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COMPARATIVE MORPHOLOGICAL, ANATOMICAL AND PALYNOLOGICAL STUDIES ON *TRIGONELLA* (FABACEAE) IN KHORASSAN RAZAVI PROVINCE (NE IRAN)

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Abstract

Morphological, anatomical and palynological characters of five species of *Trigonella* growing in Khorassan Razavi province were examined. In morphological study, the differences of vegetative and floral features were assessed. Then cluster analysis was done using UPGMA to recognize the affinity between species. Moreover PCA analysis was carried out to identify species diversity and valuable features for species identification. For anatomical research, the cross sections of the stems and leaflets were prepared and stained by differential staining. In palynological study, the pollen were extracted, acetolysed and observed by SEM. The results of cluster analysis showed circumscription between species and sections. Moreover PCA analysis indicated species diversity and useful traits for identification. The anatomical results displayed some variations in vessel arrangement and cell type of xylary fiber in stem between species. Furthermore, pollen ornamentation was variable among species and helped to identify them.

Introduction

The genus *Trigonella* L. belongs to Fabaceae Lindl. which distributed in west Asia, Europe, north and south Africa, north America, Australia and China (Mabberly 1977, Rechinger 1984, Federov 1987, Davis 1989, Chase and Reveal 2009). This genus comprises around 32 species in Iran which six species growing in NE Iran (Khorassan Razavi province) that belong to the sections *Buceratos* Boiss., *Falatulae* Boiss., *Verae* Sirj. and *Reflexae* Sirj. (Rechinger 1984, Mousavi and Khosravi 2010).

Besides morphological characters, anatomical and palynological features were evaluated to identify variations among species. In previous study, anatomical characters have been compared between two species of *Trigonella* (Ranjbar *et al.* 2012). The previous palynological studies were done on the pollen of *Trifolieae* tribe and some species of *Trigonella* (Gazar 2003, Taia 2004, Lashin 2006). Due to deficiency of information of anatomical and palynological features of Iranian *Trigonella*, biosystematical studies were done on five species distributed in NE Iran for recognition of different characters among species and their role in identification of species and sections circumscription.

Materials and Methods

Five taxa of *Trigonella* including *T. orthoceras* Kar. and *T. monantha* subsp. *monantha* Meyer from sect. *Bucerates*, *T. foenum- graecum* L. from sect. *Falatula*, *T. grandiflora* Bunge. from sect. *verae* and *T. monspeliaca* L. from sect. *Reflexae* were examined. Most samples of *Trigonella* were prepared either from fresh materials collected in the field of Khorassan Razavi province during May - July, 2011 (Table 1) or from dry specimens kept in FUMH. Voucher specimens were deposited in Islamic Azad University, Mashhad branch herbarium (IAUM). The

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morphological characters, like life form, presence trichomes on stems, the shape of stipules, leaflet, legume, floral segments and length of calyx and corolla were assessed among species. Cluster analysis was done based on vegetative and floral features by NTSYS software ver. 20.02 using UPGMA method. Also, for identification of valuable morphological characters used for taxonomy, PCA analysis was carried out by CANOCO software ver. 4.

Table 1. The locality of studied *Trigonella* species.

Species	Locality
T. monantha subsp. *monantha*	Taibad to Dogharou, 700 m, 18247, FUMH; Mashhad, Pardis, 1040 m, 37710, FUMH; Between Mazdavand- Sarakhs, 650 m, 35003, FUMH; Kalate Naderi to Darchangan, 1100 m, 28937, FUMH; Between Mashhad- Moghan, north of Sarborj, 1450 m, 21986, FUMH; Chenaran, between Abghad- Ferizi, 1300 m, 9051, IAUM; Gonabad, Sar Asiab, 1800 m, 9052, IAUM.
T. orthoceras	Mashhad, Kang mountains, 2100 m, 20990, FUMH; North of Quchan, Aslameh, 2000 and 2300 m, 9055 and 34742, IAUM and FUMH.
T. foenum-graecum	Mashhad, Kardeh village, 1100m, IAUM.
T. monspeliaca	South of Dargaz, Ghazah Ghoz mountains, 1450 m, 16437, FUMH; Dargaz, Tandoureh, between Shekarabad- Chehel mir, 2300 m, 20490, FUNH; Mashhad, Kalat, 13450 m, 9045, IAUM.
T. grandiflora	South of Saraks, Khatoun bridge, 900 m, 31862, FUMH; 9 km Sarakhs-Mashhad, 284 m, 13987, FUMH; Chenaran, Ardakan village, 1500 m, 9053, IAUM

For anatomical study, cross sections were made from the base of stems and middle part of leaflets. They were stained by green methyl and carmine, then photographed by different magnification of light microscopy (LM) ZEISS model 1.25X, CONTAX camera model 167MT. Anatomical characters such as shape of vascular bundles, arrangement of vessels, number of epidermis and cortex layer in stem and mesophyll type in leaflet, were analyzed.

For the palynological study, the pollen grains of three species were extracted from the anther, acetolised and studied by SEM at magnification 5000 and 20000 (Erdtman 1966, Moore *et al*. 1991). The characters such as P (polar axis length), E (equatorial axis length), P/E ratio and pollen ornamentation were assessed. The electro-micrographs were obtained with a Leo-1455 vp scanning electron microscope. The pollen terminology in general followed Punt *et al.* (2007).

Results and Discussion

The morphological study showed significant variations between species in terms of vexilum, wing and keel shape and apex, the shape of stipule, legume and legume nerves, life form and calyx length/corolla length. The shape of vexilum were observed cordate - obovate in *T. orthoceras* and *T. foeunm-graecum* though obovate in the others. Vexilum apex in *T. monantha* subsp. *monantha*, *T. grandiflora* and *T. monspeliaca* was obtuse while in *T. foenum-graecum* and *T. orthoceras* was emarginate. Wing apex was acute in *T. orthoceras*, *T. grandiflora* and *T. monantha* subsp. *monantha*, obtuse in *T. foenum-graecum* and retuse in *T. monspeliaca*. Legum nerves in *T. monantha* subsp. *monantha*, *T. foenum-graecum* and *T. orthoceras* were parallel while in *T. monspeliaca* and *T. grandiflora* were reticulate and oblique, respectively. Stipules shape was very different for example in *T. monantha* subsp. *monantha*, *T. orthoceras* and *T. monspeliaca* were almost sagittate. Also in *T. foenum-graecum* and *T. grandiflora* were hairy triangular- lanceolate and hairy lanceolate, respectively (Table 2). Cluster analysis of morphological characters showed species distinct circumscription. *T. foenum-graecum* posed far from the others. *T. grandiflora* was

separated from *T. monantha* subsp. *monantha*, *T. monspeliaca* and *T. orthoceras* due to difference of leaf width, corolla color, calyx hairs and the shape of leaflet margin. *T. monspeliaca* differed from *T. monantha* subsp. *monantha* and *T. orthoceras* because difference of legume nerves, stipule shape. Moreover, *T. monantha* subsp. *monantha* was separated from *T. orthoceras* due

Table 2. Morphological features of studied *Trigonella* species.

Species	Vexilum shape	Wing apex	Legume nerves	Stipule shape	Calyx length/ corolla length
T. monantha subsp. *monantha*	Obovate-obtuse	Acute	Parallel	Sagittate-dentate	1
T. orthoceras	Cordate-obovate, Emarginated	Acute	Parallel	Sagittate	<1
T. foenum-graecum	Cordate-obovate, Emarginated	Obtuse	Parallel	Hairy triangular -lanceolate	1>
T. monspeliaca	obovate-obtuse	Retuse	Reticulate	Sub sagittate	1<
T. grandiflora	obovate-obtuse	Acute	Oblique	Hairy lanceolate	1>

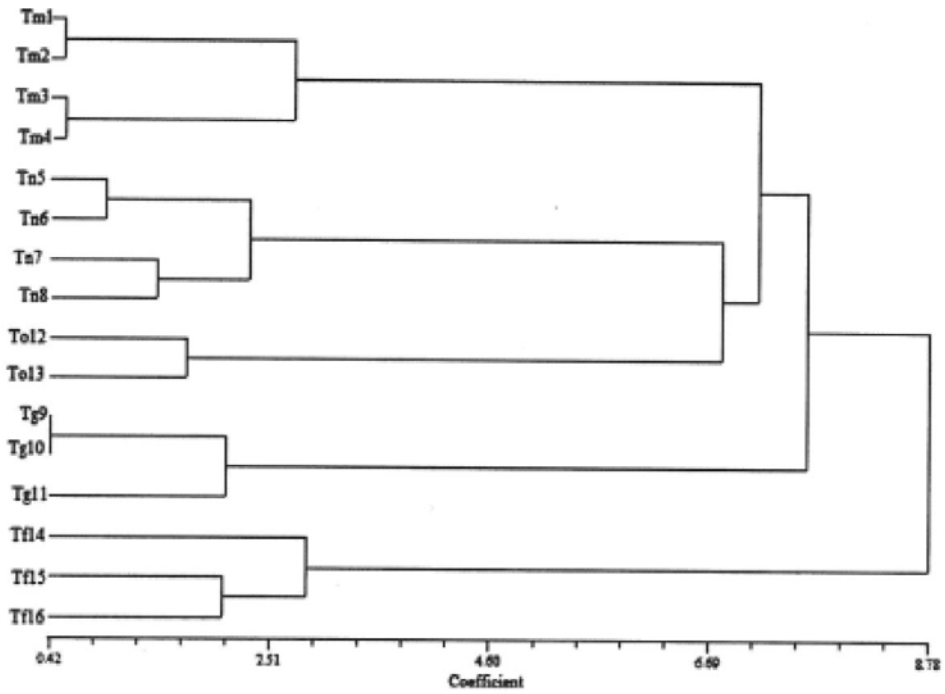

Fig. 1 A. Drawn dendrogram of cluster analysis based on morphological characters.

Tm1: *T. monspeliaca* (1, 2: Mashhad, Kalat, 3, 4: Mashhad, Zosk.). Tn: *T. monantha* subsp. *monantha* (5: Chenaran between Abghad and Ferizi, 6, 7, 8: Gonabad, Sarasiab). Tg: *T. grandiflora* (9, 10, 11: Chenaran, Radkan). To: *T. orthoceras* (12: Mashhad, Kang; 13: North of Quchan, Aslameh). Tf: T. *foenum- graecum* (14, 15, 16: North of Torbat Heidarieh, Khomari pass).

to difference in seed size, life form, the shape of corolla segments and leaflet and calyx hairs. PCA analysis indicated valuable features to identify species like life form, legum and stipule shape, calyx length/corolla length, stem and legume hairs. Moreover, distribution of specie showed no overlapping (Figs 1A-B).

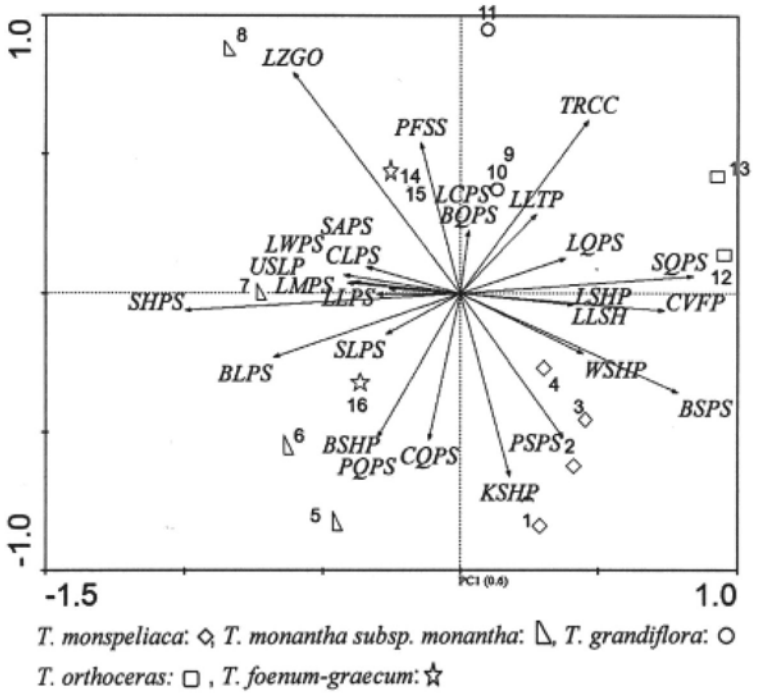

Fig. 1 B. Diagram of PCA analysis showing important traits for identification and species diversity.

LZGO: Legume shape, PFSS: Habit, TRCC: tooth length/ tube length in corolla, LLTP: Leaflet apex, LCPS: Legume curving, BQPS: Bracts trichoms, BLPS: bracts length, SQPS: Stem trichomes, CLPS: Calyx length, USLP: Trichoms on the abaxial side of leaflet, LLPS: leaf length, LWPS: leaf width, LMPS: Leaflet margin. SHPS: Stem length, SAPS: Seed width, SLPS: Seed length, PQPS: Stipule trichomes, PSPS: Stipule shape, LLSH: Leaflet shape, WSHP: Wing apex, KSHP: Keel shape, BSHP: Vexilum shape, CQPS: Calyx trichom, BSPS: Bract shape, CVFP: Legum coat nerves, LSHP: Legum length, LQPS: Legum trichom.

Anatomical results of stem, revealed discontinuous sclerid above the phloem, radial chain pore- solitary vessel arrangement in *T. orthoceras*, radial chain pore in *T. monantha* subsp. *monantha*, *T. foenum-graecum* and *T. monspeliaca*, and radial chain pore- cluster in *T. grandiflora*. Just gelatinous fiber was observed in xylary fiber of *T. monantha* subsp. *monantha.* Moreover, the cortex thickness in *T. monantha* subsp. *monantha* was less than the others. Xylem bundles were compact- wide and lax- narrow in *T. monantha* subsp. *monthana* and *T. foenum-graecum* respectively (Figs 2A-E). In leaflets cross section of studied species, mesophyll was observed dorsi-ventral (Fig. 2F). According to anatomical results, just vessel arrangement was variable among species. Presence of gelatinous fiber in *T. monantha* subsp. *monantha* stem confirmed this species was adapted to dry habitate. On the other hand Ranjbar *et al* reported, the difference were observed between stem cross sections of *T. elliptica* and *T. yasujensis.* The outline of stem transverse section and type of parenchyma tissue in peduncle were pentagonal and spongy in *T. yasujensis* while in *T. elliptica* were sinuate and palisade- spongy respectively (Ranjbar *et al.* 2012).

Palynological results displayed pollen in *T. foenum-graecum* and *T. monantha* subsp. *monantha* were prolate- sub prolate and oblong spherical in *T. orthoceras*. High variation was observed in pollen ornamentation i.e. echinate- porate in *T. orthoceras,* porate in *T. monantha* subsp. *monantha*, reticulate in *T. foenum-garecum.* Also, Taia has reported oblong- spherical and granulate pollen in *T. monspeliaca* (Taia 2004). Moreover, *T. foenum- graecum* and *T. orthoceras* had the maximum and minimum pollen size (Figs 3A- F, Table 3). Indeed, pollen ornamentation

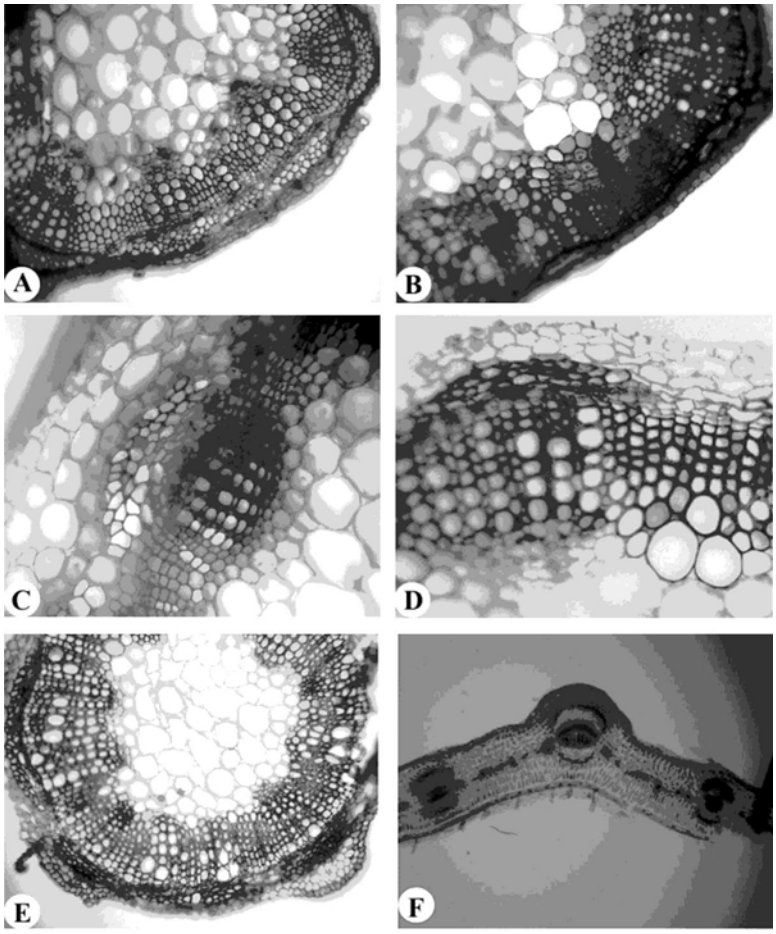

Fig. 2. Cross section of stems. (A) radial chain pore-solitary vessel arrangement in *T. orthoceras* (B-D) radial chain pore in *T. monantha* subsp. *monantha*, *T. foenum-graecum* and *T. monspeliaca*. (E) radial chain pore- cluster in *T. grandiflora*. The arrow showing gelatinous fiber. (F) Dorsi-ventral mesophyll in leaflet cross section of *T. orthoceras*.

was useful character to identify species. According to Taia's report, *T. arabica* Del., *T. anguina* Del, *T. anguina* Del., *T. stellata* Forssk. had aperture with soft margin while *T. hamosa* L., *T. maritime* Del., *T. laciniata* L., *T. monspeliaca* L. had granulate margin. Pollen ornamentation was reticulate in *T. arabica*, *T. occulata* Del. ex DC., *T. stellata*, *T. laciniata, T. maritime*, granulate in *T. hamosa*, *T. mospeliaca* and granulate- porate in *T. anguina* (Taia 2004). Based on Lashin's report, pollen of *T. foenum graecum* was prolate, tricolporate and reticulate which these characters

were similar to the present research results except pollen shape in polar view. In the present study the shape of pollen reported prolate or sub-prolate pollen. He pointed *T. stellata* had sub prolate, reticulate pollen too (Lashin 2006).

Table 3. The details of studied *Trigonella* pollen.

Species	P/E	Meso-colpium length	Ornamentation
T. monantha subsp. *monntha*	1.17	24	Porate
T. orthoceras	1.03	32	Echinate-porate
T. foeunum-graecum	1.35	36	Reticulate
T. monspeliaca	1.12	-	Granulate

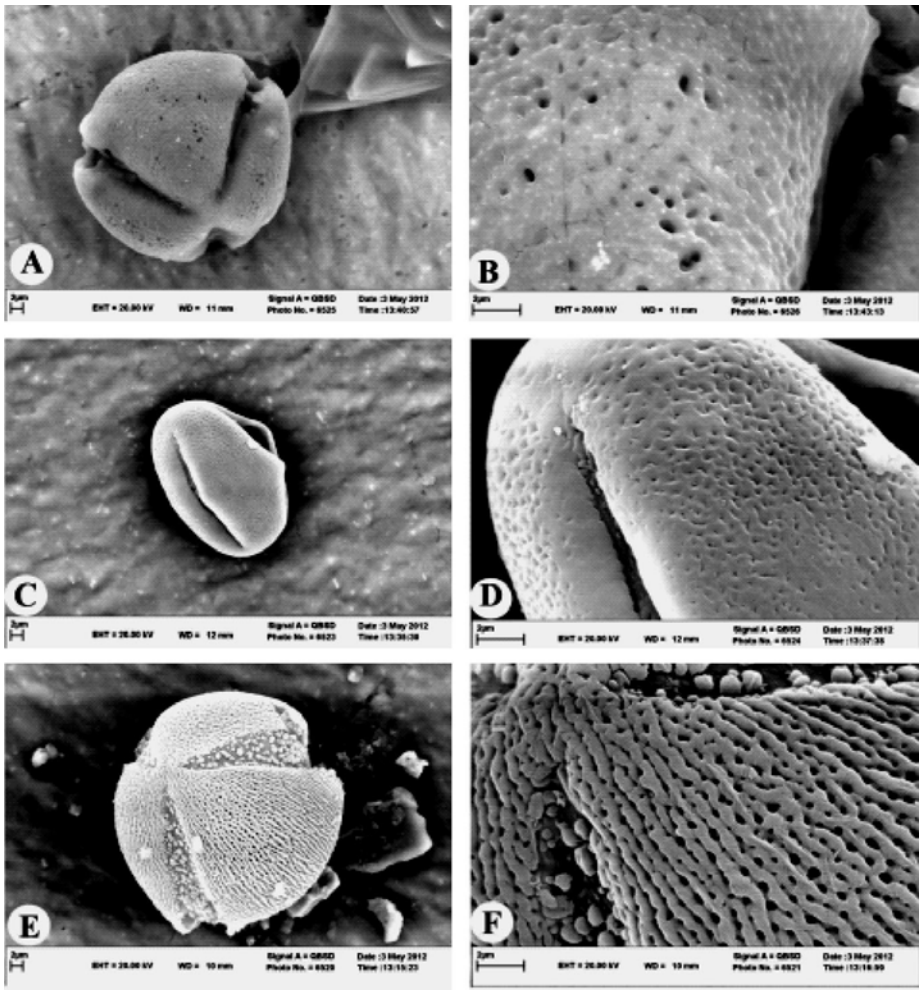

Fig. 3. The electro-micrograph of pollen ornamentation. (A,B) echinate-porate in *T. orthoceras* ×5000, 20000. (C,D) perforate in *T. monantha* subsp. *monantha* ×5000, 20000. (D,E) reticulate in *T. foenum-graecum* ×5000, 20000.

Based on above results, morphological characters and pollen ornamentation helped to identify species and their circumscription.

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