, UK, pp. 862.

Juani, R., Pastor, J., Milla, F., Alaiz, M. and Vioque, J. 2004. Amino acids composition of *Teucrium* nutlet proteins and their systematic significance. Ann. Bot. **94**: 615–621.

Manzanares, P., Gómez-Campo, G. and Tortosa, M.E. 1983. Estudiossobre el indumento de las especiesibéricasy baleáricas del género *Teucrium* L. (Lamiaceae). Anales del Jardín Botánico de Madrid **40**: 94–106.

Marzouk, R.I., El-Darier, S.M. and Askar A.M. 2016. Nutlet micromorphological characters of *Teucrium* taxa (Lamiaceae) in Libya. Phytotaxa **263**(3): 245–254.

Navarro, T. and El Oualidi, J. 2000a. Sinopsis of *Teucrium* L. (Labiatae) in the Mediterranean region and surrounding areas. Flora Mediterranea **10**: 349–363.

Navarro, T. and El Oualidi, J. 2000b. Trichome morphology in *Teucrium* L. (Labiatae), a taxonomic review. Anales del Jardín Botánico de Madrid **57**: 277–297.

Navarro, T., El Oualidi, J. and Trigo D.M. 2004. Pollen morphology of *Teucrium* (Labiatae) and its taxonomic value. Belgian J. Bot. **137**(1): 70–84.

Ojeda, F. and Díez, M.J. 1992. Pollen dimorphism in three *Teucrium* species (Lamiaceae). Plant Syst. Evol. **183**: 43–49.

Oybak-Dönmez, E. and Inceoğlu, Ő. 1988. Pollen morphology of some *Teucrium* L. (Labiatae) species. Communications Faculty of Sciences University of Ankara Series C: Biology **6**: 133–146.

Oybak-Dönmez, E., Inceoğlu, Ő. and Pinar, N.M. 1999. Scanning electron microscopy study of pollen in some Turkish *Teucrium* L. (Labiatae). Turkish J. Bot. **23**: 379–382.

Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S. and Le Thomas, A. 2007. Glossary of pollen and spore terminology. Review of Palaeobotany & Palynology **143**: 1–81.

Radulović, N., Dekić, M., Joksović, M. and Vukićević, R. 2012. Chemotaxonomy of Serbian *Teucrium* species inferred from essential oil chemical composition: the case of *Teucrium scordium* L. ssp. *scordioides*. Chemistry and Biodiversity **9**:106–122.

Siddiqi, M.A. 1985. 3: *Teucrium. In*: Jafri, S.M.H. and El-Gadi, A. (Eds), Flora of Libya. Al Faateh University, Faculty of Science, Department of Botany, Tripoli, pp. 13–30.

Valdés-Bermejo, E. and Sánchez-Crespo, A. 1978. Datos cariologicos sobre el genero *Teucrium* L. (Labiatae) en la Peninsula Ibérica. Acta Botánica Malacitana **4**: 27–54.

Velasco-Negueruela, A. and Pérez-Alonso, M.J. 1990. The volatiles of six *Teucrium* species from the Iberian Peninsula and the Balearic Islands. Phytochemistry **29**: 1165–1169.

Yasaman, S., Stefan, K., Günther, H. and Christian, B. 2016. Phylogeny of non-monophyletic *Teucrium* (Lamiaceae: Ajugoideae): implications for character evolution and taxonomy. Taxon. **65**(4): 805–822.

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